

Innovation of Food Products in Halal Supply Chain Worldwide



Edited by
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Aishah Bujang



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Preface

Innovation of Food Products in Halal Supply Chain Worldwide deals with an area of vast interest in scientific development and adaptation to halal, especially with new halal compliant and sustainable food products towards a wholesome halal supply chain. Halal products innovation is an exciting and rare scope of halal that fills a major gap in the literature. Among the chapters are newly developed food ingredients, which depict substitutions of non-halal ingredients with halal alternatives; advances in halal supply chain; traceability from farm to fork, and beyond; and incorporation of sustainability in halal food supply chain.

Are there actually enough halal supplies to meet the world's demand? There are plentiful new products and systems innovations that are Muslim-friendly but are scarcely commercialized nor highlighted in the mass. The focus of this book is to address the issues behind controversial halal ingredients, to know the advancement of available halal substitutes and replacers, to encourage the manufacturing of highly potential product to cater the world mass halal demand, and to enhance product sustainability and food security.

A key feature of this book will be that all the chapters have been written by acknowledged experts in their field; thus the book will bring together the top researchers in this essential topic of importance to a huge percentage of the world's population.

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Section 1

Introduction

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Chapter 1

Overview on halal issues

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1.1 Halal issues: an introduction

Halal industry is rapidly growing due to the increased demand for halal products and services (Azam & Abdullah, 2020; Farouk, 2013; Mumini, Veek, Luqmani, Quraeshi, & Kamarulzaman, 2018). There is also evidence to suggest that the demand for halal products by non-Muslims is growing too due to their perception of halal foods as being safer and more natural compared to counterparts' products (Bashir, 2019). DinarStandard (2019) in their report on the state of the global Islamic economy estimated that “Muslims spent US\$2.2 trillion in 2018 across the food, pharmaceutical and lifestyle sectors that are impacted by Islamic faith-inspired ethical consumption needs. This spending reflects a healthy 5.2% year-on-year growth and is forecasted to reach US\$3.2 trillion by 2024 at a Cumulative Annual Growth Rate of 6.2%.” This growth rate is impressive, because not so long ago, when halal industry is mentioned what comes to the minds of many was only the supply of meat and meat products. Today the term halal industry has gone beyond meat and food to pharmaceutical, cosmeceutical, fashion, tourism and leisure/recreation, and their associated and support industries such as logistics, transport, analytical, and finance services. According to DinarStandard (2019), this rapid growth in halal industry is fueled by the growth in the population, affluence, and the adherence of Muslims to Islamic teachings; increased digital connectivity and ethical consumerism; multinational and Islamic countries drive for economic growth; and greater awareness and enforcement of standards and certification of halal products and services. The growth and complexity of halal industry is not without its issues (see Fig. 1.1 for how complex the industry has become using just one halal research program of one research institute representing a tiny fraction of researchable issues). Many of these issues are being tackled as they arise with varying speed of implementations, using experiences from counterpart nonhalal industries, especially where the issues do not impact on the core halalness of products and services such as in management, logistics, and procurement. There are, however, halal industry issues that are long-standing, and others emerging, which require concerted and strategic effort to be resolved or avoided. These are grouped under the following categories: (1) production; (2) authentication and certification; (3) ownership and control; (4); training and capacity building; and (5) antihalal groups and economic Islamophobia.

1.2 Production-related issues

There are many issues around production impacting on the halal industry presently and for the foreseeable future. Some of these issues are briefly discussed under the following subsections.

1.2.1 Raw materials and ingredients

The production of the major raw materials requiring certification or halal assurance such as meat and associated products is largely in the hands of countries where Muslims are in minority, with the majority Muslim countries as net importers (Farouk, Pufpaff, & Amir, 2016). The situation is similar for other raw materials such as milk, cereals, pulses, vegetables, and related products, which are considered halal in their natural forms without further processing. This issue is very important as it affects the food security of halal consumers in general. The situation might have prompted the recent establishments of the Islamic Organization for Food Security (IOFS), a specialized institution of the Organization of Islamic Cooperation (OIC), created to “ensure sustainable food security in the OIC countries through

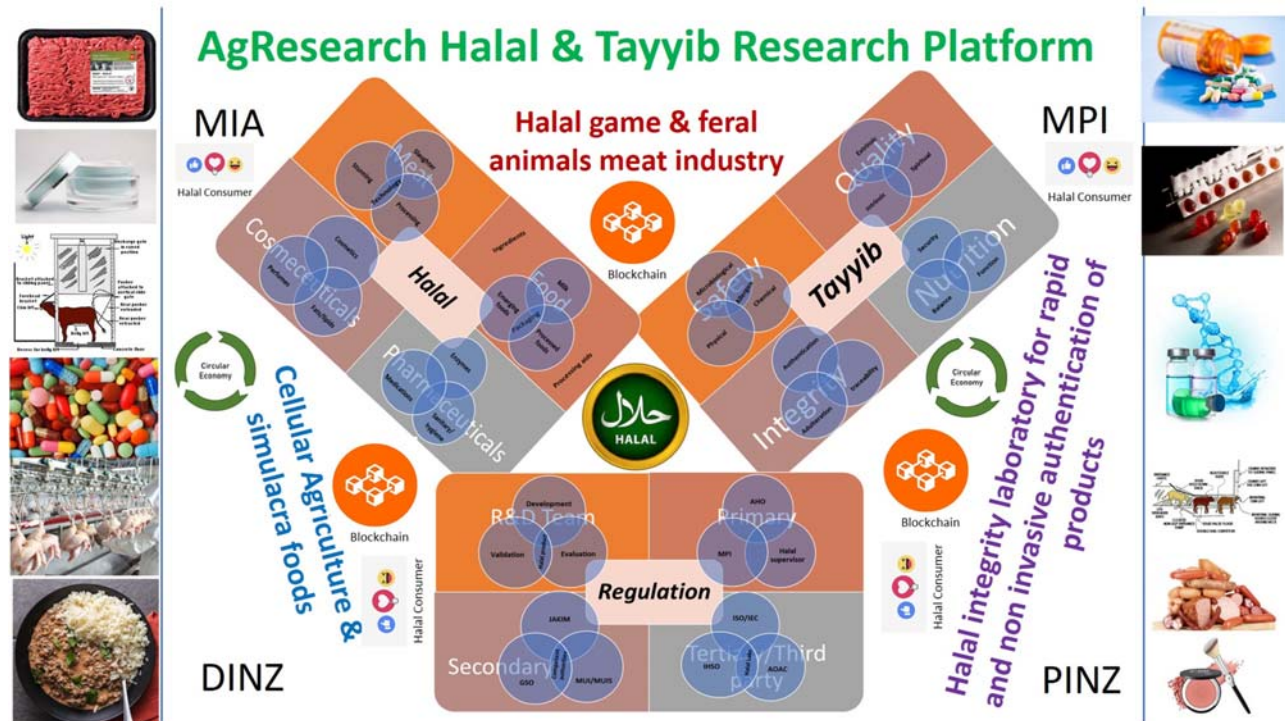


FIGURE 1.1 AgResearch Ltd, New Zealand Halal research program briefly showing the multifaceted nature of Halal and Tayyib research requirements even for a small program in a single institute (Farouk, 2022).

socio-economic development and systemic promotion of targeted programs related to agriculture, science and technology, humanitarian aid, trade and food export to the IOFS/OIC nations” (Salaamgateway, 2021). The food security of halal consumers is so important that failure to guarantee it may render the whole halal industry meaningless, especially when one considers the fact that necessity, for example, starvation, removes prohibitions and the need to consume halal (Farouk et al., 2015).

1.2.2 Preslaughter stunning in halal meat production

One of the most divisive issues in the halal industry is that of preslaughter stunning of animals and poultry in the industrial production of halal meat. The controversy pitches the proponents and opponents of stunning within and outside the Muslim halal certifiers and consumers against each other and against those who view the whole issue purely from animal welfare perspective. This has been highlighted in Farouk et al. (2014) where it was stated that “a major area of contention is the slaughter of animals without stunning prior to throat slit and exsanguination — a practice allowed in many countries, but extremely controversial with regard to animal welfare due to concerns about the stress of restraining the animal, whether the cut is painful, and whether the animal experiences undue distress while bleeding out, such as the aspiration of blood into the lungs.” A way out of this controversy has been suggested to require the harmonization of spiritual views with those that are temporal or corporeal and which are based on scientific evaluation and sincere dialog between proponents and opponents of the use of stunning in halal meat production (Farouk et al., 2014). Harmonization here does not mean accepting any process that is not halal but is about accepting the use of scientific methods where those methods do not compromise halal requirements in any ways.

1.2.3 Cellular and simulacra agriculture technologies

The production of raw materials synthetically sometimes referred to as cellular agriculture, cell-cultured meat, clean meat or in vitro meat, or food made in the laboratory, food made without farms, and almost without animals, is growing fast and companies developing these types of foods are receiving huge financial investments by rich investors and traditional food companies such as Tyson Foods and Cargill and even governments such as China (Small, 2018). These

disruptive technologies will alter the ways raw materials are produced and the ingredients that could be produced from these materials. The possibilities arising from cellular agriculture could create opportunities for Islamic countries that are presently dependent on imports for most of their foods to reduce their dependencies by adopting cellular agricultural technologies in its infancy before dominance in the field is established elsewhere. Doing that will first require ascertaining these technologies meet the spiritual and other ethical considerations pertaining to halal. This is easily resolved considering that in a comprehensive review covering various aspects of in vitro meat, [Bhat, Morton, Mason, Bekhit, and Bhat \(2019\)](#) surmise that because the production of the meat does not involve any slaughtering of animals, then as long as the initial source of the cells used in the production/culturing of the meat is halal, the meat developed from the process will be halal. The same principles apply to other cultured foods such as milk and other animal and seafood products. Recent surveys indicate consumer resistance to simulacra products due to what is known as the “yuck factor.” Whether or not the yuckiness of the products of cellular agriculture or the fact that the cells used in the production could be harvested from a living animal affect the halalness of these products in some instances should be a subject for scholars to tease out before these products become more common in the markets.

1.2.4 Halal workers’ work and wellness

An important issue in the halal industry that is hardly discussed is the work nature and wellness of halal workers particularly those involved in the primary production of halal foods such as the slaughter of animals for food. Internationally very little research has been done on slaughtermen and their wellness specifically ([Leibler, Janulewicz, & Perry, 2017](#); [Victor & Barnard, 2016](#)). Slaughterhouses present a work context with an undercurrent of violence (due to animal slaughter), persistent trauma, stringent and monotonous production routines, health hazards, and physical strain ([van Holland, Soer, de Boer, Reneman, & Brouwer, 2015](#)).

[Dillard \(2008\)](#) investigated the psychological harm suffered by slaughterhouse employees, including slaughtermen, and demonstrated that slaughterhouse workers likely suffer serious psychological trauma in their workplace. A study investigated the prevalence of serious psychological distress among slaughterhouse workers and found that workers in a US slaughterhouse experienced high rates of occupational injury, as well as stressful work conditions, and higher prevalence of serious psychological distress ([Leibler et al., 2017](#)). Data from studies conducted in the United States and other countries show that due to the physically demanding and monotonous nature of slaughterhouse work, the work environment is characterized by high staff turnover, absenteeism, and disciplinary actions ([Victor & Barnard, 2016](#)). The work is inherently dangerous, and, as workers handle dangerous cutting tools at extreme production speeds, slaughterhouses have some of the highest reported injury rates in the manufacturing industry, with injury rates as high as 20%–36% per annum, with associated ailments like carpal tunnel syndrome, “trigger finger,” back problems, tendonitis sprains, cuts, punctures, back pain “white finger” and musculoskeletal disorders such as “claw hand,” ganglionic cysts, bursitis, and arthritis ([Broadway & Stull, 2006](#); [Dillard, 2008](#); [Human Rights Watch, 2004](#); [Victor & Barnard, 2016](#)). Slaughterhouse work poses a risk to the psychological well-being of employees and cases of cumulative trauma disorder have been reported ([Dalla, Ellis, & Cramer, 2005](#)). Often slaughterhouse employees lack adequate resources to cope with the strenuous environment because of their poor socioeconomic background, lack of training, and the shortage of safety equipment at the site ([Human Rights Watch, 2004](#)).

A key aspect of personal wellness in the workplace is a condition called burnout, which is a work-related syndrome that is characterized by emotional exhaustion (i.e., a state of energy draining), cynicism (i.e., a sense of disengagement and gradual loss of concern about the contents or the recipients of one’s work), and reduced professional efficacy (i.e., feelings of incompetence). Internationally, there are very little data available on burnout among halal slaughtermen. A recent survey of halal meat workers ([Botha, Farouk, & Upsdell, 2019](#)) found that the majority (78%) of respondents said they felt a strong spiritual burden to get every halal killing right; 22% of halal slaughtermen suffered from high to very high levels of burnout and disengagement; and 27% of them suffered from high to very high levels of fatigue, raising a serious concern regarding their wellness.

Wellness is more than being healthy or not being sick, it is about achieving one’s full potential and is a journey, not an end state ([Botha & White, 2013](#)). Unwell workers cannot achieve their full potential and under slaughterhouse conditions, serious injuries and preventable deaths are at the extreme end of unwellness. Halal slaughtermen who are unwell will struggle to reach their potential and could be in danger of injury and even worse. If nothing is done about the wellness of workers in the primary sectors of the halal industry, halal food production will be affected, and the consequences will be felt right through to the pockets and stomachs of halal food consumers wherever they might be.

1.2.5 Halal-compliant financing

For devout Muslims to be properly engaged with the halal industry, particularly, the food production aspects, there must be available halal source of funding/financing for them to tap into. Currently, the low profitability of food businesses compared to other economic activities such as real estate and other frivolous activities militates against the availability of such financing for those interested in growing halal businesses particularly among the Muslims living in non-Islamic countries where most of the exported halal products are produced. Islamic finance and halal are both Islamic Shariah-compliant businesses that should be working cooperatively because for a halal business to be truly halal, all aspects of the business must be halal. Sadly, this is not always the case even in Malaysia—a leading halal product- and service-promoting country (Jaffar & Musa, 2014). The importance of halal financing for halal businesses and the Islamic principles upon which halal financing is based is explained in more detail by Ahmad and Sungit (2016) and Ismail and Noor (2016). The integration of Islamic financing and halal products and services throughout the supply chain is needed to create a strong halal ecosystem, a nexus that is currently lacking and which will require a heightened awareness and understanding of halal among all stakeholders for such a nexus to be established (Antara, Musa, & Hassan, 2016; Hassan, Rabbani, & Chebab, 2021). In addition to raising the awareness of all stakeholders, a survey of micro, small, and medium enterprises (MSMEs) in Indonesia found that the reputation and competitiveness or the cost benefit of the Islamic financing provider must be improved, and the blessing (*berkah*) associated with halal source of business financing highlighted for MSMEs to patronize Islamic finance institutions or products rather than the conventional sources of financing (Qoyum & Fauziyyah, 2019).

1.3 Authentication and certification issues

The consumption and the provision of halal foods and services to Muslims and non-Muslims alike are individual (*Fard-ain*) as well as collective (*Fard-kifayyah*) responsibilities of Muslims (Quran 2:168, 172). For this reason, making sure halal foods and services are available is first and foremost a duty before it can be considered a business. When we consider this service as a duty, then it is incumbent on Muslims and their governments and organizations to provide halal services even if it is free of charge because of the order of Allah SWT that we must consume what is halal and Tayyib. However, because a Muslim is expected to perform all his duties well and professionally as advised by the Prophet (May peace be upon him), a certain level of cost will be incurred, and associated services are required in the assurance of the halalness of the foods and services being provided. These attendant expenses are the reason for the justification of the fees charged for halal compliance services. The expenses involved have skyrocketed with the greater demand for the rapid accreditation, certification, auditing, and authentication of halal products and services (Farouk, 2013). According to Tieman and Williams (2019), the “high demand for certification services in combination with a decentralised form of accreditation and an inability to support the halal industry efficiently demonstrates cracks in the conventional halal certification model, which has become costly, inefficient, and risky from a halal perspective.” The reasons for this state of halal industry malaise are discussed in the following subsections.

1.3.1 Too many halal certifiers with no unified standard

Farouk (2013) observed that “because of the rapid growth in the volume and value of trade in meat and meat products from ritually slaughtered animals around the world and the increased demand for assurance by the consumers of these foods, a number of regulatory and certifying bodies have sprung up in both producing and importing countries to ensure the compliance of these products to the halal requirements.” In that same year, Riaz (2013) estimated the number of halal-certifying bodies to be about 111 around the world. This number is likely to have risen worldwide to 500 by 2019 (Tieman & Williams, 2019). The proliferation of halal certification bodies underscores the growth and sophistication of the halal industry; however, the lack of harmony and unified standards and recognition between these bodies is a big issue in the halal industry with multifaceted negative consequences for the industry, which need to be resolved quickly and comprehensively. As far back as 2013, while reflecting on the meat industry halal meat certification, Farouk (2013) lamented that “the lack of a unified standard — while not a major hurdle for halal red meat exporting countries, confuses meat exporters in that they have to balance the need for commercial efficiency, religious requirements, and the requirements of non-religious and consumer groups and deal with multiple standards at the same time.” With meat production largely situated in majority non-Muslim countries, the lack of a unified standard and the associated hiccups to efficiency and morale could potentially frustrate processors enough to find alternatives to halal markets for their products consequently resulting in scarcities in halal export markets.

1.3.2 Lack of appropriate rapid authentication techniques

Authenticating the halalness of primary ingredients such as raw and unprocessed meats, milk, vegetables, fruits, cereals is much easier compared to when these ingredients are further processed into finished products or when blended with other processing aids and additives in the formulation of the products needing authentication. The more complex the product the more sophisticated techniques and technologies required to rapidly verify the compliance of such products to halal requirements (Nakyinsige, Man, & Sazili, 2012). In the fast-moving world of goods and services, delays cause multiple issues and resulting consequences with negative effect on halal businesses and ultimately on the choices and pockets of halal consumers. Currently, the speed with which techniques are being developed or rapid noninvasive technologies are being developed to authenticate halal products and services is outpaced by the speed with which new ingredients, formulations, and products and services are being developed, with the gap between the two expanding by day. With the advancement in invasive and noninvasive techniques and technologies being used for other industries, the challenge is for those involved in the halal industry to adopt some of these technologies for use in the halal industry while blazing the trail in other technologies for other industries to use in return (Edwards, Manley, Hoffman, & Williams, 2021; Muller-Maatsch, Weesepeel, Roetgerink, Wijtten, & Alewijn, 2021). The developments in the industry toward closing the gap are some of the topics discussed in various chapters of this book.

1.3.3 Ownership of halal-certifying organization

The production, authentication, and certification of halal products have both physical and spiritual aspects to it (Farouk et al., 2015). Using the production of halal meat products as an example (see Fig. 1.2 for a simple farm-to-fork aspects of halal and Tayyib meat products production), most of the aspects of the production and authentication can be carried out by Muslims or Non-Muslims and by organizations own by either of the two groups. The only aspects in the whole chain that can only be carried out by Muslims or organizations wholly own by Muslims are the certification/assurance aspects of the products. The reason is the aspects of certification and assurance are closely tied to the spirituality of the certifier and his qualification to assure another person of the halal status of a product (Shah, 2016). As alluded to in a preceding section of this chapter, the responsibility of sourcing, verifying, and consuming halal primarily falls on a Muslim individual (*Fard-ain*); however, due to the separation between individuals and the production of the food he/she consumes, the responsibility of assuring such an individual that the product he/she consumes is halal can be assumed by the Muslims closest to the source of that product supply (*Fard-kifayah*) (see Wahb, 2021 for discussion on *fard ain* and *Kifayah*). For the latter Muslim to assume such a responsibility for the former, the testimony of the latter must be acceptable Islamically—based on his/her spiritual qualifications. Because only the testimony (*shahada*) of a person who believes in Allah and in *Halal* (allowable) and *Haram* (prohibited) as ordained in the Quran (verbatim words of Allah revealed to the Prophet Muhammad, may peace be upon him) and the practices of the Prophet, is acceptable when it pertains to the spiritual matters of halal and haram, thus, it is only a Muslim or a business solely

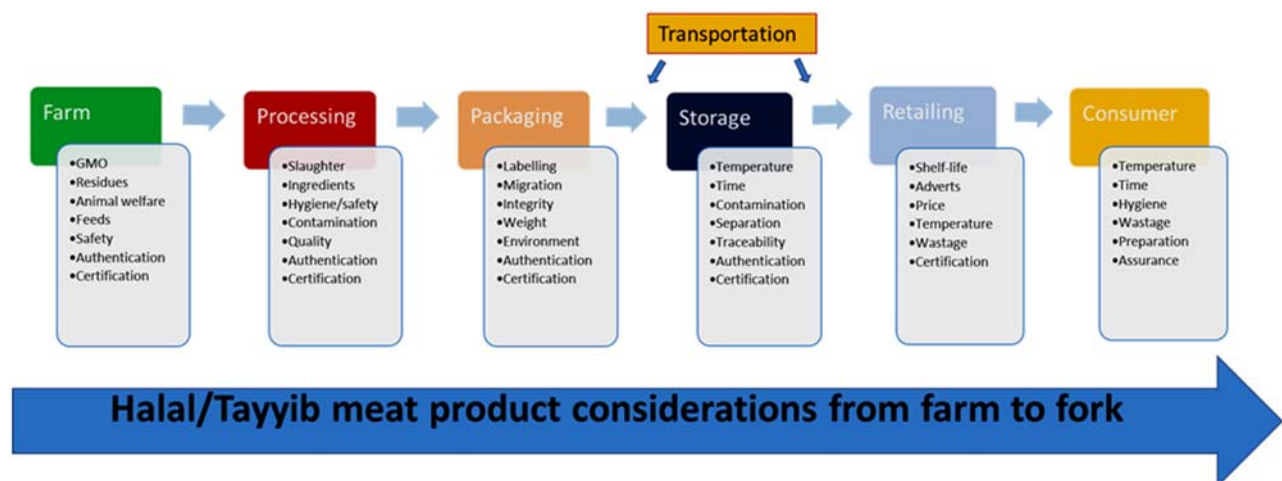


FIGURE 1.2 Various aspects of halal and Tayyib from farm to fork showing the many functions that can be undertaken by both Muslims and non-Muslims except the certification, and assurance aspects which only Muslims can undertake (Farouk, 2022).

owned by Muslims can certify halal products throughout the supply chain, for another Muslim far removed from the process to accept it as halal (refer to [Shah, 2016](#) for detailed discussion of ownership). If a person who spiritually does not believe in Allah and the Quran or the transmitted practices of the Prophet Muhammad (may peace be upon him) certifies or own a business that certifies halal meat products, then a Muslim cannot accept the product as halal and the verification of the status of that product becomes incumbent on that individual him- or herself, hence, making it impossible for a proper halal chain to be established with consumers far removed from the point of halal meat production being able to purchase halal products at outlets conveniently available to them. Simply put, if a Muslim is offered a meal containing meat or a meat product by another Muslim, the former is allowed to eat the meat or product as halal without the need to verify or even inquire as to its halalness—because if the product is not halal, the onus/sin of the former consuming the product is on the Muslim who offered it to him/her. On the other hand, if a non-Muslim offers meat or meat product to a Muslim, the latter must inquire/verify its halalness before consumption, failure to do so will incur the person a sin for eating haram product, because one should not expect a non-Muslim to eat only halal meat, or to be concerned about halalness, thus, underscoring the need to have Muslim ownership of halal certification and assurance throughout the chain of supply for halal meat consumers. The basis upon which this conclusion is drawn could be understood from the principles pertaining to halal and haram in Islam ([Al-Qaradawi, 1960](#)).

1.4 Antihalal groups and economic Islamophobia

Opposition to halal food production is growing in many parts of the world. According to [Ruiz-Bejarano \(2017\)](#), the UNESCO-UA Chair Islam, Culture and Society, “Islamophobic discourses have gone global, affecting most regions in the world, and being orchestrated from all components of society. Be it by members of the European Parliament, ordinary citizens or petty offenders, Muslims and Islam continue to be targeted, defamed, attacked, and excluded from the mainstream in many societies.” This phenomenon when it pertains to the halal certification of foods “closely parallels previous campaigns against kosher highlighting the increasing resemblance between contemporary Islamophobia and historical anti-Semitism” ([Hussein, 2015](#)). [Farouk et al. \(2016\)](#) reviewed aspects of meat and nutritional security of Muslims and Jews and reported that many ways are being used to restrict halal and kosher meat production around the world, including legislations, food regulations, insistence on labeling meats as halal and kosher at retail to enable the meats to be discriminated against, and negative social media campaigns by groups, and concluded that the food security in terms of availability, affordability, and utility of halal and kosher meat consumers is being affected due to these restrictions.

Most of the opposition to halal food production smacks of bigotry and in few cases good old crass politics.

Politically, [Bowen \(2021\)](#) observed for instance that “ritual slaughter is no longer an issue between Christians and Jews, nor Muslims, nor among cultural groups; but rather a tension between religious and secular outlooks” and went on further to say that “If halal worries provide useful ammunition for anti-Islamic politics across Europe, they also highlight the diversity of post-colonial politics; French politicians can evoke aspirations toward Republican public space devoid of signs of separatism to castigate supermarkets for showcasing their halal products; and at least since the mid-2000s, French officials have charged corner shops that stocked only halal meat with discriminating against non-Muslims.”

Economically, [Ruiz-Bejarano \(2017\)](#) observed that “systematic attempts to undermine the halal food industry made by some European Members of Parliament, claims of animal cruelty sparked by animal rights groups, bans on halal sacrifice in the meat industry, the ‘boycott-halal’ on-line campaign, alleged funding of terrorism, threats and other expressions of hatred have managed to prevent many businesses from accessing the emerging halal market.” Halal production is not under threat only in Europe or other western industrial countries but in other parts of the world too halal is increasingly coming under attack. China’s Xinjiang region launched a campaign against halal products in a predominantly Uighur Muslim region of China in the pretense of stopping “Islam penetrating secular life and fuelling ‘extremism’” ([Reuters, 2018](#)). In the Indian State of Kerala, the ruling party the BJP “demanded that the state government ban the ‘halal system’ and halal boards at restaurants” ([Sabith, 2021](#)). In Sri Lanka, “the anti-halal and anti-animal slaughtering campaigns and oppositions were found to be part of anti-Muslim sentiments intended to sabotage the economic pride of Muslims and undermine their religious renaissance” ([Yusoff & Sarjoon, 2017](#)). The damage to halal food production and the economy of Australia by the “Boycott Halal” and similar campaigns in that country is well documented ([Etri & Yucel, 2016](#); [Hussein, 2015](#); [Ruiz-Bejarano, 2017](#)).

Countries and organizations hide their bigotry behind different reasons, including animal welfare to ban halal and kosher slaughter. This might have led [Kanji \(2021\)](#) to lament the double standards of certain European countries when she wrote “three times as many European countries have enacted bans on halal and kosher slaughter as have prohibited

or pledged to prohibit maceration – the widespread practice of grinding up live un-stunned male chicks, who are treated as extraneous ‘waste’ in the production of eggs; far from being generally illegal, maceration by an ‘apparatus [with] rapidly rotating mechanically operated killing blades’ is prescribed as a standard procedure by EU regulations on the ‘protection of animals at the time of the killing,’ for ‘chicks up to 72 hours’ old.”

The challenges posed by the various forms of halal/economic Islamophobia are one of the biggest issues facing the halal industry, and the gravity of the threat is better perceived when one factors in the fact that antihalal manifestation of Islamophobia mainly emanates from the halal food surplus countries. Although the immediate impact of the threat from Islamophobia is likely to be felt by those living as minorities in those countries, the net halal food importing majority Muslim countries and their citizens will suffer too, particularly when the economic considerations that drive the current trade between the supplying and the importing countries are replaced or overshadowed by other political considerations. This is the reason a serious halal goods freedom of movement diplomacy should be entrenched in international trade agreements to ensure the flow of halal goods is not impeded by Islamophobic politicians. Alarming as it may sound, it is not a secret that there are many places in the world today where food is being used as a weapon of war (Dewaal, 2020). However, it shall be pointed out that this issue is yet to be resolved and is not elaborated in subsequent chapters. Any effort in resolving this issue is highly encouraged.

1.5 Halal training and capacity building

The rapid global growth of halal industry and the associated requirements is not presently being met with the same pace by the development of the people properly trained in the various aspects of the economy to man the industry. It is important to train skilled individuals in all aspects of the halal economy throughout the supply chain (Hashim & Shariff, 2016). There are currently many training modules designed to provide rudimentary knowledge of halal to upskill those currently working in or planning to enter the industry to enable them to discharge their responsibilities. These types of training are mostly provided by halal authorities such as JAKIM in Malaysia or LLPOM-MUI in Indonesia, or the many halal certification bodies around the globe. While these types of training are important in supporting the industry, more structured training with curriculums starting from primary and secondary schools and not only at tertiary levels are needed if the human capital capable of advancing all the fields of knowledge required to grow the industry is to be developed (Ibrahim, Jamaludin, Kartika, Hashim, & Zubairi, 2022). There are presently few dedicated halal training institutes attached to universities and polytechnics mainly in Malaysia and Indonesia and other Asian countries producing trained individuals. A comprehensive list of some of these institutes are found in Zain, Muhamad, Yaacob, and Ahmad (2017) and Akim, Heryadi, Dewi, & Hermawan, (2018). To encourage the institutes currently providing halal training to continue providing such services and to get more universities and higher institutions of learning around the globe to establish courses in halal, all the actors in the halal industry must insist on hiring only those who are qualified graduates from these institutes to work in all sectors of the industry.

1.6 Conclusion

This chapter reviewed some of the issues facing the halal industry. The issues are multifaceted, and the industry is mainly using existing tools to manage the issues. Many of the challenges and solutions to the halal industry, especially on halal food alternatives and halal supply chain sustainability, are discussed in the various chapters. This chapter concentrated on some of the perennial ones, including raw materials supply, lack of unified standards for authentication and assurance, ownership of certification bodies, shortage of trained halal manpower, lack of halal financing for entrepreneurs in majority non-Muslim countries, economic Islamophobia, and the work and wellness of halal workers and its implications to halal food supply. This chapter concludes that for the halal industry to continue to grow sustainably and to properly cater to its consumers, each of these issues must be taken seriously and solutions found soonest. Resolving these issues will require team approaches by experts on each of the rings in the halal supply chain to ensure that whatever products or services the halal industry offers are halal based on our primary sources of law—the Quran and the Sunnah of the Prophet (PBUH).

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Halal food product innovation according to Shariah law

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2.1 Introduction

The halal industry is unquestionably one of the world's fastest expanding. It has attracted not only Muslim countries but also non-Muslim countries, due to its economic potential. The fundamental driver of the halal business is rising demand from Muslim customers and communities seeking products and services that adhere to Islamic principles. However, the market is no longer limited to Muslims, as non-Muslims recognize the value of halal goods and services. Halal is in demand for more than just food and beverages. Industry operators have assured that their products are of high quality and, most significantly, that they fit the requirements of Islamic law to meet the demands of the halal market segment's buying public. Some manufacturers go even further by obtaining halal certification, which necessitates strict adherence to halal certification bodies' (HCBs) guidelines and procedures, such as the Department of Islamic Development Malaysia (JAKIM), the Indonesian Halal Product Assurance Organizing Agency, the Pakistan Halal Authority, and so on (JAKIM, 2020).

In addition to learning their rights, Muslim halal consumers should verify that their consumption practices are consistent with Islamic ideals. Islam, like many other religions, prescribes a set of dietary rules for its adherents. In general, Islamic dietary law distinguishes between permissible (halal) and prohibited (haram) foods and beverages (haram). According to some academics, these laws aim to bond followers together as a coherent group and build a distinct Islamic identity. The dietary restrictions governing approved and banned foods are very easy for Muslims; however, the laws dictating how allowed food animals are killed are less so (Riaz & Chaudry, 2018).

In terms of food laws, Islam and Judaism are very similar, even if Quranic law focuses on establishing disparities between Jews and Muslims in many other areas. Dietary restrictions are likely to be similar because these Abrahamic religious communities have similar ethnic histories. Muslims are permitted to consume only that which is "good" (Quran 2:168), which is defined as food and drink that is pure, clean, healthy, nourishing, and agreeable to the palate. Everything is permissible (halal) save what has been specifically forbidden; in some instances, even otherwise forbidden food and drink can be consumed without the act being deemed a sin. A "law of necessity" (*dharurah*) in Islam permits banned activities to occur if there is no feasible alternative. If there was no halal accessible, it would not be regarded as sinful to ingest normally banned food or drink in the event of possible famine. However, necessity should always be the starting point for any innovation; remember the old adage, "Necessity is the mother of invention." The fact that there are so many sophisticated and complex consumer products on the market today attests to worldwide advances in science and technology. It also represents a shift in a specific consumer segment's viewpoint as well as their increasing needs. Halal items have seen a lot of increase recently. It is currently deemed essential not just to produce technologically advanced inventions, but also to ensure that such items adhere to Islamic teachings.

2.2 Product innovation in Islam

Islam promotes innovation. The introduction of a new idea, method, or device is referred to as "innovation." It also entails devising a more effective technique for achieving objectives. Innovation and invention are essential components

of human life, and so of Islam. Actively providing solutions is just a response to Allah's commands and Islamic teachings. According to Allah subhanahu wa ta'ala (SWT),

٢٩ أَلَّا يَلْبَسَ أُولَٰؤُا وَلِيَتَذَكَّرَ ءَايَتِهِ لِيَذَّبَرُوا مَبْرُكَ إِلَٰهِكَ أَنْزَلْنَاهُ كِتَابَ

(This is) a blessed Book which We have revealed to you, (O Muhammad), that they might reflect upon its verses and that those of understanding would be reminded.

(Surah Sad, 38: 29)

Muslims must develop and generate new solutions or systems that adapt to changing lifestyles, technology, and social and political circumstances. There is no need to avoid innovation as long as Islam's vehicle continues to advance and human life progresses toward civilization and modernity. Problems and barriers arise as a result of improvements, developments, and advancements. Taking on these challenges may necessitate even more innovation (Lever, 2013).

Instead of being complacent, Muslims are encouraged to learn, engage, and lead the road to success. The believers were described by Allah as follows:

١٤ الصَّالِحِينَ مِنْ ءُولَٰئِكَ الْخَيْرَاتِ فِي وَيُسِرُّوْنَ الْمُنْكَرَ عَنِ وَيَنْهَوْنَ بِالْمَعْرُوفِ وَيَأْمُرُونَ بِالْإِحْسَانِ وَالْيَوْمَ بِاللَّهِ يُؤْمِنُونَ

They believe in Allah and the Last Day, and they enjoin what is right and forbid what is wrong and hasten to good deeds. And those are among the righteous.

(Surah Ali-Imran, 3: 114)

2.3 Principles of the *Halalan Toyyiban*

Halal is an Arabic noun whose verb form is *halla*, *yahillu*, *hillan*, which means to liberate, release, dissolve, and enable. It has two meanings in the realm of Islamic jurisprudence: first, it refers to anything that does not result in a person being penalized for doing so; and second, it refers to everything that can be done in accordance with Shariah law. The word halal comes from the Arabic word *halalan*, which means “permissible” or “lawful.” Halal also means “permitted by Shariah law without the imposition of a penalty on the perpetrator.” Haram, which means “forbidden, unlawful,” is the polar opposite of halal.

Because the root *toyyib* (from *toyyiban*) implies “good,” *halalan toyyiban* denotes that the product is both permissible to consume and helpful to the customer. The concept of *halalan toyyiban* is not new. It was a central halal principle that appeared at least four times in the Quran. Verse 168 of Surah al-Baqarah, for example:

٦٨ إِنَّهُ الشَّيْطَانُ خُطُوهُ تَتَّبِعُوا وَلَا طَيِّبًا حَلَالًا أَلَّا تَرْضَىٰ فِي مِمَّا كُلُوا النَّاسَ يَأْتِيهَا

O mankind eat from whatever is on earth [that is] lawful and good and do not follow the footsteps of Satan. Indeed, he is to you a clear enemy.

(Surah al-Baqarah, 2: 168)

This verse demonstrates Allah SWT's order to eat, utilize, and utilize products that are not only halal according to *fiqh*, but also hygienic, safe, and high quality. Surah al-Maidah (5:88), Surah al-Anfaal (8:69), and Surah an-Nahl are other verses that mention *halalan toyyiban* (16:114).

Halal and *toyyib* are complementary. *Toyyiban* emphasizes the importance of ensuring the quality and safety of food before eating it, so preparing halal food without considering whether it is *toyyib* will result in fatal consequences. To summarize, halal denotes the fulfilment of Shariah requirements that lead to the permissibility of an object or activity, whereas *halalan toyyiban* denotes a more comprehensive approach. As a result, within the halal industry, a clearer distinction between the two concepts is required.

The *halalan toyyiban* concept is considered a more thorough approach that aims to adhere to international standards, making it universally accepted. As a result, the relevant parties must put in more effort to promote the integration of halal and *toyyiban* into the halal certification processes of many countries. *Halalan toyyiban* may encourage halal market participants to produce goods or services that are not only halal-certified but also contain wholesome ingredients. For example, nutritious products that contain halal ingredients but do not contain any substance that could jeopardize human health and life may be certified as *halalan toyyiban* by the certifying authority if they meet all of the *halalan toyyiban* standards' requirements.

2.4 Haram: forbidden food and drinks

Muslims are obligated by their faith to abstain from specific foods. This is considered to be for the sake of health and cleanliness, as well as to follow Allah's commands. The following meals and beverages are strictly prohibited (haram) in the Quran (2:173, 5:3, 5:90–91, 6:145, 16:115):

As previously said, haram is an Arabic phrase that means “forbidden.” Haram acts are forbidden in the sacred texts of the Quran and the Sunnah, and they remain forbidden no matter how good one's intentions or purpose are. Dietary restrictions are considered to assist one's knowledge of the divine will in Islamic law.

Consumption of flowing blood is forbidden for Muslims. Meats that are considered *haram*, such as pork, dog, cat, monkey, or any other haram animal, can only be considered lawful in cases when a person's life is in danger and such meat can save them. However, if one has access to nonimpermissible food, these meats are not considered a necessity or permissible. All carnivores with fangs are considered haram, including lions, tigers, wolves, dogs, and cats. All talons-bearing birds (such as hawks, falcons, vultures, and eagles) are considered haram. Donkeys that have been domesticated are considered haram. Mice, scorpions, and snakes are examples of haram animals. All reptiles, amphibians, and rodents are considered haram. Any animal that has died before being slaughtered according to Islamic law, or that has not been slaughtered properly, is considered haram. Any animal murdered in the name of anyone other than Allah is also forbidden, as is anything made from a human body part. Similarly, intoxicants, or *khamr*, are also forbidden in Islam. Even in relation to non-Muslims, the Prophet prohibited the dealing, export, import, and gifting of intoxicants. According to a hadith attributed to Abu Hurairah Raḍiya 'llāhu 'an-hu (RA),

قَدْ إِنَّهَا: وَسَلَّمَ عَلَيْهِ اللَّهُ صَلَّى النَّبِيُّ فَقَالَ حُرِّمَتْ وَقَدْ عَامَا، إِلَيْهِ فَأَهْذَاهَا خُمُرٌ، مِنْ رَاوِيَةٍ عَامَ كُلِّ وَسَلَّمَ عَلَيْهِ اللَّهُ صَلَّى النَّبِيُّ يُهْدِي كَانَ رَجُلًا أَنْ
بِهَا يُكَارَمُ أَنْ حُرِّمَ حَرَمَهَا الَّذِي إِنَّ: قَالَ الْيَهُودُ؟ بِهَا أَكَارُمُ أَفَلَا: قَالَ: «بِيعَهَا حَرَمَ شَرِبَهَا حَرَمَ الَّذِي إِنَّ»: فَقَالَ أَبِيعَهَا؟ أَفَلَا: الرَّجُلُ فَقَالَ: «حُرِّمَتْ
«الْبَطْحَاءُ فِي شَنْهَا»: قَالَ بِهَا؟، أَصْنَعُ فَكَيْفَ: قَالَ: «الْيَهُودُ»

There was a man who always presented a pouch of wine to the Prophet PBUH every year. And in one year, he presented it to the Prophet PBUH again, but now it has been prohibited. Then the Prophet PBUH said to him: “Wine has been prohibited.” The man then asked: “Then, can I sell it?” The Prophet PBUH said: “The God who prohibit drinking it has also prohibit selling it.” The man asked again: “Can I give it to the Jews then?” The Prophet PBUH answered: “The God that prohibit it also prohibit giving it to the Jews.” Then, the man asked: “What should I do with it then?” The Prophet PBUH answered: “Throw it down the sidewalk.”

Musnad al-Humaidi (1064). 2/229

It is forbidden for a Muslim to work in or own a business that sells intoxicants. It is forbidden for a Muslim to sit at a table where alcohol is served. According to qiyas—an analogical reasoning technique applied to the inference of legal concepts from the Quran and the Sunnah—heroin, cocaine, marijuana, and any other intoxicating substances are also prohibited.

2.5 Mashbooh, mushtabahat: questionable or doubtful

A gray area known as *mashbooh* exists. If one does not know whether a meal or drink is halal or haram, it should not be ingested. Any food or drink that is classed as *syubhah*, which means “questionable” or “dubious,” falls into the gray area and does not clearly fall into the halal or nonhalal categories. Gelatine, which can be derived from plants or animals, is an example of a *syubhah* component. If the gelatine comes from plants, it is halal, but if it comes from pigs or animals that are not slaughtered according to Islamic law, it is not.

Muslims should refrain from taking food or drink until its *syubhah* status is determined. The Prophet (PBUH) has provided guidance on *syubhah* issues. Bukhari, Muslim, Abu Daud, Ibn Majah, and Darimi all relate the following:

فِي وَقَعَ الشُّبُهَاتِ فِي وَقَعَ وَمَنْ وَعَرَضَ لِدِينِهِ اسْتَنْبَرَا الشُّبُهَاتِ اتَّقَى فَمَنْ النَّاسُ مِنْ كَثِيرٍ يَعْلَمُهَا لَا تَأْتِ، مُشْتَبِهَةٌ وَبَيْنَهُمَا بَيِّنٌ، وَالْحَرَامُ بَيِّنٌ، الْخَلَالُ
الْحَرَامُ

What is Halal is clear. And what is Haram is also clear. And in between those two is a dubious area in which many people do not know about. So, whoever distanced himself from it, he has acquitted himself (from blame). And those who fall into it, he has fallen into a state of Haram. (Bukhari and Muslim)

[Hadith No. 6, 40 Hadith an-Nawawi]

Between halal and haram, *syubhah* is a contentious and delicate topic concerning which some people have doubts, confusion, and unresolved thoughts. This could be due to different interpretations of a rule or different levels of authority. Muslims should avoid these *mushtabahat* to avoid committing genuine haram, according to Islam. Muslims may disagree on whether or not meat slaughtered by people of the book or monotheistic people like Christians and Jews meets this criterion. In short, a good practice is this: when in doubt, leave it out (Rahman, 2018).

2.6 Slaughtering of animals according to Islamic rights

Halal dietary guidelines determine which foods are permissible for Muslims to consume. These rules can be found in the Quran and hadith books (the traditions of Prophet Muhammad S.A.W.). Shariah is the name given to Islamic law, which has been interpreted by Muslim scholars over time. The fundamental principles of Islamic law remain constant; nevertheless, their interpretations and applications may vary depending on time, place, and circumstance. Biotechnology, unusual sources of components, synthetic materials, and developments in animal slaughter and meat processing are just a few of the concerns that Muslim scholars are grappling with (Aghwan & Regenstein, 2019).

Because, in the Islamic belief, life is holy and one must kill only with God's permission to meet one's rightful need for food, significant care is paid to the method in which animals' lives are taken to supply food.

Muslims slaughter their animals by a quick cut through the animal's throat with mercy, while reciting "In the name of God, God is Most Great" (Quran 6:118–121). Before being slaughtered, the animal should not suffer in any way and should not see the blade. The knife must be razor-sharp and free from any blood of a previous slaughter. Before eating, the animal's blood must be completely drained. This type of meat is known as *zabihah*, or simply "halal meat."

Fish and other aquatic meat sources, which are all considered halal, are exempt from these regulations. Unlike Jewish dietary law, which considers only aquatic life with fins and scales to be kosher, Islamic dietary law considers all aquatic species to be halal.

Some Muslims would refuse to consume meat if they are unsure about how it was murdered, even if they know the animal was killed humanely. They also think it is important that the animal has been properly bled, because else it would not be safe to eat. Some Muslims in largely Christian nations, on the other hand, believe that one can consume commercial meat (apart from pig, of course) and just say God's name while doing so. This viewpoint is based on a Quranic scripture (5:5) that declares that Muslims may eat the meals of Christians and Jews.

Major commercial meatpackers are increasingly establishing certification processes to ensure that their products adhere to Islamic dietary guidelines. Meats that have been properly slaughtered and labeled "halal certified" are available to Muslim consumers. With the halal food sector accounting for 16% of the global food supply and likely to rise, it is safe to assume that halal certification from commercial food producers will become increasingly commonplace over time.

In the Quran, Chapter 5, Verse 3, it says:

Forbidden Unto you (for food) are carrion and blood and swine-flesh, and that which hath been dedicated unto any other than Allah, and the strangled, and the dead through beating, and the dead through falling from a height, and that which hath been killed by (the goring of) horns, and the devoured of wild beasts, saving that which ye make lawful (by the death-stroke), and that which hath been immolated unto idols. And (forbidden is it) that ye swear by the divining arrows. This is an abomination. This day are those who disbelieve in despair of (ever harming) your religion; so, fear them not, fear Me! This day have I perfected your religion for you and completed My favour unto you and have chosen for you as religion AL-ISLAM. Whoso is forced by hunger, not by will, to sin: (for him) Lo! Allah is Forgiving, Merciful.

2.7 The basic principles of halal, by al-Qaradawi, in the book *al-Halal wa al-Haram fi al-Islam*

Some basic Islamic knowledge related to halal, including the basic principles of halal, should be known to develop a good halal policy. The role of the jurist is only limited to explaining what Allah and the Prophet Muhammad have identified to be halal or haram. Thus the Quran and Sunnah have declared what is halal and Muslims are required to accept it as lawful. Likewise, nobody has the authority to declare any food, drink, dress, or trade and business to be haram or unlawful. In fact, everything in the universe was created for the benefit of humans and can be used by them. This fact is derived from the legal maxim (*maqasid shariah*) method, which states that everything is permissible for everyone. There are 11 principles of halal and haram as follow:

- Principle 1: The fundamental premise is that all things created by Allah are permissible, with a few exclusions. Pork, blood, meat from animals that died for reasons other than proper slaughtering, food dedicated or immolated to someone other than Allah SWT, alcohol, and intoxicants are among the exceptions.

- Principle 2: Allah alone has the authority to make something legal or illegal. No human being, no matter how pious or powerful has the power to change things on his or her own.
- Principle 3: Prohibiting what is permitted, and permitting what is prohibited, is similar to ascribing human partners to Allah. This is a major sin that will cause one to be expelled from the Islamic faith.
- Principle 4: Impurity and harmfulness are the primary reasons for things being prohibited. A Muslim is not permitted to inquire as to why or how something is unclean or harmful in Allah SWT's prohibitions. Some reasons may be self-evident, while others may be obscure. Some of the obvious reasons to a person of a scientific mind are as follows:
 - Because the decay process produces chemicals that are harmful to humans, carrion and dead animals are unfit for human consumption (Awan, 1988).
 - The blood of an animal contains pathogenic bacteria, metabolic products, and toxins (Hussaini & Sakr, 1984).
 - Swine can carry pathogenic worms into the human body. *Trichinella spiralis* and *Traenia solium* infections are common (Awan, 1988).
 - Pork fat's fatty-acid composition has been said to be incompatible with human fat and biochemical systems (Sakr, 1991).
 - Intoxicants are thought to be damaging to the neurological system, impairing the senses and human judgment, creating social and familial issues, and even death in some cases (Al-Qaradawi, 1984).
 - These reasons and other, similar explanations may sound reasonable to a layperson, but they become more questionable under scientific scrutiny. If the meat of dead animals was prohibited due to harmful chemicals in decaying flesh, then dead fish would have been prohibited. If pork contains *Trichinae*, beef might contain *Escherichia coli*. If pork fat is bad, so are trans fatty acids. The underlying principle, it seems, behind the prohibitions is not scientific reason, but the divine order "forbidden unto you are..." appears to be the underlying principle behind the prohibitions, rather than scientific reason.
 - Swine can carry pathogenic worms into the human body. *Trichinella spiralis* and *Traenia solium* infections are common (Awan, 1988).
 - These and other similar theories may appear acceptable to the untrained eye, but scientific analysis casts doubt on them. Dead fish would have been outlawed if the meat of dead animals was prohibited due to toxic compounds in decaying flesh. Beef may have *E. coli* if pork includes *Trichinae*. Trans fatty acids are just as harmful as hog fat. The divine order "forbidden unto you are..." appears to be the underlying principle behind the prohibitions, rather than scientific reason.
- Principle 5: What is allowed is sufficient, and what is forbidden is unnecessary. Allah forbade only what was superfluous or dispensed with, and provided better alternatives. People may survive and live better without eating unhealthful carrion, pork, or blood, as well as the root of many vices: alcohol.
- Principle 6: What is conducive to what is prohibited is prohibited in and of itself. If something is forbidden, everything that leads to it is forbidden as well.
- Principle 7: It is against the law to misrepresent something that is illegal as legal. It is illegal to use flimsy justifications to consume something that is illegal, such as drinking alcohol for ostensibly medical reasons.
- Principle 8: A person's good intentions do not make something illegal acceptable. When a believer's permissible action is accompanied by a good intention, it is considered an act of worship. In the case of haram, no matter how good the intention or how honorable the purpose is, it remains haram. Islam forbids using haram means to achieve a commendable aim; rather, it requires that not only the goal be honorable, but that the means used to achieve it be legal and legitimate. According to Islamic law, only just means can be used to secure what is right.
- Principle 9: Things that are in doubt should be avoided. Between what is plainly legal and what is definitely illegal, there lies a gray area. This is the realm of "what is questionable." Muslims who avoid questionable things and stay away from what is illegal are considered pious in Islam. "The halal is clear, and the haram is clear," Prophet Muhammad said. Between the two, there are ambiguous issues about which individuals are unsure whether they are halal or haram. If someone avoids them to protect his religion and honor, he is safe; however, if he engages in a part of them, he may be doing something haram.
- Principle 10: Everyone is prohibited from doing things that are illegal. Islamic laws apply to people of all races, creeds, and genders. A privileged class does not receive preferential treatment. In reality, there are no privileged classes in Islam, so the issue of preferential treatment does not arise. This idea holds true not only between Muslims but also between Muslims and non-Muslims.
- Principle 11: Exceptions are required by necessity. The list of things that are prohibited in Islam is quite short, but the emphasis on following these prohibitions is very strong. At the same time, Islam is not blind to life's challenges, their magnitude, or human weakness and its ability to deal with them. A Muslim may, for example, eat prohibited

food in sufficient quantities to remove the necessity and thus to survive if he or she is forced to do so by necessity (Regenstein & Chaudry, 2000).

In essence, these 11 basic principles of halal serve as a comprehensive guideline, particularly for jurists working to find new *fatwa* decisions in the rapidly evolving halal product market. As such, only hygienic, safe, and high-quality products are allowed for Muslims' consumption, and the public in general.

2.8 Halal standards as an effort toward *Halalan Toyyiban*

In comparative evaluation, a standard is a degree of quality or attainment that is used as a measure or a benchmark of a norm. It is typically used to determine product safety or to ensure that products and materials are tailored to their intended use, that they encourage product and service interoperability, that they enable trade by removing trade barriers, and that they foster common knowledge of a product.

A *toyyib* food is “good,” however, not all good food is *toyyib*. Halal standards and procedures help people understand what it means to be halal. This is not only a halal issue; international standards like the Good Manufacturing Practice (GMP) and the Good Hygiene Practice (GHP).

While GMP and GHP have long been used in western countries, halal standards promote good halal practices, such as forbidding fraud, forgery, oppression, *gharar* (uncertainty) in business, unequal scales, and the use of fake addresses and labels, as well as any practices that are illegal.

To carry out its authority, halal standards are based on Shariah law and supplemented with components of modern science and national regulations. It is halal to slaughter a chicken by severing both its *halqum* (trachea) and *marikh* (esophagus tubes) in the context of *zabihah*; however, it is not *toyyib* if the slaughterer then places the chicken on a surface contaminated with germs and bacteria. Halal certification would not be provided in this case.

Halal certification allows for practices like “from farm to fork,” which is a popular food production and marketing strategy in many western countries. Raw materials, food processing, handling, and additives, as well as packaging, tools, storage, transportation, and other parts of the halal food chain, will all be thoroughly inspected before halal certification is awarded.

Halal standards were created to aid the halal industry (or firms seeking certification from a specific halal body) in meeting halal requirements to become certified or renew an existing certification. Standards developers, who are usually based in particular countries, create these standards. The Department of Standards Malaysia (DSM), the National Standards Body, and the National Accreditation Body, for example, cooperate with diverse stakeholders to promote halal standards through reputable services for global competitiveness. The DSM is in charge of developing other standards and ensuring the safety and quality of any production process, in addition to developing halal standards. The authorized body establishes standards to ensure that the halal industry adheres to the regulations that have been established to meet the criteria for obtaining a specific certification.

Halal food standards have been produced by a number of countries and certification organizations. Malaysia and Indonesia, for example, were among the first countries to establish halal standards and certification procedures to assure that Muslim consumers would trust and accept food manufactured by non-Muslim manufacturers in their countries.

The halal food standard includes practical instructions for the food business, including nutrient enhancers, for the preparation and processing of halal food. It establishes the ground rules for food goods and food enterprises, and it is utilized as the basis for certification by halal certification organizations or agencies while taking into account additional requirements to complete the certification process. It should be noted that not all countries have the same halal food standards. Some have criteria that are comparable to others, while others have requirements that are different.

Four primary halal standards divisions represent their different geographical areas (Abdallah, Rahem, & Pasqualone, 2021). The first is the Halal Assurance System (HAS), issued by the Indonesian Council of Ulama's Assessment Institution for Foods, Drugs, and Cosmetics; the second is DSM's MS 1500:2019 Halal Food—General Requirements (Third Revision); the third is the 993:2015 standard issued by the Gulf Cooperation Council's Standardization Organization (GSO); and the fourth is the 1:2019 standard issued by the Organization of Islamic Cooperation (OIC) (SMIIC).

The Lembaga Pengkajian Pangan, Obat-obatan, dan Kosmetika (LPPOM) is an Indonesian organization dedicated to halal certification in the food, drug, and cosmetics industries. The LPPOM was founded in 1989 to help Majelis Ulama Indonesia (MUI) as a halal-certifying authority in Indonesia, Asia's largest market for halal food items. Its members are qualified in chemistry, biochemistry, food science and technology, and agro-industry. A company must adopt a HAS to acquire halal certification, which ensures the continuity of halal production procedures for the duration of the certificate's validity.

In 2019 the MS 1500 standard, which was first published in 2000, received its third revision. This standard gives the food sector in Malaysia-specific guidelines on how to prepare and handle halal food. The halal certification is given by a federal authority, specifically JAKIM, the Malaysian government's religious department. The Malaysian requirements have been cited by the Codex Alimentarius Commission as a model for halal certification and as a foundation for halal standards in many other nations.

The GSO, which was founded in 2001 and has been in operation since 2004, includes national standards and specifications departments from the Arab Gulf states (Saudi Arabia, Qatar, Kuwait, Oman, Bahrain, and the United Arab Emirates). Through specialized technical committees, the GSO issues Gulf Standards and Technical halal rules. Each GSO member country's national standardization organizations are required to adopt the GSO standards, removing any conflicting national standards. As a result, GSO norms became national standards throughout the Arab Gulf (GSO, 2019).

The halal certificate accompanying meat and meat (or other food products) imported into any of the Arab Gulf countries must be examined by the consulate of the importing country. This verification is necessary to ensure that the certificate issuer has been accredited by the relevant agencies in accordance with the GSO standard's requirements. The certificate certifies that the slaughtering and production were carried out in line with the GSO standard and national laws and regulations. To prevent counterfeiting, meat and meat products must have a food-grade stamp applied by the certificate issuer or an Islamic center or institution, or better, a systematic traceability system from farm to fork.

The OIC is a group of countries in which Islam plays a large role, including those where Islam is the state religion, those where Muslims make up a majority of the population, and those where Muslims are a minority but play an important role. Uganda, Benin, Togo, Mozambique, the Ivory Coast, Gabon, Cameroon, Suriname, and Guyana fall within the latter type. Currently, the OIC includes 56 member countries scattered over 4 continents, with the majority of them located in North Africa, the Middle East, and Central Asia (WorldData.info, 2022). Egypt, Saudi Arabia, Iran, Iraq, Turkey, Pakistan, Bangladesh, Nigeria, Algeria, and Indonesia are the 10 largest food markets in the OIC. The SMIIC, which was founded in 2010, is the designated organization for the establishment of standards under the OIC. Its goal is to create a single framework in which all OIC member states employ the same certification, accreditation, and laboratory services standards and techniques to enable economic exchanges. SMIIC works closely with a number of other organizations, including the Emirates Standards and Metrology Authority and the Turkish Accreditation Agency (SMIIC, 2022). However, member nations (such as Indonesia and Malaysia) that have already developed and accepted their own halal food standards may not recognize the OIC/SMIIC norms.

2.9 Harmonization of halal standards

There have been several initiatives to harmonize existing halal standards, as well as certification and accreditation methods. Despite the fact that these efforts are to be credited with conceiving the idea of a transnational cooperative organization, no unification has yet been achieved—for the simple reason that several of these organizations (named a “council,” “forum,” or “alliance,” depending on the individual case) have been formed (Wan Hassan, 2007) (WorldData.info). There is still a long way to go before a universal halal standard is established.

The first meeting of the OIC's Economic and Commercial Cooperation Standing Committee (COMCEC) in 1984 sparked the concept of establishing a dependable system for standard harmonization among Islamic governments (SMIIC, 2022). The Standardization Experts Group for Islamic States was formed for this purpose in 1985, and its efforts resulted in the SMIIC Statute being approved at the 14th COMCEC Meeting in 1998.

The World Halal Food Council (WHFC), created in 1999 on the LPPOM MUI's suggestion, was another attempt to harmonize the halal market with the goal of creating a global halal standard. Around 20 halal certification agencies from around the world joined the WHFC in 2011. It was suggested that a minimum core standard (covering a number of fundamental issues like pork and alcohol prohibition) be established, with specific, additional requirements being applied to each country. The WHFC is still working on contentious issues surrounding halal dietary laws (World Halal Council, 1999).

A similar attempt was made during the 2006 World Halal Forum by forming the International Halal Integrity Alliance, an international organization aimed at developing a global halal standard to protect the halal industry's integrity (Halim & Salleh, 2012).

The global standardization of accrediting standards in the halal sector was also taken into account. The International Halal Accreditation Forum (IHAF), a network of Halal Accreditation Bodies (HABs), was established in 2016 as a step forward in this direction. The accreditation of HCBs is a crucial step in ensuring their objectivity and reliability during the certification process. Acting as a certification and accreditation agency at the same time may result in conflicts of

interest. A few of HCBs, on the other hand, have not completely accepted the concept of accreditation and function as unofficial HABs. A total of 10 component members attended the IHAF's first meeting, including Australia, New Zealand, the United Kingdom, the United States, and Spain. The number of members grew to 27 HABs, which operate in both OIC member and non-OIC member nations (IHAF, 2020).

2.10 Conclusion: halal product innovation

The food and beverage market is not the only place where innovation can be found in this enticing industry. Finance, agriculture, education, consumer goods, and cosmetics are all included, yet the list is never ended.

Halal innovations include the use of technology in slaughterhouses to simplify the slaughtering process and alleviate animal cruelty concerns voiced by animal rights organizations. This has increased the pace of the slaughtering process, given the operator a considerably safer working environment with set protocols, and, most significantly, made the process more sanitary. These enhancements improve its dependability and use the *toyyiban* notion outlined earlier in this study.

The development of more reliable and effective (but clean) soil amendments and fertilizers is another example of halal innovation (Kassim, Hashim, Hashim, & Jol, 2014). Although traditional fertilizers are not classified as halal in Malaysia's certification procedure, there are alternate techniques of utilizing beneficial microorganisms for soil amendments and to break down natural wastes such as fruit peels into fertilizers for plants and agribusiness.

Biotechnological technologies such as the polymerase chain reaction for the detection of any pork-derived DNA considerably improve and simplify the detection of nonhalal components.

A holistic strategy capable of creating a user-friendly quality system, consisting of manpower, process, rules, and lab analysis, is an example of innovation. Consumers' rights to informed choice, credibility, and halal certification are protected by this system, which is backed up by each country's acts, rules, and regulations. This opens up chances for Muslim and non-Muslim producers to make halal products, positively benefiting the halal value chain both locally and globally. Elements of innovation include technologies that improve the process, safety, traceability, quality, and simplicity of halal certification for manufacturers; this is accomplished through online registration, ingredient declarations in databases, and apps designed to make customer verification easier. Verify Halal and Smart Halal are two such apps.

The manufacturing of genetically modified (GM) foods is the subject of the third set of fatwas. A Special Conversation in the Fatwa Committee of the National Council for Islamic Affairs in Malaysia (1999) explored the use of biotechnology in food and drink. Using DNA generated from swine sources, for example, was considered to be unnecessary because there are plenty of other halal ingredients that may be substituted. As a result, a resolution was passed prohibiting the use of pig DNA in the production of commodities, food, and beverages:

The use of biotechnology in the processing of pig DNA products, food, and beverages is opposed to Islamic law and is "haram," and the use of biotechnology in the processing of pig DNA goods, food, and beverages has not yet reached the level of emergency because other ingredients are still available.

Also, nowadays, GM food sources are being used in an expanding number of food products. As a result, the Fatwa Committee of the National Council for Islamic Religious Affairs Malaysia (2011) decided that, because genetic manipulation entails the transfer of plant and animal genes of either halal or nonhalal origin to yield the desired characteristics as food or medicine, consumption of nonhalal GM foods is prohibited, as is the use of substances that are banned or harmful to humans and the environment. Naturally, using clean livestock created by GM methods is permitted, as long as the animals are slain according to Islamic law (Asa & Azmi, 2018).

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Section 2

Product and Processing Innovations

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Modification of plant fats and oils as lard alternatives

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3.1 Introduction

Lard is an animal fat that is extracted from the adipose tissues of swine. Until the development of plant-based shortenings, lard was among the popular fat ingredients used in food applications. Particularly, lard along with its hydrogenated form was used as raw material for producing shortenings of different kinds (Marikkar, Yanty, Musthafa, & Miskandar, 2022). Food industries incorporated lard-based shortening in baking while some ethnic groups preferred to use it as a frying medium (Marikkar, Mirghani, & Jaswir, 2016; Nur Illiyin et al., 2014). Lard was preferred over tallow as a shortening for baking of biscuits, dripping sandwiches, snacks, and moon cakes (Yanty, Marikkar, & Miskandar, 2012). A food analysis report recently showed that some of the commercial biscuit formulations contained lard as an ingredient (Yanty, Marikkar, & Abdulkarim, 2014). The unique liquid-to-solid ratio of lard was believed to be the reason for its inclusion in bread-making industry (Nur Illiyin et al., 2014). The performance characteristics of lard during food processing as well as flavors imparted on fried products are said to be other reasons (Marikkar & Yanty, 2014). All these happenings raised serious concern among the consumers over the authenticity of ingredients in commercial food formulations.

The issue of inclusion of lard in food has been one of the outstanding issues, causing uneasiness amongst the followers of certain religious faiths (Nina Naqiyah, Marikkar, & Dzulkifly, 2013; Yanty, Marikkar, Abdulkarim, et al., 2014). It is well-known that the Islamic and Orthodox Jewish religions prohibited the consumption of pork, lard, and other related products. The research evidence showing the negative health implications of pork and lard consumption has been given elsewhere in the literature (Rashood, Shaaban, Moety, & Rauf, 1996). Owing to this situation, there has been a growing interest among researchers to develop halal authentication methods and formulate high-quality halal food to ensure consumer health and successful domestic and international trade (Nina Naqiyah, Marikkar, Mirghani, Nurulhidaya, & Yanty, 2017). Although effort to produce alternative halal gelatin as replacement for porcine gelatin has already been underway for some time, the emphasis given for the development of plant-based substitute for lard was abysmally low. Nonetheless, as a proactive approach, some preliminary efforts were taken in the early 2011 to study the solidification and melting characteristics of *Madhuca longifolia* seed fat to identify its potential utility as an alternative for lard (Marikkar & Yanty, 2012). The outcome of this study was promising and paved the way for further initiatives of formulating plant-based substitutes for lard by others (Marikkar, Yanty, Peciulli, Miskandar, & Chiavaro, 2018; Nur Illiyin et al., 2014; Yanty, Marikkar, Shuhaimi, & Miskandar, 2014; Yanty, Marikkar, Shuhaimi, & Miskandar, 2017). Investigations in this regard were continued on palm oil (PO), palm stearin (PS), cocoa butter (CB), enkabang fat, soybean oil, canola oil, guava seed oil, and so on and so forth.

Producing novel fat products through blending different oils and fats is generally the easiest and most economical way to avoid costly processes involving chemical treatments. At times, fat products produced through chemical methods might not comply with the requirements set by halal regulations. Any effort in this regard would therefore require some meaningful criteria for assessment. Particularly, analytical approaches used should be comparable to those adopted in the assessment of *trans*-free margarines (Miskandar & Nor Aini, 2010), structured lipids (Norizzah, Chong, Cheow, & Zaliha, 2004), and CB equivalents (Jahurul et al., 2014; Wassell & Young, 2007). Generally, researchers have paid much attention to aspects such as fatty acid and triacylglycerol (TAG) compositions, melting and crystallization

behavior, solidification and polymorphic characteristics in their assessment of any novel fat formulations (Jin et al., 2018; Miskandar & Nor Aini, 2010). Profiling the fatty acid and TAG compositions would be essential to help determine the nutritional status of formulated fats. It is because the nature of distribution of fatty acids in the glycerol backbone is tightly connected with human nutrition. As pointed out in several previous reports, thermal analysis by Differential Scanning Calorimetry (DSC) has been one of the useful approaches to determine a variety of thermal properties involving fat modification (Yanty, Marikkar, & CheMan, 2013; Yanty, Marikkar, Shuhaimi, 2013; Nur Illiyin et al., 2014; Nur Illiyin, Marikkar, Shuhaimi, Mahiran, & Miskandar, 2013). Other parameters such as solid fat content (SFC) measurements by pulse Nuclear Magnetic Resonance (NMR) spectroscopy were proven to be useful to monitor the changes in hardness as a function of the percentage solids at different temperatures (Noor Lida, Sundram, Siew, Aminah, & Mamot, 2002). The objective of this chapter is to elaborate strategies adopted in formulating plant-based substitutes for lard through modification of locally available resources in Malaysia.

3.2 Triacylglycerol composition of lard-alternative lipids

The preliminary step of lard-alternative formulation has been the comparison of the chemical and physical characteristics of lard with those of various naturally occurring plant lipids to screen out the best possible list. All plant lipids are generally permissible for consumption under halal and kosher food regulations. PO, avocado fat (AVO), PS, and CB are included in this scheme by giving consideration for cost factor and ready availability in Malaysia (Yanty et al., 2012). Important considerations were given to TAG distributional pattern of above-mentioned plant fats as TAG species are the basic building blocks of any lipid that determine the physical and functional properties. According to a number of previous reports, the predominant TAG molecular species of lard were 1-palmitoyl-dioleoyl glycerol (POO) (20.67%), followed by palmitoyl-oleoyl-linoleoyl glycerol (POL) (20.0%) and StPO (12.52%) (Nurjuliana, Che Man, & Mat Hashim, 2010; Rashood et al., 1996; Yanty, Marikkar, Che Man, & Long, 2011). With reference to lard, AVO extracted from Malaysian cultivars was one of the potential candidates having had general compatibility in TAG distributional pattern. As shown in Table 3.1, the TAG composition of AVO contained POO (22.76%) and POL (19.29%) as major TAG molecules, but very little amount of StPO (0.57%). It was hence assumed that AVO had the closer similarity to lard among these plant fats to become the best raw material for the formulation of lard alternative. Next to AVO, mee fat (MF) was considered to be closest in compatibility to lard based on TAG composition. Although MF had POO as its predominant TAG, its second most abundant TAG molecular species was 1-stearoyl-dioleoyl glycerol (StOO) (Marikkar, Ghazali, & Long, 2010). In compatibility with lard, it was found to have POO (25.64%), POL (6.78%), and stearoyl-palmitoyl-oleoyl glycerol (StOP) (14.86%) as other TAG molecular species (Table 3.1). These figures suggested that MF can also be one of the potential candidates as raw material for the preparation of lard-alternative fat. In PO, PPO (31.61%) and POO (24.76%) were found to be the predominant TAG molecules. Nonetheless, its potential use in the formulation of lard alternative stands only after AVO and MF. The fractions of PO such as PS and palm olein could be considered for blending purposes after assessing their suitability. In the case of CB, it showed greater differences from the TAG distribution pattern of lard owing to the predominant presence of TAG molecular species such as StPO and StOSt (Table 3.1).

With regards to fatty acid composition, lard is reported to have low amount of saturated fatty acids (SFAs) than unsaturated fatty acids (USFA) (Marikkar, Alinovi, & Chiavaro, 2021; Nurjuliana et al., 2010; Yanty Marikkar, Che Man, et al., 2011). In similarity with lard, plant fats such as AVO, MF, and PO were also found to possess low amounts of SFAs than USFAs. These characteristic features are indicative of the positive attributes of AVO, MF, and PO to serve as main raw materials for the preparation of lard-alternative fat. CB, on the other hand, possessed more SFA (64.25%) than USFA (35.75%) due to the presence of higher proportions of stearic and palmitic acids. Based on this, it was clearly suggested that blending either AVO or MF with another fat in an appropriate ratio is a good option to get a fat blend simulating the properties of lard. Both PS and CB could become auxiliary components of this formulation.

Slip melting point (SMP) and iodine value (IV) are generally considered the most important quality indices of oils and fats. As the physical nature of the lipids is dependent on them, these two parameters are used internationally for quality assurance purposes (AOAC, 2007). As shown in Table 3.1, all plant lipids mentioned earlier were found to possess SMP values higher than that of lard, except AVO whose SMP value (30°C) was found to fall within the range of lard. IV of lard was found to be 73.76 while those of AVO, CB, PO, and MF were 84.30, 34.00, 54.00, and 61.10, respectively. Although none of the plant fats was found to exhibit IV exactly similar to that of lard, the closest value was displayed by AVO. This gives the supposition that a fat blend simulating the IV of lard would be prepared through blending AVO with either PO or MF in an appropriate ratio.

TABLE 3.1 Triacylglycerol compositions of lard-alternative lipids.

	Avocado butter ^a	Cocoa butter ^b	Palm oil ^b	Mee fat ^b	Lard ^b
SMP	30.00	35.66	30.50	35.25	27.50
IV	84.30	34.00	54.00	61.10	73.76
LLLn	1.87 ± 0.00 ^a				1.54 ± 0.21 ^a
LLL	0.85 ± 0.07 ^a				0.68 ± 0.21 ^a
OLL	3.23 ± 0.02 ^b	—	—	0.88 ± 0.02 ^c	4.68 ± 0.08 ^a
MMM	—	—	0.21 ± 0.01 ^a	—	—
PLL	4.21 ± 0.00 ^b	0.27 ± 0.00 ^e	2.08 ± 0.03 ^c	0.83 ± 0.02 ^d	7.05 ± 0.06 ^a
MPL	—	—	0.54 ± 0.01 ^a	—	—
OOL	9.00 ± 0.03 ^a	—	1.62 ± 0.02 ^d	5.00 ± 0.14 ^c	6.93 ± 0.04 ^b
POL	19.29 ± 0.06 ^b	0.85 ± 0.01 ^e	9.96 ± 0.01 ^c	6.72 ± 0.00 ^d	20.00 ± 0.27 ^a
PPL	4.03 ± 0.06 ^b	1.55 ± 0.00 ^e	10.19 ± 0.01 ^a	2.08 ± 0.01 ^d	2.62 ± 0.04 ^c
OOO	11.42 ± 0.01 ^a	0.69 ± 0.01 ^c	3.97 ± 0.02 ^b	11.06 ± 0.01 ^a	4.33 ± 0.21 ^b
POO	22.76 ± 0.02 ^c	2.27 ± 0.02 ^e	24.76 ± 0.01 ^b	25.64 ± 0.04 ^a	20.67 ± 0.11 ^d
PPO	12.43 ± 0.00 ^c	18.08 ± 0.01 ^b	31.61 ± 0.01 ^a	10.39 ± 0.01 ^e	10.63 ± 0.01 ^d
PPP	2.88 ± 0.01 ^b	0.26 ± 0.01 ^d	4.77 ± 0.03 ^a	0.33 ± 0.01 ^c	0.38 ± 0.00 ^c
StOO	0.52 ± 0.01 ^e	2.98 ± 0.00 ^c	2.72 ± 0.02 ^d	12.05 ± 0.04 ^a	3.62 ± 0.04 ^b
StPO	0.57 ± 0.02 ^e	40.78 ± 0.1 ^a	5.65 ± 0.01 ^d	14.86 ± 0.01 ^b	12.52 ± 0.12 ^c
PPSt	0.11 ± 0.01 ^e	0.41 ± 0.01 ^c	0.92 ± 0.01 ^a	0.33 ± 0.01 ^d	0.81 ± 0.00 ^b
StOSt	—	29.35 ± 0.01 ^a	0.52 ± 0.01 ^c	6.36 ± 0.21 ^b	0.83 ± 0.01 ^c
StStSt	—	0.40 ± 0.05 ^b	—	0.23 ± 0.01 ^c	1.31 ± 0.01 ^a
Others	6.83 ± 0.04	2.11 ± 0.14	0.48 ± 0.01	3.24 ± 0.03	1.41 ± 0.33
UUU	26.37	0.69	12.68	16.94	18.16
UUS	46.78	6.37	40.06	45.24	51.34
USS	17.03	89.76	47.97	33.69	26.60
SSS	2.99	0.66	5.90	0.89	2.50

Each value in the table represents the mean of three replicates. Means within each row bearing different superscripts (a-e) are significantly different ($P < .05$). O, Oleic; P, palmitic; L, linoleic; Ln, linolenic; St, stearic; U, unsaturated; S, saturated; USS, disaturated; SOS, 1,3-distearoyl glycerol; StOP, stearoyl-palmitoyl-oleoyl glycerol; SOO, 1-stearoyl-dioleoyl glycerol.

^aYanty et al. (2012) and

^bYanty, Marikkar, and Long (2011b).

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3.3 DSC thermal profiles of lard-alternative lipids

Multiple uses of DSC in thermal analysis of oils and fats have been documented by several research groups (Marikkar, 2015). As shown in Fig. 3.1, DSC cooling curves of various plant fats are compared with that of lard to screen out the best possible list. The cooling profiles of lard is represented by *curve-e*, while those of avocado butter, CB, PO, and MF are represented by the *curve-a*, *curve-b*, *curve-c*, and *curve-d*, respectively. According to Fig. 3.1, the onset of crystallization (T_{onset}) of lard was 18.25°C while those of avocado butter, CB, PO, and MF were 23.93°C, 17.01°C, 18.90°C, and 26.86°C, respectively. This comparison shows only PO was found to exhibit onset of crystallization

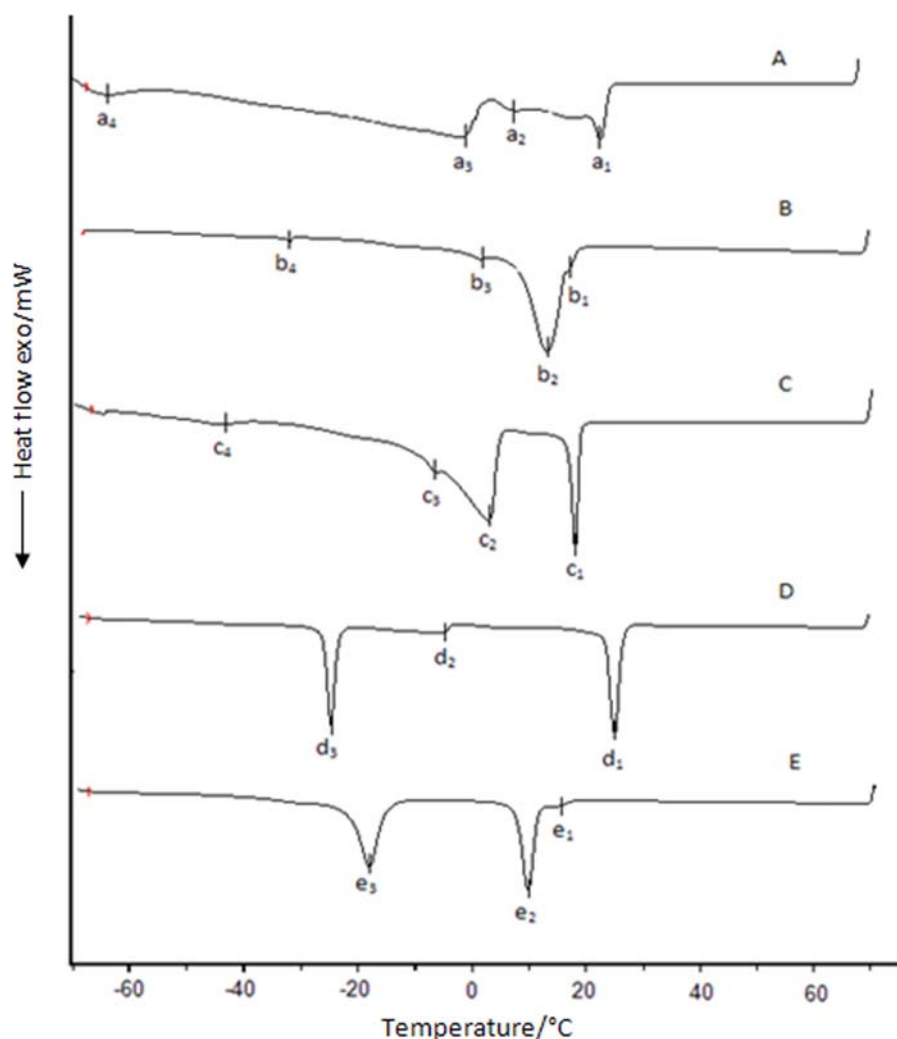


FIGURE 3.1 DSC cooling curves of avocado butter (*curve-a*), cocoa butter (*curve-b*), palm oil (*curve-c*), mellee fat (*curve-d*), and lard (*curve-e*). Reproduced with permission from Yanty, N.A.M, Marikkar, J. M.N., Miskandar, M.S. (2012) Comparing the thermo-physical characteristics of lard and selected plant fats *Grasas y Aceites*. 63, 328–334. Copyright CSIC 2012.

value (18.90°C) somewhat closer to that of lard (18.25°C). As an important attribute, both lard and MF (*curve-d*) were found to display thermal transitions in both high- and low-temperature regions in their respective cooling curves. This similarity between them would be evidently seen as their compatibility in thermal behavior. On the other hand, the cooling curve displayed by CB (*curve-b*) was considerably different from those of the other three plant fats as it had a single exothermic thermal transition at 13.40°C. The cocrystallization of TAG molecular species within a narrow temperature range has been attributed to this peculiar single thermal transition behavior of CB. This thermal feature rules out that CB could not become as the major base material for preparation of lard-alternative fat, but instead it can be useful as an auxiliary ingredient in the formulation.

DSC melting curves of the four plant fats are compared with that of lard as shown in Fig. 3.2. The melting profile of lard is represented by *curve-e*, while those of avocado butter, CB, PO, and MF are represented by *curve-a*, *curve-b*, *curve-c*, and *curve-d*, respectively. Based on the results shown in Fig. 3.2, the end-set of melting (T_{endset}) of lard was 35.70°C while those of avocado butter, CB, PO, and MF are 44.74°C, 29.48°C, 39.28°C, and 38.86°C, respectively. Among the plant fats, only MF is found to display T_{endset} value (38.86°C) closely related to that of lard (35.70°C).

As shown in Fig. 3.2, both lard and MF were found to have both high- and low-melting transitions. CB, on the other hand, was found to display a single melting transition, which existed in the high temperature region above 10°C. As pointed out earlier, this could be probably due to the comelting of the TAG species of CB within a narrow temperature range. This thermal feature further confirmed that it could not be used as a main base material for preparation of lard-alternative fat, but instead it could become an auxiliary component in the formulation.

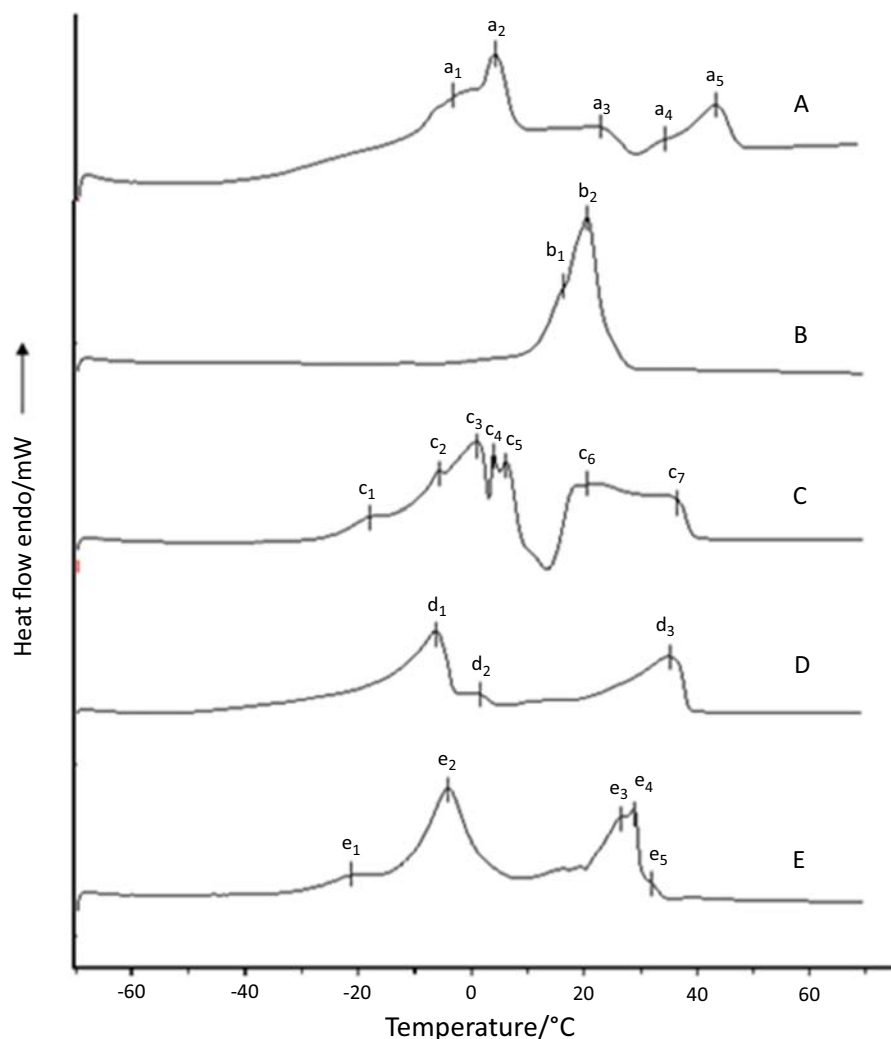


FIGURE 3.2 DSC heating curves of avocado butter (curve-a), cocoa butter (curve-b), palm oil (curve-c), mee fat (curve-d), and lard (curve-e). Reproduced with permission from Yanty, N.A.M., Marikkar, J.M.N., Miskandar, M.S. (2012) Comparing the thermo-physical characteristics of lard and selected plant fats *Grasas y Aceites*. 63, 328–334. Copyright CSIC 2012.

3.4 Solidification profiles of lard-alternative lipids

Investigations on the SFC profiles of oils and fats helped in the past to make important decisions with regard to their functional properties targeted for different end uses (Marikkar et al., 2010). Hence, this has been widely used in the effort taken to formulate a substitute for lard (Marikkar et al., 2018; Nur Illiyin et al., 2014; Yanty, Marikkar, Shuhaimi et al., 2014; Yanty, Marikkar, Shuhaimi et al., 2017). As shown in Fig. 3.3, the SFC profiles of the various plant fats and lard were compared within a specified temperature range to devise strategies of blending ratios. This showed that the SFC profiles of the plant-based fats, namely, CB, PO, MF, and avocado butter at 0°C were 93.99%, 68.63%, 33.1%, and 18.02%, respectively, while that of lard was 30.8%. Among the plant fats, the closest similarity to lard at 0°C in SFC was shown by MF, but AVO, on the other hand, displayed SFC values always lower than those of lard throughout the temperature region (Fig. 3.3). Further, both CB and PO were found to have SFC values higher than that of lard in between the 0°C and 20°C. In this temperature range, MF was found to display an SFC profile closely similar to that of lard. On the other hand, SFC profile of PO was found to become equal to that of lard in the temperature range between 25°C and 40°C. Interestingly, the SFC values of PO, MF, and lard were closely similar to each other at 25°C. It is believed that the changing nature of the SFC profiles of plant fats and lard was mainly due to differences in their TAG molecular composition (Table 3.1). Meanwhile, some similarities seen between lard and MF in the distribution of TAG molecular species could be responsible for the apparent similarities in the SFC values of them within the range of 0°C–25°C (Table 3.1). Although AVO and lard were found to possess POO and POL as their predominant TAG molecules, the proportion of triunsaturated (UUU) TAG molecules of AVO (26.37%) (Table 3.1) was considerably higher than that of lard (18.16%).

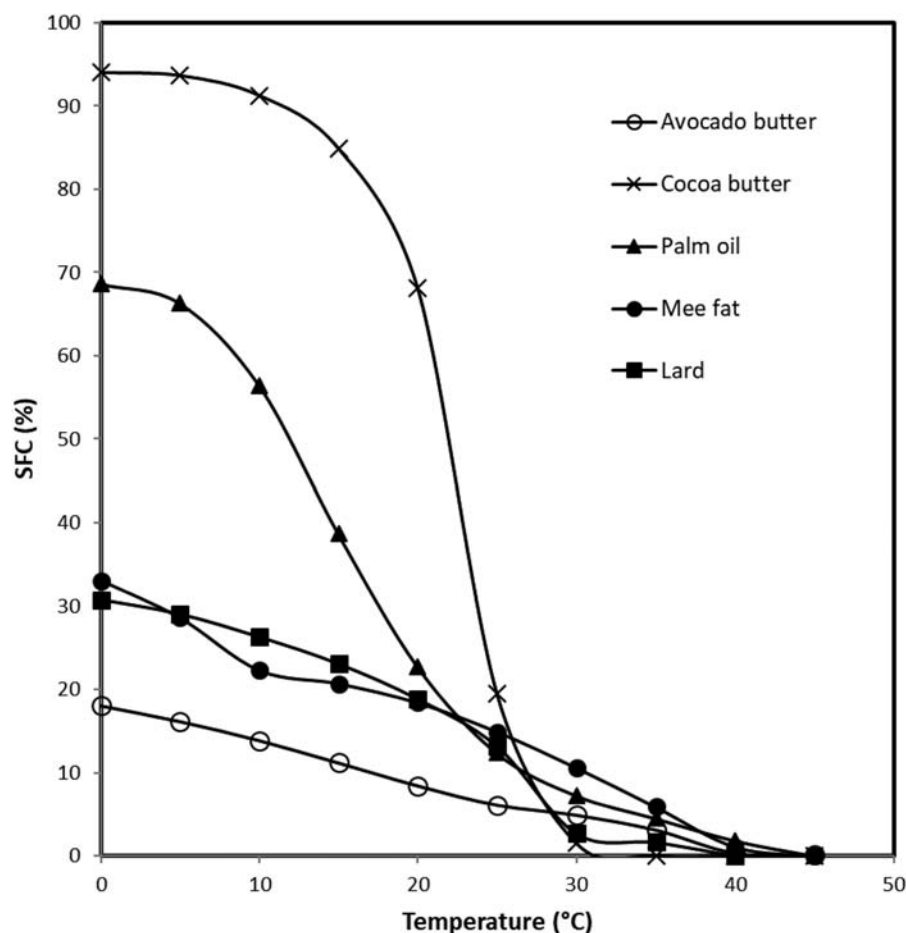


FIGURE 3.3 Solid fat content profiles of selected plant-based fats and lard. Reproduced with permission from Yanty, N.A.M, Marikkar, J.M.N., Miskandar, M.S. (2012) *Comparing the thermo-physical characteristics of lard and selected plant fats* *Grasas y Aceites*. 63, 328–334. Copyright CSIC 2012.

TABLE 3.2 Lard-alternative formulations by fat blending.

Blends	Alternative plant fats/oils	Ratio	References
Binary	Mee fat:palm stearin	99:1	Marikkar and Yanty (2012)
Ternary	Avocado fat:palm stearin:cocoa butter	84:7:9	Yanty, Marikkar, Shuhaimi et al. (2017)
Quaternary	Palm oil:palm stearin:soybean oil:cocoa butter	38:5:52:5	Marikkar et al. (2018)

On the other hand, in greater part of the temperature region, PO and CB were found to possess higher SFC values when compared to those of lard. This could be because both CB (89.76%) and PO (47.97%) were found to possess enhanced proportions of disaturated (USS) TAG molecules in comparison to lard ([Table 3.1](#)). Other than this, the proportions of diunsaturated (UUS) (6.37%) and triunsaturated (UUU) (0.69%) TAG molecules of CB were extremely low. This has been in accordance with the finding reported in several previous studies ([Yanty, Marikkar, & Shuhaimi, 2013](#)). Based on these facts, blending either PO or MF with AVO in an appropriate ratio would provide a lipid mixture simulating the solidification behavior of lard.

3.5 Lard-alternative formulations by fat blending

The lard alternatives are innovative, with promising potential especially under halal and kosher regulations. [Table 3.2](#) shows some lard-alternative formulations obtained by fat blending.

3.5.1 Binary blends of mee fat:palm stearin

Fat blend formulation by binary mixing has been reported by several research groups. In an effort to formulate a substitute for lard, Marikkar and Yanty (2012) compared the chemical compositions and SFC profiles of MF and lard. The findings of this study provided the necessary impetus for formulating three fat blends by mixing PS with MF in proportions ranging from 0.5% to 2% (w/w) (Yanty, Marikkar, Shuhaimi et al., 2014). Although both of these exhibited compatibility in terms of solidification at some temperatures, they displayed disparity at certain other temperatures. In addition, IV of formulated blends of MF:PS was significantly lower (54.27–57.81) ($P < .05$) than that of lard (73.76). When the proportion of PS mixed into MF increased from 0.5% to 2%, the proportions of POL, POO, 1-stearoyl-dioleoyl glycerol (SOO), and 1,3-distearoyl glycerol (SOS) were found to decrease in such a way that the proportions of diunsaturated TAG molecular species lowered from 43.13% to 34.65% (Table 3.1). Concurrently, the proportions of disaturated TAG molecular species increased, leading to the increase of palmitic acid in the overall fatty acid distribution. This changing composition made the SFC profiles of MF and lard to become similar at most temperatures, but some deviations are still seen at certain other temperatures (Fig. 3.3). This leads to the supposition that blending MF with another suitable fat could possibly minimize the observed deviations in SFC profile of MF. Owing to this reason, Yanty, Marikkar, Shuhaimi et al. (2014) evaluated binary mixtures composed of MF and PS in different ratios with regard to their solidification behavior. Out of the three binary blends formulated, MF:PS (99:1) displayed the closest compatibility to lard within the range of 0°C to 40°C despite their slight differences in fatty acid and TAG compositions (Table 3.2).

3.5.2 Ternary blends of avocado fat:palm stearin:cocoa butter

At times judicious mixing of three plant-based fats has yielded novel fats with multiple uses. In this respects, a plant-based substitute for lard by making ternary blends with AVO as major component with PS and CB being minor components has been attempted by Yanty, Marikkar, Shuhaimi et al. (2017). When studying properties of novel fat blends, degree of unsaturation is an index that becomes considerable importance as it is indicative of the oxidative stability of food lipids. IV estimations showed that AVO:PS:CB blends had slightly lower IV (65.47–70.27) ($P < .05$) than lard (73.76). This could be probably due to additions of PS and CB into AVO causing slight increments in the amounts of palmitic (from 30.37% to 33.08%), stearic (from 1.30% to 4.57%), and oleic (from 43.64% to 44.04%) acids with concurrent decreases in the amounts of linoleic acids (from 17.45% to 14.28%). Previously, Yanty, Marikkar, Che Man et al. (2011) reported that SFC values of AVO were comparably lower than those of lard with the specified temperature range. This leads to the supposition that the SFC level of AVO could be enhanced to the level of lard by incorporating an appropriate amount of fat like PS and CB as they are hard fat substances. According to several previous reports, both PS and CB were hard fats and remain as complete solids at room temperature (Marikkar & Ghazali, 2011). Yanty, Marikkar, Shuhaimi et al. (2017) evaluated three different ternary blends made out of these fats and concluded that AVO:PS:CB (84:7:9) was the blend displaying closest similarity to lard in terms of some physical properties (Table 3.2). The SFC profiles of this blend and lard were found to display the least differences throughout the temperatures, including 0°C, 5°C, 20°C, 25°C, 35°C, and 40°C.

3.5.3 Quaternary blends of palm oil:palm stearin:soybean oil:cocoa butter

PO is a semisolid fat that has been used worldwide for its multifarious food and nonfood applications. Nevertheless, PO cannot become a direct substitute for lard due to occurrence of both PPO and POO in high amounts, which would cause unusually high solidification profile (Marikkar et al., 2018; Siew, 2002). Experimental observations showed that the SFC values of PO were found to be higher than that of lard within the temperature ranges of 0°C–20°C and a tremendous gap existed between them particularly at the beginning as well as end point of melting. For instance, the SFC profiles of lard and PO at 0°C were 30.8% and 68.63%, respectively, and hence tended to become 0% at 40°C and 55°C, respectively. It was assumed that blending an appropriate amount of liquid oil such as soybean oil (SBO) with PO would help to adjust its SFC values to become roughly similar to those of lard at almost all temperatures. As such, PO, PS, SBO, and CB were mixed to formulate a set of quaternary blends to identify a fat blend simulating the properties of lard (Marikkar et al., 2018). The formulations were made by making both PO and SBO as major components and PS and CB as minor components. Among the three formulated quaternary blends, PO:PS:SBO:CB (38:5:52:5) was found to display the closest similarity to lard in terms of some physicochemical parameters, SFC profiles, and shortening characteristics (Yanty, Marikkar, Miskandar et al., 2017). The SFC profile of this fat blend showed the least differences throughout the temperature range, which include 0°C, 5°C, and 25°C.

3.6 Future prospects and challenges

Food industry is one the single largest industries expanding rapidly worldwide. The recent trend shows that ethnic and religious foods have increasingly become an inevitable part of the total food industry. This is mainly due to the ever-increasing migration from Asia, Africa, and the Middle East to America and other Western countries. This trend coupled with globalization has opened up many doors for new market segment for religious and vegetarian foods devoid of animal components. In these countries, religious and ethnic foods occupy a small but rapidly expanding niche in every level of the food chain. As a result, there has been a growing recognition for ethnic and religious foods in Canada, North America, Australia, New Zealand, and other Western European countries. This is evidently seen from the incorporation of ethnic and religious foods as a study component of the food science curricular of some universities. Although world major religions do not hold exactly the same ideology, they do embrace some similarities with regard to food consumption and dietary habits. As for instance, they do take a common stand in prohibiting the consumption of pork and lard by their adherents. Consequently, any innovation leading to technology development on pork-free products or alternatives for lard will be welcomed by all communities. In this context, some resistance from the existing piggery industry cannot be ruled out owing to the risk posed to their supply chain on pork products and lard.

The plant-based fat substitutes for lard are novel innovations, showing high potential in replacing lard extracted from porcine sources. As the technology involved is simple, it will easily fulfill the call for a healthy fat substitute for animal fats, including lard. This can be utilized widely in the “halal” food production, bakery, meat, cosmetic products, and so on. As all raw materials of the lard substitute formulation are of plant origin, they will have additional advantages of being rich in fat-soluble vitamins and other phytonutrients (Muguerza, Ansorena, & Astiasaran, 2003; Raihana, Marikkar, Amin, & Shuhaimi, 2015). However, cost and ready availability of the fat ingredients used such as AVO, MF, and CB are a real concern. This is mainly due to the imbalance between supply and demand as the plantations producing these fat ingredients are mainly limited to Asia and Africa. Apart from this, ready availability of lard-alternative fats to some ethnic minorities living in certain countries might not be possible right now due to low demand or smaller market segment existing over there.

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Umami sources in flavorings and seasonings: halal approach

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4.1 Introduction

Food consumed by Muslims must meet Islamic dietary guidelines known as halal. Halal word comes from the Arabic, meaning lawful, justified and allows to be consumed under Islamic law (Kamali, 2013; Miskam et al., 2015). Umami sources can be obtained from plant, animal, and fermentation sources through direct utilization of food material, traditional processing or involving industrial production (Marcus, 2007). Halal status is critical when processing involves the use of nonhalal substances, slaughtered animal, enzymes of animal origin, and fermentation processes utilizing nitrogen sources of animal origin (Jais, 2019).

Flavorings and seasoning are important ingredients to enhance the taste of food. Flavoring refers to an ingredient that changes the original flavor of the food while seasoning enhances the taste of the food. The tasty curry noodle has curry and shrimp flavors while the addition of sodium glutamate enhances further the taste of the prepared food (Thomas, Boobyer, Borgonha, van den Heuvel, & Appleton, 2021). Foods containing umami taste have been favored for centuries. Some natural foods contain umami substances, such as mushroom, meat, seafood, vegetables, and cheeses. Bouillon in Europe, fish and soya sauces in Southeast Asia, and dashi broths in Japan are well-known examples of foods with umami sources used as taste booster in cooking (Ninomiya, 2015). In addition to sour, sweet, salty, and bitter taste, umami and kokumi taste have been known as the fifth and sixth basic tastes (Briand & Salles, 2016). Umami enhances the characteristic tastes of savory foods where in its absence, the unique taste of the food disappears. Its sensation spreads across the tongue and lingers longer than any other taste. Umami taste was scientifically introduced as one of the basic tastes in 1985 (Ninomiya, 2002). L-Glutamate taste receptor, taste-mGluR4, which regulates the “firing” of taste-receptor cells, was discovered (Chaudhari, Landin, & Roper, 2009). Nelson, Chandrashekar, and Hoon (2002) later discovered that an amino acid receptor, T1R1 + 3, was highly stimulated by L-glutamate. Kokumi on the other hand is characterized by the sensations of thickness and of mouthfulness besides having a pleasant taste (Li, Zhang, & Lametsch, 2020; Maehashi, Matsuzaki, Yamamoto, & Udaka, 1999; Maruyama, Yasuda, Kuroda, & Eto, 2012). The kokumi taste induces the response of Ca²⁺ receptor cells and thus enhances the intensity of salty, sweet, and umami tastes (Yang et al., 2021). Umami and kokumi are powerful substances found in cheese, beef broth, beans and yeast, onion extract, corn sauce (Charve, Manganiello, & Glabasnia, 2018), preserved egg yolk (Gao et al., 2021), and chicken hydrolysate (Zhang, Wang, Jiang, & Jin, 2019). Majority of kokumi-enhancing molecules are dipeptides and tripeptides (Zhang et al., 2021).

The synergistic action between glutamate, inosinate, and guanylate in enhancing umami taste has influence the formulation of savory food craving desire loved by consumers and manufacturers. The well-known seasoning, monosodium glutamate (MSG) formulated in Japan in 1909 opened a wealth of flavor-enhancement opportunities as commercial umami source. Umami taste was further enhanced by the discovery of the nucleotide, inosine-5'-monophosphate (IMP), also known as disodium inosinate, from dried bonito tuna in 1913 by Shintaro Kodama, and the nucleotide guanosine-5'-monophosphate (GMP), also known as disodium guanylate, from shiitake broth in 1960 by Akira Kuninaka (Lindemann, Ogiwara, & Ninomiya, 2002). Umami is also elicited by a few other amino acids mainly

aspartate, many short peptides, some organic acids (lactic, succinic, and propionic acids), and possibly other compounds (Amino et al., 2017).

Thus umami substances are consumed daily as natural and processed foods (Gabriel, Ninomiya, & Uneyama, 2018). Processed food may contain diversified types (amino acids and nucleotides), combinations and concentration of umami compounds. These varying combinations of umami substances in daily consumption contribute to significant differences in the perceived umami taste (Ninomiya, 2015). Novel taste of food continues to be formulated to enhance the umami taste by smart combination of natural food containing free glutamic acid, IMP, and GMP at threshold level (Marcus, 2019). It is a common practice to supplement cooked or processed food with umami compounds where desirable interactions with food components occur. It generates amplified and lasting sensations, far greater than any single ingredient can create. It is to express the power of enhancement of taste when these ingredients (glutamic acid, IMP and GMP) were present together.

The complexity of ingredients in halal value chain requires a thorough understanding of food ingredients, classification of food additives, functionality of food additive, origin of food additives, application of additives in processed food, halal food additives, E numbers, and so on and so forth, with umami products included. The halal origin of and functionality of various types of ingredients is determined in accordance to the guidelines to the Malaysian Standard MS 1500:2019. Determination of halal ingredients is facilitated by a decision tree method normally executed at each step of procurement, processing, handling, and storage and transportation of the desired product. The ingredients are classified as either critical, semicritical, or noncritical based on criteria stated in the MS 1500:2019 and Meat Protocol. The noncritical ingredient thus does not require halal certification. This includes identification of halal critical control points (HCPs), characteristics of HCPs, halal analysis of critical control points, halal storage, halal logistics, and distribution (Manan, Azemi, Abdul, & Sarip, 2020). Apart from reviewing some available halal umami sources, this chapter exhibits the example of a halal management system for this product.

4.2 Umami sources

4.2.1 Traditional food

Traditional and local foods in Asian regions are tasty, mainly contributed by the umami-rich preparations. In Asia, umami is found in both animal- and plant-based foods, mainly in fermented seafood products, fermented beans and grains, mushrooms, and tea. Traditional Malaysian fermented condiments such as *belacan* (fermented shrimp paste), *budu* (fermented fish sauce), *cencaluk* (fermented shrimp), and *taucu* (fermented soybean paste) are the common umami source (Hajeb & Jinap, 2015; Khairunnisak, Azizah, Jinap, & Nurul Izzah, 2009). In the Philippines, several traditional fermented food and seasonings with umami taste such as fermented rice–fish mixture, fermented rice–shrimp mixture, fish paste, fish sauce, and soy sauce are consumed. In traditional Japanese cooking, fish and seafood, konbu seaweed, shitake mushroom, vegetables, and soybean products are used to enhance the umami taste (Gabriel et al., 2018). In China, several types of cuisine use dried mushroom, fermented products, seasonings, and soup stocks in creating umami taste (Nakayaman & Kimura, 1988). In Korea, salted fermented fish is commonly used either for a side dish or a seasoning to provide umami taste (Koo et al., 2016).

4.2.1.1 Animal-based umami sources

4.2.1.1.1 Marine-fermented products

In Southeast Asia, seasonings containing fish sauces are commonly used (Yimdee & Wang, 2016). The major ingredients in fish sauce production are fish and salt, where the ratio between these two varies depending on the formulation (Natteewan et al., 2017). The fermented fish products are available in sauce or paste form. These products develop umami taste by the partially hydrolyzed protein, amino acids, nucleotides, and salt, yielding a reddish brown color and a distinctive smell. Fish sauce is marketed with various names in different countries; *nampla* in Thailand, *budu* in Malaysia, *bakasang* in Indonesia, *shottsuru ishiru* in Japan, *ae-jeot* in Korea, *nouc-mam* in Vietnam, and *patis* in the Philippines (Lopetcharat, Choi, Park, & Daeschel, 2001). Innovative methods include the addition of exogenous proteases and *koji* for the promotion of proteolysis in underutilized fish species (Giyatmi & Irianto, 2017). Different ingredients are also added during the fermentation of fish flesh: application of bacteria isolated from the fish-sauce mash (*moromi*); proteolysis by pure enzymes, for example, bromelain, visceral enzymes, soy sauce *koji*, and *koji*-mold, lactobacilli. Sauces made by controlled fermentation of fish have a better taste and savory aroma without unpleasant smell in comparison with the traditional fish sauces (Natteewan et al., 2017) (Table 4.1).

TABLE 4.1 Halal concern of animal-based umami substances.

Umami source	Umami ingredient	Origin	Halal concern
<i>Nampla</i>	Fish sauces produced by the fermentation of anchovies for 6–12 months contain high levels of peptides, amino acids, and nucleic acids and exhibit a strong umami taste. High-technology application involves the use of starter cultures and protease enzymes that not only produce consistent and quality product but also shorten the fermentation	Thailand	Hygiene practices Nonhalal enzyme origin Nonhalal culture medium for propagation of microbes.
Fish sauce	Fish sauce exhibited intense umami taste and high content of glutamate (259.2 mg/100 mL) of the fish sour.	Taiwan	Hygiene practices
<i>nuoc mam</i>	Traditional fish sauce as condiments consisted of glutamic and aspartic acids, threonine, alanine, valine, histidine, proline, tyrosine, cystine, methionine, and pyroglutamic acid. Glutamic acid content of fish sauce reaches 575 mg/100 mL after hydrolysis.	Vietnam	Hygiene practices
<i>Bakasang</i>	Fermented fish sauce produced by fermenting whole sardines for about 3–6 weeks. Alanine, isoleucine, glutamic acid, and lysine were prominent amino acids in <i>bakasang</i> . High glutamic acid is related to umami taste imparted from <i>bakasang</i> .	Indonesia	Hygiene practices
<i>Patis</i>	Seasonings in the Philippines with strong umami taste. Glutamic acid was the main free amino acid in <i>patis</i> . Fish or shrimp paste is made by the fermentation process of whole fish or shrimp in the presence of 20%–25% salt under ambient conditions. Fish sauce is a yellow or clear liquid extracted through <i>patis</i> , the complete hydrolysis of fish/salt mixture for 9–12 months.	Philippines	Hygiene practices
<i>Terasi</i>	A fermented shrimp paste with considerable levels of free glutamic acid and umami taste is a concentrated extract made from fish. It is used as a flavor enhancer in various Indonesian dishes.	Indonesia	Hygiene practices
<i>Petis udang</i> , <i>Petis kupan</i>	<i>Petis kupan</i> is a concentrated clam paste flavored with sugar. <i>Petis udang</i> is shrimp paste produced by boiling the heads and shells of shrimp. They contain free amino acids, such as glycine, alanine, and glutamic acids which are very high.	Indonesia	Hygiene practices
<i>Belacan</i>	<i>Belacan</i> is made from fermented tiny shrimp. It has salty and umami taste with strong shrimp odor. Dishes containing <i>belacan</i> show meaty flavor which best represents umami taste. Glutamic acid content is in the range of 20 mg/g in <i>belacan</i> .	Malaysia	Hygiene practices
<i>Dashi</i>	Boiled extract of dehydrated bonito, <i>konbu</i> , and/or <i>shiitake</i> mushroom. These stocks are very rich with umami substances (MSG and MP) and other taste-active components, including histidine and lactic acid.	Japan	Nonhalal cross-contamination
<i>jeotkal</i> , <i>sikhae</i> , and <i>aekjeot</i>	Fermented salted fish, fish fermented fish with cereal and fish sauce. Korean fish sauces contained low glutamate and high proline, glycine, and alanine which are sweet amino acids.	Korea	Nonhalal cross-contamination

MP, Inosine monophosphate; MSG, Monosodium glutamate.

Source: Adapted from Hajeb P, Jinap S. (2015). Umami taste components and their sources in Asian foods. *Critical Reviews in Food Science and Nutrition*, 55(6), 778–791. doi: 10.1080/10408398.2012.678422.

4.2.1.2 Plant-based umami sources

4.2.1.2.1 Fermented soybean product

Fermented soya beans are the main source of seasonings and savory condiments from plant origin. Soy sauce is commonly used as umami ingredient in cooking in Asian countries. Soy sauce is described as a liquid condiment which is used to add flavor and color to the Oriental diet. The method of production of a good quality soya sauce by genuine

TABLE 4.2 Halal concern of plant-based umami substances.

Umami sources	Umami ingredient	Origin	Halal concern
<i>Koikuchi</i> , <i>tamar</i> and <i>shiro</i>	<i>Koikuchi</i> has a sharp aroma and a deep brown color, is obtained when equal ratio of soybeans and wheat is used. <i>Tamari</i> has a greater viscosity and less aroma than <i>koikuchi</i> with a darker brown color, is made using soybeans with smaller amount of wheat. <i>Shiro</i> is produced with a high ratio of wheat to soybeans, with lighter color. The <i>koikuchi</i> makes up 90% of the Japanese soy sauce. Salty and umami are characteristic tastes of all types of Japanese soya sauce.	Japan	Nonhalal cross-contamination
<i>chiang-you</i>	Light or fresh soy sauce (<i>shengchou</i>) is used as seasoning and dark or old soy sauce (<i>laochou</i>) is used in cooking. Further process, including adding caramel color, drying, to obtain dark sauce.	China	Nonhalal cross-contamination
<i>Kicap</i>	A mixture of caramel, sugar to the extracted moromi mash of soya beans. Available in three forms, <i>Kicap Masin</i> , <i>Kicap Manis</i> , and <i>Kicap Lemak Manis</i> , formulated to contain 17% salt and not less than 0.6% total nitrogen. L-Glutamic is the main umami contribution.	Malaysia	Hygiene practices
<i>Ketcap</i>	Method of brewing produces L-glutamic acid, L-phenylalanine, and L-tyrosine in Indonesian soy sauce which are responsible for the intense umami taste of soy sauce.	Indonesia	Hygiene practices
<i>Kanjang</i>	A unique fermentation method using fermented soya bean block, incubation in brine.	Korea	Nonhalal cross-contamination
<i>Eoyukjang</i>	It is produced by fermenting meat and fishes with high concentrations of salt. Hydrolysis of the fishes and meat protein yield free amino acid, peptides, and ammonia is produced. Mixed sauce provides the strong and complicated umami taste which is stronger than that of soy sauce.	Korea	Nonhalal meat Nonhalal cross-contamination
<i>Doenjang</i> , <i>kochujang</i> and <i>cheonggukjang</i>	Fermented soya bean pastes are available in the form of <i>Doenjang</i> (soybean paste), <i>kochujang</i> (hot pepper soybean paste), <i>cheonggukjang</i> (fermented soybean paste by <i>Bacillus subtilis</i>). These products are very rich in umami taste with high concentrations of glutamic acid and aspartic acid.	Korea	Nonhalal cross-contamination Nonhalal culture medium for propagation of microbes

Source: Adapted from Hajeb P, Jinap S. (2015). Umami taste components and their sources in Asian foods. *Critical Reviews in Food Science and Nutrition*, 55(6), 778–791. doi: 10.1080/10408398.2012.678422.

fermentation of soya bean requires a long time, ranging from 6 to 10 months. There are different types of soy sauce, depending on its origin, composition, and method of preparation (Diez-Simon, Eichelsheim, Mumm, & Hall, 2020) (Table 4.2).

4.2.2 Commercial preparation

4.2.2.1 Umami and kokumi compounds

The typical taste of MSG that can be found naturally in different foods such as cheese, meat, vegetables, and seafood can also be produced commercially through fermentation process. Similarly, other important umami substances that belong to the group of purine-5'-nucleotides: IMP, GMP (Bicas et al., 2016; Yamaguchi, Ninomiya, 2000) can also be produced by commercial processes. Numerous peptides, generated by proteolysis of proteins originated from plant or animal products, can be obtained by chemical synthesis, elicit an intense umami taste (Lioe, Takara, & Yasuda, 2006). These peptides contain glutamyl or aspartyl residue such as Gly-Asp, Ala-Glu, Gly-Asp-Gly, Val-Asp-Val. The octapeptide Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala, hydrolyzed from beef exhibits umami taste properties (Dang, Gao, Ma, & Wu, 2015).

The discovery of synergistic effects between different umami-tasting molecules and the potentiation of MSG umami taste by IMP and GMP leads to the formulation of trio mix containing the three components for seasoning formulation and condiments. They are available in powder, granulate, and cube forms with various flavors and spices added to suit cooking desires. The trios are also added in canned products, cooking paste, and snack food to boost the palatability and acceptance of ready to it food.

The use of fermentation to produce seasonings, nucleotides, and MSG satisfies the vegetarian market with umami flavor enhancers derived from plants. Fermentation also offers a route to natural flavor enhancers as there is enormous consumer demand for natural flavorings (Paula Dionísio et al., 2012).

4.2.2.1.1 Soya sauce

Typical method of soya sauce production involves fermentation of soya bean and wheat, beginning with solid-state fermentation at koji stage followed by fermentation in brine solution, known as moromi, normally not less than 6 months to yield a sweet-smelling soya extract. Numerous modifications to shorten the maturity period of soya sauce fermentation are available. As production of soya sauce involves a two-step fermentation process, innovation mainly involves koji and moromi stage. Innovation at koji stage in one example involves maceration of koji mass to create a larger surface area, hence exposes to cell wall degrading enzymes, amylase, and proteolytic enzymes, thereby providing an enhanced substrate concentration for fermentation at moromi stage; while at moromi stage, fermentation is enhanced by reduction of salt in brine solution, increase in incubation temperature, addition of starter cultures, and separation of lactic acid and yeast fermentation in different tanks. To an extreme, immobilized enzyme and microorganisms are used for rapid and continuous soya sauce production. In modern production, a bioreactor is used where larger volume of moromi extract is produced under controlled parameters, including aseptic environment, temperature setting, starter culture inoculation, intermittent aeration, and lower brine concentration (Devanthi & Gkatzionis, 2019; Diez-Simon et al., 2020).

In Malaysia, batch fermentation under open environment is still common where a fiberglass tank of 2000–2500 L accommodates 1150–1200 kg koji with 24.5% brine to fill up the volume of the tank. Fermentation of moromi is carried out for 6–12 months to achieve the desirable flavor, aroma, and high total nitrogen (TN of at least 1%) of the fermented extract. A long period is required to allow the slow buildup of halophilic lactic acid bacteria naturally followed by yeast fermentation after the pH has dropped. Variation in quality of the fermented soya extract depends on culture selection at koji stage, the microorganisms present in the moromi, slight brine concentration range, and duration of fermentation. Starter culture (sometimes referred to as seed culture) for koji preparation is propriety to the manufacturer, being passed over generations or being bought from selected koji seed producers to produce consistent product quality. Koji stage provides all the necessary enzymes, including amylase, protease, and cell wall degrading enzymes for the next-stage fermentation to be carried out successfully by a consortium of microorganisms. Lower brine concentration reduces fermentation time slightly but is only possible with strict hygiene practice and clean environment (Sassi, Wan-Mohtar, Jamaludin, & Ilham, 2021).

4.2.2.1.2 Monosodium glutamate, inosine-5'-monophosphate, and guanosine-5'-monophosphate production by fermentation process

MSG is manufactured by fermentation of carbohydrate source from corn, sugarcane, or tapioca using the bacterium, *Corynebacterium glutamicum*, which is capable of producing high yields of L-glutamic acid. Ammonia is used as the nitrogen source and oxygen is provided by passing compressed air into the fermenting vessel. The L-glutamic acid is released by the microorganism into the fermentation medium and recovered by downstream crystallization (Mahmood, 2014; Sano, 2009). Ajinomoto (Japan), CheilJedang Group (Indonesia), and Daesang (Korea) have been producing this amino acid by fermentation for many years. In addition to the amino acid MSG, these companies also produce the nucleotides disodium IMP and disodium GMP by fermentation. Selected microbial strains, such as *Escherichia* or *Bacillus*, capable of overproducing MSG and nucleotides, are utilized for their production. IMP and GMP can also be produced by the enzymatic degradation of RNA with phosphodiesterase (Baines & Brown, 2016).

4.3 Halal process and execution in umami production

4.3.1 Standards and halal references

JAKIM being the competent authority in Malaysia stipulated by law for halal certification has established its functions to implement the halal certification system. Foreign Halal Certification Bodies recognized by JAKIM are institutions appointed by JAKIM to ratify halal status based on Malaysia Standard (MS) and practices. Hence, JAKIM recognition

program for international halal bodies is the most stringent and sought-after bilateral halal system recognition program in the world. The iconic Malaysia halal logo is the most sought-after, globally recognized hallmark that serves as the halal benchmark worldwide. It promotes opportunities in halal-related business for both products and services covering various halal economic sectors: manufacturing, tourism, financial, food and cosmetic, clothing and apparel, and pharmaceutical. The development of global halal market trend further emphasizes the critical requirement of an up-to-date system for effective global promotion and marketing, and thus the requirement for authentic halal certification ([Abdallah, Rahem, & Pasqualone, 2021](#)).

With the framework of Malaysian Halal Assurance Management System ([JAKIM, 2020b](#)) as the backbone of halal execution, the Halal Certification Manual Procedure ([JAKIM, 2020a](#)), and series of halal standards, the halal compliance status of registered products is assured through halal certification. [Table 4.3](#) includes among the pertinent references developed and utilized for halal execution.

4.3.2 Halal risk management through Malaysian halal management system

The principles of MHMS are somewhat similar to Hazard Analysis Critical Control Points (HACCP) approaches with the added value of monitoring and control of halal status throughout the supply chain via Malaysian Halal Assurance

TABLE 4.3 References used as guidelines for halal operation.

No.	References	Title	Publishers
1	Department of Standard Malaysia (2019f)	Malaysian Standard: MS1500: 2019 (Halal food: General requirements third revision)	DSM ^a
2	Department of Standard (2019b)	Malaysian Standard: MS 2424:2019 (Halal pharmaceuticals—General guidelines first revision)	DSM ^a
3	Department of Standard (2019a)	Malaysian Standard: MS 2634:2019 (Halal cosmetic—General guidelines first revision)	DSM ^a
4	Department of Standard (2012)	Malaysian Standard: MS 2200–2:2012 (Islamic consumer goods—Part 2: Usage of animal bone, skin and hair—general guidelines)	DSM ^a
5	Department of Standard (2019c)	Malaysian Standard: MS 2400–1:2019 (Halal supply chain management system—Part 1: Transportation—General requirements first revision)	DSM ^a
6	Department of Standard (2019d)	Halal supply chain management system—Part 2: Warehousing—General requirements (first revision)	DSM ^a
7	Department of Standard (2019e)	Malaysian Standard: MS 2400–3:2019 (Halal supply chain management system—Part 3: Retailing—General requirements first revision)	DSM ^a
8	Department of Standard (2014a)	Malaysian Standard: MS 1900:2014 (Shariah-based quality management systems—Requirements with guidance first revision)	DSM ^a
9	Department of Standard (2009)	Malaysian Standard: MS 2300:2009 (Value-based management system—Requirements from an Islamic perspective)	DSM ^a
10	Department of Standard (2013)	Malaysian Standard: MS 2393: 2013 (Islamic and halal principles—Definitions and interpretations on terminology)	DSM ^a
11	Department of Standard (2014b)	Malaysian Standard: MS 2565: 2014 (Halal packaging—General guidelines)	DSM ^a
12	Department of Standard (2015a)	Malaysian Standard: MS 2594:2015 (Halal chemicals for use in potable water treatment—General guidelines)	DSM ^a
13	Department of Standard (2015b)	Malaysian Standard: MS 2610:2015 (Muslim friendly hospitality services—Requirements)	DSM ^a

(Continued)

TABLE 4.3 (Continued)

No.	References	Title	Publishers
14	Department of Standard (2017)	Malaysian Standard: MS 2627:2017 (Detection of porcine DNA—Test method—Food and food products MS 2594:2015)	JAKIM ^b
15	JAKIM (2020b)	Malaysian halal management system	JAKIM ^b
16	JAKIM (2020a)	Malaysian halal certification procedure manual	JAKIM ^b
17	Department of Islamic Development Malaysia (2011)	Malaysian protocol for the halal meat and poultry productions	JAKIM ^b
18	Food Act 1983 and Food regulations 1985 (Act 281) (1985)	Arrangement of regulations	MDC Publisher

^aDepartment of Standard Malaysia.^bJabatan Kemajuan Islam Malaysia.

System (JAKIM, 2020b). It is as an internal mechanism to monitor, control, improve, and prevent any noncompliance in halal production.

Application of MHMS requires the formation of internal halal committee that builds and implements MHMS. This approach leads to the human capacity building of halal competent and certified halal executive that enables to conduct internal halal audit and periodical review of the system. It also allows halal materials control and implements traceability of finished products. As training is essential event, latest issues and statutory requirement are always updated.

Product specification, product process flow, and premise layout requirement are among the essential documents in MHMS. Ingredients for product formulation can be listed from the information obtained from the product specification and can be identified as critical, semicritical of noncritical for halal certification requirement. The HCP can be determined from the process flow of product formation, whereby the limit, control, and monitoring can be done, sharing the concept of HACCP. Layout of the premise ensures the preferred processing flow preventing cross-contamination and inefficiency.

4.4 Innovative trends in halal food industry

4.4.1 Case study: chicken hydrolysate the halal way

With the establishment of halal management system, a complete list of application guidelines, up-to-date categorized halal standards, and the availability of numerous certified halal products globally, application of halal is easier and faster than the early days with lower cost implications. Halal is now a branding of safe and wholesome food and preferred by consumers alike. An example of halal seasoning derived from chicken is highlighted to illustrate the ease of halal preparative steps for a successful application.

Chicken is one of the highest sources of raw protein that contribute to varying type of amino acid which differs in concentration among the parts. Chicken feet and neck contains high amount of protein, when hydrolyzed contributes to high glutamic acid and sweet amino acids that provide savory taste on food. The chicken hydrolysate can be produced by dual enzyme treatment using alcalase and flavourzyme, yielding peptides and amino acids which are not only beneficial to dietary contribution but potentiate umami taste of food preparations (Table 4.4).

The efficacy of the newly formulated seasoning was tested on grilled chicken for its umami contribution. Marinated chickens using the new food seasoning were compared with two other sets of chicken using commercial seasonings and blank which consisted of maltodextrin and spices. All chickens were marinated for a night in chiller before being grilled. Chickens were grilled for 15 minutes using air fryer. The marinated chickens were served to the panelist. Each grilled chickens were cut into small pieces with the same size and arranged in different bowls with coding. Panelists were given three different types of marinated grilled chickens and distilled water along with a 9-scale Hedonic form to be filled during evaluation (Aziz, 2020).

The formulated food seasoning enhanced the umami flavor of marinated chicken since it contained high glutamic acid, alanine, and glycine. Umami is the savory taste that represents the amino acid glutamate and widely used in food to enhance the flavor of savory product (Methven, Dermiki, Suwankanit, Kennedy, & Mottram, 2014). ANOVA

analysis indicated that the formulated food seasoning was the best food enhancer where the mean value for the chicken hydrolysate on umami attributes was the highest. Attribute on sweetness achieved the highest mean and were significantly different compared to both other samples. Chicken neck and feet contained the highest amount of sweet amino acids (glycine, alanine) and umami taste (glutamic acids) compared to both breast and thigh (Araújo et al., 2018).

Such new product utilizing chicken hydrolysate consisting of naturally derived amino acids without the addition of additives is potential to be marketed to Muslim customers. However, as it involves an animal source, the halal status needs to be verified. A summary of preparative steps required prior to halal application is shown in Table 4.5.

TABLE 4.4 Formulation of food seasoning (Aziz, 2020).

Ingredients	Amount
Feet ^a (hydrolysate)	1000 mL
Neck ^b (hydrolysate)	1000 mL
Sugar	10.75 g
Salt	10.75 g
Powder spices	1.35 g

^aFeet (1.3-g L-glutamic acid, 31.60-g L-alanine, 3.99-g L-glycine).
^bNeck (0.77-g L-glutamic acid mg, 6.41-g L-alanine, 6.10-g L-glycine).

TABLE 4.5 Essence of documentation for food seasoning halal certification application.

Checklist category	Relevant note	Status	Halal documentation
1. Product specification	Product description, product profile, product information, nutritional information, microbial limit, allergens, storage conditions	Provides information on ingredient list, and their origin	Halal certificate for relevant ingredients and raw material
2. Ingredient for seasoning formulation			
Chicken parts	Animal source	Critical—Decision tree analysis	Halal certificate (halal slaughtering)
Water	Natural source. Involve processing steps which may include the use of nonhalal filtering aids	Critical—Decision tree analysis	Halal certificate
Sugar	Plant source but uses activated carbon in processing which may originate from animal source	Critical—Decision tree analysis	Halal certificate
Salt	Sea origin	Not critical—Decision tree analysis	Preferable to have halal certificate
Spices	Plants sources	Not critical—Decision tree analysis	Preferable to have halal certificate
Proteolytic enzymes	Microbial sources which may be cultured in nonhalal medium	Critical—Decision tree analysis	Halal certificate
3. Processing/storage	Formulation, preparation, processing, handling, storage and transportation are in accordance with the MS 1500:2019; Malaysian Food Act (1983), and Food Regulation (1985)	Requirement of product specification and process flow. HCP analysis and MHMS plan development	Part of MHMS (2020) file documentation

(Continued)

TABLE 4.5 (Continued)

Checklist category	Relevant note	Status	Halal documentation
4. Premise, equipment, machineries, and utensils	Premise setup, equipment, machineries, and utensils are in accordance with the MS1500:2019	Premise layout diagram. Physical separation of premise with nonhalal line	Part of MHMS (2020) file documentation
5. Packaging and labeling	In accordance with the MS 1500:2019; Malaysian Food Act (1983), and Food Regulation (1985)	Halal packaging material and no misleading claims	Part of MHMS (2020) file documentation
6. Human resource	Internal halal committee and adequate Muslim workers, trained in halal principles	Halal executive to lead halal activities, development and implementation of MHMS	Part of MHMS (2020) file documentation
7. Documentation	Development of HAS file	Verified by management	Complete documentation of HAS file submitted to JAKIM after online HALAL application

HAS, Halal Assurance Management System; *HCP*, Halal control point.

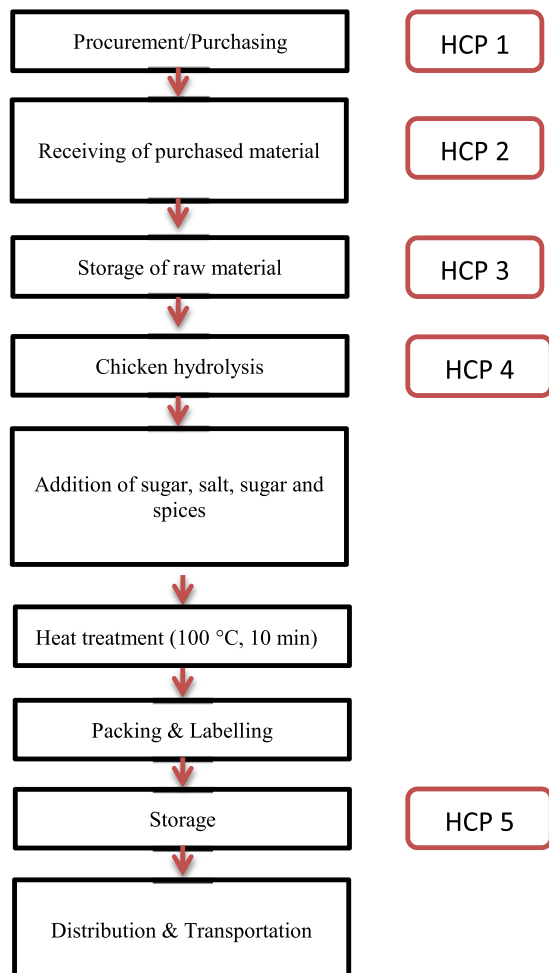
**FIGURE 4.1** Process flow for food seasoning production.

TABLE 4.6 Halal control point (HCP) risk management plan summary.

HCP no.	Process step	Control limits	Monitoring				Corrective action	Verification	Records
			What	How	When	Who			
1.	Procurement/purchasing	Only halal material.	List of halal material to be purchased	Approved halal supplier or halal product	Procurement process	Procurement/purchasing department	New supplier	Valid halal certificate	List of ordered material and valid halal certificate issued by competent authority
2.	Receiving of purchased material	Only halal material	1. Items received 2. Supplier's invoice	Checking details	Upon product arrival	QC/ warehouse officer	1. Halal executive to reject uncertified halal product 2. Return to supplier immediately	1. Checking stock through warehouse management system. 2. Physical inspection for nonhalal product presence at critical product storage area	1. Incoming form 2. Invoice/DO
3.	Storage of raw material	Only halal material	No mixing of nonhalal material	Physical separation	Upon receiving	Storekeeper	Reject nonhalal material	Categorize list of stored material	Approved list of stored material
4.	Chicken hydrolysis	Only halal material	Halal chicken, enzyme, salt, sugar and spices	Halal certificate	Before processing	Operator	Reject noncertified material	Audit by halal executive	Processing sheet
5.	Storage of finished product	Only halal material stored together	Packed seasonings	Physical separation	Upon storage	Storekeeper	Reject nonhalal material	Categorize list of stored material	Approved list of stored material

The vital part of MHMS is the summary plan which controls the risk of nonhalal threat. This requires the process flow for the manufacturing of the product of interest. The process flow provides the identification of several points which are necessary to control so that no contamination of product with nonhalal sources occur, known as HCP. Monitoring of each step is feasible after control limit has been set where corrective action will in place whenever there is a deviation. The concept is like HACCP where verification and documentation are an essential component of halal management (Kohilavani, et al., 2013). The process flow is also matched with the layout of the premise to ensure the proper flow of material, equipment, workers, and finished products to avoid cross-contamination. A typical process flow to produce the food seasoning is shown in Fig. 4.1.

Each of the identified HCP is then elaborated in a summary plan (Table 4.6) where it will be followed, monitored, and verified. The efficacy of the summary plan is determined by the compliance level during internal and external audits. This approach allows continuous improvement on halal compliance after several reviews on the summary plan.

With the monitoring of the determined HCPs, the nonhalal threats for the preparation of chicken-based seasoning can be prevented at many levels starting from purchasing steps, followed by acceptance of raw material and ingredient, handling, processing, and storage before distribution (JAKIM, 2020b). The integrity of halal umami is thus assessed by adequate measures reflected in the completed documentations, periodical site visits, audits, and occasional sampling of products. Upon fulfilment of the requirement and complete documentation, successful online application is executed within a short period.

4.5 Conclusion

Fish and plant-based umami sources have been easily obtained from fermentation of noncritical halal ingredients such as fish and soya bean product both traditionally and through industrial production. The halal authentication upgrades the hygiene and safety aspects of the final products. However, umami sources from animal meat such as chicken require stringent assessment. With the implementation of Halal Assurance Management System, production of halal umami sources can also be produced from poultry. Moreover, high-technology application using enzyme is already applicable with ease as the availability of halal ingredients, chemicals, and enzymes generated simultaneously with the Halal Assurance Management System.

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Chapter 5

Engineered meat and its acceptability

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5.1 What is engineered/cultivated meat?

Ever since the discovery of cells and the idea that cells constitute the basic elements of all life forms, mankind has been fascinated by the idea of growing cells outside of the body (i.e., in vitro). The origin of in vitro cell culture lies with Harrison's success in growing frog-derived nerve cells in a lymph fluid (Harrison, 1906). An intern in Harrison's lab sent by Alexis Carrel refined the cell culture method for avian and mammalian cells (Burrows, 1910) by substituting lymph fluid with serum, a blood-derived product. The friendship of Alexis Carrel with Winston Churchill led the latter to comment in 1932 in his book "Thoughts and Adventures" for the first time on the possibility to use cell culture for meat production.

5.1.1 Cells

For cultivated meat, this cell culture technology is used to create meat in vitro (Post, 2012). For this, starter cells are required. Specifically, to cultivate muscle, these starter cells consist of a muscle precursor cell, the so-called satellite cell (SC), as well as a precursor cell that can generate fat tissue, such as the fibro-adipogenic precursor (FAP). Both types of starter cells can be taken from a muscle from a living animal, for instance, through a needle biopsy. The SCs are then propagated in vitro to large numbers and are shaped and assembled to create muscle fibers. Likewise, fat precursor cells are grown and differentiated into fat tissue. The two tissues are then combined in a minced meat mixture and shaped into a hamburger or in any other format (Fig. 5.1).

SCs and FAPs are adult stem cells as opposed to embryonic stem cells. Adult stem cells have a limited life span and replication capacity, so using them requires recurrent biopsies from live animals. From a process point of view, but also ethically, this is suboptimal. However, continued scientific improvements may lead to increased proliferative capacity of these adult stem cells and thus to a meaningful reduction of the number of biopsies required to grow meat. Alternatively, one can use stem cells or cell lines, such as embryonic stem cells, induced pluripotent stem cells (iPSCs)

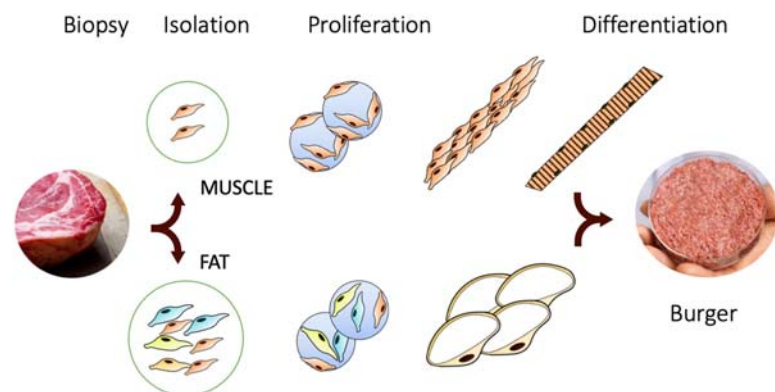


FIGURE 5.1 Cultivated meat starts with a muscle biopsy from which muscle and fat precursor cells are isolated. These cells are expanded to large numbers through proliferation. After that, they are differentiated into muscle and fat tissues that are recombined into, for instance, a hamburger.

or immortalized cells that have a much higher replication capacity and could therefore offer an advantage (Post et al., 2020). The advantages of adult stem cells on the other hand are that no genetic modification is required to harvest them and that their differentiation into full-blown muscle or fat is relatively easy.

As some of the religious ethical considerations on cultivated meat are related to the retrieval and identity of starter cells, a more in-depth discussion of the different alternatives is presented here.

5.1.1.1 Embryonic stem cells

Embryonic stem cells are taken from an early embryo, when the embryo is no more than two cell types surrounding a fluid-filled center, typically 6–8 days after gestation. The cells have an almost infinite capacity to proliferate and can differentiate into any type of tissue. The conditions to retrieve and culture embryonic stem cells are well defined for mouse and humans, but for many species, they still need to be developed or optimized (Ezashi, Yuan, & Roberts, 2016). To date, mouse and human embryonic stem cells have been exclusively used for scientific research. For domesticated animals such as cows and pigs, scientists only since very recent are able to maintain embryonic stem cells (Gao et al., 2019; Zhao et al., 2021). Also here, these cells are only used for research and currently are not applied to cultivate meat. As embryonic stem cells are not committed to any specific differentiation pathway, they typically need more specific triggers to differentiate into muscle or fat tissue. In addition, safety issues with embryonic stem cells such as their ability to induce teratoma's (malignant tumors consisting of many cell types) limit their medical application and may be an obstacle for regulatory approval of meat applications as well. Using cells derived from embryos for food application might also be an unsurmountable barrier to public acceptance of cultivated meat.

5.1.1.2 Induced pluripotent stem cells

In 2006 Takahashi and Yamanaka presented a new technology to turn regular tissue cells back into stem cells that are very similar to embryonic stem cells (Takahashi & Yamanaka, 2006). This “reprogramming” technology includes the insertion of four genes (Oct-4, KLF-4, cMyc, and Sox2) that are typically associated with undifferentiated stem cells and thus relies on genetic modification of the cells. The scientific importance and the potential applications for medical diagnosis, drug development, and therapy were underscored by the Nobel Prize for Medicine awarded to Yamanaka. iPSCs have since then been generated from many species, including pigs and cows (Ezashi et al., 2016; Su, Zhu, Salman, & Tang, 2020). To date, many reprogramming efforts for pig and cow cells have not resulted in fully reprogrammed iPSCs yet, although the level of reprogramming might still be sufficient for food applications. When fully successful, iPSCs present the same issues as embryonic stem cells in that their differentiation into muscle and fat is more cumbersome than for adult stem cells. They also share with embryonic stem cells the ability to form teratomas. Finally, the fact that genetic modification is still required to produce iPSCs may also prove an additional obstacle for consumer acceptance and regulatory approval.

5.1.1.3 Immortalized cells

Immortalized cells are adult tissue cells, such as muscle cells, that on the basis of spontaneous or induced mutation, have adopted the ability to proliferate indefinitely. Most cell lines that are commercially available, such as the mouse muscle cell line C2C12, belong to this category. For experimental purposes, these cell lines are easy to work with. Unfortunately, no such lines exist for bovine or porcine muscle cells. Several methods have been developed to create immortalized cells, the most common one being transgenic insertion of telomerase (TERT) (Cudré-Maroux et al., 2003). This is a genetic modification. Successful TERT insertion while maintaining the capacity for the genetically modified cells from cows or pigs to differentiate into muscle cells has not been described yet. A second method to generate cell lines is to rely on spontaneous mutations or induced mutations, either randomly in the DNA or more targeted to specific areas where mutations are thought to be more productive. This is a huge trial-and-error expedition. The final method is to edit the DNA in specific areas to achieve a more precise and less invasive genetic alteration. When immortalized cells have been identified for bovine, porcine, or avian muscle cells, they could become a very productive source of precursors for cultivated meat requiring even less animals for production than adult stem cells.

To date, adult stem cells such as SCs and FAPs, and iPSCs are primarily considered for cultivated meat (Fig. 5.2).

5.1.2 Cell culture

Once cells are harvested or produced, they need to be expanded to huge numbers. Cells are grown in a fluid, called medium, that contains nutrients, minerals, vitamins as necessary building blocks for new cells. Medium traditionally

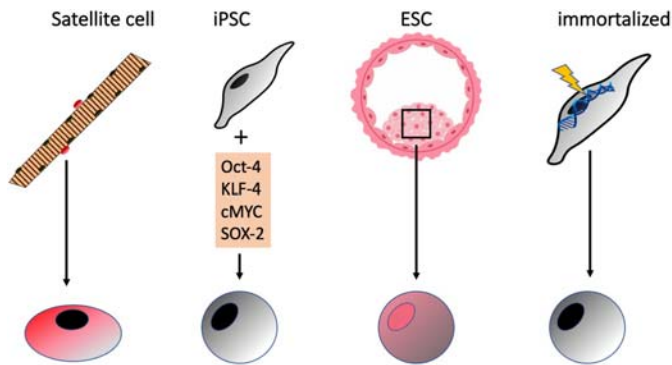


FIGURE 5.2 Cell types that are considered for cultivated meat. *ESC*, Embryonic stem cell; *iPSC*, induced pluripotent stem cell.

also contains serum that was successfully introduced in cell culture since 1910 (see earlier). Serum is a problematic component of medium for a number of reasons, including ethical concerns: it is harvested from unborn calves. It is also expensive and limited in supply (Brindley et al., 2012). When cultivated meat becomes a success and reduces the number of cows to grow meat, the supply of serum would even be less.

The search for serum-free media started in the medical field, specifically in vitro fertilization, out of a need for a more defined medium to grow embryos (Gardner & Lane, 1998). Since then, many serum-free media have been developed for different cell types and species. Muscle cells have proven to be a particular challenge for serum-free media as they are highly serum dependent, requiring up to 20% serum, where most cells thrive on 5% or even less. Recently, serum-free media have been described for bovine muscle cells (Kolkmann, Post, Rutjens, van Essen, & Moutsatsou, 2020) and it is expected that in less than 5 years, the use of serum is completely phased out in cultivated meat applications and perhaps in biomedical research in general. Moreover, the medium will be chemically defined, meaning that all components are fully defined in terms of chemical composition and not based on ill-defined animal or human extracts.

To culture cells in a serum-free medium, a set of proteins is typically necessary to sustain growth. These proteins are made using a recombinant technology, identical to how insulin is produced today (before the advent of recombinant technology, insulin came from pig and cow pancreas extracts). Technically, recombinant technology could be considered genetic modification, in this case of the microorganisms such as bacteria or yeast that produce the protein. Although the production system is genetically modified, the end product is not as it is a protein that is identical to the natural protein it substitutes. Proteins, unlike genes, do not have the capacity to spontaneously multiply or integrate in the DNA of the recipient. During the formulation of the end product, care is taken to remove any potential remnant of the original microorganism by which it is produced. This technology has therefore been accepted for pharmaceutical applications and many food applications, such as insulin and enzymes (e.g., transglutaminase or “meat glue”) used in food manufacturing and curing.

Although it is still a challenge to source a sufficiently cheap and scalable medium for cultivated meat applications, the underlying technology and science is well established.

5.1.3 Tissue

During the initial proliferation phase, intended to grow as many cells possible, SCs and FAPs, do not have the specific nutritional value yet that is typically associated with them. Proliferating SCs contain very little protein and hardly any specific muscle proteins, and FAPs do not contain fat yet. To achieve those mature muscle and fat cell states, cells need to be differentiated into muscle cells and fat cells, respectively. Differentiation of cells to muscle and fat is required to achieve all of its taste, structure, and cooking functionality as well as its nutritional value. A slightly different medium will initiate differentiation, but usually cells are also brought into a different environment that allows them to make a tissue. To this end, a specified number of cells are held together by a gel or scaffold that facilitates tissue formation. Gels are soft and allow the cells to dominate the final shape of the tissue, whereas scaffolds are strong and force cells to follow a predefined shape. Both consist of biomaterials that can be natural such as alginate, plant protein hydrolysates, or synthetic (Post et al., 2020). Biomaterials can be selected from a wide range of biomaterials that have been developed and extensively tested for medical applications of tissue engineering.

When cells, supported by a gel, form tissue, its size is limited to less than a mm in diameter. When tissues become bigger, the diffusion of oxygen and nutrients to the center of the tissue will be insufficient to keep the cells alive and

differentiating. This system is therefore limited to the production of minced meat such as hamburgers, sausages, or ingredient to minced meat dishes.

To make full-thickness cuts of meat that are thicker than 1 mm, a scaffold material is necessary to force muscle and fat cells into a predefined shape. In addition, a channel and perfusion system is required that allows perfusion of oxygen and nutrients to and removal of metabolic waste products from every part of the tissue (Post, Rahimi, & Caolo, 2013). This is technically more difficult than minced meat, but the principles and components of manufacturing and the end product are not necessarily different.

Most cultivated meat efforts are currently focusing on minced meat or cells as an ingredient, few steps are already taken into the direction of complex, full thickness, pieces of meat.

5.1.4 Benefits and downsides of cultivated meat

Meat is a highly valued yet extremely resource-intensive part of our diet. As a result of the innate metabolism of domesticated livestock animals, the conversion of animal feed into meat is inefficient. This is worst for cows, whereas fish have the most efficient feed-to-meat conversion (de Vries & de Boer, 2010). The low conversion ratio in combination with a high global demand for meat currently already requires 70% of arable land for crop production designated for animal feed (FAO, 2006). At the same time, global demand for meat is projected to increase by 70% over the coming decades, suggesting that this demand surpasses peak production of meat using conventional livestock husbandry. In addition, livestock agriculture appreciably contributes to greenhouse gas emission, reportedly up to 20% of total anthropogenic emission (FAO, 2006). There are also increasing societal concerns about the industrial use of animals for our food, attested by the steady growth of the number of vegetarians and vegans in postindustrial societies (Tan, Conner, Sun, Loughnan, & Smillie, 2021).

Other issues that are associated with industrial animal farming and current meat processing are the large-scale use of antibiotics and the appearance of contagious diseases that occasionally transmit to humans, so-called zoonoses.

Cultivated meat can theoretically solve many and perhaps all of these concerns. By optimizing culture conditions and introducing recycling of medium, a high conversion rate of feedstock into muscle protein or fat can be achieved. Cell and tissue culture is a closed system that is more amenable to recycling than open pasture animal husbandry. For cows and other ruminants specifically, the inherently inefficient gastric fermentation is bypassed in cultivated meat. The final conversion ratio will be in large part determined by the amount of processing that feedstock from plant-based proteins and sugars needs to make the available for cell metabolism during cell and tissue culture. Bypassing gastric fermentation in ruminants has the additional advantage of eliminating methane emission by ruminants. Methane is a greenhouse gas that has a much more potent effect on climate change than carbon dioxide. Some anticipatory life cycle analyses have been conducted to estimate the potential effect of cultivated meat on use of resources such as land and water, deployment of energy, greenhouse emission, and fine particle matter. These analyses are anticipatory as no definitive large-scale production method of cultivated meat has been established yet. To fully evaluate the environmental impact of technology, a detailed outline of the production has to be present. The anticipatory life cycle analyses therefore rely on some assumptions that still need to be affirmed. Yet, they do provide a rough estimate of gains to be made by cultivated meat and more important they give insight into where in the process the gains can be made. A recent report by CE Delft positions the environmental impact of cultivated meat between that of chicken meat and Tofu (Pinke & Odegard, 2021), provided that the energy comes exclusively from renewable sources.

Not considering ritual slaughter, animal slaughter is no longer necessary to obtain meat and depending on the multiplicity factor (Melzener, Verzijden, Buijs, Post, & Flack, 2021), much less animals are needed to produce meat. The multiplicity factor is how many meat in cow equivalent one can get from one donor animal and is thus a measure of the projected reduction in animals needed to meet global meat demand. Less animals mean less intense farming and will likely improve living conditions of animals.

Like in livestock agriculture, antibiotics are often used in cell culture to avoid cultures becoming infested with bacteria. However, with proper aseptic technique, the use of antibiotics will not be necessary (Verbruggen, Luining, van Essen, & Post, 2017). Finally, cell and tissue culture occurs in a closed and hygienic or even aseptic environment and is thus not vulnerable to contamination with bacterial, viral, or parasitic microorganisms that can cause zoonoses.

Are there downsides to cultivated meat? First and foremost, it needs to be established that cultivated meat is safe to eat and has no short or long-term undesired health effects. Being the result of a very different production process, safety assessment and regulation need to be carefully designed. In most countries, cultivated meat is considered a novel food, so that regulatory approval needs to be in place before the product can enter the market.

The theoretical downsides of cultivated meat are of socioeconomic and cultural nature. Livestock agriculture is practiced at many scales from small-scale rural farming to large industrial feed lots that are operated by a highly centralized

market dominated by a few large companies. Cultivating meat is potentially a disruptive force that not only affects large companies but also rural villages and families. For sure, livestock farmers will need time to adapt to a new situation where their role will be different or no longer necessary. At the same time, new opportunities and jobs will rise when cultivated meat will be at industrial scale. The technology of cultivating meat can develop into something that is user-friendly, so that small-scale production will be a theoretical possibility. It is uncertain, however, to what extent economy of scale is necessary to produce cost-effectively.

When animals are replaced by a resource-efficient system to grow meat, the efficiency of agriculture will increase. Grasslands that are now being used exclusively to feed cows, for instance, in Uruguay, will likely no longer be maintained and will turn back into forest. Alternatively, the land is used to produce crops other than the relatively protein-poor grass that can be used as input for cultivated meat. This will inevitably reshape the country.

Finally, when cultivated meat becomes an accepted alternative for conventional meat, it will change the way people think about meat and likely also about animal slaughter as a prerequisite for obtaining meat. Tolerance for these conventional practices will likely diminish, potentially threatening the practice of, for instance, ritual slaughter. Two other theoretical threats are that people will start to eat more meat thereby off-setting the initial environmental benefits and that conversion to the even more environment-friendly plant-based alternatives will slow down.

5.1.5 Acceptance of cultivated meat

5.1.5.1 General

Since 2011 a large number of surveys carried out in different populations across the world provided detailed information on the acceptance and its course over the years, nicely summarized by Bryant et al. in 2019 (Bryant & Barnett, 2018). While some outcomes vary between the studies, it is clear that when people understand what cultivated meat is, their acceptance increases. General acceptance in these studies varies between 25% and 60% of respondents accepting the concept of cultivated meat. Surveys are limited in relevance when it comes to predicting purchasing behavior when the products become available in stores or restaurants. Only one study involved a tasting session albeit it with a “fake” cultivated meat product (Rolland, Markus, & Post, 2020). In this study, all 193 participants ate the piece of meat that they were led to believe was cultivated and acceptance increased dramatically after information on cultivated meat and its potential benefits was provided, and even more after the piece of meat was eaten.

Benefits of cultivated meat are understood and apparently appreciated by respondents to surveys but concerns are also expressed. Concerns that are expressed revolve mostly about the expected quality of cultivated meat or its healthiness and cultivated meat being unnatural.

5.1.5.2 Special interest groups

It is conceivable that there are special groups that are either more or less accepting of cultivated meat, such as certain religious groups or consumers with self-imposed diet restrictions like vegetarians or vegans. In a study on cultivated meat acceptance in the United States, India, and China (Bryant, Szejda, Parekh, Deshpande, & Tse, 2019), religious background was listed as a variable. A subanalysis revealed 49%–68% of Muslims find cultured chicken, beef, or lamb appealing. Cultured pork only appealed to 28% and was also consumed by a 30% minority of surveyed Muslims (Bryant, 2020). For the denominations Judaism, Hinduism, and Buddhism, the appeal of cultivated meat from different species paralleled actual consumption of their conventional counterpart. For Hindus, for instance, cultured beef and cultured pork are appealing to only 19% of the participants whereas the appeal for cultured poultry and lamb was much higher (64%–68%) correlating with the higher consumption of conventional poultry and lamb. The average percentage of respondents who were accepting cultivated meat ranged from 33% in the United States to 62% and 63% in China and India, respectively, suggesting that adherence to a denomination did not affect acceptance of cultivated meat much.

Many speculations are made on the willingness of vegetarians and vegans to eat cultivated meat. Little information is available on this topic although predictably some studies show that cultivated meat is not appealing to this group (Valente, Fiedler, Sucha Heidemann, & Molento, 2019; Wilks & Phillips, 2017). From a motivational point of view, that is understandable as their dietary choices are already beneficial from an environmental and animal welfare perspective. On the other hand, a group of vegetarians and vegans may continue to crave for meat and for them cultivated meat might be a way to reconcile their principles with giving in to this craving. Most likely, and supported by the limited data available, this is true for a small minority of consumers who limit themselves largely or exclusively to a plant-based diet.

In summary, the acceptance of cultivated meat in special interest groups seems to reflect the consumption pattern that they show for regular meat, which is largely unaffected by religious beliefs.

5.1.6 Criteria for halal approval

In the halal perspectives, Islam has established several rules for identifying what foods are halal and haram. In general, every food is halal with the exception of those that are prohibited by Islamic law, such as blood, pigs, and animals that are not slaughtered in accordance with Islamic law, which has been stated in Surah al-Ma'idah, verse 3.

Therefore, in determining the halal status of the cultured meat, it must be ensured that the process of taking the tissue complies with the guidelines set by the Islamic law. Debates on this topic were mostly based on several arguments and it must therefore be studied in light of a number of factors.

First, if the tissue is taken from the limbs of the animal, then the animal must be classified as an animal that is halal to eat such as cows, goats, and the like and slaughtered in accordance with the teachings of Islam.

The described method to produce cultivated meat was originally designed to use small muscle biopsies from living animals that are not being slaughtered. The process of taking tissue from a living animal, whether embryonic or not, and preparing food from it is clearly not compatible with halal regulation (Hamdan, Post, Ramli, & Mustafa, 2018). According to the religious scholar, the tissue collection can be deemed as taking part of an animal that is severed or separated from it. If the tissue is taken while the animal is alive or dead without being slaughtered, then it is deemed as a carcass and illegal to be eaten. On the other hand, if it is taken from a dead animal that was slaughtered according to sharia, then it is halal to eat.

Although currently not practiced, it is conceivable that the process is changed in such a way that the animal is ritually slaughtered and that all of its muscle stem cells are being harvested. If one would be able to capture all the SCs from one cow and all can be used for meat production, only 100 cows would be needed to replace the current annual beef production of 300 million tons [based on 0.5 g biopsy leading to 5000 kg (Melzener et al., 2021) and 250 kg of “biopsy” from one animal]. This option is technically very challenging, but conceivable, and perhaps even acceptable to the general public and policy makers.

Alternatively, religious scholars might be able to argue that:

1. A 0.5-g biopsy from a living animal under “surgical” conditions with local anesthesia and aseptic technique is sufficiently different from the practice that is forbidden by Islamic ruling.
2. A 0.5-g biopsy is not a chewable size piece of meat.
3. With cell culture practices of the last 100 years, a piece of tissue from a living being that can be kept alive should therefore not consider being dead. This argument presumably also holds for CHO cells that were originally derived from Chinese Hamster Ovaries—hence the name—and that are increasingly being used to make lifesaving drugs such as insulin.

Were that argumentation to be successful and accepted by Muslim jurist, the currently envisioned practice of using biopsies of living animals could become accepted as Halal.

Second, the other Islamic ruling that is also relevant for cultivated meat is that blood or blood products may not be eaten. With the current practice of cultivating meat, that is no longer an issue as the industry has widely adopted the standpoint that serum cannot be used to cultivate meat. There are many nonreligious arguments in favor of that standpoint, the most insightful one being that successful scaling of cultivated meat will deplete the source of serum, so that peak serum (Brindley et al., 2012) will be reached quite early in the process.

These two major conditions for cultivated meat to be considered halal can therefore be met, opening the possibility for acceptance by the Muslim population.

5.2 Conclusion and recommendations

Cultivated meat is a technically challenging solution to a serious problem: the future production and consumption of meat and its negative externalities if realized through conventional animal agriculture. The coming years will be crucial for the development of cultivated meat into a scalable and cost-effective alternative to conventional meat. It is important to include the Muslim community and Muslim scholars during this process as halal compliance depends on how the technology develops. Once compliant, it is expected that the Muslim community will accept cultivated meat equally to the non-Muslim community.

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Product innovation: palm oil fat in plant-based meat

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6.1 Introduction

Meat analog or meat substitutes are products that are made from nonmeat and/or sometimes nondairy ingredients, which are available in many forms, including sausages, burgers, breaded nuggets, meatballs, pizza, and taco toppings or fillings, and also including fish and seafood products (Joshi & Kumar, 2015; Kazir & Livney, 2021). Due to the high demand of meat substitutes, extensive research and development works are conducted to improve not only the nutritional value but also the texture of these products to mimic its meat counterparts.

Meat products such as sausages, patties, and frankfurters are basically made up of meat and fat, besides other ingredients such as nitrate, flavoring agent, and others, have become an important part of modern diet in many communities. There are many types of sausages such as fresh, cooked, smoked, and fermented sausage. According to Essien (2003), consumption of sausage in the United Kingdom is still common despite the series of meat safety issues within Europe.

Meat alternatives have been introduced to satisfy the trends and demands of healthy and delicious meat-free food for the vegetarian and others. Meat alternatives can be defined as food products that give esthetic qualities and/or chemical characteristics that are quite similar to some types of meat (Kumar et al., 2017). Soya protein, mushroom, wheat gluten, egg albumen, carbohydrates, gums are some of the meat alternatives used for meat-free products (Kumar et al., 2017).

As a type of processed meat, sausages have many issues related to health concerns. First, consumption of meat products may increase the risk of cancer, where consumption of 30 g of processed meat a day may increase the risk of colon cancer (Larsson & Wolk, 2006) (as cited in Kumar et al. (2017)). Besides, the addition of nitrate and nitrite into sausages can form carcinogenic compound nitrosamine when cooked at high temperature therefore also increase the risk of getting cancer. Moreover, they are also high in fat that can lead to obesity if excessively consumed. Therefore it is significant to develop plant-based ingredients to address health concerns and provide assurance to consumers. In recent years, new product development in the meat products using alternative meat protein sources to produce meat products is increasing. As such, many plant-based meat alternatives have been successfully used. As such, many plant based meat alternatives like burgers, sausages, mince, nuggets and seafood alternatives are readily available in the retail market shelves together with their meat counterparts (Curtain and Grafenauer, 2019; Alessandrini et al., 2021).

6.2 Market size of plant-based meat products

Meat analog or plant-based meat is a rapidly growing food segment globally in the food market, both in retail and food service industries. The growing demand of alternative proteins in food products is supported by consumer concern on environmental sustainability, animal welfare, global shortage of animal protein, strong demand for wholesome and religious (halal) food, and various economic reasons. Allied Market Research reported that the global meat substitute market was measured at USD 4.51 billion in 2019 and is estimated to hit USD 8.82 billion by 2027. In another analysis by Fortune Business Insight, the market is expected to grow to USD 10.8 billion by

2028. Therefore extensive development and innovation works are conducted globally anticipated to benefit from the lucrative opportunities in the future. Nevertheless, with the consumer expectation for engineered meat to be almost indistinguishable from traditional meat products, with similar nutritional properties and sensory characteristics, this will be big challenges for the manufacturer to fulfill the consumer expectation. To achieve the high acceptance, the food industry currently studies to improve the quality of meat alternatives, especially for the structural and sensory properties.

Aside from protein, vegetable fat is found to be an important ingredient that is able to contribute to the overall eating quality of plant-based meat that helps to improve palatability, flavor release as well as being able to contribute to the feeling of satiety. In this chapter, the potential of using palm oil as an animal fat replacer in comparison to other vegetable fat sources and its acceptability are discussed. Furthermore, palm fat being a plant source is also accepted as a product to replace animal fat by some consumers due to religion and health concerns.

6.3 Driving factors of plant-based meat products

The world population is growing exponentially and is predicted to reach 9 billion by 2050 (Bonny, Gardner, Pethick, & Hocquette, 2015). Due to the rapid increase in global demand for meat, livestock farming has been keeping up to produce enough supply. Unfortunately for future generations, the world may not be able to meet future demands for meat due to limited land and water resources, increase in animal welfare issues, and worrying climate changes and environmental impacts (Alexander et al., 2017).

Over the recent years, the livestock industry has raised concerns over its environmental impacts. Based on recent data (Lowder, Marco, & Bertini, 2021; Poore & Nemecek, 2018), the livestock industry accounts for 77% of global farming land which includes pastures used for grazing with arable land used to grow crops for animal feed. Despite taking up most of the agricultural land, the industry only produces 18% of the world's calories and 37% of total protein. The situation is shockingly different for the crop industry. Excluding animal feed, the industry only needs the remaining 23% of agricultural land to produce 82% and 63% of global calorie and protein supply, respectively.

According to the same source, the livestock and fisheries account for 31% of global greenhouse gas emissions and since human-edible and cereal crops are each supplemented in the diets of monogastric and ruminant species, respectively, an additional 6% are produced in crop production for animal feed. Not only that, the industry is also responsible for significant water use for feed crop irrigation. Additionally, the effluent from the industry is blamed as a major cause of freshwater pollution. Crop production for direct human consumption on the other hand accounts for 21% of food's emissions.

Meat consumption has been associated with increased risks of heart disease and type 2 diabetes (Micha, Michas, & Mozaffarian, 2012), stroke (Kaluza, Wolk, & Larsson, 2012), and certain cancers—particularly colorectal (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2018). This triggers the consumer's need to transition to a healthy lifestyle or the change in eating habits as it is deemed to improve health and cure illnesses (Fehér, Gazdecki, Véha, Szakály, & Szakály, 2020). Furthermore, meat analogs are in high demand and the plant-based ingredients incorporated into these products will also benefit consumers with strict dietary practices such as vegetarians, vegans, and those that prefer meat-free diets due to religious reasons (Joshi & Kumar, 2015). Food safety concerns have also risen as pathogenic contamination of animal origin can not only be risks to consumers, but also to workers in the operational premises as they may be exposed to zoonotic pathogens (Fitch, Hricko, & Martin, 2017). Many bacterial pathogens such as *Salmonella*, *Escherichia coli*, *Campylobacter*, and *Listeria* are found in the guts of animals and as such, may cause foodborne illnesses.

Animal welfare problems are not exempted from the livestock industry dilemma. The industrial livestock production is designed to produce as much meat, eggs, and dairy products as possible, at minimal cost and as such, most farmed animals are raised in crowded premises, often in confined crates or cages with no access to the outdoors and are unable to roam freely and exhibit natural behaviors (Pew Commission on Industrial Animal Farm Production, PCIAFP (2008)). Although the consumption of seafood has been associated with many health benefits, there is not enough global seafood supply for everyone to consume (Thurstan & Roberts, 2014). Due to these pressing concerns, the need to produce meat alternatives as protein sources has become an urgency.

6.4 History of plant-based meat

By definition, conventional meat is traditional meat from farm animals, while plant-based meat analogs are made of plant and fungus proteins. By extracting proteins found in plants, plant-based meat analogs can be produced

TABLE 6.1 Plant protein sources used in making plant-based meat analogs (Asgar, Fazilah, Huda, Bhat, & Karim, 2010).

Plant	Protein
<i>Fusarium venenatum</i> (Filamentous fungus)	Mycoprotein
Legumes	Glycinin, Vicilin
Oil seeds	Legumin, Albumins, Globulins, Glutelins
Wheat, rye, and barley	Gluten (Gliadins, Glutenins)

Source: Adapted from Asgar, M. A., Fazilah, A., Huda, N., Bhat, R., & Karim, A. (2010). Nonmeat protein alternatives as meat extenders and meat analogs. *Comprehensive Reviews in Food Science and Food Safety*, 9, 513–529.

(Bonny et al., 2015). Meat analogs are defined as food products that mimic the esthetic, organoleptic, and chemical characteristics of traditional meat products. Some of the main sources of these analogs are wheat, soybean, legumes, oil seeds, and fungi (Table 6.1).

In our history, plant protein is one of the oldest sources of food. In 965 CE, tofu was first consumed in China which was referred to as “mock lamb chops” or “the vice mayor’s mutton.” Several other products such as seitan, yuba, and tempeh were introduced in the following decades in different regions around the world. In 1876 the term “substitute for meat” was first used in a catalog for Japan’s International Exhibition at Philadelphia in a description of tofu and miso (Shurtleff & Aoyagi, 2014).

Tempeh is a fermented product made from soybean that originated from Indonesia and has been widely consumed. It is made from dehulled soybeans that undergo solid-state fermentation with *Rhizopus oligosporus* which encourages the growth of its hyphae into the cotyledon and therefore binds the soybean together and produces a whitish yellow cake (Taylor, 2011). Tempeh is produced in four main steps which starts from soaking dehulled soybeans, boiling, followed by inoculating them with *Rhizopus* sp. and lastly incubating at room temperature (Astuti, Meliala, Dalais, & Wahlqvist, 2000). Besides, it can also be incubated as a solid bed at 30°C–37°C for 40–48 hours (Mo et al., 2013).

Meanwhile, soybean curd, which is also known as tofu, is a traditional product that is well-known in Asian food culture and it mainly consists of protein and fat (Cui, Zhao, & Lin, 2015). It is a product of coagulations of soymilk, white or pale yellow in color and has a soft texture likely similar to firm yoghurt. It is commonly known as salt or acid-coagulated water-based gel, whereby gel networks trapped the soy lipids and proteins inside (Kohyma, Sano, & Doi, 1995).

Traditionally, meat analogs are low in lipid content. However, modern meat analogs contain more lipid content than traditional ones as the modern analogs tend to imitate the composition of traditional meat products (Bohrer, 2019). In fact, oftentimes the modern meat analogs meet the macronutrient composition of conventional meat products (Kumar et al., 2017). Moreover, the newer generation of meat substitutes is modified to mimic the appearance, texture, taste, and aroma of authentic meat products by using various technologies (He, Evans, Liu, & Shao, 2020). Examples that are worth mentioning are with the use of soy leghemoglobin and beet juice or powder in Impossible Foods and Beyond Meat products, respectively, to create the red color and “bleeding” effects of raw meat. The use of soy leghemoglobin in plant-based burgers is to contribute to the flavor and aroma of meat during cooking (Fraser, Brown, Karr, Holz-Schietinger, & Cohn, 2018).

According to Bashi, McCullough, Ong, and Ramirez (2019) and Essien (2003), new product development in the meat products sector began to target alternative protein sources and other ingredients to produce sausages since the meat industry is influenced by the food safety issues thus making people lose confidence to consume meat as a source of protein. Fernandez (2012) also claimed that public health messages have been encouraging people to change from animal protein to more plant-based protein for protein group consumption. Besides, since years ago, there has been rising demand for using nonmeat components to produce enhanced quality and healthier meat products (Bashi et al., 2019). The increase in numbers of individuals with a flexitarian diet influenced the development of the newer generation of plant-based meat alternatives. A flexitarian diet is a diet where individuals consume meat occasionally to improve nutrient density and reduce environmental impact due to meat consumption (Bashi et al., 2019). As meat eaters prefer the taste of meat, most often the taste and flavor of traditional meat alternatives are less enjoyed, leading to the use of technologies to form meat-like textures and appearance (He et al., 2020).

Some of the meat alternatives that can be found in the market are summarized in Table 6.2.

TABLE 6.2 Some of the products available in the market.

Type	Example	Reference
Emulsion	sausages/frankfurters/bologna/deli “meats” ham	Kyriakopoulou, Dekkers, & van der Goot (2019)
Finished Product	Burgers/patties, meatballs, nuggets, fish & seafood minced meat analogues	Kolodziejczak et al. (2022)
Resembling raw meat	Chopped meat analogues, whole muscle meat analogues	Kolodziejczak et al. (2022)

As mentioned in Leahy, Lyons, and Tol (2010) (cited in He et al., 2020), the growing community of vegetarians and vegans influenced the further development of meat analogs, especially in Western countries and increased the market demand for meat alternatives leading to the popular demand of texturized vegetable protein (TVP). Many modern meat analogs can be categorized as ultraprocessed foods which are manufactured with little or no whole foods, and with processed ingredients or substances that are extracted or refined from whole foods (i.e., protein isolates, oils, hydrogenated oils and fats, flours and starches, and variants of sugars and refined carbohydrates) (Bohrer, 2019). According to Essien (2003), protein imitation from vegetable sources such as soybeans has been frequently used as meat extenders, analogs, or replacers thus have big potential in vegetarian sausage choices. Kumar et al. (2017) have listed out some of the key ingredients to be used during preparation of meat alternatives or meat analog which are soya protein, mushroom, wheat gluten, egg albumen, carbohydrates, and gum, and last flavoring and other miscellaneous compounds. Fat is another crucial component for the development of plant-based meat as it contributes to the overall juiciness, flavor, texture, and mouthfeel of the product (Bohrer, 2019).

6.5 Ingredients in plant-based meat

6.5.1 Types of protein and their functions

Plant protein is one of the most important ingredients for the development of plant-based meat as it contributes to the main texture and functional properties. Besides physical functionality, combinations of plant proteins are also added to plant-based products to create a nutritionally balanced amino acid profile. The plant proteins are processed and refined to improve their functionalities and mixed with other ingredients to form a meat-like texture (Rubio, Xiang, & Kaplan, 2020).

Soy is a common plant-based protein that is used in processed meat products (Taylor, 2011). Soy protein has been extensively used to partially or completely replace meat as its nutrient content is comparable with the meat and also gives lower possibility of cardiovascular diseases (Kumar et al., 2017). It is rich in calcium and alpha linoleic acid therefore provides a good source of nutrition for vegetarians as well as providing a good replacement for meat products (Wardlaw & Kessel, 2002). Besides, it also can act as stabilizer and emulsifier as it has the ability to enhance texture and give good water-holding capacity to the final product. Soy protein can be used in the forms of soya flour, soya bits, isolated soy protein, spray dried soya milk, and texturized soy proteins (Qin, Wang, & Luo, 2022).

Soybean is a part of a vegetarian diet that contains all of the essential amino acids required in the human diet (Henkel, 2000). It is also a good plant-based alternative that has been successfully incorporated to many processed meat. Tempeh and soybean curd are soybean by-products that can serve as meat alternatives as they have the meat-like flavor profiles (Babu, Bhakayaraj, & Vidhyalakshmi, 2009; Taylor, 2011). Besides, they have B vitamins, including folate and vitamin B12 that are lacking in meat (Lukito, 2001) (as cited in Taylor, 2011).

Many plant-based meat alternatives such as TVP and isolated soy protein have successfully been used in sausage or other meat products; however, little or no information is available on the effect of addition of tempeh and soybean curd on the physicochemical and sensory properties of sausage.

Taylor (2011) reported that years ago a study by (Kuo, Wang, Peng, & Ockerman, 1989) showed that tempeh has been previously incorporated into ham; besides, a processed fish product containing tempeh was created and patented (Gyorgy, 1972). Tempeh can be served as a meat alternative among the vegetarians for the meat-like flavor profiles and it is rich in protein and B vitamins that are normally lacking in vegetarian diets (Taylor, 2011). Besides, Taylor (2011)

also reported that there is a potential for the new products development by partially or fully incorporating tempeh in meat products with aims on improving health benefits.

Rekha and Vijayalakshmi (2013) claimed that “tofu is meat without bone,” as it is a versatile meat or cheese alternative due to its bland taste and porous texture. Besides being cheap, it has a lot of nutritional value, including excellent sources of proteins, minerals, and polyunsaturated fatty acids, and contains no cholesterol. There are a lot of studies that use tofu as alternatives in meat products. One of the previous research hamburgers that are substituted with tofu and the outcome shows the taste is similar to hamburger, but with lower calories and fat (Ohyama et al., 2013). Therefore the incorporation of tofu in sausages may give advantages on improving physicochemical and sensory properties of sausage.

Another common plant protein ingredient in plant-based meat is seitan (wheat gluten). Seitan is a protein derived from wheat and has a fibrous texture when hydrated.

Other than soy protein, mushroom is also used as meat alternatives due to its meaty flavor because of the presence of sulfur-containing amino acids and glutamic acid in fungi which has been demonstrated in Quorn® myco-protein fungus (Trinci, 1994) (as cited in Kumar et al., 2017). Besides, mushroom flavor of fungal protein is better than the beany flavor of textured soy protein.

6.5.2 Fat (function, types, comparison with animal fat in meat and plant-based meat products)

Fat plays a main role in creating great mouthfeel of a food product where it can contribute to the juiciness, tenderness, and flavor release (Kyriakopoulou, Dekkers, & van der Goot, 2019) in which similar functionality is applied for animal fats (Calkins & Hodgen, 2007). The presence of fat within the meat tissues assists in lubrication of the muscle fibers, enhances the water-holding capacity, and helps in softening the meat product and thus improves the chewing sensation during eating (Savell & Cross, 1988).

The vast selection of vegetable fats and oils with different range of characteristics leads to the possibility of mimicking animal fat in plant-based meat. The combination of solid fats like coconut oil or palm oil and liquid oils, such as sunflower or canola oils are usually done to mimic the texture of animal fats (Sha & Xiong, 2020). As such, marbling appearance in plant-based meat products that is similar to meat products is also possible through the blending of saturated and unsaturated fats.

Apart from the texture and taste profile contributed by fats, the aroma of a product is also contributed by fats. As mentioned in Kaczmarek, Taylor, Piyasiri, and Frank (2021), the distinct aroma and flavor in meat is contributed by the intramuscular fat present in between the meat tissues. The different composition of fatty acids in animal fats gives its distinct flavor and aroma. However, this is not applicable to the plant-based meat counterparts, as vegetable fats and oils usually have a bland taste and have little to no aroma. Therefore the use of flavor additives is essential to create the distinct meat attributes.

Some of the most common vegetable fats and oils used for the formulation of commercialized plant-based products include avocado oil, canola oil, cocoa butter, coconut oil, corn oil, safflower oil, sesame oil, soybean oil, and sunflower oil (Sha & Xiong, 2020). Coconut oil is the most common fat substitute for meat alternatives formulation and being a natural source of solid fat, the hydrogenation (fat solidification) process can be avoided (McClements & Grossmann, 2021). Moreover, coconut oil has a sharp melting curve, where it appears solid at room temperature but melts at warmer temperature, which is a desirable characteristic for flavor release. Blends of coconut oil and liquid oils such as canola or sunflower oil can be used to achieve the right balance for meat substitute products.

6.5.3 Carbohydrate as binding agents

To further improve the product properties of plant-based meat, carbohydrate-based ingredients are added as the binding and texturizing agents. Many sources of this ingredients are used for the manufacturing of meat analogs which comes from starches or flours and vegetable gums, such as, methylcellulose, acacia gum, xanthan gum, carrageenan that are used to improve product consistency, texture, stability, and form (Kyriakopoulou, Dekkers, & van der Goot, 2019; Bohrer, 2019).

Another meat alternative that is greatly used in the food industry is carbohydrate gums such as hydrocolloids, maltodextrins, and pectin as texture-modifying agents in many different types of products. For example, starches and maltodextrins are polymers of glucose which form gel and mimic fat-like texture when hydrated (Kumar et al., 2017).

6.5.4 Other additives

Coloring and flavoring ingredients in plant-based meats are important as it can improve the appearance and enhance its physical qualities. Good taste and appearance are important sensory attributes as it determines the acceptability of a product. A plant-based meat is also expected to behave like meat (beef) where it appears reddish color and turns brownish once it is cooked. Different approaches have been used to stimulate this characteristic. For example, the company Impossible Foods uses an iron-binding protein identified from the roots of soybean plants—soy leghemoglobin—which changes color during cooking that is similar to meat. Other approaches used as natural ingredients like beetroot powder/extract, pomegranate fruit powder, tomato paste, paprika oleoresin, carrot extract, and many others are used to imitate the reddish color of meat (Kyriakopoulou, Dekkers, & van der Goot, 2019; Sha & Xiong, 2020; Bohrer, 2019). Furthermore, flavor additives are added to plant-based foods to enhance the taste of meat due to the lack of flavor from the ingredients used. Not only can it enhance the meaty profile, but also mask the beany off notes from the plant proteins. Yeast extract, mushroom, garlic and onion extract, and various herbs and spices are used to enhance the flavor of plant-based products.

To avoid nutritional deficiencies due to the adoption of plant-based diets, nutritional additives such as vitamins and minerals are incorporated into the formulation of plant-based foods. Vitamin D, omega 3 fatty acids, vitamin B12, calcium, iron, or zinc fortification is useful as the levels for these are usually lower in individuals who follow a plant-based diet (Tuso, Ismail, Ha, & Bartolotto, 2013). Moreover, the use of unsaturated oils in the majority of plant-based meat products also makes products more prone to oxidation. Therefore antioxidants and preservatives are usually added to prolong the shelf life of these products.

6.6 Palm fats in plant-based meat

6.6.1 Palm oil fractions and solid fat composition

Palm oil is a vegetable oil derived from the mesocarp (pulp) of the fruit of oil palms (*Elaeis guineensis*). The kernel (seed) of the fruit produces palm kernel oil. Both palm oil and palm kernel oil have different chemical properties. The fatty acid composition of these oils differs where palm kernel oil contains higher levels of saturated fatty acids (more than 80%) than the palm oil (50%). Palm oil has high content of oleic acid (C18:1) and linoleic acid (C18:2) and also natural antioxidants such as vitamin E tocotrienols giving it excellent oxidative stability. Palm kernel oil has a high level of lauric acid and a sharp melting profile, making it suitable for confectioneries and other specialty fats (Dian et al., 2017). This determines the functionality of the oils in food applications. For example, the palm kernel oil is commonly used as a cocoa butter substitute in confectionery as it has similar attributes as cocoa butter and coconut oil while palm oil is widely used as cooking oil. Both palm oil and palm kernel oil can be separated into two fractions: olein and stearin which are the liquid and solid fractions of each oil. This intriguing aspect of the oils from the oil palm fruit is due to the fact that they are naturally semisolid. Both palm and palm kernel stearin and olein are separated using fractionation technique, which is a thermomechanical process and uses the difference in crystallization properties of the fats' triacylglycerols into a low melting fraction and a high melting fraction (Pande, Akoh, & Lai, 2012).

Palm stearin, the harder fraction of palm oil, has a higher proportion of saturated fat with a melting point from 45°C to 50°C with palmitic acid content ranging from 49% to 68% and oleic acid content from 24% to 34%. Due to the high melting point of fat, it is normally used as the hard stock for soft margarine manufacturing (Sue & Pantzaris, 2009 as cited in Pande, Akoh, & Lai, 2012). The fat can undergo a second fractionation process which yields another fraction called palm mid fraction (PMF) that can be used as a cocoa butter equivalent for confectionery products. Due to the wide range of properties palm stearin possesses, it allows food manufacturers to have a wide choice of materials for their fat formulations with unsaturated oils in products (Pande, Akoh, & Lai, 2012). Moreover, it exists as a solid fat naturally and hydrogenation process is avoided which can prevent the formation of trans fats. On the other hand, palm olein is the liquid fraction of palm oil that is filtered out during fractionation. Palm olein contains higher amounts of oleic acid (39%–45%) and linoleic acid (10%–13%) as compared to palm oil (Pande, Akoh, & Lai, 2012). It is liquid at room temperature with a melting point of 18°C–20°C and has different grades based on its iodine value for different applications. Similar to palm stearin, it can be further fractionated to produce PMF; however, the PMF product from stearin fraction is more preferable for confectionery applications. Another product of fractionation of palm olein is a more unsaturated olein called superolein with 43%–49% oleic and 10%–15% linoleic acids (Sue & Pantzaris, 2009) making superolein is more “unsaturated” or more liquid fraction of olein. Superolein can be used as salad dressing or mayonnaise substitute when blending with other vegetable oils such as soybean or corn oil (Dian et al., 2017).

Palm kernel oil and its fractions, on the other hand, have different properties compared to palm oil. Palm kernel stearin, through the hydrogenation process is made into cocoa butter substitute, which is used for cocoa butter alternatives in the confectionery industry for a cheaper fat alternative. However, limited research was conducted in the evaluation of palm kernel oil and its fractions in the plant-based meat segment. Moreover, the minor components in palm oil may also be beneficial to enhance the food application industry. Carotene is naturally found abundantly in crude palm oil and can be added as value toward product development. Red olein, one of the products from palm, is produced through low-temperature processing, can be a possible ingredient to boost the health benefit of plant-based meat products. Red olein is also considered a natural antioxidant due to the high amount of vitamin A and can be a great source for an antiinflammatory ingredient. Another great component in palm oil is the abundance of tocotrienol and tocopherol or vitamin E, which has high antioxidative properties. This could prolong the oxidative stability of the oil and simultaneously increase the shelf life of a product.

6.6.2 Use of palm oil and palm kernel oil as animal fat replacer

Animal fat has come a long way as a major constituent that helps impart flavor and texture in the production of processed meat products such as burgers, frankfurters, and nuggets. Despite the common use of animal fat in processed meat, or even processed food in general, it is associated with many health issues related to diet. It is mostly due to the contents of its cholesterol and trans fatty acid (Ospina-E, Sierra-C, Ochoa, Pérez-Álvarez, & Fernández-López, 2012). By substituting animal fat with vegetable fat, these issues can be addressed and any dietary risks can be reduced or eliminated.

Palm oil and its fractions such as palm olein and palm stearin have been reported to be suitable vegetable fats to be used as animal fat replacer. For example, it has been found that the blend of palm olein and palm stearin as the fat component in chicken frankfurters has sensory properties comparable to those of a commercial sample. In fact, the fat and water separation issue in the meat batter was eliminated by the presence of palm olein (Tan, Aminah, Zhang, & Abdul, 2006).

Vural and Javidipour (2002) found in their study that interesterified palm oil was able to partially replace beef fat in the formulation of frankfurters. In the same year, another study (Hsu & Yu, 2002) showed that partial palm oil substitution is suitable for making low-fat emulsified meatballs, or *kung-wans*. The balanced (50% saturated fatty acids and 50% unsaturated fatty acids) of palm oil can contribute to the formation of organoleptically acceptable properties of the meat products. According to (O'Brien, 2009) palm oil is similar to animal fats such as lard and tallow in terms of its physical properties—which makes it an ideal alternative for animal fat replacement in meat products such as sausages and meat patties.

Abd Hamid and Hassim (2018) conducted a study to determine the optimum amounts of vitamin E-enriched palm fat, oats, and xanthan gum in a vegetarian nugget formulation formulated in a preliminary study. The plant protein constituted in the formulation is seitan, a chewy protein-rich food made from wheat gluten. In the study, palm fat played a major role in influencing oil absorption during frying, as well as firmness and toughness of the nugget. It was found that a higher content of oil resulted in higher content of vitamin E. The use of the seitan also influenced the oil absorption of the nugget during frying where less oil was absorbed. This is mostly because the gluten (seitan) made the dough more elastic and hence, less absorptive of oil. Xanthan gum also played a part in reducing oil absorption by the nuggets in addition to its role as the binding agent. The study concluded that the optimum formulation for the nugget consisted of 54.8% seitan, 6.4% vitamin E-enriched palm fat, 6.8% xanthan gum, and 10% oats. The nuggets of this formulation were well received during a sensory evaluation and hence have a lot of market potential.

Meanwhile, an invention by Trottet et al. (2018) describes the use of liquid oil such as palm oil to create a meat analog food product that has the appearance, texture, and taste of meat, with low amount of starch and/or flour. The amount of liquid oil is around 2%–15%, and any oil could be included such as palm oil, fractionated palm fat, fully or partially hydrogenated or interesterified palm oil soybean oil, corn oil, sunflower oil, high oleic sunflower oil, olive oil, canola oil, safflower oil, peanut oil, cottonseed oil, coconut oil, almond oil, hazelnut oil, or rape seed oil. The resulted nonmeat analogs were produced using extruder barrel and found to have the appearance, texture, and taste of meat after incorporating liquid oil.

Undeniably, a limited amount of research has been conducted on the use of palm fats into plant-based meat; however, quite a number of studies were done and there is increasing trend to use palm fats as a meat fat replacer in various products. The possibility of using palm fat in plant-based meat and its performance toward the product can be explored. In addition, the use of palm oil and its fractions have been mentioned in several publications (Kyriakopoulou, Dekkers, & van der Goot, 2019; McClements & Grossmann, 2021). Therefore, the possibility of using palm fat in plant-based

meat and its performance toward the product can be explored further. Palm oil also being naturally semisolid and having the solid and liquid fraction be separated through a nonhydrogenation process can lead to endless food development options in plant-based segments.

6.7 Conclusion

The use of palm oil is not much explored in the development of plant-based meat. However, some findings showed that the ability of palm oil to be used as an animal fat replacer introduces possible developments for solutions in the plant-based segment. Moreover, the multifaceted properties of the oil palm fruit to produce various forms of oil and fat open up a great opportunity for palm oil or palm kernel oil to penetrate the plant-based market. The presence of minor compounds such as carotene, vitamin E, and others can also add benefits to the food manufacturing industry.

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Palm oil–based emulsifier: halal and sustainable

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7.1 Introduction

About 75% of the world's emulsifier is supplied by mono- and diacylglycerols (MAGs and DAGs) (Feldes, de Oliveira, Block, & Ninow, 2013). Due to their surface–active properties, MAG and DAG are frequently used as food emulsifiers to adjust techno-functional properties like viscosity, creaming, and foaming stability, primarily in the production of bread, pastry, margarines, ice cream, and other milk products. The most common methods for producing MAG and DAG emulsifiers are by transesterification of triacylglycerols (TAGs) with glycerol, for example, from hydrogenated palm oil, or direct esterification of glycerol with fatty acids (FAs) (Norn, 2015).

E471 emulsifiers are mixtures of MAGs, DAGs, TAGs, and free fatty acids (FFAs) with different and highly variable compositions, as well as by-products such as glycerol and inorganic components utilized for neutralization (Oellig, Brändle, & Schwack, 2018). The halal status of emulsifiers is occasionally questioned because MAG and DAG can be derived from lipids from either vegetable or animal sources (Riaz & Chaudhary, 2004).

Alternatively, emulsifiers can be obtained from palm oil materials. Indonesian Oil Palm Research Institute has produced many kinds of MAG and DAG utilizing basic material from palm kernel oil, olein, and stearin fractions of palm oil (Hasibuan, Hardika, & Ijah, 2015). Palm oil is a healthy alternative to trans-fatty acid (TFA)-containing hydrogenated fats, which have been shown to cause major health problems (May & Nesaretnam, 2014). This property is paramount to produce an excellent emulsifier. To date, besides nutritionally beneficial, the Roundtable on Sustainable Palm Oil–certified plants stated that sustainable palm oil production needs to be legal, economically viable, environmentally appropriate, and implement socially beneficial management and operations (RSPO, 2020).

Additionally, there were some claims of E471 in chocolate, as well as some commercial coffee and mayonnaise brands, was produced from animals. The fact that some manufacturers failed to declare the origin of the E471 emulsifiers used in their formulation was the main reason for these rumors becoming popular on social media (Marikkar & Yanty, 2018). Consequently, halal certification was introduced in Malaysia and worldwide to overcome this challenge, among others. To complement halal audits and monitoring, many detection methods on nonhalal products, including emulsifiers, were developed (Nasyrah, Marikkar, & Dzulkifly, 2014; Nizar, Marikkar, & Shuhaimi, 2015).

Hence, this chapter would enlighten the readers on an overview of emulsions and emulsifiers, application of emulsifiers in food and nonfood products, sustainable palm oil as emulsifiers, halal certification for palm oil, and detection methods to differentiate halal and nonhalal emulsifiers.

7.2 Overview of emulsifiers and emulsion

Emulsifiers are well-known additives that stimulate the formulation and commonly act as a stabilizer that maintains the emulsified state for emulsion. Emulsifiers are amphiphiles which means, it comprises hydrophobic part that is a long-chain FA and hydrophilic part that can be charged or uncharged. The emulsifier's hydrophobic part dissolves in the oil

phase, while the hydrophilic part dissolves in the aqueous phase, resulting in a dispersion of minute oil droplets. Food emulsifiers are also surfactants that react with other polar and nonpolar substances often found in food matrices due to their amphiphilic character (Faergemand & Krog, 2005).

Surfactants are effective emulsifiers, dispersing, and foaming agents because of their surface activating capacity (Orthoefer, Kim, and Hasenhuettl, 2019). They lower the surface tension of aqueous media (such as air–water) as well as the interfacial tension of liq–liq (such as oil–water or water–oil) and liq–solid (such as wetting phenomena) systems. They aid polar chemicals' solubility in organic solvents. Surfactants are the active chemicals in soaps and detergents, and they are often utilized to remove oily materials from a given medium. The hydrophilic and lipophilic balance contributes to the suppression of the interfacial tension due to this nature in creating boundary between oil and water phase (Sourav, Susanta, Ghosh, Saha, and Bidyut, 2015).

The term hydrophilic–lipophilic balance (HLB) is created to quantify the balanced polarity of the surface–active molecules (Lauridsen, 1976). Hasenhuettl (2019) specified that the HLB value determines whether hydrophilic or lipophilic groups are more prevalent. Food emulsifiers have a range of values between 2 and 16. High HLB emulsifiers dissolve in water, while low HLB emulsifiers dissolve in oil. High HLB emulsifiers are excellent emulsifiers, for example, in the aqueous phase for dissolving flavor oils. Oil-in-water (O/W) emulsions benefit from high HLB emulsifiers, whereas water-in-oil (W/O) emulsions benefit from low HLB emulsifiers. Emulsifiers with intermediate HLB values are poorly soluble in both oil and water, causing them to build up at the interface.

On the other hand, dispersion is a term used to describe systems with at least two immiscible phases. In a continuous flow, a scattered phase makes up a dispersion system (Goodarzi & Zendehboudi, 2019). An emulsion however mixes the two immiscible liquids in which one of the liquids is dispersed as minute spherical droplets in the other (Fig. 7.1) (Friberg, Larsson, & Sjoblom, 2004; McClements, 2005). The two immiscible liquids in the food industry are commonly oil and water; however, this is not necessarily essential (e.g., “water-in-water” emulsions can be made) (Norton & Frith, 2001; Norton, Frith, & Ablett, 2006). Emulsions are formed by shaking, stirring, and injection, using colloid mills, homogenizers, and ultrasonics (Usaid, Premkumar, & Ranganathan, 2014). There is a multitude of mixing sources that result in shear forces during the production of crude oils (Goodarzi & Zendehboudi, 2019).

An emulsion is formed by stirring oil and water together; however, once the stirring is stopped, the emulsion begins to break down. According to Goodarzi and Zendehboudi (2019), emulsion stability is principally determined by interactions between surface–active chemicals and water/oil interfaces. Emulsifying agents are adsorbed to the newly created interfacial film during emulsification, weakening the interfacial forces and allowing the immiscible phases to partially mix. Following the production of the initial drop, the former emulsion undergoes a series of time-dependent changes, including Ostwald ripening, coalescence, flocculation, sedimentation, and creaming (the most controversial processes).

The goal of emulsification is to keep the emulsion from breaking down because of creaming agglomeration and coalescence. Minimizing the size of dispersed particles, reducing the density difference of dispersion, and safeguarding the surface of oil droplets are all efficient ways to address these concerns. O/W emulsion, or oil droplets in water, which can be found in ice cream and milk, and W/O emulsion, or water droplets in oil, which can be found in butter and margarine, are the two varieties of emulsion. Recent advances have been made in the production of W/O/W-type emulsions, or water dispersed within oil droplets of O/W-type emulsions, and O/W/O type, an opposite type of emulsion. These multitype emulsions not only produce low-calorie products like cream with less oil, but they also help to stabilize the

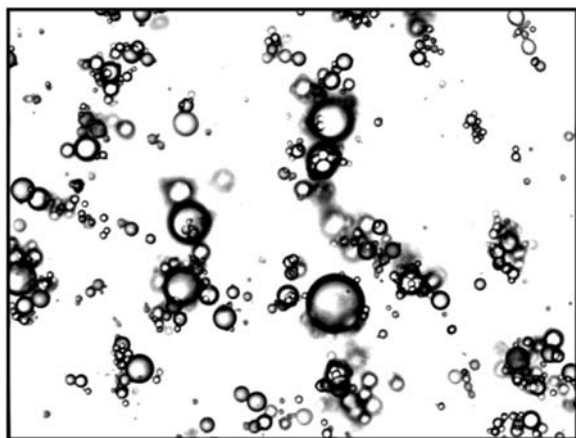


FIGURE 7.1 A photomicrograph of a polydisperse oil-in-water emulsion with oil droplets dispersed in a continuous aqueous phase. In this system, there is indication of droplet flocculation (McClements, 2007). Adapted from McClements, D. J. (2007). *Critical review of techniques and methodologies for characterization of emulsion stability*. *Critical Reviews in Food Science and Nutrition*, 611–613.

emulsion by dissolving the unstable ingredient found in the lowest region of water droplets. Seasonings and tastes can also be injected into the water droplets to improve the flavor.

Partially hydrolysis of fat/oil (TAG), partial esterification of FFAs with glycerol, and glycerolysis of fats/oils with glycerol can all be used to make MAG and DAG (Devi et al., 2008; Feltes et al., 2013; Gunstone, 1999). Controlling the hydrolysis reaction is challenging with partial hydrolysis; yields are low and considerable volumes of oils/fats are required. Another technique for obtaining MAG and DAG is partial esterification of glycerol and FFAs, although this process is reversible for rehydrolysis (Subroto, 2020). Glycerolysis is the procedure that theoretically delivers the best yield of MAG and DAG of all the methods for manufacturing them (Feltes et al., 2013). On a large scale, MAG and DAG are made by continuously glycerolysis of fat/oil with alkaline inorganic catalysts at high temperatures (220°C–260°C) and an inert atmosphere (Damstrup et al., 2005). Enzymatic catalysis can be used to make MAG and DAG, which is appealing because it does not require high temperatures (mild reaction), the lipids are not quickly damaged, and the products are simple to purify and environmentally acceptable (Subroto, 2020).

7.3 Emulsifiers in food applications

Generally, food-grade emulsifiers are composed of a water-loving end and a fat-loving end. The water-loving end would compose of FAs such as stearic, palmitic, oleic, or linoleic acid or a combination of these whereas the fat-loving end would compose of hydroxyl or carboxyl groups. Emulsifiers can be classified as follows: mono- and diglycerides; propylene glycol monoesters; lactylated esters; polyglycerol esters; sorbitan esters; ethoxylated esters; succinylated esters; fruit acid esters; acetylated mono- and diglycerides; phosphated mono- diglycerides; and sucrose esters (Zielinski, 1997).

The melting point of the FAs used to prepare the emulsifier will determine the melting point of each ester in every category. Short- and medium-chain FAs such as stearic and palmitic acid would contribute to a relatively higher melting point compared to long-chain ones such as oleic and linoleic acid. The higher the melting point, the more solid the ester will be and vice versa. Various food products may contain emulsifiers, for example, ice cream (Fredrick, 2013), cakes (Usaid et al., 2014), and candies Hasenhuettl (2019). Different emulsifiers functions differently in different food products. Table 7.1 summarizes among food-grade emulsifiers utilized in food products with specific functions.

7.4 Emulsifiers in nonfood applications

Other than food, it is interesting to note that emulsifiers, including lecithin and phospholipids, are used in nonfood applications too. Among the uses are as processing aids (Sipos, 1989), lubricants (Weimin, Yanxia, Xiaobo, & Weimin, 2013), dietary supplements (Orthoefer et al., 2019), animal feeds (Kullenberg, 1989), personal care formulations (List, 2015) masonry and asphalt, metal processing, insecticides, polymers, release agents, textiles, and water treatment/pollution, among others (Schmidt & Orthoefer, 1985). Lecithin serves multiple purposes in numerous applications. Table 7.2 summarizes the uses of emulsifiers in nonfood application and functions.

7.5 Palm oil and its derivatives as halal emulsifier

Various palm oil–based emulsifiers have been synthesized and applied in food products. Palm oil derivatives such as palm stearin, palm olein, palm kernel oil, and their blends are now utilized for emulsifier production. For example, Cheong, Hong, Yuan, & Xuebing (2007) utilized partial hydrolysis using Lipozyme RMIM lipase in a solvent-free system that was used to produce a DAG-enriched palm olein, while Yeoh et al. (2014) produced highly purified DAG-based palm olein via lipase-catalyzed glycerolysis and appropriate processing method and conditions. Next, Hasibuan et al. (2015) used emulsifier originated from palm kernel oil, palm olein, and palm stearin with monoglyceride content of 55%–60% and 30%–35% diglyceride in producing chocolate with cocoa butter substitute. On the other hand, Arum, Hidayat, and Supriyanto (2019) synthesized emulsifier from refined bleached deodorized palm stearin via chemical glycerolysis. Additionally, Subroto, Supriyanto, Utami, and Hidayat (2019) applied enzymatic glycerolysis–interesterification to synthesize structured lipids containing high MAG and DAG from a palm stearin–olein blend.

As mentioned earlier, palm oil–based emulsifiers would fit the halal requirement. Production of emulsifiers is possible from either vegetable or animal sources; hence the halal status of emulsifiers is occasionally questioned (Riaz & Chaudhary, 2004). Additionally, labeling regulations do not necessitate origin of ingredient declaration. For example, E471 defines mono- and diglycerides of FAs; however, the sources for these MAG and DAG's are not stated. Consequently, palm oil and its derivatives is an excellent material to produce halal emulsifiers.

TABLE 7.1 Food-grade emulsifiers utilized in food products with specific functions.

Food products	Emulsifiers	Function	References
Ice cream	Monoglycerides, polysorbates	– To promote nucleation of fat during aging, thus reducing aging time	Fredrick (2013)
		– Improve the whipping quality of the mix which results in reduced air cell sizes and homogeneous distribution of air in the ice cream; produce a dry and stiff ice cream as they enhance fat partial coalescence/destabilization; facilitating molding; and increase resistance to shrinkage and rapid meltdown	Cropper et al. (2013)
		– Promotes fat agglomeration and air bubbles causing thinner lamellae between adjacent air bubbles on the size and growth of crystals which confers resistance to the development of coarse/icy textures	Barford (2001)
Shortenings	MAG, lactylated monoglycerides, propylene glycol esters, lecithin, polyglycerol esters, polysorbate 60, and sodium stearoyl lactylate	– Longer shelf life, improve tenderness and taste release, reduce mixing tolerance and time, improve machinability, improve water absorption, increased volume, improve hydration rate of flour and other ingredients, enhance texture and symmetry, reduction of egg and shortening usage	Chrysam (1985), Weiss (1983)
Crumb softeners	Lactylates, SMG, MAG, DAG, hydrated distilled MAGs, polysorbate 60, ethoxylated monoglycerides (EOM), lecithin	– Less free amylose is formed post-chilling, hence less stiff gel is formed – Soft crumb that is distinctive of freshly baked bread can be preserved for longer-Crumb firming, which is related with staling and starch retrogradation, can be postponed for 2–4 days – Complexing with the starch and adsorbing onto the starch surface, emulsifiers are expected to lower the rate of water migration and therefore staling	Usaid et al. (2014), Knightly (1968), Pisesookbuntern and D'Appolonia (1983)
Cakes	Diacetyl tartaric acid esters of MAG, glycerol fatty acid esters, and calcium stearoyl-2-lactylate	– Increase volume by facilitating air incorporation, disperse shortening in smaller particles, and improve moisture retention	Usaid et al. (2014), Painter (1981), Flack (1983)
Candies	Lecithin, monoglycerides (e.g., glycerol monostearate) and diglycerides	– Eliminate fat crystal transition from alpha and beta prime configuration to beta configuration and increase shelf stability – Stimulate breakdown into small fat globules of products that consist of a dispersed phase like caramel, toffee, etc. – Offer lubrication for easier processing and utilization, in part by dispersing the fat phase. In chewing gum and bubble gum to function as plasticizers for the gum base and to promote hydration while chewing	Weyland & Hartel (2008), Hasenhuettl (2019), Hartel and Hasenhuettl (2019)

(Continued)

TABLE 7.1 (Continued)

Food products	Emulsifiers	Function	References
		– Emulsifiers control viscosity, impact fat crystallization, and moderate polymorphic changes of the lipid phase in fat-continuous confections such as chocolate and coatings – Low-fat confections frequently contain mono- and diglycerides to improve lubricity and mouthfeel – To manage aeration or to keep the product from sticking to machinery and packaging – To provide the chewing gum base with appropriate texture and antistick qualities – Softening, aid in the retention of water and hydration of the gum base while chewing, as well as the reduction of tack on the teeth – Carry colors and flavors in chewing gums, which aid in the dispersion of these key chemicals within the gum base and facilitate their release into the oral cavity during chewing – Aid in the creation of bubbles in bubble gum	

DAG, Diacylglycerol; *MAG*, monoacylglycerol; *SMG*, succinylated-monoglycerides.

7.6 Palm-based emulsifier in meeting global trend

It was reported that the three largest emulsifier trends are nonpartially hydrogenated vegetable oils (PHVOs), nonbioengineered or nongenetically modified (GM) ingredients, and sustainably sourced palm-based emulsifiers (Gelski, 2016).

In 2018 the World Health Organization (WHO) released REPLACE, a step-by-step guide for the elimination of industrially produced trans fatty acids (TFAs). The WHO recommendation for a maximum TFAs threshold in products is no more than 2 g of TFA per 100 g of fat or oil. The threshold is proportional to the amount of fat in a product, not its finished weight. For example, a product that contains 10 g of fat can contain no more than 2% TFAs of that 10 g fat. The common practice in the food industry prior to this initiative is to hydrogenate vegetable oils partially.

Hydrogenation of vegetable oil is a process that simply turns liquid or soft oils—high polyunsaturated FAs oils like sunflower, canola, and soybean—into something with more solid fat in the presence of a catalyst. The degree of hydrogenation of unsaturated oils influences the final consistency of the product. When the process is done partially, the end product gains some saturated FA qualities but still retains some levels of unsaturated FAs. The melting point of the end product increases to the point where a solid is present at room temperature, and therefore it is now more stable for various applications.

However useful the process is, hydrogenation, when partially done, is a harmful process that causes the formation of TFAs. As confirmed by the Joint WHO/FAO Expert Consultation on diet, nutrition and the prevention of chronic disease (Nishida, Uauy, Kumanyika, & Shetty, 2004; World Health Organization, 2018), TFAs have adverse effects on blood and coronary heart diseases. In response to the REPLACE initiative, the food industry in many regions around the world has been putting efforts in phasing out the use of PHVOs from their products—among them are PHVO-based emulsifiers. Fortunately for the food industry, palm-based emulsifiers have come to the rescue. It offers similar benefits to PHVO-based emulsifiers because of its FA profile.

As palm-based emulsifiers are nonbioengineered and non-GM food, they have become an excellent choice to meet the market trend. In 2016 a survey conducted by Pew Research Centre (2016) found that one-in-six (16%) Americans are concerned with GM foods as they predominantly believe that GM foods pose health risks. It was also perceived that

TABLE 7.2 Emulsifiers in nonfood applications with specific functions.

Nonfood application	Function	Properties	References
Lubricants (lecithin)	As an anticorrosive lubricant in turbine or spindle engines Multifunctional lubricating oil additive	An oxygen scavenger with strong molecular polarity, soy lecithin can quickly adsorb onto metal surfaces and form a protective barrier that isolates the metal surface from the surrounding water, resulting in good antirust performance in a variety of base stocks	Weimin et al. (2013)
Processing aids (lecithin)	Aids surfactants to spread and stabilize the oil-based paint and coatings	As grinding aid to help with pigment wetting and dispersion, reduce grinding and mixing time, stabilize pigment by reducing sedimentation, and aid in pigment dispersion after storage	Sipos (1989)
Pharmaceuticals (phospholipids)	Employed in liposomes and other oral, cutaneous, and parenteral applications.	As wetting agents, emulsifiers, and builders in pharmaceutical technology, as well as components of mesophases such as liposomes, micelles, mixed micelles, and cubosomes	Hoogevest and Wendel (2014)
Pharmaceuticals (lecithin)	Dietary supplements for cardiovascular health, liver and cell function, fat transport and metabolism, reproduction, and child development, physical performance and muscle endurance, cell communication, memory, learning and reaction time, arthritis, skin and hair health, and treatment of gallstones	n.d	Orthoefer and List (2006)
Animal Feed (lecithin)	To stabilize products and provide antioxidant characteristics Improve pet's health	n.d	Kullenberg (1989)
Agriculture (lecithin)	Plant protection Foliar fertilization Reduce the quantity of xenobiotic pesticides released into the environment A biorational antifungal pesticide	Used as a contact product to minimize fungal spore formation by activating plant defenses and having a direct impact on spore germination. The common regulation (EC) No 889/2008 in Annex IIa of this fundamental substance recognizes lecithins as a fungicide in organic farming at the EU level	Ghyczy, Imberge, and Wendel (1987), Bauer, Ghyczy, and Osthoff (1986), Jolly, Vidal, and Marchand (2018), Bohinc, Znidarcic, and Trdan (2016), EU (2016)
Personal care (lecithin)	Cleansers, moisturizing liquid makeup, beauty lotions, lipstick, skin penetration enhancers and performing cremes nail enamel and hair conditioner	Improve the ease of application and emollience of lipsticks, to modify the films produced by face masks through increased elasticity, to protect hair in heat waving compositions, to add to creams to maintain the elasticity of nails, to produce bubble lathers in shaving creams, and to act as a synergist to phenolic antioxidants, and to act as an emulsifier in denture pastes in cosmetics	Fazwi, Iyer, and Mahjour (1988) Succi, Gunderman, Fottiu, and Kabacoff (1981), Spitzer, Marra, Osipow, and Claffey (1982), Baker (1989), Sagarin (1957)

GM foods are very likely to bring problems for the environment along with health problems for the population as a whole. In another survey by [Cui and Shoemaker \(2018\)](#), the percentage of respondents in China who opposed GM food was on the rise, and they recommended that significant effort is needed to overcome that trend. Efforts in food security, poverty reduction, and transgenic commercial planting by the Chinese government were brought to a halt in recent years due to the sensitive nature of the GM food issue among the public. As far as the global food security is concerned, although it is predicted that there will be a growing interest in the adoption of bioengineered foods, the general public still opts for nonbioengineered ones as they are less “frightening.”

Due to the increasing global demands for edible oils and biofuel feedstocks, and its high profitability, the oil palm industry is very likely to expand. Other vegetable oils such as soy, sunflower, and canola are only 10%–20% as efficient as palm oil on a per-hectare basis and would therefore require larger areas of cultivation to produce similar benefit ([Basiron, 2009](#)). In fact, in 2018, 300 million hectares were devoted to oil crop production in which palm oil accounted for 6% only (19 million ha) despite producing more than 30% of global oil supply ([Figs. 7.2 and 7.3](#)).

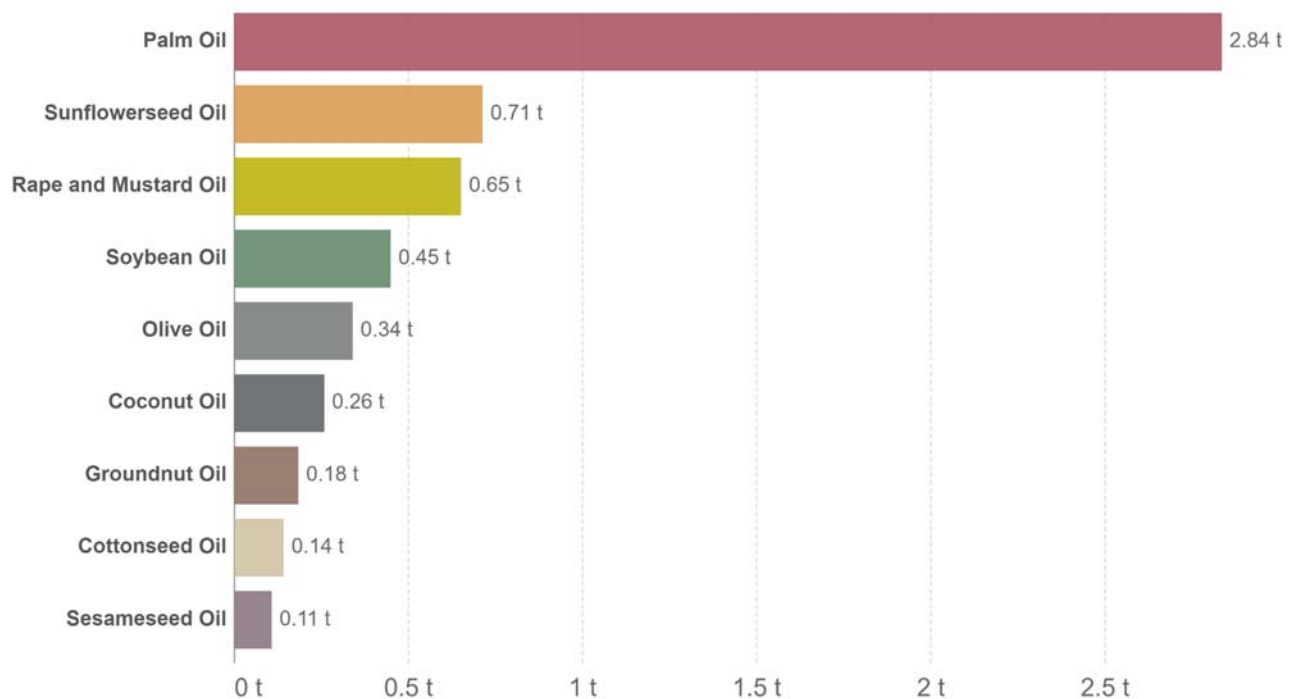
Evidently, from the data gathered, palm oil production is the most land-efficient vegetable oil requiring the least area of land but producing the most oil per hectare. To ensure a sustainable supply chain, according to an analysis by [Obaideen \(2020\)](#), there are 11 certification schemes focusing on sustainability in which 7 of them are available for palm oil ([Table 7.3](#)).

In the analysis, three of the seven sustainability supply certifications available for palm oil were developed specifically for palm oil, whereas, for soybean oil, only two of the five available certifications for soy were developed specifically for the soy industry. The only certification present for rapeseed is not even specifically for rapeseed. The same can be said for sunflower where the two existing certifications were not developed for sunflower specifically. This greatly implies that the production of palm oil and its derivatives are heavily governed and scrutinized. For manufacturers and consumers, it can definitely be assured that palm oil is certified as sustainable.

Oil yield by crop type, 2018

Global oil yields are measured as the average amount of vegetable oil produced per hectare of land. This is different from the total yield of the crop since only a fraction is available as vegetable oil.

Our World
in Data



Source: Calculated by Our World in Data based on data from the UN Food and Agriculture Organization (FAO)
OurWorldInData.org/crop-yields • CC BY

FIGURE 7.2 Oil yield by crop type, 2018. *Our World in Data.*

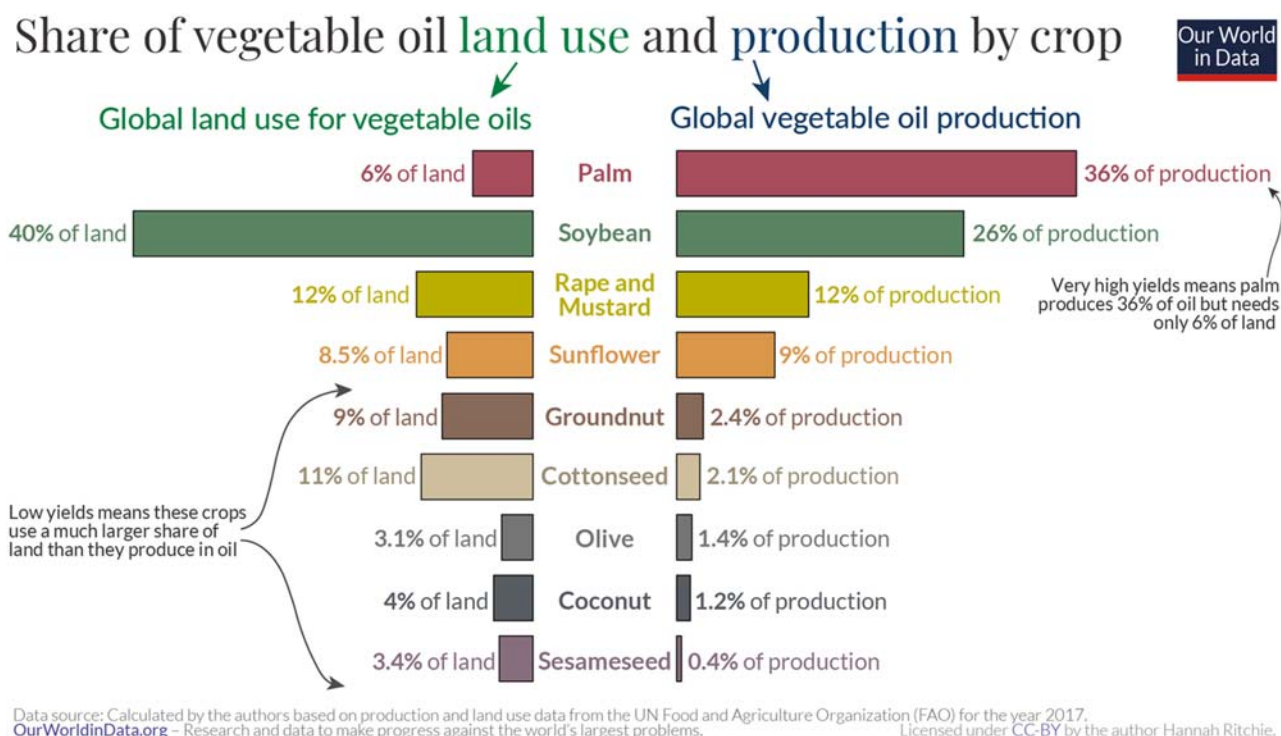


FIGURE 7.3 Share of vegetable oil land use and production by crop, 2018. *Our World in Data.*

TABLE 7.3 Sustainability certification schemes adopted for major vegetable oils.

Certification schemes	Palm oil	Rapeseed oil	Soybean oil	Sunflower oil
RSPO	/			
MSPO	/			
International Sustainability and Carbon Certification	/	/	/	/
Indonesian Sustainable Palm Oil	/			
Roundtable on Sustainable Biomaterials	/			
High Carbon Stocks Approach	/			
Sustainable Agriculture Network	/			/
Round Table on Responsible Soy			/	
ADM Responsible Standard			/	
Sustainable Farming Assurance Programme—Non-Conversion			/	
ProTerra			/	

MSPO, Malaysian Sustainable Palm Oil; RSPO, Roundtable on Sustainable Palm Oil.

Source: Adapted from Obaideen, K. (2020). *Contribution of vegetable oils towards sustainable development goals: A comparative analysis* (pp. 45–52.). Central Jakarta, Jakarta: Ministry of Foreign Affairs of The Republic of Indonesia.

Palm-based emulsifiers have been in the hot seat as far as the beauty industry is concerned. However, this is mostly due to the growing concern that the production of palm oil has led to the destruction of native rain forests. A company based in Germany, which has had a focus on cosmetic ingredients from sustainable resources, established production of the widely used glyceryl stearate citrate based on palm oil free starting materials (McDougall, 2011). Due to the perception that palm oil causes deforestation, manufacturers tend to miss the superior quality of palm-based ingredients.

7.7 Halal authentication for emulsifiers

E 471 emulsifiers are authorized as food additives by Commission Regulation (EC) No 1333/2008 ([The European Parliament & the Council of the European Union, 2008](#)), and they are allowed to be used without a maximum restriction; nevertheless, they should not be used in greater concentrations than are acceptable. E 471 emulsifiers are mixtures of mono-, di-, and triesters of fatty acids composition (FAC) of edible oils with glycerol and low levels of free FA ([The European Commission, 2012](#)). According to this regulation, the total amount of mono- and diesters (=MAG and DAG) in the emulsifier product must be greater than 70%. An AOCS standard gas chromatography (GC) method was published for the analysis of MAG and DAG in vegetable oils with E 471 emulsifiers ([American Oil Chemists' Society, 2017](#)).

Although plant lipids were the most commonly used raw material for partial acylglycerol synthesis, animal fats were also used. Cheong and coworkers demonstrated the potential value of lard (LD) as a raw material for the manufacture of partial acylglycerols ([Cheong, Hong, Yuan, & Xuebing, 2007](#)). Even though the utilization of animal fat in industrial products could be beneficial for the animal carcass industry, the use of animal-derived ingredients in food might not be desirable due to food taboos based on certain religious restrictions. [Table 7.4](#) exhibits potential detection methods of halal and nonhalal emulsifiers.

Ahmad Nizar and coresearchers determined the fatty acid composition by GC-mass spectrometry (MS) and $\delta^{13}\text{C}$ carbon isotope ratio by EA-IRMS of MAG and DAG derived from individual animal fats. The results concluded that the $\delta^{13}\text{C}$ values of MAG and DAG of LD were significantly different from those of MAG and DAG derived from chicken fat, beef fat, and mutton fat. Further chemometrics approach by principal component analysis (PCA) showed fatty acids, namely, stearic, oleic, and linoleic as the most discriminating parameters to distinctly identify MAG and DAG derived from different animal fats ([Nizar et al., 2015](#)).

Nasyrah and team studied FAC and DSC thermal profiles of MAG and DAG isolated from six commercial emulsifiers available in Malaysia and compared them with those of MAG and DAG derived from LD. The study demonstrated that the FA distribution patterns of MAG and DAG in commercial emulsifiers are distinctly different from those of MAG and DAG derived from LD. As the nature of the DSC profiles displayed by MAG and DAG derived from LD is distinctly different from those of MAG and DAG extracted from commercial emulsifiers, finding differentiation between them by direct comparison is easier ([Nasyrah et al., 2014](#)).

Previously, the team concluded that PCA from FAC derived from GC-FID and heating and cooling profiles from DSC could distinctly classify MAG and DAG of plant lipids from those derived from animal fat ([Nasyrah et al., 2014](#)).

TABLE 7.4 Detection techniques for halal and nonhalal emulsifiers.

Food matrices	Detection technique	Highlight	References
Animal fats	EA-IRMS, GC-MS, and PCA	Different $\delta^{13}\text{C}$ values of MAG and DAG among species	Nizar et al. (2015)
MAG, DAG	DSC, GC-FID, and PCA	FAC of MAG and DAG in commercial emulsifiers are distinctly different from those derived from -lard (LD)	Nasyrah et al. (2014)
Partial acyl glycerol of lard and other plant oils	GC-TOF-MS	DB17ht as the primary column and SLB-5Ms as the secondary column for the separation of individual partial acyl-glycerol	Indrasti et al. (2010)
Food-based emulsifier	LC-APCI-MS	Quantification of MAG and DAG of fatty acids (E471) and qualitative evaluation of MAGs and DAGs esters of fatty acids (E472)	Suman et al. (2009)
Animal- and plant-based emulsifiers	DSC, GC-FID, and PCA	PCA from FAC derived from GC-FID and thermal analysis from DSC showed that it was possible to distinctly classify MAG and DAG of plant lipids from those derived from animal fats	Nasyrah et al. (2012)

DAG, Diacylglycerol; GC-TOF-MS, gas chromatography time of flight-mass spectrometry; LC, liquid chromatography; MAG, monoacylglycerol; PCA, principal component analysis; GC-FID, gas chromatography with flame ionization detection; DSC, differential scanning calorimetry; LC-APCI-MS, liquid chromatography/atmospheric-pressure chemical ionisation mass spectrometry; LD, -lard; FAC, fatty acid composition.

Indrasti and coworkers used GC time-of-flight MS to discriminate partial acylglycerols of LD from those of sunflower oil, corn oil, butter, and palm oil employing two types of nonorthogonal columns, namely, DB17ht as the primary column and SLB-5Ms as the secondary column for the separation of individual partial acyl-glycerol. 3-Monopalmitoyl-sn-glycerol (MG 3-C16) was the highest concentration in LDs, butter, palm oil while monostearoyl-sn-glycerol (MG C18) in corn oil and 1,3-dilinoelrac-glycerol (DG C18:2c) in sunflower oil. PCA accounted 82% of variance using a combination of PC1 and PC2. The presence of monostearoyl-sn-glycerol (MG C18), 3-monopalmitoyl-sn-glycerol (MG 3-C16), 1,3-dilinoelrac-glycerol (DG C18:2c), 1,3-dipalmitoylglycerol (DG 1,3-C16), and 1,3-dielaidin (DG C18:1t) caused differentiation of the samples tested (Indrasti et al., 2010).

Suman used liquid chromatography coupled with MS (LC-MS) to quantify some food emulsifiers composed of MAG and DAG in complex food matrices (Suman et al., 2009). Recently, Oellig et al. (2018) described a high-performance thin-layer chromatography with fluorescence detection method for determining MAGs, DAGs, TAGs, and FFAs in E 471 emulsifiers, as well as a response factor system for quantification. From the examples given, most commonly, GC and high-performance LC are used to separate the compounds, and detection is mainly performed by MS to gain high selectivity and sensitivity.

Changes in the relative composition and dose of the emulsifier have a significant impact on the product structure, particularly the viscosity qualities. As a result, maintaining the composition of the applied emulsifier is critical to ensuring long-term stability and high product quality. To manage the composition of emulsifiers, reliable and simple approaches are necessary (Oellig et al., 2018). Additionally, these methods could control the traceability of emulsifiers especially in the area of halal.

7.8 Conclusion

After being chosen as 1 of the 12 National Key Economic Areas (NKEA) Malaysia to fuel the nation's economy, the adoption of the Economic Transformation Program gave the oil palm industry a new focus. The palm oil sector NKEA aims to increase upstream productivity and downstream expansion while focusing on the oil palm industry's long-term sustainability. The exploitation of palm oil and its derivatives as halal and sustainable emulsifiers in downstream activities such as in food processing is apt. This chapter briefly highlighted some concepts behind emulsifiers, functions of emulsifiers in food and nonfood products, as well as an overview on different derivatives of palm oil used to produce halal emulsifiers. There were also some indications on palm oil as sustainable source for emulsifiers, as well as detection methods for nonhalal emulsifiers. It is hoped that this chapter gave a quick information on plausibility of Malaysia as among the top producer of halal and sustainable palm oil-based emulsifier.

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Chapter 8

Enzyme, the cheese case

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8.1 Introduction

For centuries, cheese production is known to be one of the oldest applications of enzymes and the major step that has been used in its production is enzymatic coagulation of milk. There was indication of cheese making descended upon cave paintings around 5000 BCE (Shah, Mir, & Paray, 2013). Cheese products are said to be highly concentrated and very rich in lipids and proteins, minerals such as phosphorus and calcium, and essential amino acid (Sánchez-Muñoz et al., 2017). Historically, the enzyme preparations used to make cheese have been extracted from the ruminant's stomachs and this process has been used to this day (Shah et al., 2013). This extracted enzyme from the ruminant's stomachs is called rennet enzyme.

It is located at the abomasum of the young ruminants which is the fourth stomach, mostly from calves. Calf rennet (CAR) contains very high concentration of chymosin which is also known as rennin, which could account for up to 95% of the total proteases found in young calves' abomasum extract. Due to its ability to separate milk into curds and whey, rennet, which is known as a proteolytic enzyme, is very important for cheese production. The coagulation process begins with the addition of this enzyme by enzymatic cleavage of the k-casein phenylalanine (105)-methionine (106) peptide bond, followed by casein micelle destabilization, aggregation, and formation of clots resulting in a three-dimensional matrix of proteins (Ben Amira et al., 2017).

As the demand for cheese products has increased by a factor approximately about 3.5 since 1961, the enzyme used as rennet enzyme supplied for cheese production is reduced due to the limited availability of ruminant stomach (Shah et al., 2013). In addition, the cost of growing CAR has prompted the quest for alternative milk coagulant enzymes that would replace satisfactorily CAR in cheese production. In addition to the high price of CAR, it also affects a number of religious factors, such as the halal status of obtaining CAR for Muslims and vegetarian consumers, which restricted their food choices (Ben Amira et al., 2017).

Substitutes that are essential in meeting cheese production requirements include microbial, recombinant, and plant-based enzymes (Shah et al., 2013). Plant-based enzymes are one of the alternatives for animal-based enzyme substitution. The law of cheese production is the enzyme's ability to clot milk under appropriate conditions to be recognized as a proteolytic enzyme (Shah et al., 2013).

Seeking an alternative for milk coagulant enzyme must be fulfilled to increase global demand for diversified and high-quality cheese production. This can be one cheese product that can be embraced without any restrictions by all groups of people. Plant extracts have been used since ancient times as a milk coagulant agent in cheese production. The use of vegetable coagulant is found primarily in countries of the Mediterranean, West Africa, and Southern Europe. Spain and Portugal have recorded the greatest variety and development of cheeses using *Cynara sp.* as the coagulant based on plants (Shah et al., 2013). Bromelain is one of the plant-based enzymes other than *Cynara sp.*, which has been reported as an alternative to CAR. It was foreseen as an effective substitution of rennet enzymes in cheese production and also provides added value such as increasing the cheese product's nutritional content.

Bromelain enzyme is capable of coagulating milk as it is a proteolytic enzyme as well. Unlike papain, it is also used in soft cheese processing and produces good results. Most studies found that papain has been used as a milk coagulant enzyme to replace rennet enzyme, but bromelain has some potential characteristics for several factors compared to papain enzyme. Bromelain enzyme can digest 1000 times its weight of protein (Bahmid, 2013). Despite the potential

characteristics mentioned earlier, there is still minimal bromelain enzyme used in cheese production. Furthermore, the analysis to be carried out to know the optimal state of the enzymatic bromelain condition is the concentration of the enzyme, the incubation period, and the temperature range for cheese production.

8.2 Cheese

Cheese is a commonly consumed commodity, rich in lipids and proteins, minerals such as calcium and phosphorus, and essential amino acids (Sánchez-Muñoz et al., 2017). Cheese is the most complex food among the dairy products with chemical, biochemical, and microbiological processes involved. It is a solid or semisolid food product made from cow, ewes, goats, or other mammals' milk. There are over 400 cheese varieties produced worldwide, created by variations in milk origin (geographic district or mammalian species), fermentation and maturing conditions, as well as pressing, size, and shape. Today's most cheese is made from the milk of cows.

Since prehistoric times, cheese has been made and is one of the oldest food products in the world. People have been raising animals for milk for thousands of years, turning their surplus milk into cheese. The transformation of milk to cheese includes many distinct but interrelated processes, including coagulation, acidification, synthesis (separation of whey from cheese), dehydration, molding, pressing, and salting (Enwa, Avbunudiogba, & Godstime, 2013). Cheese is the concentrated form of milk that contains most of the calcium, protein, fat, and vitamins that man needs for the development of strong bones and healthy living, making it an important item in almost all people's diets.

8.2.1 Soft cheese

The different flavors and textures of cheese are the result of variations in the milk type, the amount of fat in the milk, the bacteria used to ferment the milk, and the processing conditions. Cheeses can be ripened or unripened, soft or semi-hard, and hard or very hard product (Budreckiene & Struzeckiene, 2014). For this entire group, soft cheeses are the easiest to make and are popular in many countries as it is a traditional food. Soft cheeses made from raw milk lead to a variety of flavors that some consumers view favorably and are also correlated with manufacturing (Westling et al., 2016).

Soft cheeses are also called cottage cheese, paneer, and cream cheese. Soft cheese is a fresh acid-curd cheese that is fermented at pH values of ~ 4.5 – 4.8 with lactic acid bacteria (Gutiérrez-Méndez et al., 2019). Colloidal calcium phosphate (CCP) is solubilized in casein micelles during acidification of milk. If the pH drops below 5.0, most CCP will dissolve, dissociating micelles from casein. When the pH in the milk reaches a value of ~ 4.6 , the negative surface load on casein micelles is reduced sufficiently to form a gel structure (Gutiérrez-Méndez et al., 2019). The acid-milk gels' firmness can be enhanced either by preheating milk or by adding a small amount of rennet. Milk heat treatment above 69°C denatures the related whey proteins [β -lactoglobulin (β -lg) and β -lactalbumin (β -la)] associated with casein (Gutiérrez-Méndez et al., 2019). The aggregation of β -lg with β -casein reduces the net repulsive charge between caseins, thereby improving the protein–protein interactions and the strength of the gel (Gutiérrez-Méndez et al., 2019). On the other hand, the addition of a small amount of rennet (chymosin) at the beginning of fermentation induces a stronger network of proteins that enhances the strength of milk gels. Chymosin hydrolyzes caseins that generate para-casein (mostly κ -casein) and glycomacropeptide (Gutiérrez-Méndez et al., 2019).

8.2.2 Milk coagulant

Milk coagulation is induced by several enzymes and can be divided into two phases that are common enzymatic hydrolysis of κ -casein (at the Phe105–Met106 bond) and para- κ -casein aggregation in the presence of calcium ions (Leite Júnior, Tribst, Ribeiro, & Cristianini, 2019). Ideally, milk coagulant enzymes should be highly specific and have little general proteolytic activity (PA) to obtain high-yield cheeses with a minimum bitter flavor or fragile texture.

Milk coagulation is the main step in cheese production and can be achieved through a number of proteolytic enzymes from different sources, such as different animal species, microbial proteinases, and proteinases extracted from fruits and plants. CAR is still the enzyme most commonly used in cheese production. Nevertheless, increasing cheese consumption and a worldwide depletion of calf coagulants have prompted a hunt for alternative milk coagulants (Say & Guzel, 2016).

8.2.3 Rennet

Rennet or milk coagulants are preparations for enzymes that have been used in cheese-making processes for thousands of years. During cheese ripening, rennet is involved in milk coagulation and proteolysis, which is very significant in cheese production. Rennet proteolysis influences the flavor and texture of the cheese. Mixture of proteolytic and lipolytic enzymes is found in rennet, including chymosin, lipase, and pepsin (García-Gómez, Vázquez-Odériz, Muñoz-Ferreiro, Romero-Rodríguez, & Vázquez, 2019). Its activity is essential for the coagulation of enzymatic milk. It is used to turn liquid milk into a soft gel during cheese making, usually referred to as curd (Camin et al., 2019). The most commonly used rennet in cheese production is CAR (An, He, Gao, Zhao, & Zhang, 2014).

Rennet is an enzymatic preparation that has traditionally been extracted from young ruminants' abomasum (fourth stomach), mainly from calves (Moreno-Hernández et al., 2017). The first type of rennet discovered, which originates from the abomasal mucosa of newborn or adolescent ruminants, primarily veal and lamb balls, is called rennet (Camin et al., 2019). CAR contains a high chymosin concentration, which can account for up to 95% of total proteases in abomasum extracts of young calves (Moreno-Hernández et al., 2017). It contains three genetic variants of chymosin: A, B, C (10%–90%) and pepsin, APs (Camin et al., 2019). This proportion, however, decreases with the age of animals and pepsin becomes the predominant enzyme in abomasum extracts of adult cattle (Moreno-Hernández et al., 2017).

Rennet helps in the degradation of casein proteins by breaking the covalent peptide bonds between phenylalanine and methionine. The enzymatic cleavage will cause the casein micelles that are aggregated to be destabilized and obtain a three-dimensional protein matrix containing the curd (García-Gómez et al., 2019). Proteolysis' main role is being responsible for the formation of curd. It is also linked to the release of amino acids as precursors of catabolic reactions that release flavor compounds (García-Gómez et al., 2019).

8.2.3.1 Microbial enzyme

In some countries, microbial coagulants have not been accepted for regular cheese manufacture because they are believed to result in a reduced yield and a lower quality product. Microorganisms, including *Rhizomucor pusillus*, *R. miehei*, *Endothia parasitica*, and *Irpex lactis*, have been extensively used as sources of microbial rennets. Most of the coagulants from microbial sources that have been investigated appear to be extracellular in nature and thus these microbes are likely candidates for elaboration of milk coagulants. Consumer acceptance of cheeses made with microbial rennets does not pose any problems as such products have been classified as “vegetarian” cheeses.

8.2.3.2 Plant enzyme

Nevertheless, the worldwide rise in cheese production, combined with reduced supply and increasing CAR costs, has led to the search for alternative milk coagulant enzymes as suitable replacements for rennet. Today, the cost of the production of cheese is very high, as the price of rennet enzyme is very expensive and limited (Bahmid, 2013). There are modern alternatives such as bacterial fermentation or genetically modified microorganisms (Hernández-Mancillas et al., 2017). Most microorganisms' extracellular proteases are close to chymosin and are therefore possible alternatives to rennet (Hang et al., 2016). In addition, some religious influences (Islam and Judaism) and others linked to some consumers' vegetarianism have limited their use considerably (Amira, Besbes, Attia, & Blecker, 2017).

Because of their easy availability and quick purification processes, plant rennets have become a topic of growing interest in the cheese industry. In addition, the use of plant proteases in cheese production encourages vegetarians to be more acceptable and can improve their nutritional intake (Amira et al., 2017). Using plant-based enzyme for the production of cheese can also minimize cost production and reduce fruit waste wastage, making the world more sustainable and affordable. Indeed, one of the main sources of solid municipal waste is fruit waste, which has been an increasing environmental problem (Budreckiene & Struzeckiene, 2014).

Plant-based coagulants have been researched as a potential replacement for CAR, albeit only a few are utilized in commercial cheese (Gutiérrez-Méndez et al., 2019). Since ancient times, plant extracts have been utilized as milk coagulants in cheese making. Vegetable coagulant cheeses are mostly found in nations in the Mediterranean, Southern Europe, and Western Africa. Portugal and Spain use *Cynara sp.* as the vegetable coagulant for the largest variety and production of cheeses. For the processing of Portuguese Serra and Serpa cheeses and Spanish Los Pedroches, La Serena, and Torta del Casar cheeses (from ewes' milk), as well as Los Ibores cheese (from goat's milk) and Flor de Guía cheese (from a combination of ewes' and cow's milk), *Cynara sp.* extracts were used (Shah et al., 2013). Extracts from *Calotropis procera* (Sodom apple) were used in traditional cheese production in West African countries such as the Republic of Benin and Nigeria (Shah et al., 2013).

Plant rennet's enzymatic activity is mostly associated with the action of APs or those with residues of serine and cysteine (Amira et al., 2017). In fact, the use of various types of plant proteases in cheese technology affects the level of degradation of the milk protein matrix, resulting in differences in the sensory properties of cheese (Amira et al., 2017). However, due to their excessive proteolytic nature or low milk coagulation activity, most plant proteases are not suitable for milk coagulation (Gutiérrez-Méndez et al., 2019). This has reduced their use in cheese production due to lower cheese yield and taste and texture deficiencies. The search for new possible plant-based milk coagulant enzymes is therefore underway to make them industrially usable and support the growing global market for diversified and high-quality cheese (Shah et al., 2013).

There was a study conducted by Gutiérrez-Méndez et al. (2019), *Solanum elaeagnifolium* was used in the manufacture of cream cheese as a milk coagulant. The result was that this plant-derived protease had a lower ratio of milk coagulant activity to PA (MCA/PA) than chymosin, but a higher MCA/PA than other plant proteases reported. Another study was also conducted by Hernández-Mancillas et al. (2017). It was found that *Bromelia pinguin* proteases have milk coagulation activity in a wide range of temperatures, with milk coagulation times comparable to commercial chymosin. Enzymatic extract showed a maximum activity of milk coagulation at high temperatures that could be related to its thermostability. After extended incubation time, cysteine and serine proteases were responsible for the main milk coagulation activity and demonstrated excessive casein proteolysis. An important new source of proteases for biotechnological processes is the strong concentration of milk coagulant proteases in *B. pinguin* fruit (Hernández-Mancillas et al., 2017).

Nasr, Ahmed, and Hamid (2016) also stated in their journal that they managed to develop a simple purification technique to generate substantial amounts of effective milk coagulant enzymes from sunflower seeds as a cheap milk coagulant preparation for cheese production. Furthermore, the partially purified enzyme showed great specificities of milk coagulation and curd formation comparable to that of commercial rennet, indicating its potential as a rennet substitute or additive in the cheese industry (Nasr et al., 2016).

On the other hand, Bromelain is a general name for a sulfhydryl group containing proteolytic enzymes obtained from the pineapple plant *Ananas comosus* (Dubey, Reddy, & Murthy, 2012). One of the major fruit crops in many countries is pineapple (*A. comosus* L. Mer). It is also an herbaceous tropical plant containing a special enzyme with high levels of pharmacology. The bromelain enzyme complex of *A. comosus* is known for its medical application. Bromelain is sold as a nutritional supplement in some developed countries to “promote digestive health” and as an antiinflammatory medication. In addition, proteolytic enzymes such as bromelain inhibit the action of cholera toxin and are also chosen as food processing enzymes (Truc, Thanh, & Muoi, 2010).

A bromelain enzyme in the different number of pineapple plants can be obtained on the stalk, skin, leaves, fruit, or stem. It can be reached from its fruit, especially its flesh, with a high bromelain content of 0.080%–0.125% (Bahmid, 2013). To determine the state of bromelain in pineapple, bromelain should be obtained through the method of isolation and the determination of the function of the enzyme.

The growth of the biotechnology industry has encouraged the development of engineering enzymes, particularly on industrial cheese, to reduce the cost of production (Bahmid, 2013). Presently, the cost of making cheese is very high because rennet enzyme is costly and has limited availability. A bromelain, like renin (rennet), papain, and fisin, is a proteolytic enzyme that all can hydrolyze protein and agglutinate milk. This bromelain can replace other similar enzymes. Fisin, papain, and bromelain are sulfhydryl proteases capable of hydrolyzing the peptide bond that connects protein to amino acid (Bahmid, 2013).

Bromelain is a proteolytic enzyme that can be used to coagulate the casein. Like papain, it can also be used in the manufacturing of soft cheese. Some studies have been conducted using papain as an enzyme in the coagulation of casein in soft cheese production, but to fulfill the demand for enzyme that is very large, bromelain enzyme can be an alternative enzyme. Bromelain has the same ability as papain, it can digest 1000 times its weight of protein. However, the use of bromelain enzyme in soft cheese production is still minimal (Bahmid, 2013).

There was one study about soft cheese making using bromelain enzyme conducted by Bahmid (2013). The type of cheese developed in this research was soft cheese, as its water content was above 40%. The 50°C incubation temperature treatment gives the best outcome to protein content, while 55°C incubation temperature treatment gives the best outcome to water content, fat content, and yield. Treatment with 0.4 U/mg substrate enzyme concentration also yields good protein content, while 0.5 U/mg substrate enzyme concentration yields good water content, fat content, and yield (Bahmid, 2013).

8.3 Case study: Optimization of milk-clotting activity and rheological monitoring in soft cheese production using plant enzyme

8.3.1 Optimization of yield and milk-clotting activity

Response surface methodology was used to determine the optimum condition for the factors affecting soft cheese production using bromelain enzyme. The effects of the three (3) independent variables of X_1 (bromelain concentration), X_2 (incubation temperature), and X_3 (incubation time) using five levels of central composite design (CCD) on the yield and milk-clotting activity of soft cheese were determined.

It was found that the optimum conditions for the target goal with a bromelain concentration of 0.81 U/g, incubation temperature of 53.02°C, and incubation time of 1.93 hours as well as for the minimum goal with a bromelain concentration of 0.42 U/g, incubation temperature of 49.88°C, and incubation time of 3.42 hours were feasible to be carried out. Meanwhile, the optimum condition for the maximum goal with a bromelain concentration of 0.92 U/g, incubation temperature of 50.98°C and incubation time of 1.32 hours was not feasible to be carried out. The actual yield and milk-clotting activity of soft cheese ranged from 20.2 to 31.56 g and 913.24 to 1626.02 (SU/mL), respectively, with different variables of factors combinations. Optimization condition was set up at target goal as it showed the highest response of yield (32.82 g) and milk-clotting activity (1582.50 SU/mL).

8.3.2 Evaluation of proteolysis

Research by [Mendez et al. \(2019\)](#) on cream cheese made from *S. elaeagnifolium* has confirmed the protease ability to hydrolyze caseins but not whey proteins. Protease from the fruits of *S. elaeagnifolium* was able to hydrolyze caseins, but not whey proteins. Four types of caseins: α_{s1} -, α_{s2} -, β -, and κ -casein were hydrolyzed by the plant-derived coagulant. Nevertheless, the plant coagulant may have produced peptides smaller than 10 kDa. The plant-derived coagulant, on the other hand, did not hydrolyze the whey proteins (β -lg and α -la) or generate a mild hydrolysis that could not be detected by SDS-PAGE analysis. The proteases of whey proteins are also less susceptible to hydrolysis than caseins.

S. elaeagnifolium also showed a clot ability as much as 978.36 U/mL while bromelain enzymes used in this experiment have a clot ability as much as 1574.8031 SU/mL. This result shows that bromelain enzyme is a better plant coagulant than *S. elaeagnifolium*. Based on [Bala et al. \(2012\)](#), it stated that molecular weight of bromelains ranged between 24.5–37 kDa which may also help in better hydrolyzing of casein because its molecular weight falls perfectly with casein molecular weight and also has a near molecular weight with chymosin. This research shows that plant coagulant does not have the same characteristic as rennet enzyme on the specific hydrolysis on κ -casein which helps in the production of cheese. A good milk-clotting enzyme is characterized by a high specific caseinolytic activity and a low general PA, since the proteolysis strongly affects the sensory properties of cheese ([Shah, Mir, & Paray, 2014](#)).

8.3.3 Viscoelastic properties

Monitoring the evolution of rheological properties is one of the means for measuring gel formation during coagulation. Parameters such as the elastic or storage modulus (G'), which is a measure of the energy stored per oscillatory cycle and reflects the behavior of the sample as an elastic solid; the viscous or loss modulus (G''), which is a measure of the energy dissipated per cycle and indicates the behavior of the sample as a viscous liquid. Both storage and loss modulus were used to evaluate the viscoelastic properties of bromelain enzyme soft cheese.

Soft cheeses have properties of solid gel. The overall spectra for bromelain enzyme cheese show an increase in G' and G'' as frequency increased. It was observed that $G' > G''$ and crossover modulus value occurred. During milk coagulation, the viscosity and storage moduli (G') of the curd sharply increase with coagulation ([Alavi & Momen, 2020](#)). The intensity of the G' increase is determined by the strength and number of bonds formed among casein particles as well as the structure and spatial distribution of casein strands in the gel network.

8.4 Demand for halal cheese

The global sale of halal cheese can be estimated by the Asia Pacific Market, where halal-labeled cheese is estimated to be worth USD 284.6 million. Cheese demand is increasing as a result of dietary changes and an increase in exports to Muslim countries, particularly in the Middle East and Southeast Asia ([Cochrane, 2016](#)). The market for halal cheese has been driven by halal certification. Some Muslim-majority countries require halal certification for all food products, and cheese is one of the most popular fast-food ingredients, which is rapidly expanding globally as a result of the

adoption of western culture. As a result, halal cheese can easily penetrate countries that require halal certification ([Futuremarketinsights.com, 2022](#)). Interestingly, in Malaysia, cheese is the least frequently purchased dairy product, with 61% indicating they never purchased cheese ([Boniface & Umberger, 2012](#)).

Malaysia is a country which is multiethnic and multicultural. It is important to note that the majority of Muslims and their positions and desires are protected by the constitution that has made Islam the country's official religion ([Asa & Azmi, 2017](#)). The Muslims are very cautious about their faiths and beliefs in this diverse society, especially when it comes to food. People usually choose food based on their sense of taste, desire, and affordability. Nonetheless, Muslims must determine whether or not food is acceptable in Islam, because a Muslim's life focuses on the principle of halal and haram. The basic guidance on halal food law can be found in the Quran and Sunnah themselves ([Asa & Azmi, 2017](#)).

According to Pew Research Center ([Lipka, 2017](#)), the global Muslim population is estimated at approximately 1.8 billion, representing 24% of the world's population. By 2025 the Muslim population is expected to reach 30% of the world's population ([Perdana, Jan, Altunişik, Jaswir, & Kartika, 2018](#)). The halal market is considered to be very lucrative and one of the world's largest industries, but the industry itself is extremely untouched. According to Reuters (2016), the global Halal Food Division industry was estimated at around \$1.17 trillion and revenues from halal-certified food and drink products were estimated at around \$415 billion in 2015 ([Perdana et al., 2018](#)). Halal industry is showing a high level of growth, showing strength in both business and commerce. It is no longer associated with religious values per se but extends as a global symbol of quality assurance and lifestyle choice ([Perdana et al., 2018](#)).

Halal company is the latest trend in the world market. Halal market is one of the fastest-growing industries in the world, with more than 3 billion people in the Muslim population ([Nurrachmi, 2018](#)). It covers the financial, tourism, service, transportation, and food sectors. Food is important in human life and halal food's market potential with Islam is very promising as the fastest-growing religion in the world ([Nurrachmi, 2018](#)).

As reported in the Global Islamic Economy Report in Thomson Reuters (2014), halal food is one of the world's largest consumer markets ([Nurrachmi, 2018](#)). In addition, Muslims invested 16.6% of total global food spending, ultimately making the halal food industry one of the largest food markets in the world. Previous studies show that the growing global Muslim population contributes to the emergence of halal on the global market. Global Muslim population growth will rise by 35% from 1.6 billion in 2010 to 2.2 billion in 2030 ([Nurrachmi, 2018](#)). Therefore the halal food market will continue to dominate the global food market because Muslims must eat halal food regardless of whether they live in Muslim-majority or -minority societies.

Today, the awareness of consuming halal food comes not only from Muslims, but from non-Muslims as they believe buying halal products is safer. It is also why halal's food industry is very attractive to focus on food preservation and freshness. This situation facilitated the emergence and development of the halal food industry with widespread acceptance by non-Muslim customers who consider halal as clean, hygienic, value, and nutritious ([Nurrachmi, 2018](#)). Developed countries like Japan, the United Kingdom, and Australia, which rely mainly on automotive and electronic production, have seized this opportunity. The halal food segment is believed to be the catalyst for the growth of other potential sectors negatively affected by the economic turmoil ([Nurrachmi, 2018](#)).

8.5 Conclusion

To meet the growing global need for diverse and high-quality cheese production, a replacement for milk coagulant enzyme from plant was researched. Milk-clotting enzyme from plant sources would be useful as an alternative to chymosin in the production of soft cheese. Bromelain enzyme at its optimum condition yields approximately 32.82 g of soft cheese with significantly higher milk-clotting activity (1582.50 SU/mL) although it can be improved further, to suit the demand for halal cheese worldwide. Plant based enzyme as milk coagulant in cheese is competitive and potentially well received among different religious and cultural groups.

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Chapter 9

Gelatin Substitute

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9.1 Gelatin

Gelatin is a class of biopolymer which is widely used in the modern manufacturing industries especially in food processing, pharmaceutical, cosmetic, and photographic products because of its unique functional properties. Gelatin is a source of protein that comes from large amounts of collagen and is most widely used in food ingredients due to its application including enhancing elasticity, consistency, and stability of food products (Lva et al., 2019). It is one of the major hydrocolloid products obtained from the hydrolysis of a protein collagen. This is because the characteristic proteins include at least 33% amino acid glycine and 22% proline (Said, 2020). Other study mentioned that collagen is the most abundant protein in animal body that constitutes 20%–25% of the total protein in mammals and has a unique amino acid composition together with two modified amino acids, hydroxylysine and hydroxyproline (Sultana, Ali, & Ahamad, 2018).

Fig. 9.1 depicts the composition of gelatin in terms of amino acids. When collagen is heated above its transition temperature, the heating reaction generates a mixture of protein and peptide fractures of different sizes. Thus gelatin can also be defined as partially hydrolyzed collagen (Lva et al., 2019). The extraction of gelatin from collagen frequently requires a boiling state or a hydrolysis reaction (sometimes enzymatically assisted) to produce a flavorless and colorless substance, famously known as a gelling agent in food production (Alipal et al., 2021).

As mentioned by Said (2020), food technology has developed the foods that can be used as a source of nutrition in the 21st century while also having good functional properties. Gelatin is one of the most valuable proteins in food and its application that act as emulsifier, stabilizer, wetting agent, refined material, biodegradable packaging film, microencapsulation agent that showed a good package of functional properties. According to Alipal et al. (2021), gelatin can substitute fat and carbohydrate due to its high protein content in certain nutritionally balanced foods. Hameed, Asiyanbi-H, Idris, Fadzillah, and Mirghan (2018) stated that gelatin is a hydrocolloid that has a variety of uses, including gelling, thickening, foaming, plasticizing, texturing, and binding. In general, two different types of gelatin may be produced based on the pretreatment of collagen: type A gelatin and type B gelatin. Type A is an acid treatment gelatin, which is an isoelectric point at pH 6–9 and is most used for the less covalently cross-linked collagen found in pig skin.

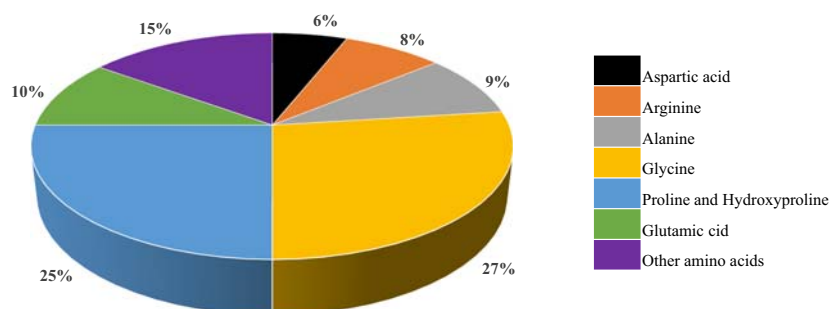


FIGURE 9.1 Amino acid composition of gelatin (Kommareddy, Shenoy, & Amiji, 2005).

Meanwhile, type B is an alkaline treatment gelatin which is an isoelectric point at pH 5 and can be applied to more complex collagen found in bovine hides. The nanoparticles in type B gelatin showed higher cross-linking degree, resulting in slower degradation rate compared to the nanoparticles in type A gelatin (Aramwit et al., 2015). Overall, because of the functional amino acid group and terminal amino acids, as well as the carboxyl group, gelatin as a protein exhibits both acidic and basic activities (Alipal et al., 2021).

9.2 Sources of Gelatin

In the food industry, most of the hydrocolloids are extracted from plants and, in general, only gelatin is extracted from animals such as pigs, cows, and fish as the primary sources of gelatin. The production of gelatin from various collagen sources of animal origin which undergone partial hydrolysis is well established in the literature (Hashim, Ridzwan, Bakar, & Hashim, 2015; Mutalib et al., 2015). Due to some scholars supporting the family of hydrocolloids, particularly plants labeled as “veggie gelatin,” hydrocolloids and gelatin have frequently been confused that leads to fraudulence and mislabeling of food products. Not all hydrocolloids are plant based, and gelatin is not a naturally occurring family of hydrocolloids; in contrast, it needs to be extracted from animal parts. It is important to note that plant sources of gelatin are nonexistent, and there is no chemical correlation between gelatin and other materials referred to as vegetable gelatin or plant gelatin (GMIA, 2012). Therefore the commercially viable source of collagen is demineralized cattle bone (ossein), pig and bovine hides, and never be plants (Alipal et al., 2021).

Chemically, gelatin is a collagen-hydrolyzed substance which is the main protein in certain mammalian species’ hides, smooth connective tissues, and bones. As a result, the raw materials for gelatin production are typically derived from various animal by-products, primarily bovine (cow-based) and porcine (pig-based) (Sultana et al., 2018), some were from fish (Lva et al., 2019), poultry, and camels or even from amphibians species such as frog (Karnjanapratum, Sinthusamran, Sae-leaw, Benjakul, & Kishimura, 2017) and salamander (Jin et al., 2019). It is reported that usually 29.4% and 23.1% of these gelatins comes from bovine hide and bones, 46% from porcine skin, and about 1.5% from fish (Alipal et al., 2021). Furthermore, due to the low cost and widespread availability of bovine and porcine gelatin, they are widely used all over the world.

Nowadays, fish gelatin has also received much attention not only because it is a reliable alternative to the other types of gelatin but also due to its unique functional properties and applications (Karim & Bhat, 2009). Gelatin extracted from fish scales and skin is a biopolymer that contains 85%–92% protein, water, and mineral salt. The partial hydroxylation condition of high temperature, alkali, acid, and enzyme can produce fish gelatin (Lva et al., 2019).

On the other hand, plant-based gelatin or “veggie gelatin” is a term coined by Lestari, Octavianti, Jaswir, and Hendri (2019) that pursues an alternative to animal-based gelatin such as Konjac (a gelatin used in Japanese cuisine) and Yam as suggested by Lestari et al. (2019), capable of producing gelatin from plant hydrocolloids. This veggie gelatin may be derived from agar, carrageenan, pectin, xanthan gum, modified corn starch, and celluloid. However, as explained by Alipal et al. (2021), despite the abundant gelatin alternatives developed, the rapid and low-cost manufacture of mammalian-based gelatin, particularly from rapid-breeding animals like pigs, remains tough to surpass. As an alternative, due to the widespread industrial production of fish by-products around the world, fish-based gelatin was more favorable at present compared to the plant-based gelatin. In the production of food gelatin, it is necessary to completely remove lipids from raw materials, which greatly complicates the technological process. Therefore, to obtain high-quality gelatins, it is advisable to use the skin of fish with a low-fat content, such as cod, haddock, pollock, hake, and so on and so forth. In addition, it is possible to improve the thermal and rheological properties of gelatin derived from cold-water fish by modifying gelatin, for example, using complex-forming ionic polysaccharides (Derkach, Kuchina, A. V., Kolotova, & Voron’ko, 2019).

9.3 Chemistry and Structure of Gelatin

Gelatin’s remarkable gelling capability has piqued the interest of numerous food scientists throughout the world. Understanding the structure, including the chemical composition, has become critical in elucidating the mechanism of gelation and explaining its other functional properties (Djagny, Wang, & Xu, 2013). Chemically, gelatin is made up of 18 varieties of complex amino acid, calcium binding 57% of glycine (amino acids are also potential calcium-binding ligands in relation to calcium absorption from food during digestion), proline, and hydroxyproline are the major compounds, while the remaining ca. 43% are other distinguished amino acid families such as glutamic acid, alanine, arginine, and aspartic acid as shown in Fig. 9.1. Gelatin is composed of 25.2% oxygen, 6.8% hydrogen, 50.5% carbon, and

17% nitrogen, while gelatin has a mixture of single and double unfolded chains of hydrophilic character (Hanani, Roos, & Kerry, 2014).

Based on chemical composition, gelatin is different from collagen. In collagen, the triple helix structure consists of three α -chains (Fig. 9.2) while the gelatin structure consists of three different chains (Fig. 9.3). These chains are α -chains, β -chains, and γ -chains (Said, 2020). Also explained by another study, gelatin consists of different polypeptide

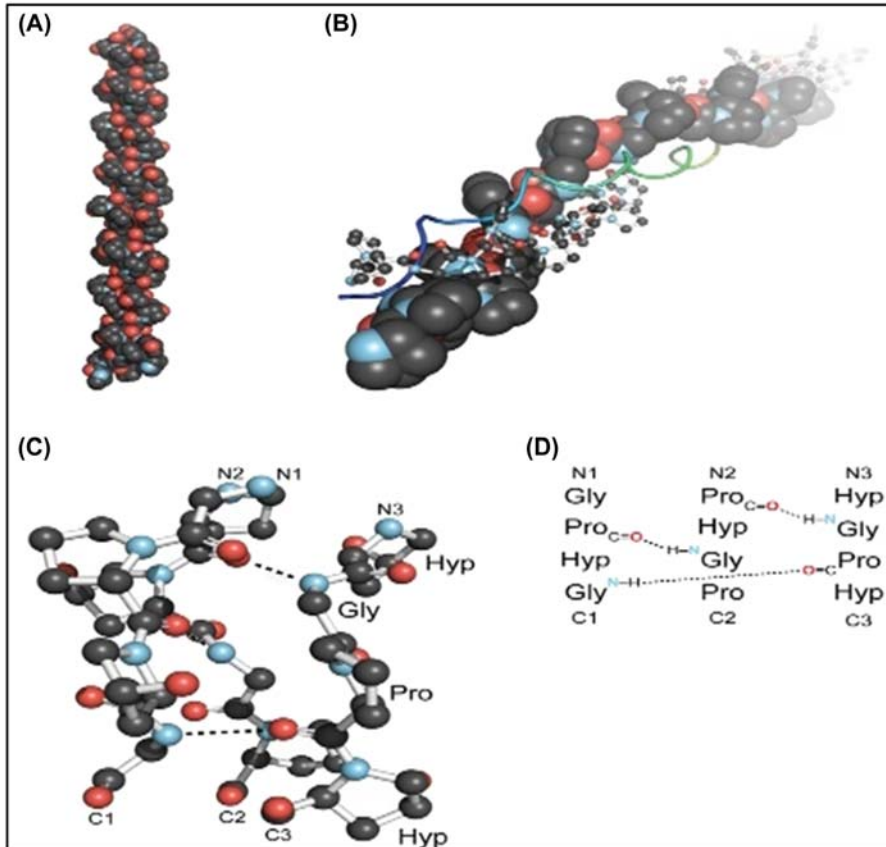


FIGURE 9.2 Overview of the collagen triple helix (A) first high-resolution crystal structure of a collagen triple helix, formed from (Pro-Hyp-Gly)₄–(Pro-Hyp-Ala)–(Pro-Hyp-Gly)₅. (B) View down the axis of a (Pro-Pro-Gly)₁₀ triple helix [PDB entry 1k6f] with the three strands depicted in space-filling, ball-and-stick, and ribbon representation. (C) Ball-and-stick image of a segment of collagen triple helix, highlighting the ladder of interstrand hydrogen bonds. (D) Stagger of the three strands in the segment in panel c. (Shoulders & Raines, 2009).

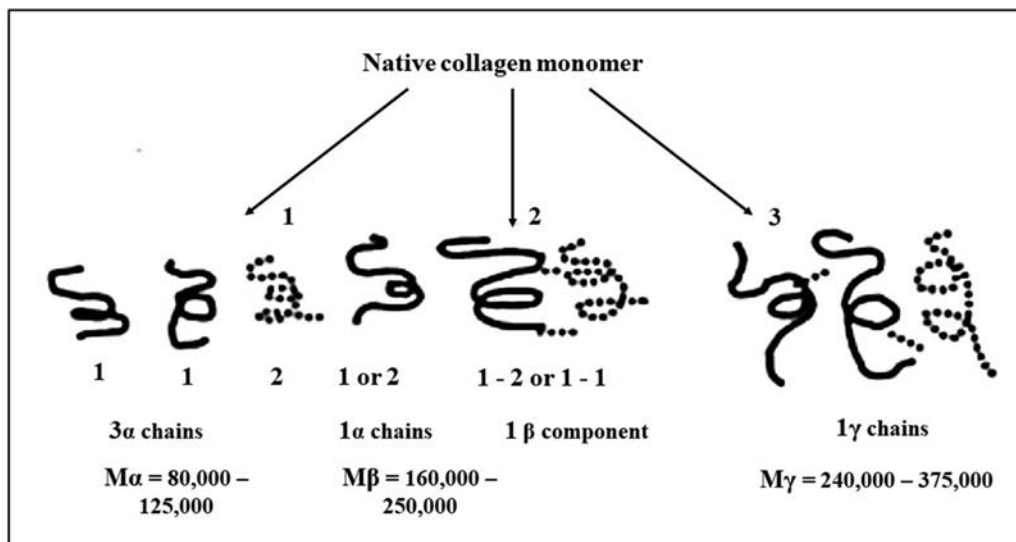


FIGURE 9.3 Possible paths of collagen conversion to gelatin (Mariod & Fadul, 2013).

chain such as α -chains (one polymer/single chain), β -chain (two α -chain covalently cross-linked), and γ -chains (three covalently cross-linked α -chains) with a molar mass of around 90×10^3 , 180×10^3 and 300×10^3 g/mol, respectively (Fig. 9.3). The presence of oligomers with increasing numbers of α -chains becomes more complex and difficult to read (Alipal et al., 2021). Thus it becomes necessary to separate these molecular weight fractions. Fig. 9.4 depicts a modern picture of gelatin structure.

According to Said (2020), gelatin is the result of combining several polypeptide chains to form a triple helical conformation. Each of the three chains in the triple helical conformation requires around 21 residues to complete one spin. Gelatin consists of rows of 50–1000 amino acids that are bonded together. Type I collagen is produced from the skin and bones consisting of two $\alpha 1$ (I) chains and one $\alpha 2$ (I) chain. In the two chains, each has a molecular mass of 95 kD with a width of ≈ 1.5 nm and a length of ≈ 0.3 μ m. Interchain bonds are formed into the hydroxyl group bonds between the amino acid hydroxyproline with carbonyl peptides forming hydrogen bonds with water molecules. Higher levels of proline produce a stronger gel. The chemical structure of gelatin has been presented in Fig. 9.5. The strength of the gel is directly related to the high hydroxyproline content of the structure of protein molecules. Similar structures and properties of gelatin among animals can occur and, hence, can create difficulties in determining the specific source.

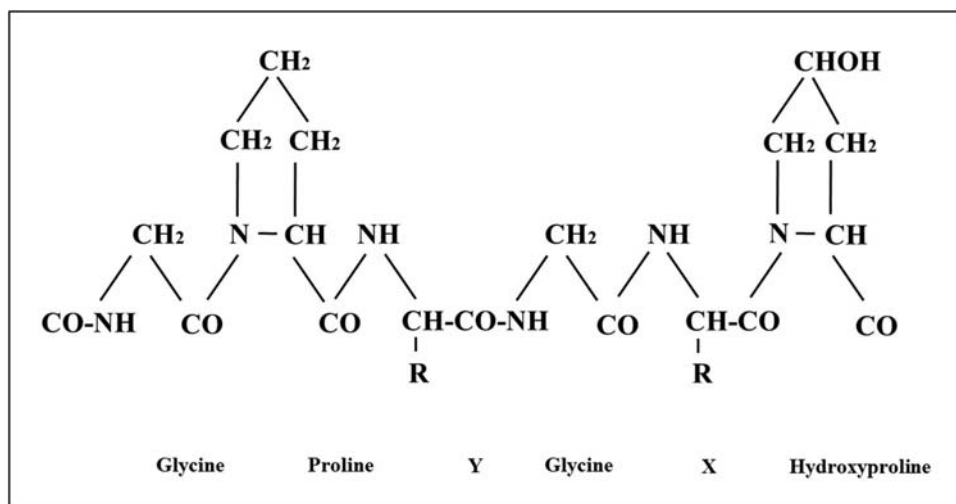


FIGURE 9.4 Chemical configuration of gelatin (Mariod & Fadul, 2013).

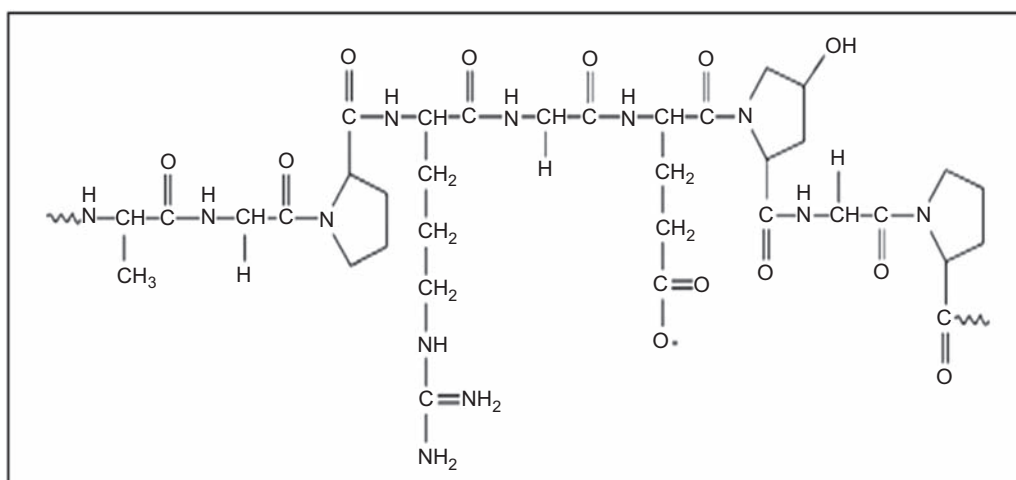


FIGURE 9.5 The chemical structure of gelatin (Chaplin, 2022).

9.4 Types of Gelatin Substitutes

The issue of gelatin alternatives has recently gained increased interest especially within Europe with the emergence of the bovine spongiform encephalopathy (BSE) virus that has infected cattle. This resulted in the banning of gelatin and beef sales from the United Kingdom to other European Union countries, from 1996 until recently, and has heightened consumer knowledge of the source of gelatin, forcing certain producers to incorporate gelatin alternatives when formulating existing or new processed food.

Most hydrocolloids are polysaccharides, which are inherently heterogeneous species in terms of chemical structure and molecular weight distribution. Molecular weight and weight distribution play a critical role for the solubility, viscosity, and gelation of polysaccharides. There are many and varied molecular structures and interactions among polysaccharides can lead to unique functionalities for very specific applications. Plant-based gelatin or “veggie gelatin” replacer is normally developed from plant hydrocolloids. Hydrocolloids have been extensively used in different food products as thickeners (soups, gravies, salad dressings, sauces, and toppings), water retention agents, stabilizers, emulsifiers, and gel-forming agents (jam, jelly, marmalade, restructured foods, and low-sugar per calorie gels). They also can be applied for inhibiting ice and sugar crystal formation in ice cream and the controlled release of flavors (Goff & Guo, 2019). Some of the commonly used hydrocolloids are listed in Table 9.1.

The polysaccharides-based gelatin alternatives generally have less flexible molecular backbones, leading to higher host viscosity than gelatin. There are notable exceptions, such as xanthan/locust bean gum gels, which are thermoreversible, but which have relatively high host viscosities, even compared to polysaccharide (Mariod & Fadul, 2013). Some hydrocolloids considered for gelatin replacement include, mixed high-methoxyl/low-methoxyl (LM) pectin gels, which is not considered a good candidate as a gelatin alternative, since it forms thermally irreversible gel and requires a low pH and high-soluble solids. However, LM pectin appears to be more flexible in terms of manipulation of gelling conditions, although at high sucrose concentrations. Modified starch/wheat fiber gel is another gelatin alternative that is a combination of a dual modified starch and wheat fiber gel to replace gelatin in yogurt. The starch-to-wheat-fiber-gel ratio was critical, with the optimum ratio at 60% starch to 40% wheat fiber gel. Yogurts with gelatin replacer showed higher stability against storage temperatures over 20°C. Carrageenan is a new iota carrageenan extract by using a new, proprietary extraction process developed to be used for gummy-type, or molded candies. The new iota carrageenan-based products allow for shorter conditioning times, easier demolding and alternate molding processes (Mariod & Fadul, 2013).

However, the most challenging task in obtaining gelatin alternatives from plant sources is to obtain the thermoreversible property of mammalian gelatin which is the melt-in-the-mouth quality (Lestari et al., 2019). It has remained difficult to beat the rapid and low-cost production of mammalian-based gelatin especially from rapid-breeding animals such as pig (Alipal et al., 2021).

TABLE 9.1 Examples of hydrocolloids used in various food categories (Goff & Guo, 2019).

Food category	Hydrocolloid used
Salad dressing	Xanthan gum, PGA, modified starch, MCC, guar gum
Muscle food	Modified starch, carrageenan, konjac glucomannan, alginate
Bakery products	CMC, fenugreek gum, guar gum, konjac gum, xanthan gum
Bakery fillings	Locust bean gum, guar gum, pectin, alginate, PGA, cellulose derivatives, konjac gum, xanthan gum, carrageenan, agar, gellan gum
Frozen dairy desserts	Guar gum, locust bean gum, carrageenan, xanthan gum, alginate, cellulose derivatives, pectin, gelatin
Culture dairy products	Modified starch, locust bean gum, guar gum, gelatin, carrageenan, xanthan gum
Restructured foods	Alginate

CMC, Carboxymethyl cellulose; MCC, microcrystalline cellulose; PGA, propylene glycol alginate.

9.5 Functional Properties: Gelling and Surface Behavior

Gelatin is truly remarkable in terms of its many functional properties in food applications. A variety of examples are given in [Table 9.2](#). The functional properties of gelatin are related to their chemical characteristics such as gel strength, viscosity, melting point, and ability to form a gel ([Said, 2020](#)).

The most important properties of gelatin can be classified into two groups (1) properties associated with their gelling behavior such as gel formation and thickening or viscosity enhancing and (2) properties related to their surface behavior, which include emulsion and foam formation and stabilization, adhesion and cohesion and microencapsulation and film-forming capacity ([Gareis, 2007](#)). Gel formation, viscosity, and texture are closely related properties determined mainly by the structure, molecular size, and temperature of the system. Gelatin has good potential properties at a lower cost. However, gelatin is less able to provide adhesion ([Kavoosi, Dadfar, & Purfard, 2013](#)). Consumers prefer gelatin made from land-based cattle because of its viscosity and better gel strength, as well as the fact that it conforms to the melting point ([Cho et al., 2004](#)). The unique properties of gel from gelatin that comes from animals are melted in the mouth which is not owned by the type of gel from plant groups (polysaccharides) such as starch, alginate, pectin, agar, and carrageenan. Gel from animals is colorless and has no taste for a softer texture than gels from polysaccharides ([Baziwane and He, 2003](#)).

According to [Mariod & Fadul \(2013\)](#), the gel strength depends on gelatin concentration and the ability to form a gel. Gelatin is capable of forming and stabilizing hydrogen bonds with water molecules to form a stable three-dimensional gel. The need to analyze the characteristics of the gel has resulted in the concept of gel strength, also known as bloom strength. Gelatin is popular for its thermally reversible gelling properties with water (e.g., during the production of table jellies). Gelling may be defined as the “water-absorbing property” of any given product. An example in this case is the addition of gelatin to canned ham before cooking. On cooking, the exudates from the meat are absorbed by the gelatin and appear as a gel when the can is opened. In confectionery, gelatin is used as the gelling binder in gummy products, wine gums, and products of this type. Type B gelatin with gel strengths from 125 to 250 is commonly used for confectionery products. Type A gelatin with low gel strength (70–90) can be used for the fining of wine and juice.

Collagen and gelatin surface properties are based on the presence of charged groups in the protein side chains, and on certain parts of the collagen sequence containing either hydrophilic or hydrophobic amino acids. Both hydrophobic and hydrophilic parts tend to migrate toward surfaces, hence reducing the surface tension of aqueous systems and forming the required identically charged film around the components of the dispersed phase, which can be additionally strengthened by gel formation. Type A gelatin, with a relatively high isoelectric point ($pI \geq 7.0$), is suitable for creating

TABLE 9.2 Functional properties of gelatin in foods ([Mariod & Fadul, 2013](#)).

Function	Food application
Gel former	Gelled desserts, lunch meats, confectionery, pate
Whipping agent	Marshmallows, nougats, mousses, souffles, chiffons, whipped cream
Protective colloid	Confectionery, icings, ice creams, frozen desserts, and confections
Binding agent	Meat rolls, canned meats, confectionery, cheeses, dairy products
Clarifying agent	Beer, wine, fruit juices, vinegar
Film former	Coating for fruits, meats, deli items
Thickener	Powdered drink mixes, bouillon, gravies, sauces, soups, puddings, jellies, sirups, dairy products
Process aid	Microencapsulation of colors, flavors, oils, vitamins
Emulsifier	Cream soups, sauces, flavorings, meat pastes, whipped cream, confectionery, dairy products
Stabilizer	Cream cheese, chocolate milk, yogurt, icings, cream fillings, frozen dessert
Adhesive agent	To affix nonpareils, coconut, and other items to confections, to bond layered confections together, to bind frostings to baked goods, to bind seasonings to meat products

oil-in-water emulsions with a positive charge over a wider range of pH values than is possible with conventional protein emulsifiers, such as soy, casein, or whey proteins. As is often the case with gelling properties, the emulsion capacity of gelatin from fish species is frequently lower than that from mammals. Apart from the distribution of charge, an important criterion in selecting a suitable gelatin type is gel firmness, because, at the same temperature and concentration, the higher the gel firmness, the firmer the gel-like protective sheath is around the oil droplets (Baziwane and He, 2003).

Gelatin is also used in the processing of marshmallows due to its excellent film-forming properties that help to stabilize the foam on cooling and because the product is normally not acidified, it needs to have much lower moisture content (<85% solids) than gummy products (76% solids) to avoid mold growth during storage (Alipal et al., 2021). Furthermore, gelatin gives a thickener property that also provides appropriate mouthfeel or better known as viscosity to the final product (Gómez-Guillén et al., 2011), for instance, flavoring sirups and canned soups. Next, gelatin is amphoteric and has no net charge at its isoionic point (pI). At its pI, acid groups, from gum arabic, for example, can react with the basic groups of gelatin to form an insoluble gelatin–arabate complex, which can be precipitated around emulsified oil droplets, forming microencapsulated oil (Piacentini, Giorno, Dragosavac, Vladislavljević, & Holdich, 2013). The formed microcapsules are hardened with formaldehyde drying. This process has been used in the food industry for encapsulating flavors.

Among other types of hydrocolloids, gelatin is most widely used on the market due to its vast and multivariate functional utility such as stabilizing, bioprotective films, and gelling agents in food production and confectionery (Saha & Bhattacharya, 2010). By far, gelatin is actually a multivariate, functional substance in today's life (Alipal et al., 2021). Nevertheless, the properties of gelatin which are superior and advantageous in the food industry led to emergence of multiple types of plant hydrocolloids, as gelatin imitation. Table 9.3 shows some examples of gelatin replacers and selected properties, in comparison to gelatin. Table 9.4 summarizes some suitable application for gelatin replacers. Cattle bones, pig skins, and bovine hides are traditional sources of gelatin production, although some of the customers will reject this source of gelatin due to religious and health issues that are of constant concern. Therefore aquatic gelatin harvested from marine-based animals such as fish fins, skins, scales, and bones would be a promising alternative to these mammalian gelatins (Said, 2020).

9.6 The Factors that Drive the Rise of Plant-based Gelatin Replacers

The unique properties of gelatin are useful in food manufacturing (Bae et al., 2008), for example, as thickener, emulsifier, plasticizer as well as gelling, foaming agents, texture improver, moisture retention, and binding agents (Rakhmanova, Khan, Sharif, & Lv, 2018). Previous sections highlighted many different gelatin replacers available with different properties and uses. Interestingly, the global gelatin business has also shown a rising demand for veggie gelatin. The usage of nonanimal-based food ingredients by vegan and vegetarian consumers is the key reason for this growth. As a result, items derived from plants, such as agar, carrageenan, pectin, and guar gum, experienced an upsurge (Businesswire, 2017). Besides the usefulness of physical, chemical, and functional properties as the main reason of gelatin replacers, there are three other factors to be considered, including the plant protein as alternative source of diet, religious, or cultural food requirement and allergens due to seafood consumption.

Recently, increasing attention in plant- and collagen-derived protein as an alternative dietary source has stemmed from global concerns on environmental sustainability. This is irrespective of plant protein having a weaker anabolic profile due to a lower essential amino acid profile (e.g., reduced leucine concentration) and poor digestion (versus animal) (Deane, Bass, Crossland, Phillips, & Atherton, 2020).

More than 80% of commercial gelatins are derived from swine bones and skins, which are forbidden in halal and kosher products (Mutalib et al., 2015) or cows in Hinduism (Uddin, Hossain, Sagadevan, Amin, & Johan, 2021). Animal origin of gelatin determines the acceptability of gelatin products. For instance, according to halal and kosher food laws, porcine material is not acceptable in any dietary items. Therefore gelatin from porcine sources is not allowed for Muslims and Jewish communities. Moreover, due to the outbreak of fatal neurodegenerative disorder BSE, the use of bovine gelatin in food, feed, and pharmaceutical products creates increased concern among regulatory authorities (Cai, Gu, Scanlan, Ramatlapeng, & Lively, 2012). However, for beef gelatin to be considered halal or kosher, the concerned animal must be slaughtered according to the respective religious ritual.

Next, the rise in the consumption of seafood has led to increase in allergic reaction, hence restricts consumption of marine-based gelatin/collagen (Carrera, Pazos, & Gasset, 2020). To guarantee consumer safety, several regulations have been implemented (European Commission, 2017), such as the labeling of food allergens that are introduced intentionally (EFSA, 2014). However, some products on the market could contain traces of allergens due to accidental cross-contaminations during the food manufacturing processes. The route of exposure is not only restricted to ingestion but

TABLE 9.3 Comparison of frequently used hydrocolloids-rheological properties (Schrieber and Gareis, 2007).

Hydrocolloids	Gel formation	Thickening effect	Transparency of the gel	Cold-water solubility	pH stability
Gelatin	+++ Thermoreversible, difference in melting/ gelling temperature low	+++	+++	0 With the exception of cold- water-soluble instant gelatins and gelatin hydrolysates	++
Agar-agar	+++ Thermoreversible, difference in melting/ gelling temperature high	+++	+	0	++
Alginates	bbb (with calcium)	+++	+++	+++	+
Carrageenan	Kappa/iota: bbb (with cations) Lambda: 0	++	++	++	++
CMC	0	+++	—	+++	++ (pH 3–11)
Gum arabic	0	+	—	+++	++ (pH 4–9)
HPMC	+++ (Gel formation on heating)	+++	+	+++	+++ (pH 1–10)
Locust bean gum	0	++	—	+	++ (pH 3–11)
Modified starches	+++	+++	+	0 With the exception of physically modified starches	++
Native starches	+++	+++	+	0	+
Pectin	+++ — Low methoxy plus Ca^{2+} thermally irreversible — High methoxy plus sugar + H^+ thermoreversible	++	+++	++	+ — High methoxy: — pH 2.5–4.5 — Low methoxy: — pH 2.5–5.5

CMC, Carboxymethyl cellulose; HPMC, hydroxypropyl methylcellulose.

KGaA. Qualitative assessment: 0 = none; +++ = high.

Source: Reproduced with permission from Schrieber, R., & Gareis, H. (2007). *Gelatin handbook*. Weinheim: Wiley-VCH Verlag GmbH & Co. Copyright Wiley-VCH Verlag GmbH & Co.

also includes manual handling and inhalation of cooking vapors in domestic and occupational environments (Jeebhay et al., 2019).

The shift to plant protein as a dietary alternative, religious or cultural food requirements, and allergies from seafood intake, an anticipated demand for halal/kosher gelatin arises. Companies are now working to develop gelatin-free goods that do not employ mammalian gelatin as a processing aid or an ingredient. Patents for fish gelatin synthesis and many published methods for fish gelatin extraction (Jeevithan et al., 2014; Lassoued et al., 2014) resulted from the hunt for new gelling agents to replace mammalian gelatin. In addition, several research and patents on the development of gelatin alternatives or substitutes based on plant hydrocolloids such as starch/modified starch (Muller & Innerebner, 2021) and pectin (Staples & Rolke, 2015), and galactomannan (Karl, 2014) have been published.

TABLE 9.4 Functional properties of gelatin in selected food applications with possible alternatives (Morrison, Clark, Chen, Talashek, & Sworn, 1999).

Food application	Desired gelatin properties	Current alternatives	Technical constraints of alternative
Desert gels ready to eat (RTE)	Clarity, elastic texture, melt in the mouth	Algin, gellan, and carrageenan	Hot viscosity, higher set temperature
High-solids confectionery	Elastic texture, clarity, low hot viscosity, low set temperature	Gellan gum blends, carrageenan systems, thinned-starch systems	Set temperature and hot viscosity, texture elasticity gels
Foamed confectionery-marshmallows	Whipping/aeration agent, foam stabilizer, elastic texture	Gellan/starch/emulsifier blends, modified starch/emulsifiers	Textural constraints-low elasticity and/or high set temperature
Low-fat spreads	Elastic gel texture, fat-like melt mouthfeel, emulsion stabilization	Sodium alginate/gellan/inulin/simplesse/maltodextrin/gum blends	Cost competitive, but good application for alternatives
Stirred yogurt	Creamy mouthfeel, gelled network prevents separation or syneresis	Gellan/modified starch/xanthan/LBG/pectin/modified starch	High viscosity and high set temperature in culture/production process
Desserts mousses	Whipping agent, creamy consistency, low set temperature	Alginate/starch blends	Current production process, stored prior to aeration chilling
Sour cream	Smooth texture, creamy mouthfeel	Gellan gum with modified starch	High set temperature during processing

Source: From Morrison, N. A., Clark, R. C., Chen, Y. L., Talashek, T., & Sworn, G. (1999). *Gelatin alternatives for the food industry*. In K. Nishinari, F. Kremer, & G. Lagaly (Eds.), *Physical chemistry and industrial application of gellan gum* (pp. 127–131). Heidelberg: Springer-Verlag, with kind permission of Springer Science + Business Media.

9.7 Regulatory Aspects and Future Outlook: An Overview

From the consumption perspectives in today's market, according to the report of Grand View Research, the estimation for market size of gelatin will reach 5.0 billion USD in the year of 2025. By the end of 2027, the gelatin market size is predicted to be USD 6.7 billion, with a Malaysia's compound annual growth rate (CAGR) of 9.29% (Alipal et al., 2021). Key players of gelatin production and manufacturing include (but not limited to) Nitta-Gelatin, Rousselot and Weishardt (PRNewswire, 2021). A confluence of factors, including rising demand for convenience and functional foods, increased health consciousness, the expansion of end-use sectors, and growth across emerging economies, is propelling the global market forward (PRNewswire, 2021). Porcine content caters 46% of the total production of gelatin, bovine hide at 29.4%, while pork and cattle bones stand at 23.1% (Gómez-Guillén et al., 2011).

Previously, the occurrence of BSE in the 1980s limits the utilization of bovine by-products for gelatin in Europe (EU Legislation, 2001), leading to almost 90% of porcine-origin edible gelatin (Sudjadi, H. S. Sepminarti, & Rohman, 2016). However, since 2016, Commission Regulation (EU Legislation, 2016) amending Annex III to Regulation (EC) No. 853/2004 of the European Parliament and of the Council as regards the specific requirements for gelatin, collagen, and highly refined products of animal origin intended for human consumption highlighted that it is necessary to ensure that raw materials for the production of gelatin and collagen for human consumption come from sources that meet the public and animal health requirements (EU Legislation, 2016). In addition to the high protein content and ease of water absorption (PRNewswire, 2021), this regulation lends higher demand for gelatin from bovine hide. Today, the expansion of the porcine gelatin market is fueled by rising demand for nutritional and sports products in the food and beverage industry (PRNewswire, 2021).

Gelatin in general meets the United States Pharmacopeia (USP) and European Pharmacopoeia standards, while FDA has approved it as generally recognized as safe (GRAS). Internationally, there are several legislations that can be found in relation to gelatin. In EU 2092/91, Annex VI, gelatin is listed under "Processing aids and other products which may be used for processing of ingredients of agricultural origin" in Section B and under "Ingredients of Agricultural Origin Which Have Not Been Produced Organically" in Section C. Meanwhile, Codex Alimentarius—Guideline for the

Production, Processing, Labelling, and Marketing of Organically Produced Foods CAC/GL 32–1999 listed gelatin under the Substance for Plant Pest and Disease Control and plant and animal and under “processing aids which may be used for the preparation of products of agricultural origin.” On the other hand, Ministry of Agriculture, Forestry and Fisheries of Japan has allowed gelatin in food additives under the Japan Agricultural Standard. In Canada, the Canadian General Standards Board National Standard for organic agriculture (CAN/CGSB-32.310–99) has permitted gelatin as a clarifying agent.

In regards to halal gelatin, few countries have come out with separate regulations or legislation, such as Turkey (OIC; SMIIC, 2021), and Malaysia (JAKIM, 2021). In Turkey, Technical Committee on Halal Food Issues published a new standard: OIC/SMIIC 22:2021—Halal edible gelatin—Requirements and test methods; the new standard provides information about the specific requirements and test methods for halal edible gelatin (OIC; SMIIC, 2021). The standard, which contains Islamic laws as well as technical standards, is intended to serve as a guide for producing halal and safe edible gelatin for human consumption. In the same year, for the purpose of verifying the source of products and raw materials based on collagen and gelatin, the Department of Islamic Development Malaysia as the coordinator with the State Islamic Religious Departments (JAIN) through the Malaysian Halal Certification Implementation Committee Meeting No. 3 of 2020 which convened on December 9, 2020 has agreed that there is a need for a specific mechanism in the matter of sampling products and raw materials based on collagen and gelatin to be coordinated for its implementation among Malaysian Halal Management Systems (JAKIM, 2020) applicants and holders throughout the country. This is in conjunction with the need for a halal certification from the Halal Certification Body as well as a Certificate or Deoxyribonucleic Acid (DNA) analysis results that are recognized as main conditions in the application for halal certification in collagen and gelatin based products (JAKIM, 2021).

9.8 Conclusion

In a nutshell, this chapter explains the various sources of gelatin, its functional properties, the adaptation of gelatin replacer properties to gelatin, the impetus of gelatin replacers, hydrocolloids and polysaccharides, misconception on “veggie gelatin,” and an overview on gelatin rulings and regulations. Advancement and dynamics on food science and technology have catered to the needs of today’s consumers on gelatin alternatives and this review could serve as a reference guide for the production and application of gelatin substitutes in academia and industry.

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Alternative biomanufacturing of bioactive peptides derived from halal food sources

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10.1 Introduction

Food is classified as halal (permissible), haram (forbidden or prohibited), or Mashbooh (unpermissible) (doubtful) (Azam, Abdullah, & Abdul-Razak, 2019; Mohd Nawawi et al., 2020). Halal food is clean, hygienic, and compatible with human health and benefits (Izberk-Bilgin & Nakata, 2016). Khattak et al. (2011) enlisted the scientific findings on Islamic dietary law and principles: (1) dead animals are deemed inappropriate for human consumption due to the decaying process; (2) which creates certain adverse substances; the blood that is pumped out of the body contains toxins, pathogens, and some metabolic products; (3) swine serve as a vector for disease transmission to humans; and (4) intoxicants are hazardous because they affect the nervous system and cause a loss of senses.

Bioactive peptides made from animal or plant protein are one way to create bio-based nutritious foods. These products could be made using enzyme hydrolysis, fermentation, or integrative processes. A few critical points in the sources and processes must be considered to gain legal recognition. This chapter examines the halal perspective on sources (animal or plant-based) and processes in obtaining the bioactive peptides.

10.2 Bioactive peptides as functional food ingredients

The effects of bioactive peptides on the digestive, cardiovascular, neurological, and immune systems have been studied extensively over the last decade. Peptide composition and amino acid sequence determine bioactivity, and they can be multifunctional (Piovesana et al., 2020; Sato, 2018; Udenigwe, Abioye, Okagu, & Obeme-Nmom, 2021; Wang, Ding, Du, Yu, & Liu, 2019; Wu, 2020). The mode of action differs according to the biological activity of the compound (Hajfathalian, Ghelichi, García-Moreno, Moltke Sørensen, & Jacobsen, 2018). They can then be introduced as functional components, where their bioactivity may contribute to disease prevention and control (Cicero, Fogacci, & Colletti, 2017; Lammi, Aiello, Boschin, & Arnoldi, 2019; Pangestuti & Kim, 2017).

Food concepts evolve to combat chronic illnesses such as cardiovascular disease, certain malignancies, and obesity, as well as improve certain health functions. Functional food ingredient or nutraceuticals is designed to help reduce or avoid illness risks such as cardiometabolic diseases, immune-inflammatory diseases, obesity and metabolic syndrome (Chibisov et al., 2019; Lyu et al., 2017; Plasek, Lakner, Kasza, & Temesi, 2020). Thus any bioactive component is added to food may be classified as a functional food (Lafarga, Álvarez, & Hayes, 2017). Any health advantages derived from novel functional foods must be shown properly. The literature offers several examples of how cholesterol levels were decreased, lactose intolerance was reduced, Crohn's disease was remitted, diarrhea was relieved, and cancer cell growth was inhibited (Helkar, Sahoo, & Patil, 2017; Mijan & Lim, 2018; Murphy, Ling, El-Nezami, & Wang, 2019).

10.3 Halal food sources and bioactive peptides production

Practically, halal food sources are of land, aquatics, and plants provided that they adhere to the Islamic dietary law. The use of haram sources such as a pig or land animal (unslaughtered or slaughtered), including its derivatives products that are not according to Shariah, must be avoided in manufacturing bioactive peptides. That includes the feeds and enzymes, including microorganisms. Genetically modified organisms though it has proven to improve the output of the process, however, require a thorough examination of the source of the gene. Muslim customers should exercise caution and sensitivity regarding halal or haram products. Recent breakthroughs and their implications on the status of religious dietary law are critical (Kashim et al., 2015; Kashim et al., 2018).

Food described as “halal” is permissible, and “haram” is prohibited in Islamic dietary law. Except for poisonous, intoxicating, or harmful to one’s health, most plant items are considered halal (Regenstein et al., 2003).

Bioactive peptides are amino acid sequence segments in proteins with biological effects and nutritional value. Antimicrobial, antioxidant, antihypertensive, and immunomodulatory properties are all present. These bioactive peptides are usually 2–20 amino acid residues long, but some are longer (Hou, Wu, Dai, Wang, & Wu, 2017). The production of bioactive peptides is quite straightforward and relies mostly on hydrolysis. The process can be carried out directly or indirectly (during food processing) with the appropriate processing condition such as temperature, pH and enzyme, or microorganism concentration. The batch process is the most common way to make bioactive peptides. Though simple, the process is lengthy with low residual enzyme activity and produces a complex mixture of peptides. As a result, it reduced the potency of the protein hydrolysate measured as IC_{50} , which is the half-maximal inhibition concentration. Thus the batch process would require subsequent downstream processes to purify these protein hydrolysates to improve efficacy.

Bioactive peptides are commonly purified using a membrane-based and chromatographic approach. Immobilized enzyme reactors, membrane reactors, enzyme membrane reactors, ultrafiltration electrolysis, and ion-exchange chromatography are the purification processes used (Marciniak, Suwal, Naderi, Pouliot, & Doyen, 2018). These steps are followed to make peptides with the desired purity and activity. However, it is time-consuming and costly. As a result, low-cost, straightforward isolation with a limited number of unit actions is required. Due to a lack of prior information on the properties of bioactive peptides, it is challenging to develop adequate purification processes, particularly in finding new peptides (Marciniak et al., 2018).

The integrative approach has been thoroughly investigated to resolve the complication. Ortiz-Chao et al. (2009) initiated the integrative process for bioactive peptide processing. Combining adsorption and in situ hydrolysis of the adsorbed protein, the approach intended to produce a particular hydrolysate with the most effective antihypertensive peptides from whey protein. Further research into using an ion-exchange resin in the integrative process to create peptides with high antihypertensive activity has generated encouraging findings (Pa’ee, Gibson, Marakilova, & Jauregi, 2015). In contrast to the traditional immobilized enzyme method, the anion resin will adsorb nonhydrolyzed protein and peptides with similar charges releasing peptides of high bioactivity. Following these prepurification steps with upstream hydrolysis processes, these peptides’ product mixture will be less complex. Thus it will increase its potency. Furthermore, high-purity peptides (up to 90%) can be achieved due to the adsorption of peptides with a comparable charge to the protein source (Welderufael & Jauregi, 2010). Separating smaller peptides from enzymes and larger peptides allows non-hydrolyzed substrates, large peptides, and enzymes to be recycled, resulting in a more efficient process.

Bitterness, limited peptide bioavailability, food-matrix peptide interactions, unconfirmed molecular mechanisms, and the sustainability of resources for mass manufacturing are some of the problems that must be overcome during the creation of the bioactive peptide. As a result, this review provides an in-depth look at the current state of bioactive peptide manufacturing, including trends, patterns, and impediments. Meanwhile, the possibility for a technological leap via an alternate process is highlighted for development sustainability.

10.3.1 Animal-based protein

The food industry uses the production of protein hydrolysates and components to increase the solubility of proteins (Hayes, 2018). In addition to this functional benefit, hydrolysis is an approach that may be used to generate health benefits from protein constituents since it produces bioactive peptides, which have hormone-like health benefits when taken, as shown in Table 10.1.

Food safety and shelf life are major concerns in the food industry. Pathogenic and spoilage bacteria can contaminate food in several ways. Synthetic agents are not permitted due to their potential environmental and health hazards. So

TABLE 10.1 Bioactive peptide properties from animal sources.

Animal-based protein	Bioactive peptide properties	References
Goat whey	Antimicrobial activity	Osman, Goda, Abdel-Hamid, Badran, and Otte (2016)
Duck meat	Antioxidant activity	Wang, Zhang, Zou, Sun, and Xu (2018)
Pacific cod	Antihypertensive activity	Ngo, Vo, Ryu, and Kim (2016)

TABLE 10.2 Bioactive peptide properties from plant sources.

Plant-based Protein	Bioactive peptide properties	References
Rice barn	Antioxidant activity	Supawong, Thawornchinsombut, and Park (2018)
Fermented soybean	Anticarcinogenic activity	Badger, Ronis, Simmen, and Simmen (2005); Chen, Wang, Liu, and Chen (2017)
Sunflower seed	Antimicrobial activity	Song, Song, Jo, and Song (2013)

new natural antimicrobials are critically needed. A study done by [Osman et al. \(2016\)](#) showed that goat whey hydrolysate has antimicrobial properties against *Escherichia coli* and *Bacillus cereus*, with minimum inhibitory concentration (MIC) values of 0.09 and 0.03 mg/mL, respectively, while it had similar MIC values against *Staphylococcus aureus* (~0.02 mg/mL).

In general, biological antioxidants balance out free radicals. The presence of free radicals can also limit shelf life, produce undesirable taste and rancidity, and generate potentially dangerous reaction products. Meat protein is high in antioxidant peptides and important amino acids like methylhistidine and hydroxymethyllysine that are not found in plant protein ([Wang et al., 2018](#)). Thus, based on the study by [Wang et al. \(2018\)](#), duck meat that had been treated with Flavourzyme showed significantly improved the hydroxyl-radical scavenging, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical-scavenging, ferrous ion-chelating reducing, and 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation-scavenging activities of duck meat.

Gelatin is a widely used food ingredient. It can improve food product flexibility, uniformity, and stability. As a stabilizer, gelatin is used in dairy products and as a fat substitute to minimize calorie content without compromising flavor. Gelatin is a useful protein derived from animal cartilage, bones, tendons, and skins ([Lestari, Octavianti, Jaswir, & Hendri, 2019](#)). While pigskin is the primary source of gelatin, other sources contribute. This contains bovine skin (29%) and cow bones (46%) in the overall gelatin manufacture (23.1%) ([Sharif, 2019](#)).

Due to the rise of mad cow disease and the unacceptability of bovine and porcine-derived gelatin by Muslims, Jews, and Hindus, there is a growing interest in manufacturing gelatin from fish ([Rakhmanova, Khan, Sharif, & Lü, 2018](#)). Two bioactive peptides, GASSGMPG (662 Da) and LAYA (436 Da), are produced by gelatin derived from the Pacific cod skin showed strong inhibition of angiotensin-1-converting enzymes that are crucial in hypertension ([Ngo et al., 2016](#)).

10.3.2 Plant-based protein

A few examples of bioactive peptides from plant sources have been classified according to their biological activities ([Table 10.2](#)).

[Supawong et al. \(2018\)](#) mention that rice bran hydrolysate (RBH) was beneficial in lowering lipid oxidation by 79.8% in a frozen fried fish cake formulation. It also showed multiantioxidant activity by reducing free radicals, reactive oxygen species, and metal chelating. RBH may prove valuable as a safe and health-friendly antioxidant for the food sector, based on customer concerns about the safety and toxicity of synthetic antioxidants.

Second, according to a study conducted by [Badger et al. \(2005\)](#), fermented soybean isolate has an anticarcinogenic effect and can reduce the risk of tumor incidence by 80% through a transforming growth factor (TGF) β

pathway-activating mechanism for preventing breast cancer. [Chen et al. \(2017\)](#) supported these studies as the peptides from soybean, black soybean, adzuki bean, and mung bean meal have inhibitory effects on cancer cells at 200–600 g/mL concentrations.

Third, by exploiting sunflower seed meal as edible film, the population of *Listeria monocytogenes* on smoked duck meats packed with sunflower seed meal film dropped by 1.31 log CFU/g after 12 days of storage compared to control packaging, implying that protein/peptide films could be used as antimicrobial food packaging materials ([Song et al., 2013](#)).

Finally, bioactive peptides appear to be potential alternatives to chemical preservatives, which have been contested for a long time due to consumer health concerns ([Görgüç, Gençdağ, & Yılmaz, 2020](#)). Furthermore, it is derived from halal sources, which can penetrate the market of Muslim community worldwide.

10.4 In-silico assessments of potential bioactive peptides

Bioinformatics is the study of biological mass data through methodologies and software tools. Foodomics require bioinformatics analysis to integrate proteomics, transcriptomics, and metabolomics ([Valdés, Cifuentes, & León, 2017](#)). Bioinformatics can reduce the number of tests required to create bioactive peptides by determining their structure–activity relationship. Bioinformatics has recently been applied to analyze bioactive peptides in proteins ([Ji et al., 2018](#)). Bioinformatics, also known as in-silico analysis, involves computational methods applied to manage, curate, and interpret information related to biological systems ([Li-Chan, 2015](#)).

In addition to linking databases of protein sequences, bioactive peptides, and sensory peptides, BIOPEP has built-in software that helps anticipate proteolytic hydrolysates and allergenic peptides ([Minkiewicz, Dziuba, Iwaniak, Dziuba, & Darewicz, 2008](#)). Protein databases like UniProtKB, NCBI, and BIOPEP include protein sequences that study protein precursors. The online programs BIOPEP and ExPASy-PeptideCutter are most often used ([Lin, Zhang, Han, & Cheng, 2017](#); [Tu et al., 2017](#)). The released peptides can then be compared to bioactive peptides reported in literature and databases like BIOPEP, PepBank, PeptideDB, and BitterDB.

10.4.1 Structure implication of bioactive peptides on ACE inhibition

[Ko et al. \(2016\)](#) provided a more detailed explanation of the inhibition pattern of both peptides isolated from flounder fish (MEVFVP and VSQLTR). The MEVFVP peptide exhibited an inhibition pattern, suggesting that it can bind to the active site of angiotensin I-converting enzyme (ACE) through metal ion interaction (Zn 701). VSQLTR peptide was unable to bind to the active site of ACE but could inhibit the hydrolysis of HHL by binding to a separate site of ACE. VSQLTR interacted with Glu131, Glu202, and Leu210 through H-bond interactions that did not occur near any active site of the ACE complex, implying that noncompetitive inhibition was occurring ([Ko et al., 2016](#)).

The presence of a hydrophobic amino acid at the C-terminus, such as a proline residue, can influence the interaction between ACE inhibition complexes such as MEVFVP, which are competitive inhibitors. The noncompetitive inhibition peptides VSQLTR have a hydrophobic aliphatic amino acid at the N-terminus.

According to several studies, the size of the peptide and the location of certain amino acids on the N- and C-termini play a critical role in providing high ACE-inhibitory activity, which may have higher potency (IC₅₀ value) ([Caballero, 2020](#); [De Leo, Panarese, Gallerani, & Ceci, 2009](#); [Fang et al., 2019](#); [Ghassem, Arihara, Babji, Said, & Ibrahim, 2011](#); [Ko et al., 2016](#); [Lin, Alashi, Aluko, Pan, & Chang, 2017](#); [Pina & Roque, 2009](#); [Pripp, Isaksson, Stepaniak, & Sørhaug, 2004](#); [Sungperm, Khongla, & Yongsawatdigul, 2020](#)). The characteristic structural predominant in the C-terminal of ACE-inhibitory peptide is proline, tyrosine, and tryptophan ([De Leo et al., 2009](#)).

10.4.2 Computational approach

10.4.2.1 Molecular docking simulation

The molecular docking procedure is a computational tool that determines the structure and position of minimum energy of a protein–ligand complex will be determined by computational tools for the docking simulation ([MacLeod-Carey, Solis-Céspedes, Lamazares, & Mena-Ulecia, 2020](#)). The exploration of the guest molecule (also known as a ligand) conformational space inside the binding cavity (receptor) and the calculation of the binding energy for each expected conformation are the two stages of molecular docking ([Bitencourt-Ferreira & de Azevedo, 2019](#); [Wereszczynski & McCammon, 2012](#)).

Conformational search algorithms often use systematic and stochastic search methods. Systematic search strategies entail small changes in the structural parameters that alter the ligand conformation. By exploring the energy landscape

of the conformational space, the algorithm converges to a conformation that corresponds to the minimum energy solution. Stochastic methods investigate the energy landscape by arbitrarily altering the structural parameters of the ligands (Wereszczynski & McCammon, 2012).

Stochastic algorithms produce various solutions when exploring a wide range of conformational space. This method avoids confining the conformations at local minima, raising the probability of producing global minimum solutions (Wereszczynski & McCammon, 2012). This method also attempts to simulate the prediction of a complex containing (usually) two binding partners. A small ligand molecule is aligned inside the binding cavity of the target protein in structure-based small-molecule docking, and the resulting docking pose is evaluated by a specific scoring function (Pantsar & Poso, 2018). The driving forces for these specific interactions in biological systems aim toward complementarities between the shape and electrostatics of the binding site surfaces and the ligand or substrate (Gupta & Kumar Varadwaj, 2018; Pagadala, Syed, & Tuszyński, 2017).

Scoring functions are typically divided into three main classes. Force field–based scoring functions are based on energy terms from a classical force field. Knowledge-based scoring functions based on a statistical analysis of interacting atom pairs from protein–ligand complexes. Empirical scoring functions reproduce experimental affinity data based on binding free energy to a set of nonrelated variables. Some empirical scoring functions (also referred to as hybrid scoring functions) were developed using a mixture of force-field, contact-based, and knowledge-based descriptors (Guedes, Pereira, & Dardenne, 2018).

Empirical scoring functions are based on the idea that all relevant binding factors, such as hydrogen bonding (H-bonding), rotational/translational degrees of freedom, and polar/lipophilic effects, can be expressed in the form (preferably simple) equations. Furthermore, water-related H-bonding networks significantly impact the structure–activity relationship. Optimizing the ligand with the surrounding water network in mind may result in increased binding affinity and longer residence time. Tying up the equilibrium constant of complex formation is binding affinity, related to the change in Gibbs free energy upon binding by thermodynamic laws (Pantsar & Poso, 2018). A lower Gibbs free energy of a complex indicates that the protein–ligand complex is more stable, according to theory. For instance, the accuracy of each scoring function results from using IC₅₀ regression with each scoring function (Chen, 2015).

10.4.3 Current molecular interaction studies in ACE-inhibitory peptides

Xu et al. (2021) focused on the conformational docking of ACE-inhibiting peptides in the model of ACE was carried out using 2OC as the peptide target. The docking simulation demonstrated that all the ACE-inhibitory peptides demonstrated a high affinity for ACE and interacted in the active site to form H-bonding (Table 10.3). The stabilization of docked ligand complexes depended on hydrogen bond interactions (Attique et al., 2019).

However, the binding affinity from soy protein isolate (SPI) may contradict the binding affinity shown in Liu, Fang, Min, Liu, and Li (2018). SPI-derived peptides showed that the higher the H-bonding, the lower the binding affinity. The binding affinity of AVKVL, YLVR, and TLVGR with ACE were -3.46 , -6.48 , and -7.37 kcal/mol, respectively. It indicates that YLVR could bind tightly to ACE, regardless of the number of H-bonding formed. The ACE–YLVR complex interaction was stable and markedly stronger due to covalent cation–pi interaction. This interaction is an important noncovalent molecular interaction. The effect of cation interaction in the ACE-peptide bond appears to be comparable to that of hydrogen and electrostatic bonds, which may explain the overall stability of the complex (Liu et al., 2018). All details are summarized in Table 10.3.

It can be supported by the recent research done by (Chen et al., 2019), which compares the ACE-inhibitory peptide derived from *Oreochromis niloticus* with captopril using a molecular docking study. The ACE-inhibitory sequence was Leu-Ser-Gly-Tyr-Gly-Pro (LSGYGP). The affinity energy obtained under optimized conditions was -90.502 kcal/mol. It also generated four hydrogen bonds and two pi-alkyl bonds. All hydrogen bonds were formed at four residues of the ACE active site, which are Glu281, Asn66, Glu143, and Tyr523, with bond lengths of 5.48, 5.9, 5.54, and 5.95 Å, respectively, while two pi-alkyl bonds with a bond length of 4.8 and 5.46 Å with Val 380 and His 383. The presence of Pro residue in the last position of the C-terminal end in LSGYGP provides greater ACE-inhibitory potency for the sequence and stabilization toward the complex as Pro generated three interactions in LSGYGP. Nevertheless, the favorable salt bridges with ACE residues allow the ligands to strongly occupy the binding site, potentially counteracting ACE (Attique et al., 2019).

As a final note, in molecular docking, the results may vary from one study to another. These peptides' ACE-inhibitory properties may be attributable to one of three factors. First, due to their complete embedding within the catalytic cavity of ACE, they compete with the substrate. Second, by lying on the substrate recognition surface, they can

TABLE 10.3 The binding of ACE residue of ACE-inhibitory peptide.

Protein ACE	ACE domain	Bioactive peptide sources	Ligand	Hydrogen bond	Total number of hydrogen bond	Binding affinity (kcal/mol)	References
Testis ACE (C-domain)	2OC2	Soybean protein isolate	IY	His 353	4	− 7.4	Xu et al. (2021)
				His 513			
				Ala 354			
				His 383			
Testis ACE (C-domain)	2OC2	Soybean protein isolate	WMY	His 353	5	− 9.3	Xu et al. (2021)
				Lys 511			
				His 513			
				Gln 281			
				Tyr 523			
Testis ACE (C-domain)	2OC2	Soybean protein isolate	YVVF	His 353	6	− 9.8	Xu et al. (2021)
				Lys 511			
				His 513			
				Gln 281			
				Tyr 523			
				Ala 356			
Human ACE	1O8A	<i>Corylus heterophylla</i> Fisch. (Hazelnut)	AVKVL	Asp 415	3	− 6.48	Liu et al. (2018)
				Ser 526			
				Glu 411			
Human ACE	1O8A	<i>C. heterophylla</i> Fisch. (Hazelnut)	YLVR	Glu 384	1	−7.37	Liu et al. (2018)
Human ACE	1O8A	<i>C. heterophylla</i> Fisch. (Hazelnut)	TLVGR	Asp 415	2	− 3.46	Liu et al. (2018)
				Lys 454			

block the entry of the substrate. Third, interactions between ACE/ACE domains and ACE-inhibitory peptides induce changes in the spatial conformation of the ACE or ACE domain (Xu et al., 2021).

10.5 Current process of bioactive peptides production and its limitation

New functionalities have been attributed to food proteins since they can be considered potential sources of bioactive peptides. A great diversity of biological activities has been associated with peptides derived from different protein sources. Bioactive peptides are short sequences of amino acids present in foods or encrypted inside a greater protein but can apply biological activity once released. These peptides usually contain 2–20 amino acids in length (although most are 2–4 amino acids long) and a high abundance of hydrophobic amino acids. These molecules can be used as ingredients for developing functional foods, nutraceuticals, and cosmetics (Udenigwe et al., 2021).

Sometimes in these studies, we encounter some problem constraints. Among them, in some industries, production of bioactive peptides has not yet been achieved mainly because of high production costs and a limited number of scientific reports validating the bioactivities in vivo. Among other constraints is the use of complete hydrolysates or fractions containing peptides, which is desired for the majority of the applications, as purification of peptides is laborious and only economically viable for high-end applications such as pharmaceuticals. (Ulug, Jahandideh, & Wu, 2021).

In addition, high specificity, mild conditions, a lack of residual organic solvents and toxic chemicals in the final peptide preparations have positioned enzymatic hydrolysis as the most preferred method for producing bioactive peptides. However, the high cost of enzymes, a low yield, and limited food-grade enzymes pose the industry to seek alternatives. Furthermore, there is an increasing interest in preparing bioactive peptides using novel technologies such as high hydrostatic pressure (HHP), ultrasounds, ohmic heating, pulsed electric fields, microwave-assisted extractions, and subcritical water hydrolysis (Lafarga, Sánchez-Zurano, Villaró, Morillas-España, & Acién, 2021). These technologies are, in general, less effective than the conventional methods and thus often applied in combination with the enzymatic method. Subcritical water hydrolysis can cleave peptide bonds but lacks specificity in cleaving peptide bonds and peptide modifications (Ahangari et al., 2021). The new trend toward using novel technologies in producing bioactive peptides continues to gain momentum because they are environment-friendly, innovative, and sustainable.

Many applications of enzymes produce bioactive peptides from protein sources. However, specific studies and research on various types of enzymes and methods of bioactive peptide production are less thorough and comprehensive (Daroit & Brandelli, 2021). In addition, the active ingredient of the peptide has various roles in the human body, and the source of the active ingredient of this peptide can be obtained from plants.

Although much is known about the structure and activity of peptide-active ingredients, further studies on the relationship between the structure and activity of peptides are still lacking. Furthermore, in addition to determining their structure and activity in vitro, their therapeutic effects must be precisely determined, which will be studied comprehensively in the future. For example, hydrolysates and peptides from fish muscle and collagen and their ACE inhibitory and antioxidant activity may lead to their applications as bioactive ingredients in functional foods, conventional foods, and nutraceuticals (Ahangari et al., 2021). Many other studies on peptide-active ingredients were obtained from various food sources. However, this study is still limited and requires comprehensive research in the future.

10.6 Alternative processes

Enzymatic hydrolysis, a batch procedure, is the most typical method of producing bioactive peptides. The batch method can be used in any bioprocessing facility because it is straightforward to execute. Most batch reactors are double jacketed and equipped with a stirrer to ensure optimal temperature control and heat distribution. The product obtained from the batch method consists of a complex mixture due to the multiple hydrolyses of complex protein reducing the hydrolysate potency. Therefore some advanced processes like emerging and integration process technology were developed to solve the problems.

10.6.1 Emerging process technology

Emerging process technology is a multifaceted approach to improving process design and optimization while lowering costs. HHP, microwave, ultrasonic, sub- and supercritical fluids, and other technologies that aid in enzymatic hydrolysis have been highlighted as the most promising developing technologies in the production process of bioactive peptides.

HHP claims that pressure accelerates any process that results in a reduction in volume. Water is commonly employed as the pressure transmission fluid, and the technique can be utilized with or without heat. It is primarily a nonthermal technique that is frequently seen as cost-effective. Several research studies have investigated the role of HHP technology in protein denaturation and aggregation. In several studies, HHP has been demonstrated to improve the enzymatic hydrolysis of various protein sources, including plants, dairy, and meat. It can also help some enzymes to produce more bioactive peptides (Boukil, Suwal, Chamberland, Pouliot, & Doyen, 2018; Guan, Diao, Jiang, Han, & Kong, 2018; Homma, Ikeuchi, & Suzuki, 1994). Protein denaturation is caused by HHP treatment, which disrupts hydrophobic and electrostatic bonding of peptides but has no effect on covalent links (Mozhaev, Heremans, Frank, Masson, & Balny, 1994; Mozhaev, Heremans, Frank, Masson, & Balny, 1996; Rivalain, Roquain, & Demazeau, 2010). According to Bamdad, Shin, Suh, Nimalaratne, and Sunwoo (2017), enzymatic hydrolysis of casein protein with HHP at 100 MPa increased the degree of hydrolysis and antioxidant properties compared to 200 MPa and ambient pressure.

The efficacy of enzymatic hydrolysis and the bioactivity of antioxidant peptides are influenced by several factors, including pressure, holding time, protein supply, and enzyme. The availability of proteins and the nature and quality of the substrate and enzyme used define HHP's operational parameters. Protein aggregation can occur due to the complexity of the reactions involved in pressurization and decompression, preventing hydrolysis. The energy consumption of HHP is a crucial factor to consider, especially given the cost implications. The compressibility of the pressure medium, the holding pressure, the filling efficiency of the vessel, and the scale of the apparatus are the primary determinants of

energy consumption. Optimizing HHP settings is essential for lowering energy usage and costs. As a result, attempts to reduce operational costs and energy consumption, particularly during the pressurization phase, are being developed.

Other than HHP, the extraction of protein peptides via microwave-assisted processing is based on the movement and collision of charged ions through inter- and intramolecular friction (Wang & Zhang., 2017). This resulted in rapid heating and the destruction of membranes and protein cell walls (Jin, Xu, Li, Zhang, & Xie, 2019; Nguyen et al., 2016; Zhou, Yi, Wang, Yang, & Wang, 2018). Microwave heating increases enzymatic proteolysis and hydrolysate properties while reducing hydrolysis time. Microwave-assisted processing changes protein structure, enhancing enzyme bond accessibility and susceptibility (Ketnawa & Liceaga, 2017; Zhang, Huang, & Mu, 2019).

Several groups have identified microwave-assisted processing as a promising technique for producing bioactive peptides (Gohi, Du, Zeng, & Cao, 2019; Zhang et al., 2019). It has exceptional properties (protein solubility, oil and water absorption capacity) and aids enzymatic digestion of Australian rock lobster shells (Nguyen et al., 2016). Compared to untreated hydrolysate, the method improves the degree of hydrolysis, protein solubility, and free radical scavenging activity. In addition, ultrasonic processing is an emerging method in generating bioactive peptides (Zou et al., 2016). Ultrasound technologies with high intensity (16–100 kHz, power 10–1000 W/cm²) and low intensity (100 kHz–1 MHz, power 1 W/cm²) are commonly employed in the food sector (Wang & Zhang., 2017). Under the same sonification circumstances, Yu, Zeng, Zhang, Liao, and Shi (2014) discovered that pepsin activity was elevated, α -amylase and papain activity decreased. According to the researchers, this was attributable to alterations in the protein's secondary and tertiary structures after hydrolysis.

Furthermore, the ultrasound-assisted method has been shown to stimulate peptide release by increasing surface hydrophobicity, which increases the production of ACE-inhibitory peptides (Huang, Liu, Ma, & Zhang, 2014). It also improved enzyme dispersion and reduced aggregation, which increased enzyme activity (Kadam, Tiwari, Álvarez, & O'Donnell, 2015). According to Zou et al. (2016), ultrasound pretreatment of porcine cerebral protein followed by Alcalase digestion resulted in more peptides. Furthermore, protein hydrolysate pretreated with porcine cerebral ultrasound had a greater scavenging effect on DPPH radicals (72%), ABTS radicals (73%), and hydroxyl radicals (73%). Several factors must be evaluated and addressed, even though the potential for ultrasound processing has been shown. Its efficacy may be influenced by protein availability, unit operations, enzyme types, and process circumstances. As Ozuna, Paniagua-Martínez, Castaño-Tostado, Ozimek, and Amaya-Llano (2015) pointed out, it is also critical to characterize the ultrasound system's acoustic field to quantify acoustic energy during hydrolysis.

Besides that, sub- and supercritical fluids are alternative methods for producing bioactive peptides. Water's physical and chemical properties alter with temperature and pressure at subcritical and supercritical levels. Water's dielectric constant decreases in sub- and supercritical conditions, allowing it to interact with nonpolar substances and lowering the binding force, allowing it to dissolve more easily (Ahmed & Chun, 2018). In a sub- and supercritical state, water or another solvent has good transport capabilities and has thus shown to be a simple, cost-effective, and highly efficient technique (Ahmed & Chun, 2018). It is also considered a green technique because it does not utilize any poisonous chemicals and instead relies on water. The appropriate conditions, particularly the right temperatures, are required to produce enough functional peptides. Depending on the protein source, the ideal conditions are usually varied. Animal resources, for instance, necessitate higher temperatures and longer reaction times than plant resources.

Supercritical carbon dioxide has recently been used to make bioactive peptides. Several factors influenced the process since proteins, peptides, and amino acids are temperature-sensitive. According to Lamoolphak et al. (2006), the isolated protein increased with temperature while the amino acids decreased. When proteins are exposed to high temperatures, they can degrade. This is caused by denaturation, the production of a disulfide bond between amino acids, the liberation of polypeptides and free amino acids, the Maillard browning reaction between amino acids and carbohydrates, and the direct oxidation of amino acids.

While new approaches have shown their capacity to create bioactive peptides, there are still several obstacles to overcome. HHP, for example, caused protein unfolding and oligomer dissociation (Garcia-Mora, Penas, Frias, Gomez, & Martinez-Villaluenga, 2015; Hoppe, Jung, Patnaik, & Zeece, 2013). Furthermore, peptides generated by sub- and supercritical fluids were rendered inactive and worthless due to difficult denaturation (Chao, He, Jung, & Aluko, 2013; Leeb, Kulozik, & Cheison, 2011). Finally, ultrasonic degraded protein structures due to hydration effects and the exposure of hydrophobic groups (Kangsanant, Thongraung, Jansakul, Murkovic, & Seechamnaturakit, 2015; Uluko et al., 2014).

10.6.2 Integrative process technology

The integrative process combines upstream and downstream processing to create peptides with various bioactive characteristics continuously and simultaneously. In the purification of peptides, the adoption of the ion-exchange principle has

sparked a lot of attention. Traditional procedures, such as acid precipitation, which causes denaturation and low purity, have been superseded by ion-exchange methods for separating extremely important protein precursors (Konrad, Lieske, & Faber, 2000; Rojas, dos Reis Coimbra, Minim, Saraiva, & da Silva, 2006). The gentle nature of the ion-exchange process on proteins and peptides has provided the groundwork for further development. The ion-exchange might be integrated into the upstream process due to its simplicity and versatility.

Pa'ee's method (Pa'ee et al., 2015) combines the ion-exchange process with the adsorption of protein precursors for ACE-inhibitory peptides. The peptides are released when the adsorbed protein precursor to the ion-exchanger is hydrolyzed in situ. The method has several advantages, including a high-potency hydrolysate that requires no further purification, protease stability throughout hydrolysis, and cost-effectiveness because the process takes place in a single vessel. Furthermore, the hydrolysate produced less complicated peptides, resulting in higher potency than traditional techniques that require additional purification processes.

Pa'ee et al. (2015) and Wolderufael and Jauregi (2010) reported caseinomacropeptide (CMP) adsorption coupled with β -lactoglobulin in the early hydrolysis stage, implying CMP adsorption. The release of peptides produced from β -lactoglobulin is enabled by removing the CMP fraction and the restart of in situ hydrolysis. As a result, three powerful ACE-inhibitory peptides were discovered: Ile-Pro-Pro (IPP) and Gln-Asp-Lys-Thr-Glu-Ile-Pro-Thr (QDKTEIPT), both derived from CMP, and Ile-Ile-Ala-Glu (IIAE), both obtained from β -lactoglobulin. Due to simultaneous purification, the researchers could get less complex peptides during the process.

10.7 Future trends in halal bioactive peptides industry

The growing demand for halal bioactive peptides demands the consumption of natural resources and the revamping and creation of a viable production method for nutritional and pharmaceutical applications. The primary advantages of using bioactive peptides include their low accumulation in human tissues, high bioactivity, target biospecificity, broad therapeutic action spectrum, and low allergenicity and toxicity (Agyei, Ongkudon, Wei, Chan, & Danquah, 2016).

However, progress in converting laboratory discoveries of bioactive peptides into commercialized functional meals has been modest. This, in turn, impeded the development of the halal bioactive peptides industries. These challenges stem from peptide bitterness, food–peptide interactions, low peptide bioavailability and yield, a lack of human studies, insufficient knowledge of molecular mechanisms, and protein supply sustainability (Li-Chan, 2015).

Nonetheless, today's readily available bioactive peptides have shown their feasibility and market potential (Table 10.4). The availability of commercialized functional foods containing bioactive peptides derived primarily from halal resources demonstrates the great development potential of the halal bioactive peptides industry. Due to the incorporation of bioactive peptides, certain commercially available functional foods and ingredients offer a variety of health claims. For example, the two most well-known products, Calpis and Evolus, are fermented milk-based products developed in Japan and Finland, respectively, and have been shown to decrease blood pressure when taken orally by hypertensive patients (Rai, Sanjukta, & Jeyaram, 2017).

Bioactive peptides derived from fish proteins have also been included in various products, subsequently marketed as food in Japan for specific health purposes. This product, known as Valturon, has been shown to have ACE-inhibitory and antihypertensive properties (Harnedy & FitzGerald, 2012). On the other hand, glutamine-rich peptides in DMV International's dry milk protein hydrolysate, which is produced in the Netherlands, have been shown to have immunomodulatory activity (Chai, Voo, & Chen, 2020). Davisco Foods, US's whey protein hydrolysate incorporates glycomacropeptide with anticarcinogenic, antimicrobial, and antithrombotic properties (Dullius, Goettert, & de Souza, 2018). The commercialized bioactive peptide products are listed in Table 10.4.

Furthermore, pharmaceutical companies are actively using bioactive peptides to promote health. The worldwide peptide pharmaceutical industry is expected to grow as therapeutic peptides have recently been approved to treat various illnesses, namely, tumors, immunological disorders, blood disorders, diabetes, and obesity. Teva's COPAXONE (glatiramer acetate) is an immunomodulator used to treat multiple sclerosis (Duda, Schmied, Cook, Krieger, & Hafner, 2000). Abbott's Lupron injection (leuprolide acetate) is beneficial for cancer treatment (Brito, Latronico, Arnhold, & Mendonca, 2004). Other leading manufacturers (Pfizer, Amgen, and Roche) have successfully produced peptides and protein-based therapies in various phases of research that will be available on the market eventually for the treatment of a variety of complex disorders (Kintzing, Interrante, & Cochran, 2016).

Food regulation is one of the most significant challenge to commercializing halal bioactive peptides. Food regulation often takes time to approve the commercialization of a new food product, and it might take years in certain cases. Given bioactive peptides' novelty and potential health and nutritional benefits, it is vital to effectively convert laboratory findings to industrial applications while ensuring compliance with the regulations (Chai et al., 2020). To make a

TABLE 10.4 The commercialized products from bioactive peptides.

Product name	Manufacturer
Capolac	Arla Foods Ingredients, Sweden
Evolus	Valio Oy, Finland
Calpis	Calpis Co., Japan
PeptoPro	DSM Food Specialties, Norway
Vasotensin	Metagenics, the United States
Casein DP Pepto Drink	Kanebo, Japan
BioZate	Davisco Foods, the United States
Remake CholesterolBlock	Kyowa Hakko, Japan
Capolac	Arla Foods Ingredients, Sweden
Tekkotsu Inryou	Suntory, Japan

health claim under specific regulations, the peptide must be present in sufficient amounts in the final product to achieve the nutritional or physiological effects claimed. However, peptide identification in complex matrices is often limiting. Technological advancements and innovative approaches to detecting and quantifying short peptides would enable the commercialization of bioactive peptides as functional foods (Lafarga et al., 2017).

Because of the incorporation of bioactive peptides, the formation of carcinogenic, toxic, and allergenic substances must be thoroughly investigated to address the food safety issue. Nonetheless, the toxicity concerns presented by bioactive peptides' unknown systemic effects can be addressed by *in vitro* and toxicological investigations in animal models. Bitterness is one of the impediments to customer acceptability. Therefore reducing peptide bitterness is critical. Peptides may also react with other food ingredients, notably carbohydrates and lipids.

When considering halal, the resources of bioactive peptides must also be taken into account. Many studies focus on producing biopeptides from conventional sources such as fish, dairy proteins, and vegetables. However, valorizing waste resources is beneficial in terms of economic benefits, waste reduction, and sustainability. Meat coproducts are ideal for use as new sources of bioactive peptides due to their high protein content and minimal cost (Lafarga et al., 2017). Interestingly, recent research has opened up new avenues for acquiring peptides from plant sources and alternative and low-cost sources such as agriculture wastes, by-products, and nontraditional crops. Plant protein sources are more diverse than animal protein sources. Nonetheless, vegetable-derived peptides have several limitations, mostly due to the more complex hydrolysis and the need for heat treatment (Rizzello et al., 2016).

Advancements in *in-silico* analysis or bioinformatics allow the prediction and identification of specific peptides that are likely to display the desired bioactivity by elucidating the structure-function interaction and examining the mechanism of action. Peptide structures and bioactivity elucidation remained a significant challenge. Therefore, the bioinformatic tool is effective. In the progression of *in silico* research, molecular docking simulations are advancing to predict potential interactions of peptides with active enzyme sites. These effectively integrated methodologies will accelerate the identification and production of bioactive peptides. Many challenges remained, such as predicting cleavage sites, due to the protein's complexity of its secondary, tertiary, and quaternary structures (Hajfathalian et al., 2018). Furthermore, because of complicated protease–protein interactions, the assumption of enzymes with perfect substrate specificity may be inaccurate in some reactions (Li-Chan, 2015; Udenigwe, 2014).

Moreover, the development of a productive and cost-effective process must be emphasized. The discovery of alternative processes, such as the integrative process, enables the separation of specific protein precursors for high-potency bioactive peptides. Most crucially, the whole process, which includes adsorption, *in situ* hydrolysis, and peptide purification, occurs in a single reaction vessel. Following this breakthrough, the concept opens the door to further research, particularly into using new types of protein feedstock, such as processing by-products from the food processing industry. On the other hand, bioactive peptides extracted are often a combination of peptides. Purifying these peptides to 99% purity would not only raise the cost and decrease yields, but it would also lose any beneficial additive or synergistic effects with other peptides present in the whole hydrolysate (Ali et al., 2021). As a result, feasibility studies, including its bioactivities and peptide sequence, as well as the setup's scalability for large-scale manufacture, are critical.

Next, the production of functional foods incorporating bioactive peptides necessitates synergistic technology considerations, notably in ways that maintain the peptides' bioactivities throughout the good shelf life. Furthermore, it has been found that processing conditions might modify proteins and peptides, causing them to lose their bioactivities. Heat treatment, for example, may cause heat-sensitive peptides to be damaged, while high pHs can destroy amino acids, and storage can hasten amino acid oxidation (García & Manrique, 2018). As a result, it is critical to optimize the processing conditions to sustain their bioactivities.

Encapsulation of bioactive compounds is necessary economically and industrially because it prevents peptide interactions with the environment and/or other compounds in the food matrix; increases peptide stability, masks, or reduces the bitter taste of peptides; protects peptides against digestive enzymes; improves solubility and bioavailability; and allows the design of triggered releasing systems (Augustin & Sanguansri, 2015). Encapsulating the peptide in mono- and nano-sized coordination polymers or particles, such as polyelectrolyte microspheres and dendrimers, might reduce the peptide's poor solubility, which can solve the transport challenges to the target cell or tissues (Agyei & Danquah, 2011).

More study is required before bioactive peptides may be utilized to prevent and treat chronic diseases. There is still a need to develop a more scalable, cost-effective, and consistent manufacturing process with excellent pharmacokinetics, targeted delivery, and lower cost. These are the primary deciding and driving factors in the worldwide market for the current development and manufacturing of halal bioactive peptides as functional foods.

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Honey diastase: a natural halal enzyme and its potential application in food

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11.1 Introduction

And your Lord inspired the bee, saying: “Take you habitations in the mountains and the trees and in what they erect. Then, eat of all fruits, and follow the ways of your Lord made easy (for you). There comes forth from their bellies, a drink of varying colour wherein is healing for men.” as exemplified in *Al-Quran*, *Surah An-Nahl*, verse 68 to 69, demonstrates the encouragement by the Prophet to the consumption of honey as a halal (lawful) and *tayyiban* (good) foods. The Muslim community highly regards honey as a *sunnah* food that meets the requirement of the *halalan-tayyiban* concept. Bees extract honey from the flower nectar and saccharine exudation of plants (Singh & Bath, 1997). It is a by-product of nectar and the upper aero-digestive tract as a salivary secretion. It is gathered, concentrated, and modified through a dehydration process and stored inside a bee comb to ripen and mature into honey (Biluca et al., 2017; Singh & Bath, 1997).

Honey has been widely consumed in its unprocessed state as a natural tonic food and one of the oldest traditional medicines that has played a vital role in treating human ailments since ancient times (Chew, Chua, Soontorngun, & Lee, 2018; Hunter et al., 2021). Recently, many research studies reported the bioactivities of honey, such as antioxidant, antimicrobial, antimutagenic, antiinflammatory, anticancer, and wound healing properties (Chew et al., 2018; da Cunha et al., 2018; Hunter et al., 2021; Peláez-Acero et al., 2021). However, honey is more commonly used as a sweetening additive in food applications (Chick, Shin, & Ustunol, 2001; Kadam, Kadam, Choudhari, & Pawar, 2010). Honey offers beneficial effects to human health due to various natural chemical compounds. Furthermore, honey serves as an excellent energy source for athletes during pre- and posttraining, for body recovery after physical activity (Ilia, Simulescu, Merghes, & Varan, 2021).

Two types of honey commonly consumed in Malaysia are honeybee honey (HBH) and stingless bee honey, locally known as *kelulut* bee honey (KBH). The most common bee species of *Apis* genera that produce bee honey is *Apis mellifera*, and it is mainly found in Europe and Asia (El Sohaimy, Masry, & Shehata, 2015). Other subspecies such as *Apis dorsata*, *Apis florea*, and *Apis cerana* are distributed in Southern and Southeast Asia (Kek, Chin, Yusof, Tan, & Chua, 2014). On the other hand, of over 500 species of *kelulut* bee found throughout the world, *Heterotrigona itama* and *Geniotrigona thoracica* are among the two *Trigona* species, which are commonly reared found in Malaysian forests (Shamsudin et al., 2019). There is more than one type of genera for the tribe of *Meliponini*, that is, *Trigona*, *Melipona*, and *Scaptotrigona*. These *kelulut* bees are native to tropical and subtropical parts like Asia, Central and South America, Africa, and Northern Australia (Boorn et al., 2010).

HBH from *Apis* species are stored in hexagonal-shaped honeycombs, while KBH from *Trigona* species are deposited in small resin pots clusters close to the extremities of their nests (Kek et al., 2014). It is comparatively easier to harvest KBH than HBH because *kelulut* bee does not sting (Nordin, Sainik, Chowdhury, Saim, & Idrus, 2018). On the contrary to honeybees that are typically lost and more vulnerable to diseases, an artificial hive is always constructed to increase the honey production by manipulating the colony as *kelulut* bees are not obnoxious in selecting a place for hive building (Abd Jalil, Kasmuri, & Hadi, 2017; Kek et al., 2014).

11.2 Chemical composition

Honey is a nutritious food that contains about 200 substances (Eteraf-Oskouei & Najafi, 2013). There are many differences between HBH and KBH, where composition and variation are mainly due to the different floral sources, geographical origins, producing species, climate, and harvesting time (Biluca, Braghini, Gonzaga, Costa, & Fett, 2016; de Rodríguez, de Ferrer, Ferrer, & Rodríguez, 2004; Singh & Bath, 1997). Despite the various constituents present in honey, six main components contribute to the honey's characteristics and nutritional composition: sugars, protein, organic acids, vitamins, minerals, and heavy metals (Table 11.1).

TABLE 11.1 Chemical composition of honey.

Main component	Examples	References
Sugars	Major sugars: fructose, glucose, and sucrose	Anjos, Campos, Ruiz, and Antunes (2015); de la Fuente, Ruiz-Matute, Valencia-Barrera, Sanz, and Castro (2011); Doner (1977); Ruoff et al. (2006)
	Other sugars: turanose, nigerose, rhamnose, maltose, kojibiose, trehalose, nigerobiose, maltotetraose, maltotriose, maltulose, melibiose, palatinose, raffinose, isomaltose, erlose, and melezitose	
Proteins	Amino acids: proline, glutamic acid, aspartic acid, glutamine, histidine, glycine, threonine, alanine, α -alanine, β -alanine, phenylalanine, leucine, isoleucine, arginine, aminobutyric acid, tyrosine, valine, ornithine, lysine, serine, asparagine, methionine, cysteine, and tryptophan	Di Girolamo, D'Amato, and Righetti (2012); Hermosín, Chicon, and Cabezudo (2003); Iglesias et al. (2006); Kečkeš et al. (2013); Sak-Bosnar and Sakač (2012)
	Enzymes: diastase, glucose oxidase, catalase, invertase, both α - and β -glucosidase, and acid phosphatase	
Organic acids	Main acids: gluconic acid and citric acid	Cavia, Fernández-Muino, Alonso-Torre, Huidobro, and Sancho (2007); da Silva, Gauche, Gonzaga, Costa, and Fett (2016); Mato, Huidobro, Simal-Lozano, and Sancho (2006)
	Other acids: levulinic acid, formic acid, aspartic acid, pyruvic acid, quinic acid, butyric acid, 2-hydroxybutyric acid, malic acid, malonic acid, methylmalonic acid, glutaric acid, α -hydroxyglutaric acid, α -ketoglutaric acid, succinic acid, tartaric acid, acetic acid, fumaric acid, galacturonic acid, shikimic acid, oxalic acid, glutamic acid, glyoxylic acid, isocitric acid, lactic acid, 2-oxopentanoic acid, and propionic acid	
Vitamins	Vitamin B complex: thiamine (B1), riboflavin (B2), nicotinic acid (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B8 or H), and folic acid (B9).	Ciulu et al. (2011)
	Vitamin C: ascorbic acid	
Minerals	Macroelements: potassium (K), calcium (Ca), sodium (Na), magnesium (Mg), phosphorus (P)	Alqarni, Owayss, Mahmoud, and Hannan (2014)
	Microelements: iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), iodine (I), lithium (Li), barium (Ba), chromium (Cr), cobalt (Co), nickel (Ni), selenium (Se), and silver (Ag)	
Heavy metals	Heavy metals: cadmium (Cd), mercury (Hg), lead (Pb), antimony (Sb), and arsenic (As)	Bilandžić et al. (2011)

11.2.1 Sugars

About 75% of sugars present in honey are monosaccharides, together with approximately 10%–15% of disaccharides and trace amounts of other sugars (da Silva et al., 2016). Three patterns of sugar composition in nectar were obtained by analyzing 889 floral species, that is, high sucrose nectar, the nectar with an equal amount of major sugars (i.e., fructose, glucose, and sucrose), and increased glucose and fructose nectar. The relationship between sugars ratio and floral species causes sugar content variation in honey due to different botanical sources (Doner, 1977).

Other than major sugars, determination of sugar profiles of honey found that honey contains other sugars, such as turanose, nigerose, rhamnose, maltose, kojibiose, trehalose, nigerobiose, maltotetraose, maltotriose, maltulose, melibiose, palatinose, raffinose, isomaltose, erlose, and melezitose, in varying amounts (Anjos et al., 2015; de la Fuente et al., 2011; Doner, 1977; Ruoff et al., 2006). Sugars in honey contribute to the honey properties such as viscosity, hygroscopicity, energy value, and granulation (Kamal & Klein, 2011). The addition of honey into duck jerky samples was found to retain more moisture than other humectants such as rice sirup and sorbitol, and incorporation of increased concentrations reduced the water activity (a_w) of products with better stability achieved (Triyannanto & Lee, 2016).

11.2.2 Protein

Amino acids make up to around 1% (w/w) of the honey's constituents, and their relative proportions are based on the honey's origin (Hermosín et al., 2003). Honey contains numerous amino acids such as proline, glutamic acid, aspartic acid, glutamine, histidine, glycine, threonine, alanine, α -alanine, β -alanine, phenylalanine, leucine, isoleucine, arginine, aminobutyric acid, tyrosine, valine, ornithine, lysine, serine, asparagine, methionine, cysteine, and tryptophan (Di Girolamo et al., 2012; Hermosín et al., 2003; Iglesias et al., 2006; Kečkeš et al., 2013). The common amino acids are proline, glutamic acid, tyrosine, alanine, phenylalanine, leucine, and isoleucine, with proline being the predominant one that represents around 50%–85% of amino acids (Di Girolamo et al., 2012; Iglesias et al., 2006; Truzzi, Annibaldi, Illuminati, Finale, & Scarponi, 2014). Proline can be used as an indicator for the maturation or adulteration of honey, whereas pure honey should have a minimum proline value of 180 mg/kg (Hermosín et al., 2003). Free amino groups of amino acids may participate in the Maillard reaction with reducing sugars during heat processing, or unfavorable storage conditions may produce undesirable products that affect the quality of honey (da Silva et al., 2016).

Honey also contains diastase, glucose oxidase, catalase, invertase, both α - and β -glucosidase, and acid phosphatase (Sak-Bosnar & Sakač, 2012). Diastase is one of the most vital enzymes that determines honey quality. It consists of both α - and β -amylase, which cleaves the branched chains into shorter chains through α -1,4 linkages, at an internal site to produce dextrin, and in a stepwise trend initiating from the nonreducing end to produce maltose, respectively (da Silva et al., 2016; Sak-Bosnar & Sakač, 2012). β -Amylase (EC 3.2.1.2) is an exoenzyme that hydrolyzes the penultimate α -(1,4) linkage from the nonreducing end of the polymeric chains and releases the disaccharide maltose. β -Amylase, acting alone, can degrade amylose completely to maltose. This enzyme will not, however, attack the α -(1,6) linkages or α -(1,4) linkages close to the α -(1,6) links; hence, it degrades only the outer chains of amylopectin and leaves a large portion (called β -limit dextrins), in which the outer chains have been degraded to stubs of two or three glucose residues adjacent to the α -(1,6) linkages (Meshram, Singhal, Bhagyawant, & Srivastava, 2019).

Amylase enzyme has been present in honey for over 100 years, and it is finally acknowledged to originate from bees as salivary secretions (Babacan, Pivarnik, & Rand, 2002; Vit & Pulcini, 1996). Characterization of honey amylase found its optimum temperature and pH to be 55°C and within the range of 4.6–5.3, respectively (Babacan & Rand, 2007). The addition of honey to the starch matrixes resulted in starch thinning and a significant viscosity loss, and there was no effect on viscosity observed in cases where honey had been preheated prior to addition. This clearly explained that starch degradation had taken place due to amylase activity. Amylase hydrolyzes starch and reduces the viscosity of starch solutions; starch fragments produced in the finished gel also reduce the rate of starch retrogradation (Torley, Rutgers, D'Arcy, & Bhandari, 2004). Heat treatment at 85°C successfully reduced amylase activity, albeit it also proved the heat resistance of honey amylase, with the evidence of measurable activity at the end of the heat treatment (Babacan et al., 2002).

On the other hand, conversion of glucose into δ -gluconolactone that is subsequently hydrolyzed to gluconic acid is done by glucose oxidase originates from honeybees during ripening (da Silva et al., 2016; Karabagias, Badeka, Kontakos, Karabournioti, & Kontominas, 2014). In addition, hydrogen peroxide that has bactericidal action is also produced. The presence of gluconic acid in equilibrium with esters or lactones, and phosphate and chloride will affect the acidity of honey (Singh & Bath, 1997; Singh & Bath, 1998).

11.2.3 Organic acids

The most abundant organic acid in honey is gluconic acid. It is produced in a way as previously mentioned in Section 11.2.2. Both gluconic and citric acids are typically used as reliable parameters in differentiating floral honey and honeydew (Mato et al., 2006). Production of levulinic and formic acids from the reactions between 5-hydroxymethylfurfural (HMF) and water molecules increases free acidity concentration in honey (Cavia et al., 2007). Other organic acids present in honey are aspartic acid, pyruvic acid, quinic acid, butyric acid, 2-hydroxybutyric acid, malic acid, malonic acid, methylmalonic acid, glutaric acid, α -hydroxyglutaric acid, α -ketoglutaric acid, succinic acid, tartaric acid, acetic acid, fumaric acid, galacturonic acid, shikimic acid, oxalic acid, glutamic acid, glyoxylic acid, isocitric acid, lactic acid, 2-oxopentanoic acid, and propionic acid (da Silva et al., 2016; Mato et al., 2006).

The acidity of honey increases over time during storage or fermentation due to the transformation of sugars and alcohols into acids by the action of yeasts (Cavia et al., 2007). The presence of organic acids also contributes to the development of color and flavor in foods (Jurado-Sánchez, Ballesteros, & Gallego, 2011). Moreover, organic acids help to preserve honey against spoiling microorganisms (Suarez-Luque, Mato, Huidobro, Simal-Lozano, & Sancho, 2002).

11.2.4 Vitamins

Honey contains only trace amounts of vitamins, such as thiamine (vitamin B1), riboflavin (vitamin B2), nicotinic acid (vitamin B3), pantothenic acid (vitamin B5), pyridoxine (vitamin B6), biotin (vitamin B8 or H), and folic acid (vitamin B9). Besides vitamin B complex, ascorbic acid (vitamin C) with antioxidant effect can also be found in honey. The vitamin content is well preserved due to honey's low pH contributed by the presence of organic acids. The filtration step during honey processing may cause a loss of vitamin content as almost all pollens are removed. Besides, oxidation of vitamin C by hydrogen peroxide produced by glucose oxidase may also reduce the vitamin content (Ciulu et al., 2011).

11.2.5 Minerals and heavy metals

Macro- and microelements present in honey are potassium (K), calcium (Ca), sodium (Na), magnesium (Mg), phosphorus (P), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), iodine (I), lithium (Li), barium (Ba), chromium (Cr), cobalt (Co), nickel (Ni), selenium (Se), and silver (Ag) (Alqarni et al., 2014). Honey is known to contain these minerals in varying amounts, making them important indicators for the quality determination of honey (Bogdanov, Haldimann, Luginbühl, & Gallmann, 2007; Czipa, András, & Kovács, 2015; Solayman et al., 2016). K is the predominant element that makes up to one-third of the total minerals content in honey (Alqarni et al., 2014). Some of these macro- (K, Ca, and Na) and microelements (Fe, Zn, Cu, and Mn) play essential roles in biological systems, for example, catalysts in biochemical reactions.

Heavy metals such as cadmium (Cd), mercury (Hg), lead (Pb), antimony (Sb), and arsenic (As) may also be present in honey. These are hazardous to health if the amounts exceeded the maximum permitted limits. According to the Fourteenth Schedule Table 1 of the Malaysian Food Regulations 1985, the maximum permitted proportion of metal contaminants in honey are 1, 2, 0.05, 1, and 1 mg/kg for As, Pb, Hg, Cd, and Sb, respectively. Nevertheless, the maximum residue level of Hg is more stringent in the European Union (EU) countries, with only 0.01 mg/kg, as stated in Commission Regulation (EU) 2018/73. Evidence of increased trace element content in honey samples near industrial areas suggests that the need to quantify toxic elements in honey is necessary for human health, safety, and environmental biomonitoring (Bilandžić et al., 2011).

Variation of metals content in honey was due to different geographical origins and botanical sources, and climatic and anthropogenic factors (Bogdanov et al., 2007; Pohl, 2009). Major minerals originated from nectar-producing plants and soil, while heavy metals derived from inorganic or metallic sources. On the contrary to amino acids and vitamins, mineral elements are indestructible even though they are subjected to light, heat, extreme pH, oxidizing agents, or other factors that degrade organic nutrients (da Silva et al., 2016).

11.3 Quality parameters and wholesomeness issues

The halal issue surfaces when the wholesomeness of honey changes due to adulteration or mistreatment of processing that causes the chemical composition change. Hence, honey should be processed halal from the source to the product application throughout the supply chain. Authenticity and control of the quality attributes need to be monitored to ensure the genuine characteristics of honey. Codex Standard for honey (CXS 12–1981) has been established and

applied to honey produced by honeybees, including all styles of honey presentations that are processed and intended for human consumption (Codex Alimentarius Commission, 2001). Recently, Malaysian standard MS 2683:2017 *Kelulut* (Stingless bee) honey – Specification was also developed for KBH (Malaysian Standard, 2017). These standards aim to establish the identity and minimum essential quality requirements for HBH and KBH. The basic essential quality parameters of honey are sugars (including HMF), moisture, free acidity and pH, and diastase content.

11.3.1 Sugars

Individual concentration and ratio of fructose to glucose can be used as important indicators and quality parameters for honey. These indicators are useful for the classification of monofloral honey, and the ratio is useful in evaluating the crystallization of honey (Escuredo, Dobre, Fernández-González, & Seijo, 2014; Tornuk et al., 2013). Generally, fructose presents the highest amount in almost all types of honey. The ratio of glucose is higher than fructose in rape (*Brassica napus*) and dandelion (*Taraxacum officinale*) honey, which indicates they have a rapid crystallization due to the lower solubility of glucose in water (Escuredo et al., 2014). The ratio of fructose to glucose is generally in an average ratio of 1.2:1, but it may differ according to the source of the honey extracted. The glucose-to-water content ratio is also a good indicator of honey quality other than the sum and ratio of fructose and glucose (Kukurova, Karovièová, Kohajdova, & Bilikova, 2008). The minimum amount of reducing sugars (sum of fructose and glucose) in HBH should be 60 g/100 g (Codex Alimentarius Commission, 2001), while the maximum amount in KBH is 85 and 90 g/100 g in raw and processed honey, respectively (Malaysian Standard, 2017). Monosaccharides are present as the most common carbohydrates that make up around 65%–80% of the total soluble solids, whereas fructose and glucose are present in 38.5% and 31%, respectively.

Halal means authentic, not adulterated with other cheaper honey or worse, with sugars or water. Sucrose is another crucial quality indicator for honey for evaluation of the maturation and authenticity of honey. Sucrose may not have been completely transformed into glucose and fructose during the early stage of honey harvesting. High sucrose levels indicate improper manipulation of honey that adulterations with other materials could have been done. This could be due to the addition of cheaper sweeteners such as cane sugar or refined beet sugar or a prolonged artificial feeding of sucrose sirups to the honeybees for high commercial profits purposes (Escuredo, Míguez, Fernández-González, & Seijo, 2013; Tornuk et al., 2013). The maximum amount of sucrose in HBH should be 5 g/100 g (Codex Alimentarius Commission, 2001), while the maximum amount in KBH is 7.5 and 8 g/100 g in raw and processed honey, respectively (Malaysian Standard, 2017). According to Regulation 130 of the Malaysian Food Regulations 1985, the maximum apparent sucrose content of honey is 10%. A recent study shows that the application of ¹H NMR metabolomics combined with chemometrics has demonstrated its potential as a tool in discriminating adulterants (sugar sirup derived from C₃ and C₄ plants) in KBH (Yong et al., 2022). This accurate, rapid, and nonlaborious analysis is useful for avoiding the unscrupulous adulteration of preparing “diabetic honey” using sugar sirups that are cheaper and less nutritious.

Apart from the adulteration issue, the accumulation of HMF produced during the thermal processing of honey also contributes to the questionable *toyyiban* status. Heat treatment or thermal processing has been widely used to maintain liquid consistency for a more extended period and extend the honey’s shelf life (Scepankova, Pinto, Paula, Estevinho, & Saraiva, 2021). Nevertheless, prolonged heating or extended storage period of honey accentuate the formation of HMF, owing to the decomposition of glucose or fructose, with or without amino acids in trace amounts (da Silva et al., 2016; Szczesna et al., 2021). Therefore, the presence of HMF is an indicator of the loss of freshness in honey and is associated with the deterioration of quality. The maximum amount of HMF in HBH should be 40 mg/kg (or 80 mg/kg for honey in tropical regions) (Codex Alimentarius Commission, 2001), while the maximum amount in KBH is 30 mg/kg in both raw and processed honey (Malaysian Standard, 2017).

11.3.2 Moisture

The second-largest constituent in honey is water that ranges from 15 to 21 g/100 g, which varies according to floral source, maturation stage in the hive, processing techniques, and storage conditions (Yücel & Sultanog, 2013). The maximum moisture content in HBH should be 20 g/100 g (Codex Alimentarius Commission, 2001), while the maximum amount in KBH is 35 and 22 g/100 g in raw and processed honey, respectively (Malaysian Standard, 2017).

Moisture content in honey is considered an important parameter as it affects its physical properties such as crystallization, viscosity, flavor, taste, color, solubility, and specific gravity (Escuredo et al., 2013). The increase in moisture content of honey could happen along with the processing operations of honey and improper storage environment that absorbs moisture from the surroundings due to its hygroscopicity (Karabagias et al., 2014). Honey has water activity

(a_w) ranges from 0.50 to 0.65, whereas a_w above 0.60 is a critical threshold value that honey is susceptible to microbial attacks. The a_w values of honey increase as glucose crystallization happens, reducing the soluble solids and causing dilution of amorphous solution.

There are no limits imposed by both HBH and KBH standards currently, but the presence of high water content and osmophilic yeasts may cause fermentation which forms ethanol and carbon dioxide that affect the honey quality (Escuredo et al., 2013; Ramli et al., 2019; Tornuk et al., 2013; Yücel & Sultanog, 2013). To overcome this spoilage issue and retain the functional properties of honey, different drying methods, such as vacuum drying, superheated steam drying, and freeze-drying, have been evaluated and compared. Interestingly, the dewatering approach using either low-temperature or low-pressure vacuum drying has been found to enhance honey physicochemical and functional stability (Chen, Chuah, & Chye, 2021; Ramli et al., 2019). Besides, Honey Interlinked Dehydration and Dispenser Apparatus (HILDA) has been successfully invented to control honey's moisture at a level that meets the limit set by the Codex Alimentarius Commission (2001). HILDA could produce wholesome honey due to its role as an integrated platform for hygienic harvesting and bottling, whereby the dewatering process involves no heating (Mustafa, Yaacob, & Sulaiman, 2018).

11.3.3 Free acidity and pH

The maximum free acidity in HBH should be 50 milliequivalents acid per 100 g (Codex Alimentarius Commission, 2001). The increase in free acidity is due to the fermentation of sugars into organic acids. Free acidity could be a good indicator of the deterioration of honey. The presence of gluconic acid in equilibrium with esters or lactones, and phosphate and chloride will affect the acidity of honey (Singh & Bath, 1997; Singh & Bath, 1998). The presence of different organic acids in different proportions, geographical origins, and harvest time may affect the free acidity of honey (Tornuk et al., 2013).

The Codex Standard document for honey (CXS 12–1981) did not specify the pH range for HBH, while MS 2683:2017 set the acceptable range as pH 2.5–3.8 for both raw and processed KBH (Malaysian Standard, 2017). Microbial growth is inhibited due to the natural acidity of honey (generally between pH 3.2 and 4.5), which is lower than optimum pH for most organisms (pH 7.2–7.4) (Karabagias et al., 2014). Hence, pH could be an indicator of honey adulteration. This is evident by the significant increase of pH values in Brazilian honey than that of pure honey after the addition of high fructose sirup (Ribeiro et al., 2014). The pH values of KBH and HBH as reported by previous studies were in the range of pH 3.17–6.56 (Biluca et al., 2016; Shamsudin et al., 2019; Souza et al., 2006) and pH 3.18–5.40 (de Rodríguez et al., 2004; El Sohaimy et al., 2015; Isla et al., 2011), respectively.

11.3.4 Diastase

According to the CXS 12–1981 document, there are two types of HBH categorized based on diastase number, that is, low natural enzyme honey and general honey. Low natural enzyme honey contains diastase activity of not be less than 3 Schade, and general honey contains diastase activity of not less than 8 Schade (Codex Alimentarius Commission, 2001). However, MS 2683:2017 did not specify the range of diastase numbers for KBH.

Diastase activity is measurable and is expressed in diastase number, as indicated by the enzyme activity in 1 g of honey that hydrolyzes 0.01 g of starch at 40°C in 1 hour. Like sugars and moisture, diastase is another quality parameter that will be affected by the heat treatment. Diastase is heat sensitive (thermolabile) and is often used as an indicator for the thermal processing of honey (Subramanian, Umesh Hebbar, & Rastogi, 2007). It can also serve as an indicator of ageing (i.e., freshness indicator) that increase of temperature under inadequate storage conditions reduces the diastase activity or when honey is subjected to heat treatment above 60°C or other long and intensive heating (Kowalski, Lukasiewicz, Bednarz, & Panuś, 2012; Yücel & Sultanog, 2013).

The vast variation in diastase activity and pH is mainly attributed to the different botanical sources, producing species, geographical origins, and harvesting time (Biluca et al., 2016; de Rodríguez et al., 2004; Singh & Bath, 1997). Young nectars harvested in early spring have lower diastase content due to the low nectar concentration and high sugar content (Vorlova & Pridal, 2002). In addition, the differences in diastase activity are not uncommon, as enzymatic and metabolic pathways in phylogenetic genera could vary (Antoñ & Denisow, 2014).

Hence, how honey is processed in a halal manner (especially in logistics and warehousing) plays a crucial role in ensuring the production of wholesome honey. Methods of transport need proper temperature controls, and a blockchain food traceability system needs to be in place.

11.4 Use of honey in foods

Honey has long been considered a high-value functional food with health-promoting properties (Bugarova, Godocikova, Bucekova, Brodschneider, & Majtan, 2021; Fletcher et al., 2020). However, honey is more commonly used as a sweetening additive in food applications (Chick et al., 2001; Kadam et al., 2010). The presence of sugars at approximately 95%–99% of honey dry matter makes it possible to substitute all or part of the normal sugars, especially refined sugars, in most products (Krell, 1996). As a traditional or natural sweetener with minimal preprocessing that has been safely consumed for years, honey is a product that tends to have lower glycaemic potency than refined sugars (Edwards, Rossi, Corpe, Butterworth, & Ellis, 2016). It is a natural sweetener with medicinal qualities that makes the use of honey in foods less harmful than sugar. A sweetmeat with therapeutic values may reduce various health issues by replacing sugar with honey in desserts or sweets (Kadam et al., 2010). Interestingly, honey-sweetened products are often regarded as value-added, and consumers are willing to pay up to an additional 13% for them compared to products containing other sweeteners (Chick et al., 2001).

Examples of starch-based foods with substitution or partial replacement of sugars with honey are *khoa burfi* (an Indian food), Aonla (*Emblica Officinalis*) (an Indian fruit) preserve (Ahmad & Kumaran, 2015), cassava-wheat bread, cookies, and biscuit (Adeboye & Bamgbose, 2015; Babajide, Adeboye, & Shittu, 2014; Filipčev, Šimurina, & Bodroža-Solarov, 2014; Kadam et al., 2010). Generally, food products with honey substitution at a particular concentration are as acceptable as those prepared with sugars. Nevertheless, incorporating honey at higher concentrations causes typically undesirable changes in a food product's physicochemical properties and textural qualities. The inclusion of honey at higher concentrations increases the moisture content, affecting final products' texture. Besides, the textural changes are also attributed to the interaction effects from other minor components of honey, diastase, and water content (Babajide et al., 2014).

However, there are limitations on the degree of substitution when factors, such as cost, handling characteristics, and the natural variations in honey characteristics that change the end product, are taken into consideration (Krell, 1996). Understandably, the replacement of sugar with honey in foods causes an increase in the cost of preparation. Honey is mainly suggested as an alternative to sugar-based on its medicinal values (Kadam et al., 2010). Other than the use of honey for its sweetening effect, honey is also used in foods for other purposes. Honey addition enhances the quality of several food products that have a more pleasant flavor, taste, and color and are much more nutritious than many other sugar-based products (Durrani, Srivastava, & Verma, 2011). It was found that the addition of honey into duck jerky samples has helped to retain more moisture than other humectants, such as rice sirup and sorbitol, and incorporation of increased concentrations reduced the a_w of products with better stability achieved (Triyannanto & Lee, 2016). Furthermore, honey is also used in packaged turkey slides for shelf life extension purposes by enhancing the oxidative stability of meat. Production of oxidation products associated with off-flavor has successfully been retarded (Antony et al., 2006).

11.5 Food innovation using honey diastase

Dodol is a well-known local traditional food in Malaysia, Indonesia, Thailand, and the Philippines, and it is also a good choice of door gift during festive seasons (Karim & Bhat, 2013; Rosniyana, Hashifah, & Norin, 2010). Due to the potential commercial value of *dodol*, different ingredients have been substituted partially to improve its nutritional values (Chuah, Hairul Nisah, Thomas Choong, Chin, & Nazimah Sheikh, 2007). Nevertheless, the textural quality (i.e., hardening issue) of *dodol* associated with potential choking hazards and food waste (i.e., disposal due to short shelf life) has always been overlooked, and this real industrial issue has remained unsolved for many years.

The Malaysia Agricultural Research and Development Institute has developed an enzymatic *dodol* processing technology using enzyme, heat treatment, and effective packaging material to improve the quality and extend the shelf life of commercial *dodol* (Zahid et al., 2012). However, the increased processing cost and the questionable halal status of the amylase enzyme made the *dodol* industry hold back on this exciting idea.

To increase the nutritional values and to solve the textural deterioration issue, there was an attempt to incorporate raw honey into the *dodol* processing. HBH with a higher diastase activity has shown a more noticeable decrease in the viscosity and texture profile of glutinous rice flour gel compared to KBH in our preliminary gel study. Although the acidic KBH also reduced gels' viscosity and texture profile, the effect is less pronounced than that of HBH. To prove that the presence of diastase causes reduced viscosity and hardness, a comparison is also carried out between the unprocessed raw honey and the heated honey with 0 Schade diastase activity (Seow, Gan, Tan, Lee, & Easa, 2019; Seow, Tan, Lee, & Easa, 2020). The results have portrayed the thermolabile characteristics of diastase as evidenced by other

published studies (Babacan et al., 2002; Czipa, Phillips, & Kovács, 2019; Elamine et al., 2020; Kowalski et al., 2012) and confirmed the contributing effect by diastase.

Driven by the promising effect of diastase in the gel model, its effect on a real food system, that is, *dodol* with glutinous rice flour (i.e., starch) as the main ingredient, has been assessed. Hence, two different *dodols*, *honeydol* (incorporated with HBH) and *keludol* (incorporated with KBH), were developed using raw unprocessed honey. These food innovations were found to have a more shelf-stable textural quality (i.e., slower hardening due to the delayed starch retrogradation) apart from the additional nutritional values. The inhibited starch retrogradation is due to the hydrolyzed starch chains by the diastase that eventually slows down the rearrangement of starch molecules during storage (Torley et al., 2004). The consumers can accept both *honeydol* and *keludol* as assessed through a sensory evaluation. The addition of raw honey as one of the ingredients also gives a positive image, owing to the impression as a “Super Food” of this naturally occurring unprocessed raw honey.

It was also found that the structural breakdowns of *honeydol* and *keludol* are more rapid than conventional *dodol*, suggesting that enzymes in honey have facilitated the mastication process (Seow, Tan, & Easa, 2021). This finding is good news to those with chewing difficulty (e.g., elderly). *Honeydol* serves as a model for further development of a texture-modified elderly food. All in all, *honeydol* has successfully demonstrated the potential of honey diastase in starch-based products (i.e., confectionery), and its uses can be explored and extended in other food applications as a source of halal food enzymes.

11.6 Conclusion, challenges, and future opportunities

Honey is one of the *sunnah* foods that has been widely consumed due to its nutritional and therapeutic benefits. However, the *halalan-toyyiban* status of this “Super Food” has constantly been challenged owing to the effect of processing or intentional adulterating activities. To retain the wholesomeness of honey, the quality parameters need to be assessed and monitored. Both Codex Standard for honey and Malaysian Standard for KBH are good and reliable references that we can refer to ensure the honey meets the requirements of authentic honey.

Apart from the quality assurance issues that surface, the use of honey diastase in food innovation has also been explored and discussed. The development of this diastase-innovation (i.e., *honeydol* and *keludol*) has opened up ideas and opportunities for the underexplored role of honey as a functional ingredient in food applications rather than its conventional role as a sweetener. Nevertheless, the role of honey diastase needs to be further explored and investigated compared to commercial diastase.

Commercially available diastase presents typically in a whitish powder form and is sourced from *Aspergillus oryzae*. Unlike honey diastase, which varies according to floral source, geographical origins, and seasons, commercial diastase has a determined diastase activity (~3500 U/g) and could be more easily controlled and applied for food applications, as the enzyme powder can be diluted to desired working concentrations. Currently, commercial diastase is more commonly used in research analysis due to its similar role to amylase as a degrading starch enzyme.

Despite the uncommon practical applications of diastase nowadays, honey diastase has portrayed its potential as a candidate of a halal enzyme naturally occurring in raw unprocessed honey. On top of the functional properties and nutritional benefits that we can gain by consuming this *sunnah* food, honey can be added as a functional food additive that serves beyond its role as a sweetening agent but a texture modifier, especially in starch-based food products.

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Insects and worms as an alternative protein source in the halal food industry

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12.1 Introduction

12.1.1 Overview

The world population is expanding yearly and is predicted to yield 10 billion by 2050 (van Huis et al., 2013). The rapid expansion of the human population will increase the demand for food, including meat, which is estimated to increase by 75% between 2005–07 and 2050 (van Huis, Dicke, & van Loon, 2015). As the human population increase and the capacity of the landmass remains constant, traditional farming may become a challenging method to produce food shortly (Bessa, Pieterse, Sigge, & Hoffman, 2020). Food availability will become progressively more critical due to the limited arable land and water resources to cultivate food (van Huis et al., 2013). Traditional animal farming for meat has promoted unsustainable harvesting practices and led to deforestation, habitat loss, and animal overexploitation (van Huis et al., 2015). The current meat production contributes to many environmental harmful impacts, particularly climate change, and has become a significant source of greenhouse gas emissions (Springmann et al., 2018) (Fig. 12.1).

One of the proposed solutions that gaining attention recently is by reducing meat consumption. This can be done by including underutilized or underexploited alternative sustainable protein sources such as insects and worms (Ordoñez-Araque & Egas-Montenegro, 2021). Insects are characterized by their chitinous exoskeleton, which accounts for approximately 10% of insect weight on a dry weight basis (Belluco et al., 2013). This exoskeleton acts as external support and protection against infectious agents, parasites, predators, and trauma (Guzmán-Mendoza, Salas-Araiza, & Martínez-Yáñez, 2016). There are around 2000 species of edible insects out of over 1 million species of insects described to date. These insects can be consumed by both human and livestock animals such as cattle, fish, and poultry as their food (Imathiu, 2020) (Fig. 12.2).



FIGURE 12.1 Aquatic beetles and bugs. Source: From, Chakravorty, J., Jugli, S., Boria, M., & Meyer-Rochow, V.B. (2016). Arunachal's Adi and Apatani tribes' traditional knowledge of harvesting and using edible insects. *Journal of Insects as Food and Feed*, 1(1), 1-11.

Insect for food or feed depicts a considerable potential as a sustainable choice in future food systems. The specialty of insects lies in their nutritional values comparable to or higher than beef, chicken, or fish (Durst & Shono, 2010; Payne, Dobermann et al., 2016; Payne, Scarborough et al., 2016). Insects are rich in protein (35%–61%), lipids (13%–33%), a significant amount of “animal” fiber from insoluble chitin, mineral, and vitamin (Ojha, Bekhit, Grune, & Schluter, 2021). Moreover, insect proteins have higher digestibility (76%–98%) than plant-based proteins and are slightly less digestible than animal-based proteins (Gravel & Doyen, 2020). Insects require less land area to produce (Durst & Shono, 2010), which can offer greater advantages of being fast, easy, and inexpensive to farm to feed the ever-growing human population (van Huis, 2013). Insect farming may reduce the adverse environmental impacts via fewer greenhouse gas emissions and ammonia than conventional livestock (Oonincx et al., 2010). Insects also have a high feed conversion efficiency, about twice as efficient as chickens and pigs and more than five times as efficient as beef cattle (Durst & Shono, 2010) (Fig. 12.3).



FIGURE 12.2 (A) Bamboo caterpillars and (B) silkworm larvae. Source: From, Chakravorty, J., Jugli, S., Boria, M., & Meyer-Rochow, V.B. (2016). Arunachal's Adi and Apatani tribes' traditional knowledge of harvesting and using edible insects. *Journal of Insects as Food and Feed*, 1(1), 1-11.



FIGURE 12.3 (A) Honeybee brood, (B) wasp brood, and (C) weaver ant nest. Source: From, Chakravorty, J., Jugli, S., Boria, M., & Meyer-Rochow, V.B. (2016). Arunachal's Adi and Apatani tribes' traditional knowledge of harvesting and using edible insects. *Journal of Insects as Food and Feed*, 1(1), 1-11.

Entomophagy means the consumption of insects as food is getting accepted around the world. Preference of people on entomophagy is directed especially due to their cultural history, experience, emotion, and adaptation (van Huis et al., 2013; van Huis et al., 2015). Besides, one of the most important reasons for the acceptance of entomophagy around the globe is the location and environment to produce edible insects. The tropics offer the most insect biodiversity, while as latitude increase away from the tropics, the practice of entomophagy decreases. To date, 2300 species of known edible insects are consumed by about 3000 ethnic groups in over 100 countries mainly located in Asia, Africa and Latin America (Baiano, 2020; van Huis, 2013). Entomophagy is also cited throughout religious Islam, Christians and Jewish literature. Each religion has different stands related to insects issues such as the type of insects prohibited or permissible to be consumed (Baiano, 2020).

12.1.2 Insect consumption from halal perspective

Locust is regarded as the most popular insect consumed as food among the Muslim community, especially in Saudi Arabia, Yemen, Kuwait, Libya, and other Arabian countries. The locust eggs are deemed unique and consumed by Arabs to quench their appetite for meat (Tajudeen, 2020). Locust is the only insect permitted to be consumed among all the Muslim scholars as there are numerous clear-cut hadiths by Prophet Muhammad that supports locust consumption. Among the hadiths is from Ibn' Umar R.'Anhuma, the Prophet PBUH said, "Two kinds of dead meat have been permitted to us: fish and locusts," Sunan Ibn Majah (3218). Another one narrated by Abu Ya'fur RA said, "I asked 'Abdullah bin Abu Awfa about killing locusts and he said: I went on six campaigns with the Messenger of Allah's hand we ate locusts." (In *Sahih Muslim*, it is stated a total of seven campaigns), *Sahih Muslim* (1952), Sunan Abi Dawud (4357), and Sunan al-Nasa'ie (4357) (Tarmizi, 2018). Given that the hadiths only explain the permissible force locusts alone, imperatively, four major madhhabs (schools of thought) in Sunni Muslims have different opinions on insects other than locusts (Rahim, 2018; Tajudeen, 2020) as listed in Table 12.1.

Benefits of insect production and consumption as alternative protein portray a sustainable supply of food for the world's growing population in the future food system. However, the knowledge of the halal perspective of edible insects is still scarce and questionable. The global halal food market, which reached a value of US\$1.4 trillion in 2017 and will reach US\$2.6 trillion by 2023, is huge to be ignored by the edible insect industry (Tajudeen, 2020) to achieve sustainability in the future (Fig. 12.4).

TABLE 12.1 Halal status of insects based on schools of Sunni Muslims.

School	Muslim population (%)	Halal status of insects	Reason
Hanafi	45	All land-form arthropods are nonhalal except for locusts; marine-form arthropods are nonhalal except for shrimps	Verse of the Holy Qur'an (e.g., Surah A'raf vs 157) that prohibits the consumption of Khabeeth (malice, malignant, and filthy thing)
Shafi'i	18	All land-form arthropods are nonhalal except for locusts; all marine-form arthropods are halal	Consideration of human beings especially the customs of Arabs at the time of the Prophet (PBUH)
Maliki	15	All nonpoisonous/hazardous land- and marine-form arthropods are halal	Verse of the Qur'an (e.g., al-Ma'idah vs 93) that permit the consumption of all that is on the earth except the ones that are made prohibited by Shariah or harmful to health
Hanbali	2	All land-form arthropods are nonhalal except for locusts; all marine-form arthropods are halal	—

Source: From, Rahim, S. F. (2018). Islamic jurisprudence and the status of arthropods: As alternative source of protein and with regard to E120. *MOJ Food Processing & Technology*, 6(4), 330–340; Tajudeen, A. L. (2020). Halal certification of insect-based food: A critique. *International Journal of Islamic Business Ethics*, 5(2), 100–112.



FIGURE 12.4 Sand cricket. Source: From, Chakravorty, J., Jugli, S., Boria, M., & Meyer-Rochow, V.B. (2016). Arunachal's Adi and Apatani tribes' traditional knowledge of harvesting and using edible insects. *Journal of Insects as Food and Feed*, 1(1), 1-11.



FIGURE 12.5 Stink bugs. Source: From, Chakravorty, J., Jugli, S., Boria, M., & Meyer-Rochow, V.B. (2016). Arunachal's Adi and Apatani tribes' traditional knowledge of harvesting and using edible insects. *Journal of Insects as Food and Feed*, 1(1), 1-11.

12.1.3 Food regulatory status of edible insects and worms

Insects and worms are considered novel food because they are not regarded as a food source by a significant number of people worldwide. Albeit, in specific regions such as Asia and Africa, insects and worms are being consumed traditionally since many years ago. Currently, there are numerous insect-based food products on markets, namely, insect flour, insect cookies, and edible insect oil. Insects and worms have a high potential to grow into a sustainable food source in the future owing to their potential health benefits. Apart from health benefits, it is crucial to consider food safety issues in consuming insects or insect-based products to avoid any sort of health risks. Since insects and worms are not widely consumed, there are no established regulatory frameworks for utilizing insects and worms as in most of the food source countries. This is because, during the initial stages of developing food and food legislation, insects were not considered food or feed (van Huis & Dunkel, 2017). As the consumption of insects and worms emerges, proper regulations should be developed and implemented soon. Currently, the status of edible insects and worms from the perspective of the international and local food safety standards and regulations is stated in the following (Fig. 12.5).

12.1.3.1 Codex Alimentarius

Under Codex Alimentarius, insects are mentioned as “impurities” when presented in amounts that may cause hazards to human health. For example, under Codex Standard for wheat flour (152–1985), it has been mentioned that the general quality of wheat flour includes no presence of living or dead insects. The same standard has been highlighted in Codex Alimentarius for certain pulses where living or dead insects are mentioned as “impurities” or “filth.” In 2010 the discussion was held to develop standards for edible house crickets and cricket-based products; the work progress was terminated in 2014 due to insufficient food risk assessment data for edible insects (FAO, 2021) (Fig. 12.6).

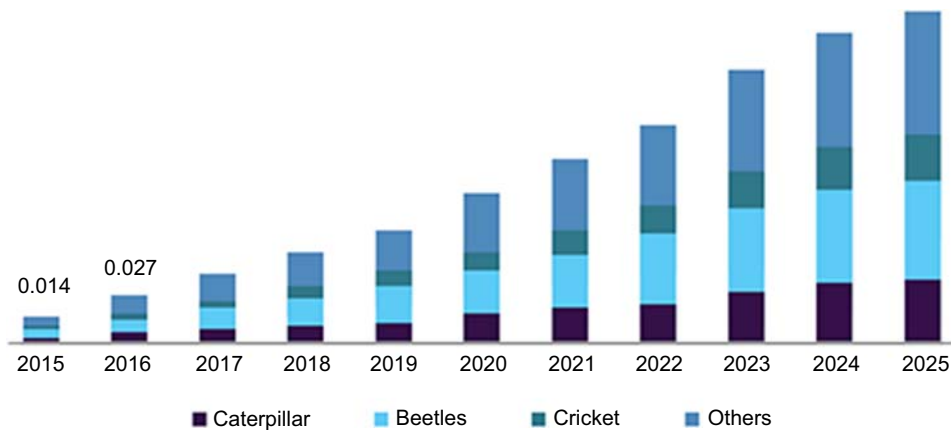


FIGURE 12.6 Market value of edible insects from year 2015 to 2025. Source: From, Chakravorty, J., Jugli, S., Boria, M., & Meyer-Rochow, V.B. (2016). Arunachal's Adi and Apatani tribes' traditional knowledge of harvesting and using edible insects. *Journal of Insects as Food and Feed*, 1(1), 1-11. <https://www.grandviewresearch.com/industry-analysis/edible-insects-market>.

12.1.3.2 The Europe Union

According to EU 2015/2283, all insects (the whole insects and their body parts) and insect-based food products have been included under the novel food section. However, in Article 29 of Regulation (EC) No. 178/20024, Europe Commission mentions that microbiological, chemical, and environmental risks due to production and consumption of insects needed to be assessed by the European Food Safety Authority where the risk assessment is inclusive all main of production till consumption steps (ESFA, 2015).

12.1.3.3 Other countries versus Malaysia food law

Some countries have included insects in their food regulations and policies. For instance, in Africa, three National Standards have been developed by the Kenya Bureau of Standards for the production of insect-based food products. The requirements and guidelines on food safety issues have been highlighted in the standard (FOA, 2021). However, in Malaysia, there are no specified food laws on insect consumption as insect-based food does not get halal status (Table 12.2). The director of Malaysian Islamic Development Department (JAKIM) claims that consumption of insects is not allowed in Islamic Law and this includes pests, flies since the insects are considered repulsive. It was mentioned that animals such as bees, scorpions, wasps and snakes are also prohibited in Islamic Law since they are poisonous (Anonymous, 2013).

12.2 Insects and worms as sources of food

12.2.1 Types of edible insects and worms

Insects and worms are used in different parts of the world as a food source as well as animal feed for centuries. Insects and worms are highly consumed in Asian and African countries compared to European countries. However, in the past few years, there is a significant increase in the consumption of insects and worms in the Western region (van der Fels-Klerx, Camenzuli, Belluco, Meijer, & Ricci, 2018). A recent study shows that about 2111 species of insects are consumed approximately in 140 countries in Asia, Africa, Australia, and American regions. In the ASEAN region, Thailand consumed up to 81 species of edible insects with 150–200 of total species from Indonesia, Lao PDR, Malaysia, Myanmar, Philippines, and Vietnam. It is worth mentioning that 57 species of edible insects are also consumed in India, Nepal, Pakistan, and Sri Lanka (Johnson, 2010). Among these species, *Coleoptera*, *Lepidoptera*, *Hymenoptera*, *Orthoptera*, and *Hemiptera* are the highly consumed edible insects and worms worldwide (Jongema, 2017; Ramos-Elorduy, 2005). Most of these examples are deemed non-halal insects and worms, except for those which are clearly stated as halal (e.g., locusts and grasshopper).

12.2.1.1 Coleoptera (beetles)

The beetles are the most consumed edible insects in the world which is 31% (Jongema, 2017). Beetles are very commonly consumed in the African region, Asia, and South America (Huis et al., 2013). Different types of edible beetles are being consumed worldwide for instance aquatic beetles, wood-boring larvae, and dung beetles. Palm weevil (*Rhynchophorus*) is the highest consumed beetle type in tropical countries (Huis et al., 2013). Beetles are consumed in different stages of their life cycle such as in larvae stage and adult stage, in which the beetle larvae are typically roasted

TABLE 12.2 Number of species of edible insects based on taxonomic group and common English name reported worldwide.

Taxonomic group	English name	Number of species
<i>Coleoptera</i>	Beetles	468
<i>Hymenoptera</i>	Ants, bees, wasps	351
<i>Orthoptera</i>	Grasshoppers, cockroaches, crickets	267
<i>Lepidoptera</i>	Butterflies, moths (silkworms)	253
<i>Hemiptera</i>	True bugs	102
<i>Homoptera</i>	Cicadas, leafhoppers, mealybugs	78
<i>Isoptera</i>	Termites	61
<i>Diptera</i>	Flies, mosquitoes	34
<i>Odonata</i>	Dragonflies	29
<i>Ephemeroptera</i>	Mayflies	19
<i>Trichoptera</i>	Caddis flies	10
<i>Neuroptera</i>	Dobson flies	5
<i>Anoplura</i>	Lice	3
<i>Thysanura</i>	Silverfish	1

Source: From, Ramos-Elorduy, J. (2005). Insects: A hopeful food source. In G. M. Paoletti (Ed.), *Ecological implications of minilivestock: potential of insects, rodents, frogs and snails*. Science Publishers.

whereas the adult beetles are toasted and grilled. The larvae and adults of *Coleoptera* order are available throughout the year; however, the adult *Coleoptera* is harvested highly between June and December which is during the dry season (Casas Reátegui, Pawera, Villegas Panduro, & Polesny, 2018). Beetles are used as an ingredient to prepare some traditional foods in Asia and Africa regions.

12.2.1.2 *Lepidoptera (caterpillars, butterflies, and moths)*

Butterflies and moths are consumed in their adult stages; however, caterpillars (larvae stage) are the most consumed worm under the *Lepidoptera* species. Consumption of caterpillars is around 18% globally (Jongema, 2017). They are widely consumed in Central Africa, South Africa, Zambia, Mozambique, Zimbabwe, and Australia. Different species of caterpillars are consumed widely and the most popular species are mopane caterpillars (*Imbrasia belina*). Bamboo caterpillars (*Omphisa fuscidentalis*) are popular caterpillar species consumed in Thailand. Generally, caterpillars are dried and stored amid food shortage periods, especially during seasonal and climate changes. The caterpillars are hand-picked, cleaned, boiled, dried, and stored to be used during a food shortage. The dried caterpillars are shelf-stable for a few months. In rural areas, caterpillars are usually harvested and marketed by some impoverished communities to generate income (Huis et al., 2013).

12.2.1.3 *Hymenoptera (wasps, bees, and ants)*

Wasps, bees, and ants are the highly consumed edible insect group. According to research conducted by Jongema (2017), the consumption of wasps, bees, and ants is about 14% worldwide. These groups of insects are very popular in Latin America and they are considered important delicacies during the festive season (Huis et al., 2013). *Hymenoptera* is consumed in all stages of their life cycle, including eggs, larvae, pupae, and adult stages. Wasps are consumed in significantly high numbers in Japan (Huis et al., 2013) while in Thailand, weaver ants are mostly consumed (Itterbeeck, van, Sivongxay, Praxaysombath, & van Huis, 2014). In Laos, weaver ants are consumed in larvae and pupae stages as f agents and to improve food texture (Payne & van Itterbeeck, 2017). *Hymenoptera* is commonly fried and sold in local markets. In Thailand, *Hymenoptera* is processed into canned food products.

12.2.1.4 Orthoptera (locusts, grasshoppers, and crickets)

The consumption of *Orthoptera* globally is about 13% (Jongema, 2017) and grasshoppers are the most consumed edible insect compared to the other insects in *Orthoptera* order. Most of the grasshopper species are edible compared to the insect species. The grasshoppers are generally harvested in the early morning when the temperature is relatively low since the grasshopper will be less active in low temperatures (Huis et al., 2013). Crickets are also consumed in many countries, especially in Asian countries. The most common cricket species consumed in Asian countries are *Acheta dome* (house cricket), *Gryllus bimaculatus*, *Teleogryllus occipitalis* and *T. nitrates*. House crickets are highly preferable in Thailand due to their softer body structure (Huis et al., 2013). *Orthoptera* is larger; hence it is easier to harvest them from their habitat (Feng et al., 2018). Generally, the *Orthoptera* are fried and skewered into bamboo sticks to be consumed and it is a very popular insect food among Asian countries especially in Japan, China, and Thailand. Besides, *Orthoptera* is also processed into other food products, for instance, crickets are processed to produce cookie flour.

12.2.1.5 Hemiptera (cicadas, stink bugs, true bugs)

Cicadas are widely found in tropical forests primarily in tree trunks. The cicadas are commonly harvested using long grasses covered with the latex of *Ficus natalensis* trees. The latex is used to stick to the wings of cicadas and they will be removed before consumption (Huis et al., 2013). In southern and northern China, cicadas are consumed by frying and roasting (Feng et al., 2018). Stink bugs are mostly consumed in Africa and Zimbabwe and are widely found on tree trunks and branches. The stink bugs are harvested traditionally by climbing on the trees and shaking the branches intensively so that the bugs fall off the tree. Once harvested, the stink bugs will be immersed in hot water and proceed to cleaning and storing (Musundire, Osuga, Cheseto, Irungu, & Torto, 2016). According to the study conducted by Musundire et al. (2016), stink bugs are composed of high amounts of antioxidants and nutrients such as crude protein, fats, and phosphorus. True bugs are widely consumed in Africa, Mexico, and Sudan and these bugs can be consumed in various stages of their life cycle such as eggs, larvae, and adult stages. True bugs are usually consumed by frying and roasting. The eggs of true bugs are highly consumed by the Aztecs (Huis et al., 2013).

12.2.2 Derived products

Commonly, the edible insects and worms are harvested fresh and prepared into food or stored as dried ingredients. Edible insects and worms are also processed into various types of food products, including flour, pasta, cookies, and bread. Since the edible insects and worms are a source of protein, they also can be processed into fitness bars and burger patties. The demand for edible insect-derived food products is significantly increasing, especially in Asian and African countries. The edible insect processing sector can be considered a developing sector and these food products can be referred to as novel food products.

12.2.2.1 Food ingredients

One of the main dried ingredients that can be derived from edible insects is multipurpose flour. The production is primarily done by processes such as drying, grinding, blending the edible insects, and processing them into edible insect flour. Different drying techniques can be used to dry the edible insects and worms, for instance, oven-drying, fluidized bed drying, and microwave drying. These techniques are used to dry solid samples and produce flour from whole insects before further processing (Gravel & Doyen, 2020). The insect flour can be used as an ingredient in baked goods such as muffins, cakes, bread, and cookies. The commonly used edible insects to produce flour are crickets, cockroaches, and termites. Besides, edible insects and worms can be used to extract edible oils. Insects consist of high lipid content primarily triacylglycerols which is the main component of edible oil extracted. The extracted edible oil is suitable for cooking and to be used in the production of other food products such as bread spread, mayonnaise, and frying oil (Sosa & Fogliano, 2017).

12.2.2.2 Ready-to-eat snacks

Edible insects and worms are ideal to produce ready-to-eat (RTE) snacks by using them as an ingredient in the food production line, using insect flour, or adding insect protein into the food products. For instance, deeply fried insects, insect chips, cereals, and cookies produced using insect flour are commonly consumed RTE snacks. The main processing steps involved in the production of RTE snacks are drying, blending, and extrusion. Generally, extruded snacks are composed of high carbohydrate content and low protein content. By producing snacks using insect flour or protein, the

nutritional value of the snacks can be increased significantly (Azzollini, Derossi, Fogliano, Lakemond, & Severini, 2018). Azzollini et al. (2018) found that the addition of insect powder into the extruded cereal-type snacks affected the physical and microstructural properties of the snacks significantly. Besides in this research, the protein content and digestibility of snacks were increased after the addition of insect flour.

12.3 Processing methods of edible insects for Muslim consumption

12.3.1 Current practice

Traditionally, the whole insects are prepared by roasting, frying, smoking, steaming, stewing, and curing for consumption (Melgar-Lalanne, Hernández-Álvarez, & Salinas-Castro, 2019). In Muslim countries, edible insects are mostly consumed and sold in raw form or traditional cooking form. For instance, locusts are consumed as savory nuts in the Middle East countries, where locusts were first boiled, sun-dried, and seasoned with salt (Emirates, 2013). In Malaysia, paddy grasshoppers (*Chortophaga viridifasciata*) are typically fried with turmeric powder and salt for consumption or to be sold on a small scale in East Malaysia with a price of MYR70/kg (USD16.74/kg) (Wan Zulkifli, 2019). On the other hand, the community in Yogyakarta, Indonesia, serves grasshopper (*Melanoplus cinereus*) as dishes for rice (Girsang, 2018).

The consumption of edible insects is less acceptable and viewed negatively in certain culture and places (Mitsuhashi, 2010). The overall experience such as taste, texture, and appearance of edible insects can be improved to increase consumer acceptance (La Barbera, Verneau, Amato, & Grunert, 2018). Consumers are more reluctant to consume whole insects (Sogari, Menozzi, & Mora, 2017) but turning insects into powder or flour could create less aversion to the consumers (Gere, Székely, Kovács, Kókai, & Sipos, 2017). Current practices have changed the edible insects to be processed into powders and flours and incorporated as a functional ingredient in food formulations (Melgar-Lalanne et al., 2019). However, there is limited information available in the literature regarding the commercialization of edible insect products in Muslim countries. Indonesia, a Muslim majority country, has commercialized mealworm oil manufactured by Biteback company (Melgar-Lalanne et al., 2019). However, this product may be aimed at non-Muslim or Muslim communities who accept mealworms as permissible to be consumed according to Maliki scholars. Another study by Asthami, Estiasih, and Maligan (2016) discussed the innovation of instant noodles from wood grasshoppers flour, owing to high instant noodles consumption in Indonesia (Asthami et al., 2016). Production of edible insect products in Muslim countries is actively studied but not yet further commercialized.

Depending on their raw material and their proximate composition, different methods and techniques are used to produce protein-enriched ingredients such as edible insect flours and protein isolates (Boye & Barbana, 2012). However, there are five general steps in creating protein-enriched ingredients: pretreatment, defatting, protein solubilization and recovery, protein purification, and drying (Gravel & Doyen, 2020). During pretreatment, the insects are usually freeze-dried or oven-dried, ground, and sieved to form a fine powder (Bußler, Rumpold, Jander, Rawel, & Schlüter, 2016; Zhao, Vázquez-Gutiérrez, Johansson, Landberg, & Langton, 2016; Zielińska, Karaś, & Baraniak, 2018). The defatting process is performed by removing fat from the samples to improve protein recovery and purity of the final products. The interaction of protein and lipid limits the protein solubility due to the hydrophobic nature of the fat (Azagoh et al., 2016; Lam, Can Karaca, Tyler, & Nickerson, 2018). The defatting step improved and increased the protein content of the insects to produce high-quality protein-enriched ingredients (Bußler et al., 2016; Choi, Wong, & Auh, 2017). Many methods are utilized in the defatting step, such as hexane extraction (Ricochon & Muniglia, 2010), ethanol extraction, methanol extraction (L'hocine, Boye, & Arcand, 2006), aqueous extraction, three-phase partitioning, mechanical pressing, and supercritical CO₂ (Boye & Barbana, 2012). However, hexane extraction is the most effective and widely used method due to its high oil recovery, usually more than 96% (L'hocine et al., 2006; Ricochon & Muniglia, 2010).

Protein solubilization and recovery involved either alkaline solubilization followed by the isoelectric precipitation (IEP), aqueous solubilization followed by the IEP, or salt solubilization method (Gravel & Doyen, 2020). The alkaline/IEP is the most common method (Boye & Barbana, 2012; Bußler et al., 2016; Zhao et al., 2016; Zielińska et al., 2018) and usually gives the highest extraction yield compared to the aqueous and salt extraction method (Karaca, Low, & Nickerson, 2011). For the insect protein isolate, both aqueous and alkaline solubilization can be used (Gravel & Doyen, 2020). The studies done by Purschke et al. (2018) obtained a *Locusta migratoria* protein concentrate of 51.7% protein yield and 82.3% purity using NaOH (Purschke et al., 2018). Yi et al. (2013) obtained protein yields and purity of five different insect species ranging from 17% to 23% and 50% to 61% using aqueous solubilization (Yi et al., 2013). Soluble proteins can be recovered by protein precipitation most commonly achieved through IEP (Zhao et al., 2016).

Protein purification is done to purify proteins from an isolate or concentrate by using the chromatographic method (Duong-Ly & Gabelli, 2014). It is popular for egg protein purification (Chang, Lahti, Tanaka, & Nickerson, 2018) and is not yet to be used on insects for food purposes. However, the method has been used on insects in the medical field (Francis et al., 2019; Gravel & Doyen, 2020). Drying is utilized both during the initial step of insect flour production and the final step to obtaining protein concentrates and isolates (Gravel & Doyen, 2020). Many techniques are used in the food industry for drying such as oven-drying, fluidized bed drying, microwave drying, and freeze-drying (Kröncke, Bösch, Woyzichowski, Demtröder, & Benning, 2018; Melgar-Lalanne et al., 2019). The most commonly used technique in insect protein processing is freeze-drying (Gravel & Doyen, 2020).

12.3.2 Food fortified with insect ingredients

Current industrial food products fortified with insect proteins are meat products and analogs, pasta, snacks, bread, and others (Kim, Setyabrata, Lee, Jones, & Kim, 2016). Insect-based products show a good insight in encouraging entomophagy but the practice is still new and not well explored in the Muslim world. According to Melgar-Lalanne et al. (2019), the most widely sold insects are cricket, grasshopper, and mealworm. Locust or grasshoppers are the only insects unanimously halal for Muslims. Table 12.3 lists locust- and grasshopper-based products that are available in the market worldwide (Melgar-Lalanne et al., 2019), suggesting a good potential of the *Orthoptera* order (grasshoppers, locusts, and crickets) for market growth. More locust-based products should be invented and commercialized, especially in Muslim countries, to encourage entomophagy in the halal food industry.

Besides, food is also fortified with insect dye as a coloring agent. Recently, there are issues in the Muslim world regarding the natural dye carmine which is derived from cochineal insects, thus used in food as a coloring agent. As for Maliki scholars, which allowed the consumption of nonhazardous insects, the utilization of carmine dye from cochineal is considered halal. However, this became an issue for Hanafi schools as insects are considered nonhalal among them. Detailed discussions such as the necessity, the health benefits of the dye and others are needed for Maliki school to render the cochineal color as halal (Rahim, 2018). JAKIM, the Halal authority in Malaysia, with most of the population being Shafi'i scholars, also has discussed this issue. JAKIM stated that cochineal is not harmful

TABLE 12.3 Locust- and grasshopper-based products that are currently available in the market worldwide.

Brand/company	Products	Country
Meat Maniac	Seasoned and flavored locusts	The United States
Crunchy critters	Chocolate covered, powder and tubes of locust	United Kingdom
De Krekerij	Variety of products including crickets and grasshoppers	The Netherlands
Kreca Ento-Food BV	Whole grasshopper, grasshopper powder	The Netherlands
Tiny Foods	Seasoned grasshoppers	The Netherlands
Bug Biters Corporation	Chocolates with grasshoppers	Mexico
Gran Mitla	Grasshopper salt	Mexico
Merci Mercado	Gourmet seasoned chapulines (grasshoppers), ground grasshoppers	Mexico
Snack-Insects	Grasshoppers. Insect-based foods such as power bars, insect pasta, and granola	Germany
Insekten Essen, Zirp Insects	Locusts	Austria
Savonia Grasshopper	Grasshopper (hot pepper, herb-garlic, sweet), Roasted hopper, Grasshopper chips	Finland
EntomaFoods	Seasoned grasshoppers	Spain
Entomos	Edible grasshoppers (lyophilized), grasshoppers Locusta	Switzerland
Next Food	Plain roasted locusts and grasshoppers	Thailand
Thailand Unique	Canned, bulk, candies, and powders of grasshoppers	Thailand

Source: From, Melgar-Lalanne, G., Hernández-Álvarez, A.-J., & Salinas-Castro, A. (2019). Edible insects processing: Traditional and innovative technologies. *Comprehensive Reviews in Food Science and Food Safety*, 18, 1166–1191.

and the carmine dye produced is beneficial. In terms of country law, the Food Regulations of 1985 stated that the carmine dye is allowed based on the Good Manufacturing Practice (GMP) Malaysia. Therefore a carmine dye derived from cochineal is permissible and the amount allowed for utilization should follow the Ministry of Health Malaysia as long it is not harmful.

12.3.3 Challenges and future direction

Islam addressed all Muslims to search for halal food. Halal is not just only about the food itself. The concept of halal requires the food to be prepared hygienically, safe, and healthy. Concerns have been raised about the lack of hygiene, quality control, and storage conditions of the insects (Melgar-Lalanne et al., 2019). One of the issues of entomophagy in the Muslim world involves the safety of the insects to consume. The authorities in Saudi Arabia have once warned the citizens to not indulge in locusts as they can be harmful and poisonous because they feed on plants sprayed by pesticides (Emirates, 2013). Due to these issues, wild harvesting could be replaced with large-scale indoor farming to maintain hygiene and quality control along with the process. Furthermore, insect farming can increase edible insect production and potentially solve overharvesting, which threatens the availability of insects (van Huis & Oonincx, 2017). In Hidalgo, Mexico, 18 species of edible insects have been reported at the risk of extinction due to overharvesting, pollution, and habitat changes (Ramos-Elorduy, 2006).

To attract more Muslim consumers to the entomophagy world, the insect-based product should comply with the halal requirement and halal certification. Halal certification can be explained as free from the animal that is prohibited for Muslims, not manufactured by filthy utensils, equipment, or machinery, no ingredients that are considered filthy by Islamic law, and not interacting with any substance that has been considered filthy by Islamic law during preparation, processing, or storage (Wahab, 2004). In Malaysia the government enacted several legislations to govern Halal food items such as the Trade Descriptions Act of 1972 (TDA 1972), the Animal Act of 1953 (updated 2006), the Food Act of 1983, the Food Regulations of 1985, the Consumer Protection Act of 1999, and the Local Government Act of 1976. According to Article 3 of the Trade Descriptions Act (Definition of Halal) Order 2011, when food or goods are labeled as halal, the foods or products do not contain any animals or substance that are prohibited to consume by Muslims, do not include any unclean ingredients, do not include intoxicate ingredients, do not contain parts of the human being or their yield that are prohibited by Shariah law, not toxic or harmful to health, not been manufactured with any impure instrument, and do not interact with any food that prohibited for consumption by Muslims during manufacturing, processing, or storing (Al-shami & Abdullah, 2021).

From these requirements, it is clear that halal-certified food encompasses all aspects of food production in terms of hygiene, contamination-free food, and nutritious food as required by Islamic law. Halal-certified food has met an international standard of food safety (Wahab, 2004). This will directly solve the issues of food safety and food hygiene, thus encouraging and increasing the confidence level of Muslim consumers to practice entomophagy. The commercialization of insects in the halal sector should further focus on producing more varieties of products that certified halal. Other countries like Thailand and Western have commercialized many grasshopper- or locust-based products (Melgar-Lalanne et al., 2019) that might be commercialized in Muslim country as well, as locust is unanimously halal among Muslim scholars. Halal-certified nonrecognizable grasshopper- or locust-based products are the potential product for Muslim consumers due to the unanimously halal status and nondisgusting insect form. Therefore more study needs to be done to improve the halal insect-based products and to gain more Muslim consumers in the entomophagy world.

12.4 Nutritional content of insects and worms

12.4.1 Proteins

Insects are predominantly composed of protein, ranging between 35% and 60% (Melo, Garcia, Sandoval, Jiménez, & Calvo, 2011) and 38%–77% dry weight (Finke, 2015). Insects are a good source of alternative protein with higher protein availability in insects than animal and plant protein such as beef, chicken, fish, soybean, and maize (Teffo, Toms, & Eloff, 2007). Proteins are organic compounds composed of essential or nonessential amino acids (Tiencheu & Womeni, 2017). Essential amino acids such as phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine, and lysine cannot be synthesized by the body and must be obtained from the diet (van Huis et al., 2013) for the functionality and proper development of the body.

According to van Huis et al. (2013), protein content of locusts and grasshoppers can be up to 28 g, silkworm up to 17 g, and cricket up to 25% (per 100 g of fresh weight). Those protein content are comparable to cattle, crustaceans,

and mollusks which contain up to 26, 27, and 18 g/100 g fresh weight accordingly (van Huis et al., 2013). The protein content in adult locusts and grasshoppers is relatively higher among insects (Finke, 2015). The fact is also supported by Ramos-Elorduy et al. (1998) that stated the red-legged locusts containing the highest protein percentage on a dry weight basis compared to other selected insects (Ramos-Elorduy et al., 1998). The increased amount of protein in locusts is due to many essential amino acids (Raheem et al., 2019), which is higher than soybean, beef, and wheat flour (Ojha et al., 2021). Table 12.4 was withdrawn and replaced with stated description. Table 12.5 was withdrawn as the information provided is repetitive.

12.4.2 Carbohydrates and fiber

Carbohydrates and fiber are formed mainly by chitin (Burton & Zacccone, 2007; Klunder, Wolkers-Rooijackers, Korpela, & Nout, 2012; Mlcek, Rop, Borkovcova, & Bednarova, 2014; Roos & van Huis, 2017). Carbohydrates also exist in the form of glycogen in insects (Mlcek et al., 2014). Chitin is a primary component of an insect exoskeleton (Roos & van Huis, 2017) and has high nutritional and health value (Burton & Zacccone, 2007). Insects contain 1%–7% of chitin (Finke, 2007). The high content of chitin, particularly fiber, interferes with protein solubility (Brogan, 2018) and contributes to low digestibility (Roos & van Huis, 2017). Locusts contain 2% chitin (Egonyu, Subramanian et al., 2021; Egonyu, Tanga et al., 2021) which is considered low among insects. The low presence of chitin gives better digestibility and protein solubility, thus improving the quality and bioavailability of dietary proteins in locusts (de Castro, Ohara, Aguilar, dos, & Domingues, 2018).

Edible insects contain carbohydrates ranging from 6.71% in sting bugs to 15.98% in cicada (Mlcek et al., 2014). The data on the fiber content of edible insects are incomparable due to the various method utilized (Klunder et al., 2012). However, chitin is considered a dietary fiber and normally represents between 2.7 and 49.8 mg/kg of fresh and 11.6 to 137.2 mg/kg of dry matter (Finke, 2007; Roos & van Huis, 2017). Soft-bodied insects are known to have less fiber than hard-exoskeleton insects (Mohamed, 2015). The carbohydrate and fiber content in locusts ranged from 4.05% to 5.51% and 14.21% to 17.09%, respectively (Mohamed, 2015). The low content of carbohydrates in locusts might be due to the low source of chitin in locusts. According to Mohamed (2015), the carbohydrate content in locusts is very low.

TABLE 12.4 Protein content in locust compared to reference protein sources.

Animal group	Species and common name	Edible product	Protein content (g/100 g fresh weight)
Insects	Locusts and grasshoppers	Adult	13–28
	Silkworm	Caterpillar	10–17
	Palmworm beetles	Larva	7–36
	Yellow mealworm	Larva	14–25
	Crickets	Adult	8–25
	Termites	Adult	13–28
Cattle		Beef (raw)	19–26
Fish (raw)	Finfish	Tilapia	16–19
		Mackerel	16–28
		Catfish	17–28
	Crustaceans	Lobster	17–19
		Prawn (Malaysia)	16–19
		Shrimp	13–27
	Molluscs	Cuttlefish, squid	15–18

Source: From, van Huis, A, Van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G., & Vantomme, P. (2013). *Edible insects. Future prospects for food and feed security*. Food and Agriculture Organization of the United Nations (171).

TABLE 12.5 Essential amino acids content in locust compared to reference protein sources.

Protein sources	Total essential amino acids (mg/g protein)
Scarab beetle	514
Mulberry silkworm	497
Bombay locust	482
House cricket	461
Flesh fly, adult	383
Western harvester ant	313
American cockroach	199
Egg white	519
Soybean	439
Beef	431
Wheat flour	350

Source: From, Ojha, S., Bekhit, A. E.-D., Grune, T., & Schluter, O. K. (2021). Bioavailability of nutrients from edible insects. *Current Opinion in Food Science*, 41, 1–9.

12.4.3 Fats

Fat is the second-largest nutrient composition in insects (Mlcek et al., 2014). Fat consists of triglycerides, which are composed of a glycerol molecule and three fatty acids. Fatty acids can either be saturated, unsaturated, or essential (Tiencheu & Womeni, 2017; van Huis et al., 2013). Unsaturated fatty acids are considered more healthful than saturated fat (van Huis et al., 2013). Insects are known for the high content of unsaturated fatty acid compared to saturated fatty acid (de Castro et al., 2018). The oils in insects are rich in polyunsaturated fatty acids. They commonly contain the essential linoleic (omega-6 fatty acids) and α -linolenic acids (omega-3 fatty acids) (van Huis et al., 2013). Polyunsaturated fatty acids have several health benefits, such as reducing the risk of diabetes and lowering blood pressure (Sirtori & Galli, 2002).

Locusts contain the essential polyunsaturated fatty acids, omega-3, and omega-6 fatty acids (Egonyu, Subramanian et al., 2021; Egonyu, Tanga et al., 2021). Grasshoppers, which are from the same group of locusts, have a 60% unsaturated fatty acid composition with linoleic and oleic acid contents of 1.4% and 44%, respectively (Heuze, Tran, Giger-Reverdin, & Lebas, 2017). This is agreeable to the studies done by Womeni et al. (2009), which explained edible grasshopper with the highest fat content of 67% compared to African palm weevil, termites, and saturniid caterpillar. The composition of primary fatty acids in edible grasshopper includes palmitoleic (28%), linoleic (46%), and α -linolenic acids (16%) (Womeni et al., 2009).

12.4.4 Vitamins and minerals

Micronutrients, such as vitamins and minerals, play a significant role in the nutritional value of food (FAO, 2011). The micronutrients in insects are highly variable, which can be present in high or low amounts depending on the insects' diet, metamorphosis stage, species, and orders (Baiano, 2020; Van Huis et al., 2013). However, they are commonly rich in potassium, sodium, calcium, copper, iron, zinc, manganese, and phosphorus (van Huis et al., 2013). For vitamins, previous data stated that edible insects contain vitamins A, B1, B2, B6, B12, C, D, E, and K (Bukkens, 2005; Mlcek et al., 2014; Raheem et al., 2019; Van Huis et al., 2013). The high vitamin content in edible insects presents a suitable alternative food source or supplement for malnourished people and animals (Tiencheu & Womeni, 2017).

Desert locusts contain calcium, iron, and zinc levels of 4–28, 1–6, and 2.4–12.5 mg/100 g, which are comparable or superior to mutton, beef, and pork (Ahmad, Imran, & Hussain, 2018; Egonyu, Subramanian et al., 2021; Egonyu, Tanga et al., 2021). Investigation of vitamin content stated migratory and desert locusts as sources of vitamin D3 (Oonincx et al., 2018), A and E (Kinyuru, 2021), which are absent or traces in beef, bacon, mutton, and pork (Ahmad et al., 2018). Migratory locusts also contain vitamin B12 5- to 10-fold higher than beef, bacon, mutton, and pork

(Ahmad et al., 2018; Egonyu, Subramanian et al., 2021; Egonyu, Tanga et al., 2021). This is good among insects since Bukkens (2005) stated that many species of edible insects have very low levels of vitamin B12.

12.5 Microbiological risk associated with insects and worms

12.5.1 Pathogen reservoir

Pathogen reservoirs are the host of harmful microorganisms. Insects are one of the pathogen reservoirs. Insects can be the vector to carry and transmit pathogens. Edible insects usually carry more mesophilic aerobes, psychotropic aerobes, spore-forming bacteria, and endospores. There are possibilities for the presence of fungi and other pathogenic organisms on the edible insects (Garofalo et al., 2019).

12.5.1.1 Bacteria

Undoubtedly, pathogenic bacteria can present in raw insects and worms. The highest abundances of pathogenic bacteria associated with edible insects and worms are *Staphylococcus aureus*, pathogenic *Clostridium* spp., and pathogenic species of the *Bacillus cereus* group (Vandeweyer, Smet, de, Loooveren, & Campenhout, 2021). For instance, a study conducted by Klunder et al. (2012) reported that boiling house cricket for 1 minute reduced Enterobacteriaceae and bacterial spores by $\sim 7 \log$ cfu/g and on mealworms $\sim 5 \log$ cfu/g, respectively. However, the counts of Enterobacteriaceae and bacterial spores on house cricket spiked during ambient storage and yielded spoilage after the second day of storage. Boiling house cricket in acidified water and turning house cricket into powder were found to increase the shelf life of the products.

Another study by Garofalo et al. (2017) stated that food spoilage and opportunistic pathogens were found in the lower count, but no viable harmful bacteria were found in the edible insect products. Nevertheless, *Listeria* spp., *Staphylococcus* spp., *Clostridium* spp., and *Bacillus* spp. were detected in edible insect products reported found in this study. The microbiological quality of different dried insects sold in South Africa exhibited high coliform counts in mopane worms and a considerable count of *Escherichia coli* and *S. aureus* count in termites. Although the overall bacterial counts of the tested edible insects and worms were lower levels, the presence of pathogens in edible insects is still a concern since it is a sign of poor food sanitation (Ramashia et al., 2020).

12.5.1.2 Fungi

Various fungal species can be found in edible insects and worms depending on the species and moisture content of insects and worms. The most common fungi types associated with edible insects and worms are yeast and molds. The fungi can be present in both living and dead edible insects and worms. Based on study by Grabowski and Klein (2017a, 2017b), *Candida albicans* were isolated from living moths of different species; however, no fungi species were isolated from living house cricket. In addition, *Issatchenkia orientalis* was isolated from living mealworms while *Geotrichum* spp. was isolated from living migratory locusts. Diverse fungi species were isolated from dead house cricket in this study. Postprocessing fungal contaminations on edible insect food products are dominantly caused by improper packaging, storing, and transportation of the products (Ozdal et al., 2012).

There are possibilities of mycotoxin-producing fungi to be found on edible insects and worms such as mealworms, crickets, and moths. For instance, mycotoxin-producing *Aspergillus* spp. and *Penicillium* spp. were isolated from crickets during the research conducted by Vandeweyer et al. (2018). The mycotoxins are very dangerous to human health and must be eliminated from edible insects before human consumption. Mycotoxins are heat resistant; thus elimination of mycotoxins from edible insects and worms requires high heating temperature. In a study conducted by Braide et al. (2011), few species of mycotoxin-forming yeasts and molds were isolated from roasted and sun-dried caterpillar larvae. Nevertheless, proper heat treatment can lower the fungal load on edible insects and worms to an undetectable level (Vandeweyer et al., 2018).

12.5.1.3 Viruses

Viruses require a host to replicate and cannot be replicated on their own. Hence, the viruses mostly cause harmful effects to humans such as transmitting various diseases. Edible insects and worms are possible vectors for virus replication. Nevertheless, the presence of viruses in insects and worms is less significant compared to bacteria and fungi. Viruses that are vectored by edible insects and worms are commonly infectious to the insects and worms themselves but harmless to human health except the viruses carried by blood-sucking insects where they highly carry viruses that

are pathogenic to humans (Dicke et al., 2020). Even so, edible insects and worms are not the reservoirs of viruses that may infect humans (ESFA, 2015). To date, insects are not proven to be the reservoirs of pathogenic viruses; yet, they can be the vectors to carry viruses that may be a threat to human health (van der Fels-Klerx et al., 2018). Further researches are required to study and understand completely the possibilities of edible insects and worms being a pathogenic virus reservoir.

12.5.1.4 Allergens

Allergens are substances or proteins which are responsible for stimulating allergic reactions in sensitive human bodies. There are many common foods containing allergens, namely, peanuts, eggs, fish, gluten, and milk. Allergies will develop if the person is sensitive to the allergen. Edible insects and worms are potential food sources to induce allergic reactions in humans. These allergic reactions are commonly induced due to the presence of panallergens of arthropods, namely, arachnids, crustaceans, myriapods, and insects (ESFA, 2015). Numerous cases of edible insects and worm allergy outbreaks were reported worldwide. For instance, there is a reported case of a 36-year-old woman from Botswana who was diagnosed with food allergy after consumption of mopane worm. After ingestion, she developed symptoms such as itchy skin rash, facial swelling, and mild hypotension, even so, although she had no record of allergic reaction in the past (Okezie, Kgomotso, & Letswiti, 2010).

The allergic reactions caused by the consumption of edible insects and worms can be classified into primary sensitization to edible insects and cross-reactivity with other allergenic species (de Gier & Verhoeckx, 2018). A study on primary sensitization conducted by Broekman, (2017) showed revealed that mealworms are capable of inducing primary sensitization in humans which can cause foodborne allergic reactions. This study also proved that primary sensitization for mealworms for individuals with no record of crustacean allergy was not induced by allergens such as tropomyosin and arginine kinase, but that other proteins, like larval cuticle protein. Meanwhile, a study by Srinroch, Srisomsap, Chokchaichamnankit, Punyarit, and Phiriyangkul (2015) showed that individuals with a record of crustacean allergy are most vulnerable to insect allergy due to cross-reactivity.

12.5.2 Potential health risks

Edible insects and worms are sustainable sources of food and possibly can provide various nutritional benefits. Nevertheless, the potential health risks of consuming insects and worms are hard to be neglected since they are susceptible to microbial growth and contamination. Food safety and food security concerns frequently arise among consumers as well as the food industry in the consumption of edible insects and worms. Numerous food safety outbreaks associated with edible insects and worms such as allergic and food poisoning reactions have been documented worldwide. Some researchers claimed that microorganisms in invertebrates are harmless to humans (Dicke et al., 2020; Huis et al., 2013). However, studies have proven that opportunist pathogenic microorganisms can be found on various body parts of insects and worms especially in guts which may lead to foodborne illnesses (Su et al., 2014). Insects and worms are vulnerable to microbial hazards; hence, it is vital to further research about the potential health risks that can be caused due to insects and insect-based food products consumption.

12.5.2.1 Food allergy

Food allergy is the body's sensitive immune reaction towards the allergens in food caused by immunoglobulin E (IgE) antibodies. Insects and worms are highly capable of causing food allergies due to primary sensitization to insect allergens. Apart from that, insects and worms may cause cross-reactivity with other potential allergenic food such as crustaceans in both sensitive groups and normal groups. Since both crustaceans and insects are arthropods, they have similar proteins or allergens in their body parts and may cause common food allergies by cross-reactivity. For instance, the muscles of both crustaceans and insects carry tropomyosin, a panallergen that is vital for muscle contraction in invertebrates (Downs, Johnson, & Zeece, 2016). Tropomyosin causes typical allergic reactions such as gastrointestinal problems, hives, nausea, and abdominal pain.

The most relevant concern for the potential allergenicity of insect foods is IgE-mediated food allergies (Downs et al., 2016). A study conducted by Wang, Calatroni, Visness, and Sampson (2011) proved that there is a strong correlation of IgE to shrimp with cockroach and dust mite exposure and sensitization. Besides, arginine kinase is also a potential panallergen in edible insects and worms. In a study by Srinroch et al. (2015) about the cross-reactivity of allergens in a small freshwater prawn and the field cricket, arginine kinase was found in both prawn and field crickets. The authors claim that it is a cross-reactive, invertebrate panallergen and thus presume that prawn-allergic patients may also

be allergic to edible insects. Several earlier studies have reported few allergenic cases due to the consumption of insects and worms. For instance, in a study conducted among insect consumers in Laos, around 7.6% of the consumers reported having allergic symptoms due to the consumption of insects such as grasshoppers and stink bugs (Barennes, Phimmasane, & Rajaonarivo, 2015).

12.5.2.2 Food poisoning

Insects and worms can be the host and vector of foodborne pathogens. Pathogens may survive on body parts of insects and worms especially in their gastrointestinal part. Apart from that, improper handling and rearing of edible insects and worms can cross-contaminate in insect-based products. Due to the presence of foodborne pathogens, the consumption of insects and worms may cause food poisoning. The most common pathogens among edible insects and worms are *S. aureus*, pathogenic *Clostridium* spp., and pathogenic species of the *B. cereus* group (Vandeweyer et al., 2021). Pathogens, including coliforms, *Serratia liquefaciens*, *Listeria ivanovii*, *Mucor* sp., *Aspergillus* sp., *Penicillium* sp., and *Cryptococcus neoformans*, were isolated from cooked and processed insect-based products as well (Grabowski & Klein, 2017a, 2017b). Some opportunistic pathogens were also found; however, the total pathogen count is lesser in processed products compared to the raw insects at the same time, the products met most of the food hygiene requirements (Grabowski & Klein, 2017a, 2017b). Grabowski and Klein (2017a, 2017b) suggest that the use of heating steps in the processing of insect-based food products can minimize the pathogen count in the final product.

12.5.2.3 Mycotoxin contamination

Mycotoxin is produced by the fungi group, and it may lead to food contamination and poisoning. Since insects and worms are potential hosts and vectors of fungi, there are high possibilities of mycotoxin poisoning due to the ingestion of insects and worms. Mycotoxin-producing fungi species such as *Aspergillus* spp. and *Penicillium* sp. have been found on reared crickets for food and feed (Vandeweyer et al., 2018). This shows that there are high possibilities for mycotoxin contamination and poisoning due to the consumption of insects and worms. Some mycotoxins are produced in the guts of fungal-infected insects and worms. The mycotoxins are occasionally hard to be eliminated from the insects and may be resistant to heat (Pradanas-González et al., 2021). Microorganisms present in the gut of insects and worms, namely, genera *Clostridium* spp., *Bacillus* spp., and *Aspergillus* spp. are capable of producing and modifying toxins other than mycotoxin (Fernandez-Cassi et al., 2019). Scientists suggest that the starvation period before insect harvesting can minimize microorganisms and toxin load since the gut content is the main cause of contamination in insects (Schrögel & Wätjen, 2019).

12.6 Consumer acceptance of edible insects and worms

12.6.1 Desirable attributes of edible insects and worms

For many years, insects and worms have been a part of the diet in Asian countries. Initially, insects and worms were cooked or consumed as a whole and nowadays many insect-based food products and food ingredients are being developed. As the world population is rapidly increasing, the production of sustainable food sources is a supreme goal of the food industry. This is the part where the development of insect-based food products comes into the picture since insects and insect-based products offer high potential to tackle the increasing demand for food. For many years, insects and worms have been a part of the diet highly in Asian countries. Initially, insects and worms were cooked or consumed as a whole and nowadays many insect-based food products and food ingredients are being developed. For instance, as discussed previously, RTE snacks, burger patties, and pasta are currently developed using edible insects ingredients, including insect flour, insect oil, and insect-based and food colorings. Although insects and worms are still under the novel food category, they possess numerous benefits for consumers' health, environment, and world economy. Some of the desirable attributes, namely, nutritional value, environmental, benefits, and economical economic benefits of insects and worms play key roles in elevating the current insect's consumption pattern to another level particularly. Furthermore, insects and worms are highly beneficial in development of new food products and food ingredients using new techniques and technologies.

12.6.1.1 Nutritional value

Awareness of consuming nutritious food keeps on rising and consumers' food choices nowadays that are highly influenced by their nutritional value and health benefits. This is very evident as many functional and nutrient-rich food

products are coming to entering the market with increasing consumer reception acceptance. Insects and worms are proven to possess several nutritional values such as high macro and micronutrient contents (Kim, Yong, Kim, Kim, & Choi, 2019). However, the composition of each of these nutrients depends on several factors such as species type, stage of insects' life cycle, and growing environment (van Huis & Dunkel, 2017). Even so, insects have high protein content in all stages of their life cycle as they are rich resources of in amino acids (Tang et al., 2019). For instance, yellow mealworm larvae consist of approximately 33% fat, 51% crude protein, and 43% true refined protein on a dry basis (Zhao et al., 2016). Research was done by Zhao et al. (2016) to study the extraction and functional properties of yellow mealworm larvae protein by freeze-drying. This study shows that yellow mealworm larvae consist of approximately 33% fat, 51% crude protein, and 43% true protein on a dry basis (Zhao et al., 2016). Furthermore, high nutrients of insects and worms were proven by processing insect-rich snacks where the macro and micronutrient contents of snacks have been reported to increase when elevated once added with insect flour was added (Azzollini et al., 2018). Besides, the exoskeleton of insects contains high amounts of chitin, which is a carbohydrate group that provides various health benefits to consumers.

12.6.1.2 *Environmental benefits*

Rearing and consumption of insects and worms are more environment-friendly as compared to those for ruminants such conventional animals such as cattle and pigs due to several factors. A study conducted to investigate the amount of greenhouse gasses emission by insects shows that the greenhouse gasses released by edible insects were only 1% of the gases released by ruminants (Oonincx et al., 2010). Greenhouse gases contribute to a significant increase in atmospheric temperature. Given that insects only emit a low amount of greenhouse gasses, the atmosphere temperature will not be rising, and this can control the risk of global warming. Moreover, the release of ammonia will be higher in meat production due to the manure and urine of livestock whereas in insects, the ammonia emission will be low (Gahukar, 2016).

Apart from that, the feed conversion ratio of insects and worms is highly efficient with fewer water footprints and land use (FAO, 2021). Feed conversion ratio refers to the ratio of the mass of feed given to the insects to the mass of insects (Alavi, Mazumdar, & Taylor, 2018). Feed conversion efficiency is inversely proportional to greenhouse gas emissions where the higher the efficiency, the lower the emission. Insects are poikilotherms as their body temperature regulation does not depend on their metabolisms (Oonincx et al., 2010) thus, lesser greenhouse gas emission. Nonetheless, the feed conversion of foodies in ability ratio does not consider dig hence, it is not completely suitable to use feed the conversion ratio to assess the environmental benefits (Halloran, Roos, Eilenberg, Cerutti, & Bruun, 2016).

12.6.1.3 *Economical benefits*

In rural areas, insects and worms provide economic benefits to the people, especially to the marginalized communities. People from marginalized communities harvest, cook, and sell edible insects as they provide a source of income (Huis et al., 2013). The insect food industries are emerging rapidly in regions with high insect consumers. As an example, cricket farming in Kenya and Uganda is significantly growing as these countries have a long history of insect consumption (Egonyu, Subramanian et al., 2021; Egonyu, Tanga et al., 2021). There are many new food products and food ingredients based on edible insects; currently in the food market and still, technologies are being developed to convert edible insects into sustainable and safe food. The rearing of edible insects is cost-efficient since no high capital is required at the same time and provides significant profit (Tang et al., 2019). As many people are concerned about the nutritional value of their food, insect-based products are expected to gain drastically high profits in the future since they are a rich source of protein.

12.6.2 **Current findings on consumer acceptance**

Apart from nutritional value, a food product must be appealing and acceptable to the consumers so that they will be interested to consume it. Edible insects and worms are still under the novel food category as they are not widely consumed yet, even so the marketability of edible insects is rocketing every day. This shows that many consumers are accepting insects and worms as a food source and their perceptions towards insect-based products are changing positively. Anyways, the degree of acceptance will differ based on the countries. Such as in Asian countries, the acceptance will tend to be higher than in European countries, due to the long history of entomophagy. In contrast, acceptance in European countries might be lower. Furthermore, sensory attributes, food safety, and neophobia play vital roles in for acceptance of edible insects, consumption acceptance by the consumers. The readiness of consumers to consider insects

and worms as food sources is still a huge question mark as people should change their mindsets and disgust towards insect-based food.

12.6.2.1 Acceptance in various regions

The familiarity of insect consumption plays a vital key where the acceptance of familiar foods is usually higher than unfamiliar foods. However, familiarity and acceptance of entomophagy do not contribute to willingness to consume insects. This has been proven by the recent survey which shows that most people are familiar with and accept the concept of entomophagy nonetheless, the willingness to try the food is still uncertainty (Modlinska, Adamczyk, Maison, Goncikowska, & Pisula, 2021). Besides, history and culture are also important factors in accepting insects and worms as food sources. Countries in Asian and African regions tend to accept insect-based food products easily as they consume insects for decades and in some regions, insects are considered traditional delicacies. In contrast, insect consumption is taboo and insects are treated as unfit foods in certain regions due to various factors, namely, culture, religion, and disgust (Shelomi, 2015). Moreover, Payne, Dobermann et al. (2016), Payne, Scarborough et al. (2016) claim that acceptance of insects is a huge challenge in regions with no current history of entomophagy to incorporate insects in food products.

12.6.2.2 Sensory aspects

Sensory aspects are crucial in consumers' acceptance of any food as it is presumed a measure of food quality and triggers the appetite. Hence, acceptance of edible insects and worms is highly associated with the sensory aspects. In a study conducted among a population in the Czech Republic, most of the respondents responded positively to taste insect-based food products even though they expressed a sense of consciousness while eating (Adámek et al., 2020). The authors mention that the sense of consciousness can be seen while the respondents consumed visible mealworm larvae on the puff pastry bars. This shows that finding suggests consumers consider the appearance of the food (appealing food) as an important criterion for acceptance. Besides, a study by Tan, Verbaan, and Stieger (2017) demonstrates that enhancing the sensory attributes of insect-based food products such as appearance and taste contributes to the acceptance of consumers. The authors claim that sensory liking of the product leads consumers to try insect-based food products.

12.6.2.3 Food security concerns

Since insects and worms are novel foods, there is a lack of legal food safety regulations about insect-based food products. Thus food safety concerns are a primary barrier to the acceptance of insects as a food source. Insects and worms are vulnerable to hazards, including biological, chemical, and physical hazards. For instance, insects and worms are capable of carrying pathogens, parasites, viruses, and heavy metals where these hazards can lead to foodborne illnesses. Studies have proven that insects and worms are susceptible to food hazards; nevertheless, proper processing and handling methods may reduce the microbiological hazards and extend the shelf life of the edible insects.

12.7 Summary and conclusion

Edible insects and worms have been consumed worldwide for centuries and their demand is increasing in the food industry. They can be a good source of protein as well as other nutrients such as fiber, phosphorus, and calcium. In rural areas, edible insects and worms are harvested in traditional methods and they provide a source of income for many marginalized communities in those areas. Edible insects and worms are crucial during the food shortage in Asia and African regions. Nowadays, many food products are being derived from edible insects and worms such as flour, cookies, cooking oil, and many more food products. However, the consumption of insects and worms is relatively low in Western countries. This might be due to the lower percentage of food shortages in Western countries compared to African and Asian countries. Besides, acceptance of food plays a vital role whereas, in Western countries, insects and worms are not widely accepted as a food source. Edible insects and worms have a high potential to evolve rapidly in the food industry due to their nutritional benefits, low environmental cost, and many other advantages. From Halal perspective, it is clear that harmonization of consensus remain a great barrier to utilize insects and worms as human food but so far, the use of insects and worms and animal feed is permissible and can be future explored.

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Food processing aids: lubricants for halal manufacturing facilities

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13.1 Introduction

Manufacturers utilize processing aids to help handle a variety of product-processing problems without having to declare them on the food label in most countries. Processing aids include everything from lubricants for equipment that comes into touch with food to peeling agents for fruits and vegetables (Al-Mazeedi, Regenstein, & Riaz, 2013). They are employed in the manufacturing of foods to provide a variety of beneficial functions and desired effects, but they are not intended to be included in the finished product's ingredients. As a result, they are not needed to be listed on the ingredient label of a product (Codex Alimentarius Standard, 2019). Over the last few decades, food safety knowledge has shifted substantially, with a greater emphasis on the materials and procedures used to manufacture meals and beverages. Lubricants used in machinery throughout food processing plants are crucial for producing high-quality and safe products, since the majority of the food we eat today has been touched by machinery. Therefore industrial equipment is fully utilized to handle and process food from picking to packing. It is utmost importance that machinery must be lubricated; hence there is a risk between the interaction of the lubricant and the food. During the last 10–20 years, the food processing business has changed drastically. Consumer sensory acceptance, food safety standards, and worldwide supply and demand are just a few of the factors that have shaped the sector into what it is now today.

13.1.1 Definitions

Before we dive into the depth of the details of this chapter, it is very important to understand the definition and terms used. This subchapter features a brief definition about the concept of lubrications, lubricants, food processing machinery, and some information on the lubricants derived from plants, animals, and synthetic lubricants.

13.1.1.1 What is lubrication?

Lubrication is the science of reducing friction and wear between two solid bodies in relative motion by interposing a lubricant between their rubbing surfaces. This is considered to be the single most vital aspect in plant and machinery maintenance, as it keeps the process plant young and contributes to a higher profitability by extending the life of wear components, as well as increasing machinery availability and reliability. A lot of effort and research has been done, which include improved lubrication, mechanization, modification of the existing lubricating system, reduction of lubricant wastage, and reduced lubricant and wear part consumption (Vishwakarma, 2015).

13.1.1.2 What are lubricants?

Lubricant is a substance that is used to control and reduce friction or wear of the surfaces in a contact of the bodies in relative motion (Harris & Kotzalas, 2006). Depending on its nature, lubricants are also used to remove heat and wear residues, supply additives into contact, transmit power, protect, and seal. A lubricant can be in a form of liquid (oil, water, etc.), solid (graphite, graphene, molybdenum disulfide), gas (air), or semisolid (fat). Liquid lubricants can be

divided into synthetic/semisynthetic, mineral, or environment-friendly (biodegradable) oils, depending on the base oil (Bart, Gucciardi, & Cavallaro, 2013). Most lubricants contain additives (5%–30%) to improve their performance.

13.1.1.3 Classification of lubricants

Lubricant is available in several forms of physical state such as solid, semisolid, and liquid form. Among the three states, liquid lubricants are the major form which generally comprise approximately 70%–99% of base oil and 1%–30% additives (Salimon, Salih, & Yousif, 2012a). According to the nature of base oil use for lubricants, they can be classified as mineral, synthetic, and biolubricant or green lubricant (Karmakar, Ghosh, & Sharma, 2017). Every type led to different properties and was proposed for different applications.

13.1.1.3.1 Mineral oil

Common industrial lubricant consists of 90% mineral-based oil and the others are additives (Vasishth, Kuchhal, & Anand, 2014). Lubricant from mineral-based basically obtained from the process of fractional distillation of crude oil. Mineral oil consists of highly number of hydrocarbon which are about 83%–87% of carbon content, hydrogen content ranging from 11% to 14% while the remaining elements are consists of oxygen, nitrogen, sulfur, and metals (Ahmed & Nassar, 2013). Mineral oil is widely used for lubricating oils because it is cost-effective and easily accessible. However, this type of lubricant is not biodegradable.

13.1.1.3.2 Synthetic oil

Synthetic oil is type of lubricant that passed through modification in chemical reaction of petroleum to produce synthetic base oil. The examples of the product are synthetic ester, silicones, and polyalphaolefins (PAOs) (Mobarak et al., 2014). The advantage of synthetic oil as is that it has molecular structure that fulfills the required properties of lubricant. However, the synthetic-based lubricant does not highly commercialize because the production cost of a new product is expensive.

13.1.1.3.3 Biolubricant

Any lubricant that is biodegradable and environment-friendly is called biolubricant. Biolubricant can be produced from oil-based resources either animal fats or vegetable oil. Vegetable oil seems to be more prominent and potentially used than animal because it is readily available, easy to extract, and lower in price (Alotaibi & Yousif, 2016). Bio-based lubricant has been developed in the Europe market for 20 years and the usage for the next few years was estimated around 18% of the total lubricant (Mendoza et al., 2011). The production of biolubricants has led to various benefits over environmental, health, and better performance of oil in comparison with the current petroleum-based lubricant (McNutt & He, 2016).

In the early year of 1980, the first biodegradable lubricants were synthesized for two-stroke outboard engines by using neopentylpolyol esters of branched-chain fatty acids as based fluids (Zulki, Kalam, Masjuki, Shahabuddin, & Yunus, 2013). This is improvement that provides modification in the structure of oil by conversion into a new ester called the polyol ester (Umaru et al., 2016).

It is an advantage to use vegetable oil or plant-based oil as the derivative for environment-friendly lubricant which possess similar lubricating properties with mineral-based lubricant. The most common materials for synthesis of biolubricant are made up of polyhydric alcohols such as trimethylolpropane, neopentyl glycol, and pentaerythritol.

There are two ways of producing biolubricant ester from vegetable oil. They are two-step reaction transesterification–esterification and combination of hydrolysis and esterification. Biolubricant that is derived from vegetable oil can be produced either from fatty acid or fatty acid methyl ester. The biolubricant ester found can improve lubricant properties in terms of oxidation stability and thermal stability (Menkiti, Ocheje, & Agu, 2017).

13.1.1.4 What is food processing machinery?

Food machinery is a comprehensive term that refers to the various components of the food processing unit, for example, food processing machines and the systems used. This food processing machinery used for food products ranges from bakery to beverages, dairy products, etc. The food processing equipment manufacturers generally aiming at conversion, that is, improving palatability, edibleness, digestibility, or storage, and extending the shelf life of food (Food Marketing Technology, 2021). They designed their machinery in a way that they are pieces of equipment which are also employed to perform preliminary or auxiliary functions, such as handling, preparation, heat processing, preservation, and packaging (Hinnou, Obossou, & Adjovi, 2022).

13.2 Why lubricants are important?

Machinery touches most of the food we consume today. Like any factory, a food processing factory is equipped with machinery full of bearings and rings to protect various rotating, shuttling, and sliding parts from the ravages of friction and mechanical loads. And like most plant equipment, food-grade machine required periodic lubrication meaning there is a possibility of interaction between the lubricant and the food itself. Awareness toward food safety has increased significantly over the past few decades, with a focus on the ingredients and processes used to produce quality food and beverage products that are safe. For companies that deal with food and drink, the health and safety of their customers are their biggest priority. High standards of hygiene and sanitation are mandatory all the way from production to the time they reach the end user. Hence, the lubricants used in machinery throughout food processing facilities are critical ([Asia Pacific Food, 2017](#)).

However, choosing a lubricant is not simple, they cannot simply slather any old sort of grease moving parts that operate near food intended for human consumption. Subsequently, food and beverage manufacturers are constantly challenged to find new ways to improve processing efficiency while meeting the highest food safety standards, including an ongoing focus on food contamination prevention. The answer to solve this problem is to use the food-grade lubricant for their machinery. Whether through leaks, excessive lubrication, or messy application during maintenance, food-grade lubricants can inadvertently become indirect food additives. It is vital for these industries to select the right lubricant for the job, but there is a vast amount of regulations and registrations that must be adhered to ([Research and Markets, 2019](#)).

13.2.1 What is food-grade lubricant?

Food-grade or food-safe lubricant is the name given to any industrial lubricant that is considered safe for incidental contact with items that may be consumed by humans or animals, as long as it does not exceed a certain concentration. Food-grade lubricants must perform the same technical functions as any other lubricant, including protection against wear, friction, corrosion, and oxidation, heat dissipation and power transfer, compatibility with rubber and other sealing materials, and, in some cases, sealing effect ([Williamson, 2019](#)). However, food-grade lubricants need to comply with a set of additional specific requirements that are (1) physiologically inert, (2) tasteless, (3) odorless, and (4) internationally approved. Definitions of food-grade lubricants can be found in the following reports ([EHEDG Update, 2003](#)):

- H-1 of the USDA
- ELGI/NLGI/EHEDG document FGLI/2001/issue 2
- DIN V 0010517
- NSF draft for ANSI standard

Furthermore, these food-grade lubricants must resist deterioration from food items, chemicals, and water/steam. They should have a neutral behavior toward polymers and elastomers, and be able to dissolve sugars for various uses in the food industry. Lubricants can also be exposed to a wide range of pollutants in the intense environment. For example, dust produced in a corn-milling environment provides the risk to the lubricants itself. Although it is not as hard as silica-based dust, it nevertheless poses a filtration challenge ([Yadav, Singh, & Negi, 2021](#)).

Other than that, the risk of water contamination is great in a meat production since it requires constant steam cleaning. Water can make up as much as 15% of the volume of some process plant's gear oils. The growth of microorganisms such as bacteria, yeast, and fungi are another component of lubrication contamination that poses a concern to food-grade lubricants. While these can be a problem in industrial settings, the risk of contamination in a food-production setting is significantly higher. This will be resulting in pollution-caused illnesses of customers, leading to a loss of trust in affected food brands and manufacturers ([Biswas, Jairath, Mendiratta, Kumar, & Bauer, 2022](#)).

Food-grade lubricants are utilized in a variety of system parts across the manufacturing and processing lines, such as gear oils, chain oils, compressor oils, hydraulic oils, and corrosion-preventative oils. Some major machining processes like turning, milling, drilling, and grinding involve lubricants and thus natural oils are preferable ([Choudhury & Muaz, 2020](#)).

13.2.2 Chemistry of food-grade lubricants

Food-grade lubricants are made up of base stocks and additives, similar to other manufactured lubricants. Food-grade lubricants are formulated from various chemical base stocks and additives depending on their different fields of application, considering which equipment and in which area they are used whether for slow or fast running machinery, at high, low, or changing temperatures, under high pressure, and under high humidity. [Fig. 13.1](#) exhibits an example of chemical modifications of vegetable oils to obtain biolubricants.

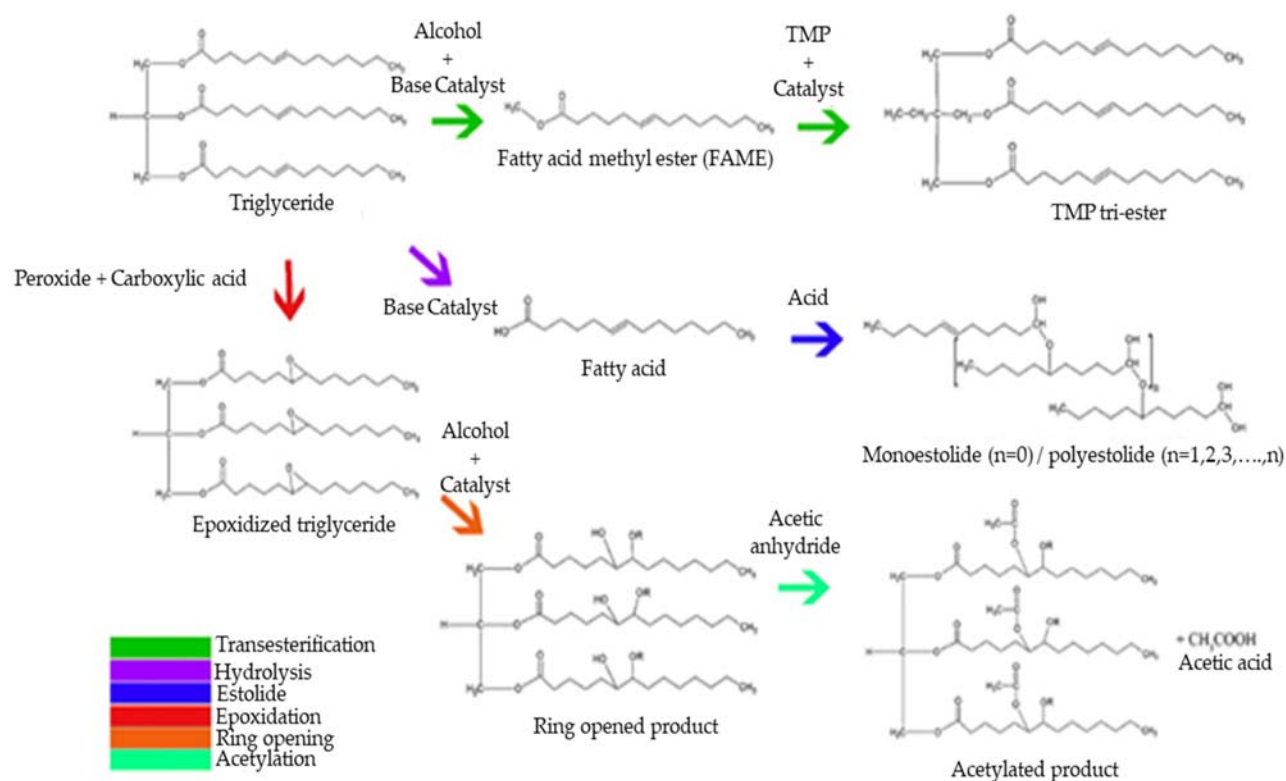


FIGURE 13.1 Chemical modifications of vegetable oils to obtain biolubricants. Adapted from Cecilia, J. A., Ballesteros Plata, D., Alves Saboya, R. M., Tavares de Luna, F. M., Cavalcante, C. L. Jr., Rodríguez-Castellón, E. (2020). An overview of the biolubricant production process: Challenges and future perspectives. Processes, 8, 257.

13.2.3 Evolution of food-grade lubricants

At the beginning of industrial food production and process plants, output was rather small as compared to today. To date, food and beverage manufacturing and processing lines operate 7 days a week, 24 hours a day, and production speed is pushed to the utmost to maximize profits. Machine breakdowns and repairs are costly; thus downtime must be maintained to a minimum. The use of the proper lubricants is critical in assuring food safety and extending the life of machinery and parts to keep repair expenses as low as possible. Early food-grade lubricants were made based on edible oils and fats, but their limitations were quickly recognized. Edible oils degrade quickly; at low temperatures, they solidify, smoke when heated, and polymerize when maintained at high temperatures (Gomna, N'Tsoukpoe, Le Pierres, & Coulibaly, 2021). Machinery requires lubricants that provide better performance while also being able to withstand harsh circumstances. As a result, additives were added to lubricants of various chemical bases to meet specific requirements (Ali, Takhakh, & Waily, 2022).

13.2.4 Example of base materials for food-grade lubricants

Many different substances can be used to lubricate a surface. The most prevalent are oil and grease. Grease is made up of oil and a thickening agent to achieve its consistency, with the oil providing the lubrication. Oils might be synthetic, vegetable, mineral, or a combination of the three. The application dictates which oil should be used, often known as the base oil. Synthetic oils can be effective in extreme situations. Vegetable base oils can be used where the environment is a concern. Other examples of base materials can be:

- mineral/white oil,
- PAO,
- esters,
- polyalkylene glycol/polyglycol,
- silicone oil,

- perfluoro polyether, and
- edible oils and fats (e.g., lard, soybean oil).

To improve the performance of the formulated product, some base materials can be mixed with each other.

13.2.5 Additives for base materials food-grade lubricants

Oil-based lubricants contain additives that enhance, add, or suppress the qualities of the basic oil. The number of additives used is determined on the type of oil and the intended application. Dr. Thomas Klein, a technical manager at LANXESS Deutschland GmbH in Mannheim, Germany, lists the eight main ingredient types utilized in food-grade lubricant formulations ([Canter, 2017](#)).

Antioxidants. Due to heat exposure, incidental food lubricants are subjected to oxidative stress in the same way as conventional lubricants are (up to 180°C). Furthermore, approved hydrocarbon base oils have no aromatic component, limiting solubility and resulting in low necessary treat rates due to high saturation. There are many antioxidants available for incidental food lubricants.

Antiwear additives. One additive available is a salt of an alkylammonium phosphate ester that also provides some rust prevention. The maximum treat rate for this additive is 0.5%.

Extreme pressure additives. Triphenylphosphorothionate, which includes just “inactive” sulfur, is the most extensively utilized member of this additive class. Because heavy-duty lubricants are rarely required in food industry applications, the lubricant industry can cope with these constraints.

Corrosion inhibitors. To deal with high-moisture situations, rust and corrosion inhibitors or metal passivators are required. Salts of alkylammonium phosphate esters and oleyl sarcosine are available as additives. Both varieties meet food contact regulations up to a 0.5% concentration.

Thickeners. This additive class is needed to enable food-grade lubricants with varying viscosity grades to be prepared. Typical thickeners are polyisobutylenes for mineral oil-based lubricants.

Tackifiers. Tackifiers are required in food-grade lubricant applications such as chain oils to increase lubricant adhesion to surfaces. High-molecular-weight polybutene tackifiers are the only HX-1 additives available.

Pour point depressants. Maleic anhydride and styrene copolymers are very effective at lowering the pour point of technical white mineral oils. They are required in applications where food is prepared at low, controlled temperatures.

Defoamers. Foam can be an issue in food manufacturing, which means that defoamers are needed to suppress it. Silicon defoamers such as polydimethylsiloxanes are approved for incidental food contact because of their low surface tension, water insolubility, thermal stability, and chemical inertness. Defoamer molecular weights must be more than 2000 g per mole, and they are highly effective in all types of approved base stocks.

The only problem with additives is that they can be depleted, and to restore them back to sufficient levels, generally the oil volume must be replaced.

13.3 Viscosity measurement of food-grade lubricants

13.3.1 Reasons for viscosity measurement

There are several reasons on why viscosity measurement is essential/required ([Anton-paar, 2022](#)):

Specific lubricants for specific applications. Food producers choose food-grade lubricants based on their specifications and intended use. A particular viscosity of lubricant is essential to enable reliable and low-wear operation of machines.

Reproducible products. Food-grade lubricant manufacturers must verify that their products have reproducible qualities. So, in addition to lubricant creation, quality control is critical. A viscosity check of the incoming lubricant guarantees that food producers get the right materials in the right quality.

Machinery health. All relevant parts must be thoroughly lubricated to ensure economical and low-wear operation to acquire the best feasible output from the unit. Contamination in the production line, such as water, dust, or debris, alters the lubricant’s characteristics. Wear of the lubricant leads to a lack of lubrication and equipment can be damaged, which leads to cost-intensive downtimes.

Requirement of condition monitoring. Monitoring the in-service lubricant is an essential part of HACCP. The degradation of in-service lubricants due to contamination is also a risk factor regarding health and hygiene (growth of bacteria and other hazardous microorganisms).

TABLE 13.1 Classification of food-grade lubricants (NSF, 2022).

Class/ category	Purpose
H1	Lubricants may be used in applications where incidental food contact may occur. Such incidental contact is limited to 10 parts per million or else the food is considered dangerous for consumption. All information of the lubricant involving base stocks, additives, and thickeners are strictly regulated based on FDA regulation, 21 CFR 178.3570
HT-1	Heat transfer fluids for cooling and heating systems that may come into touch with food or beverages. Regulated according to FDA 21 CFR 178.3570
HX-1	Ingredients for the formulation of H1 food-grade lubricants. Listed in the NSF International and regulated based on FDA 21 CFR 178.3570
HTX-1	Ingredients for the formulation of HT-1 heat transfer fluids
H2	Used for the applications in the production where there is no possibility contact with the food. Generally, most substances used in lubricant formulations are acceptable except for substances that related to toxicology
HT-2	Contrary to HT-1 heat transfer fluids, food contact is not allowed
HTX-2	Ingredients for the formulation of HT-2 heat transfer fluids
H3	Edible oils that are not intended for contact with food. Typically applied for preventing rust from equipment like hooks or trolleys. These types of lubricants need to be removed from surfaces getting in contact with edible products
3 H	Used to prevent food products from sticking on grills or other equipment. This type of category is also regulated according to FDA 21 CFR 178.3570

FDA, Food and Drug Administration; NSF, National Sanitation Foundation.

13.4 Category and classification of food-grade lubricants

Food-grade lubricants are classified according to their likelihood of contact with the food. For the lubricants in food production, National Sanitation Foundation (NSF) International categorized these lubricants in category H (nonfood compounds) as shown in Table 13.1. Apparently, this category can be divided into three main types that are H1, H2, and H3. Selection of these lubricants might be a challenging in the food processing. Therefore understanding the concept for the food-grade lubricant can be starting point for the lubricant selection. All the categories listed by NSF International are shown in Table 13.1.

13.5 Vegetable oil lubricants

Vegetable oils have long been used as lubricating oils as they can be obtained easily from various natural sources. This makes them a prime ingredient of lubricating oils in the 19th century. However, rapid industrialization came with a high requirement of lubricants which then puts pressure on the price and availability of lubricants from vegetable and animal sources. During the second half of the 19th century, mineral oils were successfully prospected and extracted and were started to be used as lubricating oils. This is also due to mineral oils being able to provide various fluids with desirable properties at lower costs.

To date, environmental protection has become very important and the demand for environmentally friendly raw materials and products has increased tremendously which includes lubricants. Vegetable oils lubricants, as mentioned previously, can be obtained from various natural sources, which makes them recognized as fast biodegradable fluid and promising for base oils (Asadauskas, Perez, & Duda, 1996).

Mineral oil-based lubricants have long been used in various kinds of applications since the beginning of industrialization. However, it is found that mineral oils with the same viscosity as those of vegetable or animal-based oils were not as effective in comparison to the latter. This is due to vegetable or animal oils and fats containing properties such as “oiliness” or “lubricity” (Ratoi, Anghel, Bovington, & Spikes, 2000). Oiliness or lubricity of vegetable oils is attributed to their ability to adsorb to the metallic surfaces and to form a firm monolayer (Huang, Tan, Chen, Dong, & Wang, 2003).

13.5.1 Sesame, coconut, and sunflower oils

Many researchers carried out detailed studies on the evaluation of physicochemical, thermal, and tribological properties of sesame oil as a potential agricultural crop base stock for eco-friendly industrial lubricants. Sesame oil was studied and compared with coconut oil, sunflower oil, and a commercially available mineral oil (SAE 20W40). Sesame seeds that were collected from Hooghly, East Midnapore, and West Midnapore districts of West Bengal, India were then extracted into sesame oil. To evaluate the tribological (wear, friction, and lubrication) properties of the oils, the wear scar diameter (WSD) and coefficient of friction (COF) were measured using a four-ball tester for COF according to the ASTM D 5183–05 standard. It was found that sesame oil was more thermally stable from the flash and fire point data, in comparison to the commercially available mineral lubricant SAE 20W40. Since the change in viscosities with temperature was small, all vegetable oils had high viscosity index (VI). Among the mentioned vegetable oils, sesame oil has the highest VI, but it is much lower than that of the mineral oil SAE 20W40. Sesame oil can be used as a commercial lubricant if the VI is further improved with the addition of proper viscosity improvers. Oil with higher VI is much more desirable because it enables the lubricant to provide a more stable lubricating film over a wider temperature range.

Meanwhile, in terms of COF, sesame oil has a lower COF compared to coconut oil and mineral oil SAE 20W40. However, the WSD of SAE 20W40 was much lower than those of vegetable oils, but sesame oil's WSD was less than that of coconut oil and sunflower oils (Woma, Lawal, Abdulrahman, Olutoye, & Ojapah, 2019). The wear can be further reduced by adding proper antiwear additives. In conclusion, sesame oil has excellent thermal and tribological properties, but the enhancements of its oxidative and rheological properties are essential as sesame oil can be considered as a potential agricultural crop base stock for industrial lubricants. It could also become the eco-friendly substitute for mineral oil lubricants.

13.5.2 Advantages and disadvantages of vegetable lubricants

For many years, people have been using vegetable oils and animal fats as lubricants. As environmental awareness has risen over the last 20 years, there has been a resurgence of interest in vegetable oil-based lubricants. In their original condition, vegetable oils can be utilized as lubricants. When it comes to manufacturing and mechanical lubrication, they offer a number of benefits and drawbacks. One of its advantages is that vegetable oils offer significantly greater lubricity than mineral oil. Other than that, it also has high VI. The VI of oil does not decrease that much when exposed to extreme heat and does not rise as when contacted with low heat just like petroleum oils do. Most significantly, vegetable oils are biodegradable which are less harmful generally, sustainable, and can help to minimize the reliance on imported petroleum oils. On the downside of vegetable oils, their original condition are lacking on the appropriate oxidative stability for lubricant applications. If the oil is not stabilized, it will oxidize fast during application in which it will turn thicker and polymerized into a plastic-like consistency. Chemical alteration of vegetable oils by using antioxidants can alleviate this issue, not to mention, they are more expensive too. Besides that, vegetable oils have a high pour point, which means that the oil loses fluidity and does not flow. For this reason, a variety of synthetic oils can be employed. Therefore the inclusion of chemical additives such as pour point suppressants as well as mixing it with other solutions with smaller pour points can all help to solve this issue.

13.6 Animal-derived lubricants

Animal lubricants are derived from the animal's fat, which are hard fats (stearin) and soft fats (lard). Animal fats are mainly used for manufacturing greases. Animal fats make up roughly one-third of all fats and oils produced. Beef tallow, pork lard, and chicken fat are some of the examples. Animal fats are appealing biodiesel feedstocks because they are significantly less expensive than vegetable oil. This is due in part to the fact that the market for animal fat is much smaller than the market for vegetable oil, as much of the animal fat produced in the United States is not considered edible by humans. Animal fat is only available as a by-product of meat processing. As a result, the amount of biolubricant made from animal fat linked through the meat chain cannot be increased. Biodiesel production from animal fat is more difficult and expensive than biodiesel synthesis from vegetable oil. This is mainly due to the presence of salts, phosphorus, sulfur, and some polymers that could not be entirely separated in the previous phase. Animal fats are divided into two types: edible and inedible fats.

Edible fats are made from fresh slaughter by-products that have passed a veterinary inspection and are obtained from well-slaughtered animals. Common edible fats include beef tallow, pork lard, goose, or duck fat. Some other edible fat is produced during the gelatine process. Pork and ruminant bones, pork skins, and split ruminant hide are the

primary sources of edible gelatine. Before they can be used in the gelatine process, these raw materials must be defatted. This fat is a by-product of the country's gelatine industry. On the other hand, inedible fats include all slaughter by-products that are obviously unfit for human consumption, such as feathers, bristles, and horns, as well as material that has been announced unfit for human consumption having followed a veterinary inspection at the slaughterhouse. These include infected meats, injured animals or legs, and meat with hematomas or inflammation. Inedible fats are used in feed, pet food, oleochemical manufacture, biodiesel production, and energy generation ([American Oil Chemist's Society, AOCS, 2022](#)). Since animal fats are highly saturated, they solidify at relatively high temperatures. As a result, biodiesel derived from animal fat has a high cloud point. Animal fat biodiesel, on the other hand, can be blended with petrol–diesel. The high cloud point of the animal fat biodiesel has little effect on the cloud point of the blend at lower blends such as B5 (a blend of 5% biodiesel and 95% petrol–diesel). In general, the saturated fatty acids found in animal fats should enable biodiesel to maintain its oxidative stability. Animal fats are low in polyunsaturated fatty acids like linoleic and linolenic acids, which are responsible for the rancidity of vegetable oils like soybean and linseed oil. However, because vegetable oils often include natural antioxidants, animal fat is not always more stable than vegetable oil in practice ([Gerpen, 2019](#)).

13.7 Comparison between vegetable- and animal-based lubricants

All lubricants made from bio-based raw materials such as plant oils, animal fats, or other environment-friendly hydrocarbons are referred to as biolubricants ([Salih & Salimon, 2022](#)). [Table 13.2](#) discusses the comparison between vegetable- and animal-based lubricants. Vegetable oil-based lubricants have long been known to be renewable and mostly biodegradable, making them a promising alternative to conventional lubricants ([Aluyor, Obahiagbon, & Ori-Jesu, 2009](#); [Syahir et al., 2017](#)). However, their direct application as base oils are limited due to low oxidative, thermal, and hydrolytic stability, poor low-temperature characteristics, and a limited range of viscosities. Chemical modification, formulation with additives, and blending with mineral oils can all help to overcome these constraints, but these increases cost,

TABLE 13.2 Differences between vegetable- and animal-based lubricants.

Parameters	Vegetable lubricant	Animal lubricant
Derived from	Soybean oil, palm oil	Lard oil, tallows oil
Advantages	1) High VI when compared to petroleum-based lubricants. For instance, soybean oil has a VI of 233 and most petroleum oils have a VI of 90–100 range. 2) When heat is introduced to these lubricants, vegetable oil-based lubricants can still sustain their high viscosity and it will not reduce as much as petroleum-based lubricants.	1) Good characteristics of wetting that will improve surface finish and can maintain a significant lubrication film. In addition, it improves lubricity in cutting oils. 2) Tallow and lanolin, similar to cosmoline, were once used as preservatives. Lanolin is a waxy substance that is produced naturally from the wool of the sheep as a barrier of protection. It is biodegradable, nontoxic, nonconductive and does not evaporate.
Disadvantages	Vegetable oil-based lubricants have a higher flashpoint which makes it more desirable to make sure the lubricants are less flammable. For example, the flashpoint for soybean oil is 326°C while the flashpoint for mineral oils is around 200°C.	Animal oil has lower oxidation stability than mineral oil. As the animal oil deteriorates, sludge is formed, and the vicious animal oil adheres to the equipment which then necessitates time-consuming cleaning.
Methods/production process	1) Involved the process of transesterification whereby it involved the reaction of triglyceride molecules with three moles of methanol which produced mixtures of glycerol and fatty acids. 2) Could be modified by altering the carbonyl groups and fatty acid chains that are supported by catalysts. Processes such as selective dehydrogenation and dimerization are possible in producing the vegetable oils.	1) Epoxidizing the fat or oil and reacting the epoxidized fat or oil with a carboxylic acid anhydride in the presence of a basic catalyst to produce a diester. 2) Hydrogenating the epoxidized fat or oil to generate mono-alcohols. 3) Acylating the alcohol functionality with acid anhydrides, acid chlorides, or carboxylic acids to produce a mono-ester.

VI, Viscosity index.

Source: Adapted from [Salih and Salimon \(2022\)](#); [Cecilia et al. \(2020\)](#); [Woma et al. \(2019\)](#); [Annisa and Widayat \(2018\)](#).

toxicity and jeopardizes biodegradability. Hence, producing a cost-effective bio-based lubricant with revolutionary combination of biodegradability and high lubricant characteristics is a big challenge (Liu, Sadula, & Vlachos, 2019).

As far as halal manufacturing facilities are concerned, vegetable oil-based lubricants are preferable compared to animal-based biolubricants. Mineral lubricants, which are primarily petroleum-based (Zainal, Zulkifli, Gulzar, & Masjuki, 2018) and are the most common lubricants, are permitted to have halal status. Plant-based oils or animal-based fats are used to make natural lubricants (Syahir, Zulkifli, Masjuki, Kalam, Alabdulkarem, Gulzar et al., 2017). Plant-based lubricants are halal; however, animal-based fats or tallow, depending on the animal source, are very crucial. Synthetic lubricants, such as hydrogenated polyolefins, esters, silicones, and fluorocarbons, are considered halal (Chang et al., 2015). Lubricants are considered halal if the manufacturing process adheres to halalan tayyiban criteria and is categorized as H1 food-grade lubricant (Alzeer, Rieder, & Abou Hadeed, 2020).

In the United States, it is noteworthy that 100% pure vegetable oil can legally contain up to 0.1% additional ingredient, which do not have to be plant-derived, allowing animal products to be used. However, because the vegetable oil and animal oil systems in the United States are kept distinct, the use of an animal-based addition is not considered standard because it would contaminate the entire vegetable oil system. The situation in other countries, on the other hand, would have to be carefully examined (Al-Mazeedi et al., 2013). It is also interesting to note that there is also a growing trend on halal-certified food-grade lubricants. Halal food-grade lubricants are manufactured in Asia, Europe, and America (Lubesngreases, 2016). Islamic Food and Nutrition Council of America and Majelis Ulama Indonesia have certified a number of food-grade lubricants (Lubesngreases, 2016; Pertamina, 2017) in the United States and Indonesia, respectively.

13.8 Conclusion

Biolubricants are defined generically as products derived from biodegradable and renewable resources. Compared to mineral oils, bio-based lubricants have a high lubricity, a high flashpoint, a high VI, and superior shear resistance. Vegetable oil is any oil derived from plants, such as canola, maize, soybean, and sunflower oils. Meanwhile, animal fats and oils are derived mostly from lard, tallow, and butterfat, whereas fish oils are derived from cod liver oil, whale oil, and salmon oil. These lubricants are nontoxic and extremely biodegradable, making them appropriate for use in food manufacturing operations. Both of these lubricants are less hazardous than mineral oil-based lubricants. Lubricants are used in a wide variety of applications but are most frequently used to reduce friction between two mechanical parts, primarily to reduce wear and deterioration of metal surfaces, as well as to ensure smooth operation and the absence of contaminants that could jeopardize the machineries or the products. Significantly, five critical factors for selecting the most convenient lubricant for use in a food operation are potential risk, material compatibility, lubricant type, consideration of base stocks, and contamination. In conclusion, based on the comparison table that is shown previously, the number of advantages of using vegetable lubricants outshines that of using animal-derived lubricants. Vegetable lubricants are biodegradable, renewable, and less toxic. It also has an excellent lubrication, high flash points, high VI, low evaporative loss, and high smoke point. These are the vital considerations when using vegetable lubricant in the food processing. Vegetable lubricants also have low oxidation stability and pour point. This is due to the presence of bis-allylic protons. Due to the great susceptibility of these active sites to radical assault, the molecules suffer oxidative breakdown, resulting in the formation of polar oxy compounds. This process finally leads to the formation of insoluble deposits and an increase in the acidity and viscosity of the oil (Erhan, Sharma, & Perez, 2006). At the end of the day, in choosing which type of lubricant that is to be used in the food processing machinery, there are many aspects that can be taken into consideration as mentioned earlier. After all, both of the lubricants are safe and are not harmful to the consumers, especially in processed food.

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Halal packaging: halal control point in manufacturing of packaging materials and halal labeling

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14.1 Introduction

In food technology, packaging plays a significant role not only in the containment, protection, and preservation of products but also serves in providing convenience and communication functions. Packaging also acts as a “silent salesman” by enticing consumers to purchase the product through its attractive shape, design, and colorful printing. Various information are printed on the outer surface of the package either to fulfill the legislative requirements or to provide instructions and additional knowledge to consumers. The convenience features such as easy opening, drinking spout, and boil-in-bag are also an added value to food packaging. With technology advancements, the common passive packaging is now upgraded to active and intelligent packaging with higher functionality and utility features such as antimicrobial and antioxidant packaging, gas and moisture absorbers, gas-controlled or -release regulator, quality, and freshness indicators.

In transport and logistics, the role of packaging is to contain and protect the goods during delivery and distribution thus eliminating or minimizing the chances for damages, spoilage, misplacement, or theft throughout the supply chain activities (Ab Talib & Mohd Johan, 2012). From the halal perspective, the packaging is expected to protect the product from any cross-contamination from nonhalal substances by protecting the product from any damages that may jeopardize the integrity of the halal status. The tracking of halal goods during transportation, logistics, and along the supply chain is required for traceability procedures in halal certification (Mohamed, Rahim, Ma'ram, & Hamza, 2016). Besides the batch number and bar codes, special devices such as radio frequency identification tags and global positioning system placed on or embedded in the product packaging will make tracking and recalling products become easier. During distribution, transportation, warehousing, and retail, the halal goods must be segregated between halal and non-halal products. For transportation a dedicated container or carrier must be used to carry a halal product. However, if the volume of halal products is small and requires container sharing, a clear separation between halal and nonhalal products needs to be set up by allocating a different section or compartment for each product (Ngah, Zainuddin, & Thurasamy, 2014). In this situation, tertiary and quaternary packaging may serve as a stern physical barrier as to provide the segregation needed during the transportation and storage of halal products.

Halal product identification is based on the halal logo printed on the packaging of the product. For food and beverages, the requirements for halal certification are focused on the halal sources of the raw materials and processing tools used to produce the product. To ensure that no cross-contamination occurs, a proper layout of the processing line with dedicated equipment, utensils, and storage is required. The sanitation and hygiene practices of the factory and among workers are scheduled and conducted diligently as well as the rights of the Muslim workers to perform their prayer must not be neglected (Malaysian Standard (MS1500), 2019; Malaysian Standard (MS2565), 2014). The use of a halal-certified logistic company in distributing the halal-certified product will be a transcendent approach for the food manufacturer in ensuring halal integrity is preserved.

It is expected that if the products produced are halal, hence the packaging used should be halal too. The possibility of cross-contamination originating from the packaging materials is known but not much attention has been made to the

issue. The raw materials, processing aids, additives, and lubricants used in the production of packaging materials can be a source of the Shariah noncompliance. The migration and leaching of toxic, carcinogenic, or mutagenic compounds from packaging materials is the *Thayyib* noncompliance that needs to be considered in selecting the packaging material suitable for halal products. To fulfill the *Thayyib* requirements, the safety characteristic of the packaging material in contact with food needs to be as inert as possible with no or minimum chemical interactions between food and packaging (Dainelli, Gontard, Spyropoulos, Zondervan-van den Beuken, & Tobback, 2008).

In 2012 a food testing laboratory (Genetic ID Europe) located in Augsburg, Germany, detected porcine contamination derived from a food package (Packaging Today, 2012). Based on thousands of polymerase chain reaction tests that were tested on halal foods, the laboratory found positive traces of porcine DNA in several samples that were traced back to stearates or lubricants from the machinery used in paper packaging production. Bill Thompson, the CEO of Genetic ID, had urged the halal food producers to be vigilant about the issue and insist on rigorous testing and certification, including the packaging. In other cases, porcine DNA has been detected contained in Cadbury chocolate produced by a Malaysian company; however, the company has disputed the result (Yu & Chan, 2020). Recently as reported by Khan and Khan (2017), Skittles Wild Berry Flavor Chocolate and Cadbury Milk Tray Chocolate have been tested positive for porcine DNA in Bangladesh. This issue has raised a perplexity on the halal-certified product (chocolate) that is contaminated with pork or its derivatives even though all the ingredients used were confirmed to be from halal sources. Therefore the most possible source of contamination is from the packaging itself as oils or lubricants that are derived from pigs are being used in the packaging production (Khan & Khan, 2017).

In Malaysia the certification of *halal* goods is overseen by a centralized government organization known as JAKIM (*Jabatan Agama dan Kemajuan Islam Malaysia*). On the other hand, a standard for halal packaging, namely, MS2565 that describes the general guidelines in the manufacturing and handling of halal packaging was established in 2014. Some of the requirements stated in the guidelines related to halal packaging are as follows:

1. Do not contain any parts or products of animals that are nonhalal by Shariah law or any parts or products of animals which are not slaughtered according to Shariah law.
2. Do not contain najis according to Shariah law.
3. Safe for human use, nonpoisonous, nonintoxicating, or nonhazardous to health.
4. For direct food contact application, it shall not be made from recycled material.
5. Not prepared, processed, or manufactured using equipment contaminated with najis according to Shariah law.
6. Do not contain any human parts or its derivatives that are not permitted by Shariah law.
7. During its preparation, processing, handling, packaging, storage, and distribution, the packaging material is physically separated from any other products that do not meet the requirements stated in items (1)–(6) or any other items that have been decreed as nonhalal and najis by Shariah law.

To certify the packaging material, the halal auditor must have a certain knowledge of the manufacturing process of packaging material. A halal auditor or inspector who has no technical background will face some difficulties in understanding and identifying the sources of noncompliance during halal inspection of the packaging manufacturing premise. Therefore adequate information and knowledge must be provided and equipped to the halal auditor. This chapter aims to provide readers with essential knowledge and information on the manufacturing process of packaging materials. The production of four main packaging materials namely paper, glass, metal, and plastics is briefly explained as well as the potential sources of halal noncompliance are also pointed out. The halal control point (HCP) is therefore proposed at a certain processing step based on the possibility of Shariah's or *Thayyib*'s noncompliance-related issues.

14.2 Halal control point for paper manufacturing

The main component used in the manufacturing of paper, paperboard, corrugated board, and similar manufactured products is plant fiber or cellulose that is derived from wood or other sources of fiber such as bamboo, bagasse, kenaf, cotton, rice/wheat straw, grass, and jute. Taking into consideration that the origin of main ingredients used in paper manufacturing is derived from plants, no potential halal issues may emerge at this point. The conversion of plant fiber or cellulose into paper is a simple process that involves disintegration and removal of noncellulosic materials, cellulose refining, pressing, and drying of the paper pulp. However, plain virgin paper has many weaknesses and is insufficient to meet the packaging requirements for food products. Virgin paper has poor barrier properties toward moisture, gasses, and macro- and microorganisms with low heat sealability and low mechanical strength (Robertson, 2013). Therefore the characteristic of this paper needs to be improved to enhance its functional properties.

Figs. 14.1 and 14.2 show the process implies in paper manufacturing that can be divided into two stages, the pulping process (Stage 1) and the papermaking process (Stage 2). Each stage is normally conducted at a separate facility and different locations. The potential source of halal noncompliance is stated as HCP at a certain processing step.

In pulping process, wood logs or other sources of fiber will be converted into stock pulp that comprises cellulose fiber. The process can be accomplished mechanically or chemically or with the combination of both. Mechanical pulping is conducted by forcing wood against a rapidly revolving grindstone, while chemical pulping uses sodium hydroxide or sodium sulfate, or a combination of calcium bisulfite and sulfurous acid (Robertson, 2013) to separate the fibers from wood. The pulp is then subjected to the digestion process to remove unwanted components such as lignin and other extractives. The process is conducted in a pressurized vessel under controlled time, chemical concentration, and temperature. After that, the pulp undergoes a bleaching process to enhance the pulp brightness and remove all traces of noncellulosic material. Certain paper products such as brown paper and paper bags are produced without bleaching. In the final steps of Stage 1, the cellulose fiber is dried and compacted as dried stock pulp and transferred to the paper-making facility.

HCP 1 is proposed at the bleaching step because of the chemicals used in the process. Chlorine bleaching was identified as the major source of polychlorinated dioxins, dibenzofurans, and absorbable organic halides which are powerful

Sources of plant fibre
(Wood logs, wood chips, other
plants source)

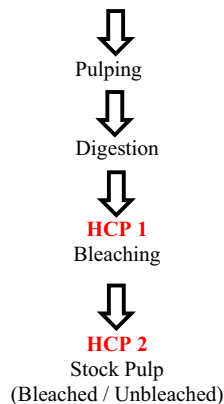


FIGURE 14.1 Proposed HCP of pulping process in paper manufacturing (Stage 1). *HCP*, halal control point.

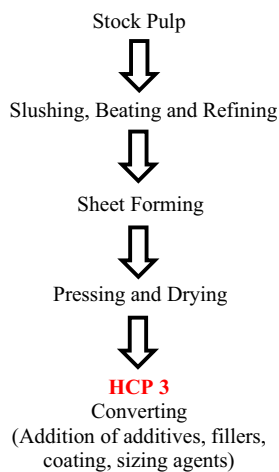


FIGURE 14.2 Proposed HCP of papermaking process in paper manufacturing (Stage 2). *HCP*, halal control point.

Paper Products
(Paper bag, carton, box, corrugated board)

toxins and carcinogens (Robertson, 2013). Nevertheless, in some countries, strict regulations have limited the production of these compounds and the method was replaced with elemental chlorine-free bleaching or total chlorine-free bleaching techniques (Bajpai, 2012). HCP 2 is suggested for stock pulp quality inspection as to ensure there is no cross-contamination contributed by lubricant oils from machinery used in the production line that is Shariah noncompliance. Most of the heavy machinery is oiled or lubricated using a cheap source of animal-derived oils. Oils that are derived from nonhalal or unslaughtered animals are categorized under Shariah noncompliance. The oil that transmits from the packaging material may emanate a concern since the oils can effectively infiltrate the food inside the package.

The papermaking process consists of repulping, beating, and refining which will diminish the fiber size and consequently alters the density, strength, transparency, and glassiness of the paper. The fine cellulose fiber will then undergo a series of presses and dryers to form paper sheets. Chemicals such as additives, adhesives, mineral fillers, and sizing agents may be added at the beater stage prior to sheet formation or the resulting sheet. The incorporation of additives is commonly conducted at the converting step as the paper sheet is transformed into desired shapes and sizes such as bags and boxes. Alternatively, multiply paperboards are produced by the consolidation of one or more sheets of liner or corrugated paper into a single layer using adhesives and subsequently converted into rigid boxes, folding cartons, beverage cartons, and similar products.

HCP 3 is proposed at the converting step due to the various additives used. At this point, halal inspection is based on the health hazard issue. The types of additives incorporated into paper sheets will rely on the final paper properties desired. Calcium carbonate, kaolin clay, and titanium dioxide are examples of fillers used. Starches, glues, alums, caseins, synthetic resins, and cellulose derivatives are the sizing agents used to render the sheet to become more impermeable to moisture, improve appearance, barrier properties, and paper strength (Dulany, Batten, Peck, & Farley, 2011; Robertson, 2013). Mineral oils, dyes (organic, inorganic, and synthetic), phthalates, adipates, and polyfluorinated surfactant (PFS) substances (Fierens et al., 2012) are among the major additive that migrates from paper packaging. PFS substances are the most widely used additive to impart oil and serve as water resistance to paper and paperboard. The PFS, including their oxidation products, is toxic and able to cause endocrine disruption (Trier, Granby, & Christensen, 2011).

HCP 4 is recommended as the final quality inspection of halal-certified packaging before going through the delivery process to food manufacturers. Similar to HCP 2 in the pulping process, the sources of oils used as machine lubricants must be Shariah compliance so that cross-contamination can be avoided. The halal aspects at HCP 4 are also related to the migration or leaching of printing ink and adhesive compounds.

In general, printing inks are made up of three major ingredients: pigments, binders or resins, and carriers. Oils and fats are often used as carriers or vehicles for oil-based inks. Lard or lard blended with other oils is commonly used to reduce the cost of printing ink production (Ramli et al., 2015). The ingredients used in making printing ink can be doubtful. A study by Ramli et al. (2015) has reported on the detection of lard retrieved from ink extracted from printed food packaging. Besides that, chemicals in printing inks could lead to kidney failure, endocrine disruption, and lung cancer (Deshwal, Panjagari, & Alam, 2019; Muncke, 2011). On the other hand, bisphenol S (BPS), a structural analog from bisphenol A (BPA) often used as a color developer on thermal receipt paper attached to the food packaging in retail food stores, is another endocrine disruptor (Pivnenko, Laner, & Astrup, 2018). Adhesives are commonly used to seal a folding carton into its shape and laminate the paper with other packaging materials. Presently, starch- and casein-based adhesives, natural rubber latex, polyvinyl alcohol emulsion, petroleum wax in combination with polymers, and tackifying resin are also used as adhesives (Robertson, 2013). These toxic chemicals' migration may leach into food products and cause health hazards in humans. Therefore if the paper packaging is to be certified as halal, the presence of these chemicals needs to be inspected and confirmed. Currently, most of the countries have their own regulations or legislation on the levels of permitted amounts for these chemicals; hence these guidelines can be used as a reference for halal inspection.

Recycled paper is sometimes added together with stock pulp in the papermaking stage. Various unidentifiable contaminants are present in the cellulose matrix thus they cannot be totally removed during the recycling process (Biedermann & Grob, 2010). There is also a possibility that recycled paper packaging may contain pork residues from its past uses. However, most of the countries have strictly disallowed the use of recycled paper that previously have direct contact with the food product for packaging applications. Based on this, the use of recycled paper as part of the ingredients in paper manufacturing should be prohibited if the company is to be halal certified.

14.3 Halal control point for glass manufacturing

Based on its copious favorable properties, glass is the best packaging material to preserve food quality. Glass is transparent, tough, chemically inert; is resistant to airborne and chemical contaminants; and does not affect the flavor or

odor of the product contained in it. Due to these properties, most of the baby foods are packed in a glass bottle. Glass is also a sustainable material since it can be reused and recycled infinite times.

The two main ingredients that are generally used in making soda–lime glass containers are silica derived from sand (SiO_2) as well as recycled or scrap glass called cullet. Other materials that are commonly used together are soda ash (Na_2CO_3), aragonite (CaCO_3), feldspar ($\text{SiO}_2\text{-Al}_2\text{O}_3$), and salt cake (NaCl). Supplementary materials such as lead, alumina, and boron are added to enhance glass's physical and mechanical properties while metallic oxides (e.g., cobalt, nickel, chromium, and iron), sulfides, and selenides are used to improve the glass optic properties (Robertson, 2013).

The glass making process involves mixing and melting the ingredients in a furnace at 1500°C . At this stage, molten glass is produced and it is cool to 1100°C in a refiner. A certain mass of molten glass called gob is then converted into the shape of a bottle or jar in a forming or molding process. After that, the formed glasses are subjected to an annealing process to remove stress points and to prevent the glass from becoming brittle. Finally, the annealed glass will undergo two surface treatments (hot-end and cold-end) to enhance its mechanical properties. In hot-end treatment, the external surface of the glass container is sprayed with a vapor containing tin or titanium in the form of a thin layer of metal oxide. The purpose is to avoid any damage to the glass surface and to improve the adhesion of the subsequent cold-end coating. Cold-end treatment involves the application of waxes, stearates, silicones, oleic acid, polyesters waxes, or polyethylene. It is done to enhance lubricity by providing a surface with a low coefficient of friction and aids the flow of containers through a high-speed filling line (Robertson, 2013).

Fig. 14.3 shows the manufacturing of soda–lime glass. In this process, two HCPs are proposed, at the cold-end treatment and the finished glass steps. For HCP 1 the origin of oils used is doubted whether it is from animal- or plant-based oils. Similarly, for HCP 2, oils are commonly applied to lubricate the heavy machinery used in the manufacturing line. Therefore any cross-contamination from the machinery lubricant should be inspected at the finishing line before packing and shipping to food manufacturers.

The use of recycled glass or cullet in glass manufacturing is to reduce the amount of new raw ingredients and to save energy costs since recycled glass melts at a lower temperature. The source of recycled glass comes from two sources; a rejected glass during manufacturing and glass containers collected from the recycling facilities. The recycling process of the used glass container consists of (1) sorting according to color and removal of any nonglass materials such as caps, closures, and labels followed by (2) thorough washing and cleaning to ensure no adhering product leftover and finally (3) breaking down into smaller glass particles or cullet.

There are no halal issues with regards to cullet usage even though the container glass was previously used to store nonhalal items such as alcohol or pork meat. Washing and cleaning will fully remove all liquids or solids while the subsequent melting at high temperatures will totally change (*istihālah kamilah*) any *mughallazah najs* into new material. However, the halal issues may arise for reusable container glass. In some beverages company, glass bottles are collected, washed, and filled up with a new beverage. According to *Mazhab Shafie*, alcoholic beverage bottles that are fully cleaned from any adhering solid or liquids are considered pure and can be used to contain new beverages (JAKIM, 2012). For any type of containers that are previously used for pork or any *mughallazah najs*, it must be

Silica sand, cullet, soda ash, aragonite, feldspar, salt cake, etc.

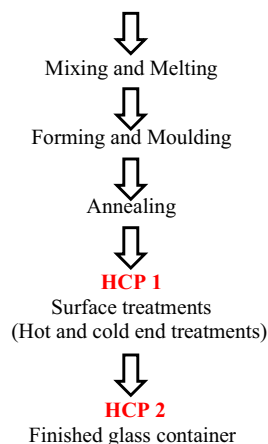


FIGURE 14.3 Propose HCP of glass manufacturing. HCP, halal control point.

washed seven times and one of these cleanings should include cleaning with soil (*sertu* procedure) before it can be used to store halal products.

14.3.1 Halal issue of ceramic manufacturing (bone china)

Another type of glass is ceramic which is widely used for making household utensils. The manufacturing of ceramics is different from glass. In general, ceramics is made from kaolin clay which is fired or baked in a kiln to achieve hardness (vitrify) and to maintain its form. A special ceramic such as bone china uses the animal bone as one of its main raw materials thus raising concerns over the halal issue (Mohd Salleh & Mohd Subri, 2018; Zulkarnian, Noor Ashraf, & Shamsbury, 2018; Rosli, 2021).

Bone china is high-grade porcelain made with 30%–50% animal bone ash mixed with feldspar and kaolin clay. The addition of bone ash contributes to its unique quality which is milky white and translucent with high mechanical and physical strength compared to the other types of ceramics. The halal issue arises the concerns on the sources of animal bone whether it is derived from halal animals slaughtered according to Shariah law or from unslaughtered halal animals or even from pig bones. Most of the bone china manufacturers such as Noritake, New Zealand, Wedgwood-Royal Doulton, United Kingdom, Narumi, Japan, Royal Porcelain, Thailand, Royal Crown-Derby and William-Edwards, United Kingdom, and many other uses cow or cattle bones as their ingredients (Mohd Salleh & Mohd Subri, 2018). Having said that, there might be some possibilities for the presence of pig bones in the ceramic since some of the manufacturers purchase the animal bone ashes from different suppliers or subsuppliers. The feasibility of mixing different animal bones may also occur during the burning or ashing process using similar furnace.

There are differences in views between *mazhabs* on halal and haram status of bone China products. The only agreement on the use of halal animal bones is only from the animal that has been slaughtered according to the Shariah law. Islamic scholars have split views in regard to the use of animal carcasses from unslaughtered animals due to their different interpretations of Shariah's arguments based on the *Qur'an*, *hadith*, *qiyās*, and *maslahah*. According to the theory of transformation of animal bones through burning (*istihālah bi al-ihrāq*), bones from unslaughtered halal animals are permissible and the decision is accepted by all *mazhabs*. However, the *istihālah* concept was not entirely accepted in the case of pig bones. For *mazhabs Shafi'i* and *Hanbali*, pig bone ash is still deemed as Shariah noncompliance even after ashing (cleansing method) since the origin is from *najs* source. However, *mazhabs Hanafi* and *Maliki* accepted the concept of *istihālah* and therefore allowed the use of bone china products (Mohd Salleh & Mohd Subri, 2018).

14.4 Halal control point for metal manufacturing

Metal containers have been long established as the packaging material of many food products due to their great versatility and excellent barrier properties. The metal container is impermeable to light, moisture, and gasses have high mechanical strength, provides a hermetic seal, opacity, and thermal conductivity, and also is lightweight and recyclable (Deshwal & Panjagari, 2020). The common metal containers used for food packaging applications are tin-plated steel, tin-free steel, polymer-coated steel, stainless steel, and aluminum. Below are brief properties of each metal container:

1. Tin-plated steel has a tin coating on both sides of the base steel that acts as a corrosion-resistant surface and provides a bright shiny appearance.
2. Tin-free steel also known as electrolytically chromium/chromium oxide-coated steel (ECCS) consists of a duplex coating of metallic chromium and chromium sesquioxide as a corrosion barrier. The surface of ECCS is less resistant to corrosion but more acceptable for protective lacquer coatings, printing inks, and varnishes than tin-plated steel.
3. Polymer-coated steel is used in cooking utensils. The famous food-safe polymer coating is polytetrafluoroethylene (Teflon) and fluorinated ethylene propylene. Polymer coating provides excellent adhesion to the base steel with greater corrosion and abrasion resistance as well as nonstick property.
4. Stainless steel is an iron alloy with a high chromium concentration that provides excellent corrosion resistance and chemical inertness. Most equipment and utensils used in food factories and food premises use stainless steel for easy cleaning and a bright appearance.
5. Aluminum is used in the form of purified alloys containing small amounts of various metals such as silicon, iron, copper, manganese, magnesium, chromium, zinc, and titanium to provide strength and improve formability and corrosion resistance. Nowadays, most of beverage cans are fabricated using aluminum alloy and thin aluminum foil is used as a common wrapping material in most households.

14.4.1 Coated-steel manufacturing

For all types of metal containers except for aluminum, the manufacturing of metal cans begins with the formation of base steel from the earth's iron ores. Fig. 14.4 shows the simplified flowchart of coated steel manufacturing.

The mixture of raw materials used in steelmaking is iron ores (hematite, Fe_2O_3 , and magnetite Fe_3O_4), solid fuel (coke), alkali fluxes (limestone and dolomite), and some scrap metals (Robertson, 2013). There is a possibility of *najis muhallazah* presence in the earth during iron ores extraction in the form of animal carcasses. This circumstance is identical to the case of animal bones in bone china products; therefore the same Fatwa decision should apply. Another consideration related to this issue is the high purity of the carbon steel plate obtained. The iron alloys used for food packaging comprise carbon content not exceeding 1% (Lee, Yam, & Piergiovanni, 2008), while other impurities such as metal oxides need to be removed as a slag (by-products).

The process begins with mixing and melting the raw materials in the blast furnace to reduce iron oxides to metallic iron and other metal elements such as carbon, silicon, and manganese. The metallic iron is then cast into ingots before undergoing a rolling process to form a thick slab. A series of hot rolling and cold rolling are conducted to obtain an iron slab with the desired thickness. The black-colored scale formed due to the hot rolling processes is then removed in the pickling process using 10%–15% sulfuric acid near its boiling point (Robertson, 2013). At this point, the safety issue of the use of acid and its storage conditions are considered an HCP 1 that must be inspected. After the pickling process, oiling is done on the iron strip as to prevent the formation of rust and at the same time, it acts as a lubricant in the subsequent process. The Shariah compliance on the oil used at this step is proposed as HCP 2. To stabilize the structure of iron ferrite in steel, the iron coils are placed in an oven for annealing process. The steel plate is then subjected to light cold rolling to impart springiness and desired surface finish. The uncoated steel plate produced at this stage is the base steel plate that will be coated with tin, chromium or polymer in the following process.

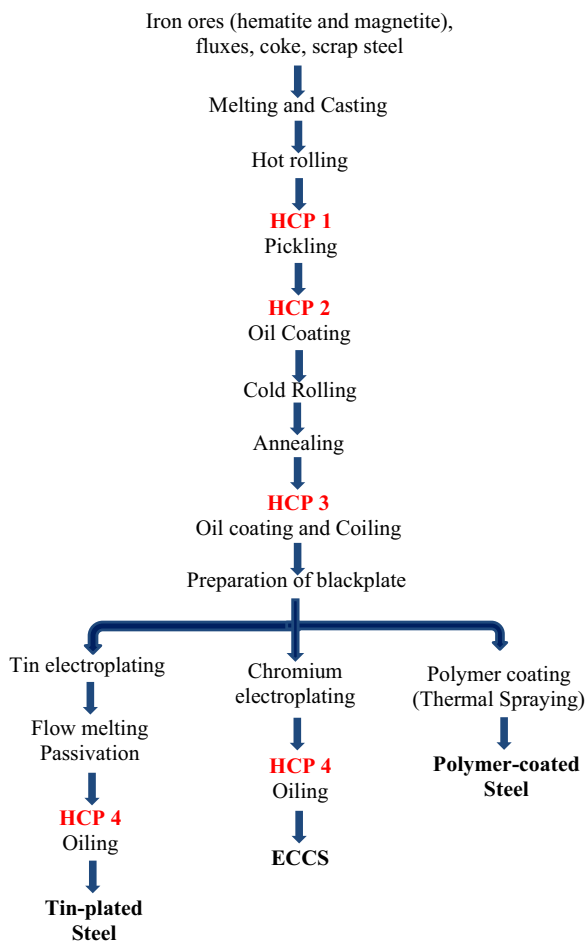


FIGURE 14.4 Proposed HCP of coated steel manufacturing. *HCP*, halal control point.

To prepare the steel-based for coating, the oiled base steel will undergo degreasing, pickling, and washing process to clean off any adhering substances and to ensure uniformity of the coating material. For tin-coated steel and ECCS, the coating is conducted using an electrolytic process. After electroplating (including flow-melting and passivation for tin-coated steel) is completed, the coated plates are lightly oiled to protect the electroplated layer and to ease the passage of the steel sheets through container-forming machines (Robertson, 2013). For polymer-coated steel, synthetic fluoropolymer (polyvinylidene fluoride, ethylene chlorotrifluoroethylene, perfluoroalkoxy alkane, and fluorinated perfluoroethylene propylene) or polyethylene terephthalate and polypropylene are thermally sprayed to form corrosion resistance and abrasion-resistant layer (Deshwal & Panjagari, 2020).

The type of oils currently use for oiling are dioctyl sebacate (DOS) and acetyl tributyl citrate (ATBC) replacing the previous cottonseed oil. HCP 3 is proposed at this stage because of the oil treatment due to its safety issue. The source of DOS is derived from natural and renewable castor beans while ATBC is produced through esterification and acetylation of citric acid with n-butanol. The halal inspection proposed at HCP 3 is based on the safety limit on the usage of oils used that must be abided by the permitted level depending on the country's regulations. Both DOS and ATBC are approved by European Commission and United States Food and Drug Administration (USFDA) as phthalate substitute plasticizers. The European Food Safety Authority has given ATBC a tolerable daily intake value of 1.0 mg/kg body-weight (Robertson, 2013) and considered it as a toxic only at high concentrations. The toxicity of these plasticizers has been investigated by Johnson (2002) before and the results obtained were contradicted. From this study, it showed that ATBC was not associated with any significant reproductive toxicity in mice. However, a study by Takeshita et al. (2011) reported that ATBC must be used cautiously as it may alter the metabolism of endogenous steroid hormones and prescription drugs at a relatively low concentration. Another study by Rasmussen, Sen, Liu, and Craig (2017) also showed that even at a low dose ATBC is detrimental to the ovary of female reproduction.

It must not be forgotten that other oils from the heavy machinery lubricants might cross-contaminate the tin plate or ECCS. Therefore ensuring no animal-based oils are used as machine lubricant would be easier than inspecting for cross-contamination at the final stage of production (HCP 4). Cross-contamination may also occur through contact between products, tools, and workers. If the use of animal-based oil lubricant is unavoidable, ensuring dedicated tools and workers can eliminate the possibility of transferring the contaminant.

14.4.2 Metal container fabrication

To shape and formed the tin-plated steel, ECCS or aluminum into metal cans for foods and beverages, metal plates must go through another process of cutting and forming. Fig. 14.5 shows the simplified manufacturing flow of metal can fabrication. The large metal plate is first to cut into a smaller plate (blank plate) based on the size of the container to be formed. The internal coating of enamel or lacquer is roller coated or sprayed to prevent interaction between the can and its contents. In some facilities, enamel coating is conducted after the can was shaped into container form.

Many types of internal enamel are used for food containers, including oleoresinous, vinyl, vinyl organosol, acrylic, polybutadiene, phenolic, and epoxy phenolic (Taub & Singh, 1997). The usage of oleochemicals in coating or enamel processing is an issue of Shariah compliance; therefore HCP 2 is proposed. Oleic acid is a fatty acid found in a variety of animal and vegetable fats and oils. The purpose of enamel or lacquer coating applied on metal cans is to slow down corrosion reaction and to prevent food items to have direct contact or reacting with the tin or steel which can lead to food poisoning. Kim, Park, and Jang (2013) found that the combination of lacquer with animal glue can be used to substitute synthetic resins. The coatings shield applied on metal can of beverage flavors may prevent interactions that can modify the organoleptic appearance of the beverage. Due to the safety aspect, typical epoxy resins containing BPA diglycidyl ether are preferred to be used as a coating on food cans. On the contrary, the application of BPA as a coating on aluminum can results in the migration to food products if it is stored at a high temperature thus would lead to health hazards (Stärker and Welle, 2019).

The shape of cans is formed using either the two- or three-piece methods. As the name indicates, a two-piece method can consist of a can body and top end. To fabricate a two-piece can, the blank plate is drawn and redrawn or the wall of the plate is drawn and ironed several times to increase the length of the can sidewall to the desired height. A three-piece method can consist of a can body, bottom end, and top end. One side of the blank plate is attached together by soldering or welding it with the other side thus forming a cylindrical shape. The bottom end is double seamed to the can body (can maker's end) leaving the top of the can open. The top end will be attached through double seaming process after the product is fill up (manufacturer's end).

HCP 1 and HCP 3 are proposed due to the process of cutting the blank plate, punching and ironing it into the metal can shapes that requires the use of lubricants. The functions of cutting fluid are to minimize friction, remove the heat

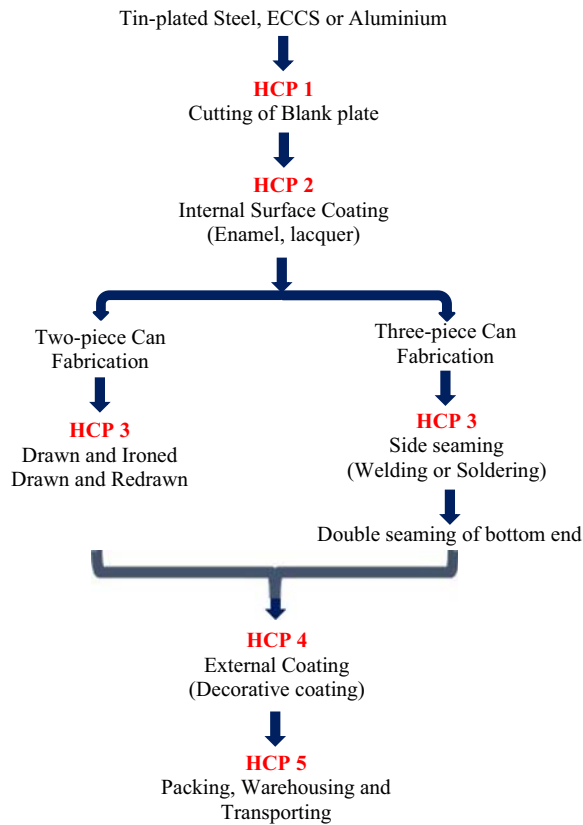


FIGURE 14.5 Propose HCP of metal container fabrication. *HCP*, halal control point.

created by the cutting activity, prevent corrosion of the workpiece and machine tool, remove machined chips from the cutting zone, and offer wheel cleaning (Bianchi, Aguiar, Canarim, & Diniz, 2013). Commercial cutting fluids are often divided into three types: cutting oils, emulsions, and synthetic/semisynthetic fluids. Cutting oils are made from petroleum, animal, vegetable, and fish oils, as well as a carefully balanced combination of additives, oxidation inhibitors, rust preventatives, antiseptics, odor control agents, and antifoaming agents (Woon, 2021).

14.4.3 Aluminum manufacturing

Earth soil is rich with metallic constituents. In the production of aluminum, the raw material used such as diaspor, gibbsite, and most commonly bauxite is also extracted from the earth. Bauxite ore contains 30%–54% alumina; therefore it must be refined to aluminum. This refining process can be accomplished in two stages. Stage 1 is the Bayer process of refining the bauxite ore to obtain aluminum oxide or alumina, and Stage 2 is the Hall–Heroult process of smelting the alumina to release pure aluminum.

In the Bayer process, bauxite ore that is mechanically crushed will be mixed with caustic soda and pumped into a pressured heated tank to dissolve the ore. The hot slurry then passes through a series of flash tanks and settling tanks. The residue or impurities consisting of silica, various iron oxides, and titanium dioxide will settle at the bottom and remove. The alumina is recovered from the solution by filtration and caustic soda is reused. The filtered alumina liquid is pumped through a series of precipitation tanks where seed crystals of alumina hydrate are added. The precipitated crystal obtained is transferred to a kiln at 1000°C to remove water molecules. Anhydrous alumina crystals are refined to obtain pure aluminum in an electrochemical Hall–Heroult process. Alumina (Al_2O_3) is dissolved in a cryolite solution (electrolyte) in a carbon-lined steel pot (cathode). A carbon electrode (anode) is lowered into the solution and an electric current is passed through the mixture to the carbon lining of the pot. The current reduces alumina into aluminum and oxygen thus combining with the anode's carbon to form carbon dioxide gas. The heavy pure aluminum will settle at the bottom of the pot and is then collected.

Fig. 14.6 shows the simplified process of aluminum from bauxite ore into foil products. No HCP is proposed at both stages of aluminum production. In addition in steel production, the presence of traces of animal carcasses may be found

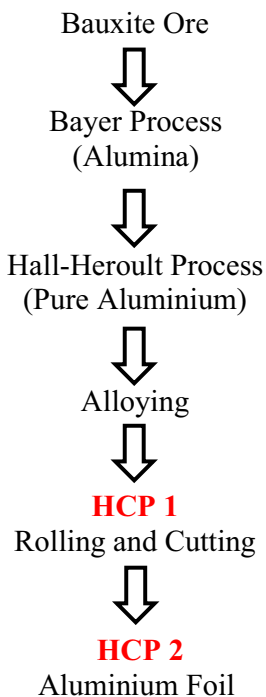


FIGURE 14.6 Propose HCP of aluminum foil processing. *HCP*, halal control point.

in the earth while extracting bauxite ore. Most of the organic materials will be destroyed during the high-temperature digestion using caustic soda while oxides of calcium and phosphorus (typical bones ash constituents) will be removed as impurities. Through this process, the purity of aluminum produced is at least 99% (Robertson, 2013). Pure aluminum is used in the manufacturing of foil and extruded containers since it is soft and easy to work on.

Aluminum foil is a thin-rolled sheet of alloyed aluminum varying in thickness from about 4–150 μm . The foil can be produced by two methods which are (1) passing heated aluminum sheet ingot between rollers under pressure several times until the desired thickness is obtained or (2) by continuous casting and cold rolling. In the rolling process of aluminum foil, kerosene is the most used coolant and lubricant for both hot and cold rolling works. Due to its flammable characteristic, a costly fire suppressant system must be installed. An alternative method to kerosene is the use of oil–water emulsion technology (Christoforou, 2013). Examples of oil–water emulsion lubricants employed for foil rolling are soluble oil emulsions and compounded oils. Emulsifiable compounds that are often incorporated in the soluble oil are waxes, cottonseed oil, and other fatty oils while the compounding agents are oleic acid, rapeseed oil, and neatsfoot oil. The amount present for these compounds is normally between 1% and 10%, depending on the application (Christoforou, 2013). HCP 1 is therefore suggested for the rolling process since some of the lubricants used may come from unslaughtered or forbidden animal sources. On the same note as HCP 1, HCP 2 is proposed for the final product due to its possible contamination on the condition that the lubricants and coolants of machinery used are not halal compliance.

14.5 Halal issues of plastic packaging material

Plastic is a generic term for macromolecular organic compounds (polymers) obtained from monomers of low-molecular-weight molecules or by chemical alteration of natural macromolecular compounds. Differences in the monomer's chemical constituent and polymer chain's structure determine the different properties of plastics such as melting point, glass transition temperature, and physic mechanical properties.

Most of the issues pertaining to plastic packaging are migration or leaching of the unstable monomer or additive compounds present in the polymer. Migration is the transfer of molecules originally contained in the packaging material (e.g., plasticizer, residual monomer, stabilizer, antioxidant, catalyst, ink) into the product. It is a complex diffusion process resulting from spontaneous natural molecular movements that occur without the assistance of external forces (Robertson, 2013). However, factors such as the plastic chemical structure, molecular weight, polarity, concentration of the migrant, types of food or beverage, interactions or compatibility between food and packaging material, surface area

to volume of the packed food, duration, and temperature of storage can influence the severity of migration ([Ossberger, 2009](#)).

In general, the manufacturing of polymers into plastic containers consists of three stages.

1. Stage 1 is the production of monomers obtained from the cracking process in petroleum refining and natural gas production. It is a chemical process that produces hydrocarbon monomers such as ethylene, propylene, styrene, vinyl chloride, and acrylonitrile.
2. Stage 2 is the polymerization of monomer into its polymer form conducted through polymerization reactions such as condensation, addition, and ring-opening.
3. Stage 3 is the conversion of polymer pellets, beads, or powder into plastic products such as bottles, trays, and films. The methods used are extrusion molding, injection molding, blow molding, or thermoforming.

The negative consequences of chemical exposure from plastic materials to human health and the environment are also one of the halal noncompliance elements. The migration of potentially toxic, carcinogenic, or mutagenic compounds from plastic into food is a known fact. Based on that if a plastic manufacturing facility or plastic products are to be halal-certified, a solid preventive measure system during production should be in place. The risk and life cycle assessment data must be inspected to ensure that no hazards can affect the environment along with workers' and consumers' well-being.

It is also important to note that the toxicity or health detrimental effects of chemicals are not only based on their concentration in the packaging material but also on the concentration of chemicals that is present in the food from the packaging material. Practically, most chemicals can be toxic if tested or consumed at high levels; however, under certain circumstances of exposure as in the diet, they may or may not be hazardous to health ([Robertson, 2013](#)). The amount of migrated chemicals in food contact substances is being regulated and enforced, yet the legislated limit might differ among countries around the world. Despite that, the permissible limit, amount of overall migration, specific migration, consumption factor, acceptable daily intake, the threshold of regulation, or other related terms used in the legislation or directive will not be elaborated on in this chapter.

Even with the strict regulations, some studies had shown that certain chemical compounds found in food products exceeded the regulated limit. A recent study by [Tian et al. \(2022\)](#) had shown that plastic-related contaminants were detected in 168 food samples (fish fillets, chicken breast, canned tuna, leafy vegetables, bread, and butter) collected in Montreal (Canada), Pretoria and Vhembe (South Africa). The contaminants measured are BPA, BPS, and seven plasticizers [di-*n*-butyl phthalate (DBP), diethyl phthalate (DEP), (2-ethylhexyl)phthalate (DEHP), di-(2-ethylhexyl) adipate, diisononyl phthalate, di-(isononyl)-cyclohexane-1,2-dicarboxylate]. [Htway, Htun, Hlaing, Lwin, and Min \(2020\)](#) revealed that the single use of low-density polyethylene (LDPE) plastic bags contained chemicals such as toluene, ethylbenzene, methyl oxirane, supraene, aldehydes, and dioxin that are toxic to human beings. Apart from that, in 2018, a global-scale study by [Luo et al. \(2018\)](#) was conducted on phthalates migration in bottled water that represented 20 countries. This study showed that the detection frequency of the targeted compounds such as DBP (67.6%), DEHP (61.7%), DEP (47.1%), benzyl butyl phthalate (36.9%), and dimethyl phthalate (30.1%) was found in more than 300 brands of bottled waters. Although the human daily intake-based risk assessment revealed that phthalates in bottled waters studied would not pose a serious health concern but the adverse estrogenic effects of phthalates in some countries such as Pakistan, Thailand, Croatia, Czech Republic, Saudi Arabia, and China appeared to be significant.

In view of the health detrimental effects and environmental pollution issues of plastic packaging, some industry has ceased the use of plastic packaging. Morrisons has become the first supermarket chain that replaced plastic packaging with recyclable paper bags for its fruit and vegetable section ([Halalfocus.net, 2019](#)). Their sales of loose fruit and vegetables were increased by 40% during the 10-month trial period in three UK stores indicating that consumers are aware and support the campaign. Likewise, Waitrose a British supermarket company also removed all plastic bags, including disposable paper cups from its stores to help the environment. Another supermarket chain Tesco has also announced to ban the use of hard-to-recycle plastic packaging by 2019 and will ultimately shift to fully recyclable packaging by 2025 ([Halalfocus.net, 2019](#)).

14.6 Halal labeling

From the marketing point of view, the package form, color, size, shape, structure, materials, graphics or image, typography, and regulatory information (product identification, brand name, labeling, and claims) are all important factors in attracting consumers' and subsequently determine the final decision at the point of sale ([Nørgaard Olesen & Giacalone, 2018](#); [Scarpi, Pizzi, & Pichierri, 2019](#); [Steenis, van Herpen, van der Lans, Ligthart, & van Trijp, 2017](#)). Packaging also

leads consumers to form perceptions of the product which serves as a critical tool to strengthen the product's image or brand (Tec  u & Chitu, 2018). Indirectly, the visual stimuli, informational elements, and functionality attributes are packaging cues that lead to the perceived quality, healthiness, naturalness, sustainability, and superiority of the product in the package (G  mez, Mart  n-Consuegra, & Molina, 2015). However, some studies point out that consumer characteristics and intrapersonal factors such as gender, age, income, cultural background, education, experience, and religion also affect the buying decision (Fernqvist, Olsson, & Spendrup, 2015; K  ster, Vila, & Sarabia, 2019; Vila-Lopez & K  ster-Boluda, 2016; Vila-L  pez, K  ster-Boluda, & Sarabia-S  nchez, 2017). For the product manufacturing company, designing or redesigning, revitalizing old design, repositioning a product, changing the target market, fulfilling legal or regulations requirements, and adopting new packaging technology are some of the packaging monetary investments necessary to ensure their product stands out and outperforms than other competitors with a similar product (K  ster et al., 2019).

Packaging design has a significant role in addressing the religious as well as the cultural sensitivities associated with the halal market (Prabowo & Aji, 2021). Apart from the halal logo image, the packaging design for halal products must also adhere to Shariah principles, such as pictures printed on the package must not contradict with the halal conceptions, and visuals should not evoke any negative feelings or create sensitivity to the Muslim community (Ab Talib & Mohd Johan, 2012).

The halal label or logo plays an important role in giving confidence to Muslim buyers thus reflecting certain merits and advantages to both marketers and consumers (Jamal & Sharifuddin, 2015; Rezai, Mohamed, & Shamsudin, 2012). The logo also serves as an explicit symbol of religious permission that the product has fulfilled the Shariah requirements and halal quality assurance through a process of halal auditing by the halal authority (Maison, Marchlewska, Syarifah, Zein, & Purba, 2018). Several studies conducted in Malaysia and Indonesia demonstrated that Muslim consumers placed halal certification as a priority in product purchasing (Amat, As'hari, & Sundram, 2014; Farhan & Andriansyah, 2016; Sukesti & Budiman, 2014). A study by Aziza, Hidayat, and Prasnowo (2020) on the influence of halal labels on Indonesian consumers showed that halal label influences their impulsive buying behavior by 52%. A study on purchase decisions of Malaysian Muslim consumers showed that 89% of respondents believed that the halal logo has more meaning and is more important than products labeled with International Organization for Standardization or similar certification (Shafie & Othman, 2006).

There are various designs of the halal logo that can be found on the packaging of halal-certified products. The design of the logo varies among countries, and even among the halal certification organizations of the same country. The Islamic Services of America a leading halal certification body in the United States and North America furnishes three different halal logo options (Fig. 14.7). For the international market, the globally recognized design with a circular seal is used while the other two are usable in the domestic US market (<http://www.isahalal.com>, 2020).

In Malaysia's retail market, various halal logos can be observed on the packaging of the halal-certified products. Due to the differences in *mazhab* or Islamic law interpretations among Islamic scholars, the Malaysian halal authority or JAKIM only approved halal-certified products that adhere to the *Shafie mazhab* of Malaysian Muslims. A total of 84 foreign halal certification bodies (FHCB) were approved by JAKIM as of December 2020 (<http://www.halal.gov.my>). It is unsure whether the halal products from the unapproved FHCB can enter the Malaysian market or not, but if they do, the Malaysian customer will have a hard time identifying the logo of the approved FHCB. Currently, due to the issue of a meat cartel in which nonhalal-certified meat was labeled as halal, JAKIM has taken a step ahead by introducing an



FIGURE 14.7 Halal logo options by the Islamic Services of America (<http://www.isahalal.com>).

TABLE 14.1 The major halal international standards in Islamic countries (Abdallah et al., 2021).

Halal Standard	Countries
OIC/SMIIC 1: 2019	56 Member countries, including Egypt, Iran, Saudi Arabia, Indonesia, Turkey, Pakistan, Nigeria, Bangladesh, and Algeria
GSO993: 2015	Saudi Arabia, Qatar, Kuwait, Oman, Bahrain, United Arab Emirates
MS1500: 2019	Malaysia
HAS23103: 2012	Indonesia

Source: Adapted from Abdallah, A., Rahem, M. A., & Pasqualone, A. (2021). The multiplicity of halal standards: A case study of application to slaughterhouses. *Journal of Ethnic Foods*, 8(1), 1–15. <https://doi.org/10.1186/s42779-021-00084-6>.

additional on-pack feature (Foodnavigator-asia, 2021). This new regulation requires all halal-certified prepackaged products from FHCB entering Malaysia to be marked or labeled with both halal logo and a QR code.

The main factors that cause the differences in the halal logo are (1) the differences in halal regulations or standards used by the halal certification authority among the Islamic countries and (2) the differences in the school of Islamic thoughts (*mazhab*), namely, *Hanafi*, *Shafie*, *Maliki*, and *Hanbali* within the Islamic jurisprudence Table 14.1 shows the major of halal international standards in Islamic countries categorized by the geographical areas. Four major halal standards and authorities in the world are the Gulf Cooperation Council Standardization Organization (GSO), the Standards and Metrology Institute for the Islamic Countries (SMIIC) standards among the Organization of Islamic Cooperation (OIC) countries (OIC/SMIIC), *Lembaga Pengkajian Pangan, Obat-obatan dan Kosmetika* by *Majlis Ulama Indonesia* and Malaysia Standard by JAKIM for Malaysia. Several attempts have been made to harmonize the halal standards but, currently, no consensus agreement has been made on a single unified international halal standard that can be established and used (Abdallah, Rahem, & Pasqualone, 2021; Mrad, 2017).

A point to ponder is that a certified halal product is identified by the halal logo printed on the product packaging. Even so, in the case of halal-certified packaging, several questions will come to mind such as how to identify halal-certified packaging? Will there be an additional halal logo on the package or will it use a different logo designed specifically for halal packaging material declaration? Having two similar halal logos on the packaging will confuse the consumers while having a new or different halal logo for packaging material could create more complexity. On the flip side, a possible misused of halal-certified packaging by irresponsible manufacturers may occur in which the halal packaging is used for nonhalal products and therefore creates a deceptive situation.

14.7 Conclusion

To be a halal-certified packaging, it is important to ensure that the raw materials, processing aids, additives, and lubricants used in the production of packaging materials must be Shariah compliant. Apart from that, the packaging materials used must be safe, clean, and nontoxic. The migration of toxic, carcinogenic, or mutagenic compounds from packaging materials is categorized under *Thayyib* noncompliance. Therefore the HCPs proposed in packaging manufacturing could help the workers to observe the process and identify the sources of ingredients or additives used. The packaging design should also encompass the concept of halal while the label of the halal logo is essential for consumers in determining and identifying the halal products.

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Section 3

Innovation in Halal Supply Chain

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The dynamics of palm oil supply chain

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15.1 Introduction

In 2020 Malaysia's palm oil (PO) industry contributes 2.7% of gross domestic product to the Malaysian economy. Being the world's second-largest producer and exporter of PO, after Indonesia, around 16.2 million metric tons of PO and PO-based products were exported around the globe, valued at RM 73.3 billion. PO has a significant share at 31.4% in the world's oils and fats production along with another three types of vegetable oils: soybean, rapeseed, and sunflower oils. It is also a major ingredient in the production of cosmetics, pharmaceutical, and nutraceutical products.

According to the report of [Amna Puri \(2022\)](#), the halal product market is expected to grow from 1.4 trillion US dollars in 2017 to around 2.6 trillion by 2024, with the largest share of the market value coming from North America. Halal market is now valued at around US \$632 billion per year, and it represents 17% of the entire global food industry. Furthermore, the demand for halal foods from the non-Muslim market is growing with global interest from non-Muslim countries such as the United States, Japan, China, Brazil, the United Kingdom, and Australia ([Omar, 2017](#)). Therefore the halal market's potential is very promising for those halal producers to disseminate their products internationally. With the expansion of the halal market, and PO's widespread use and popularity, it is pivotal to reach out to the halal target market and address any doubts.

Aside from the halal raw material, and halal-certified manufacturing processing, PO storage, dissemination, delivery, and transportation are part of the halal logistics which involves the overall supply chain network right from the origin source to final consumption, "from farm to fork" ([Ab Talib, Rubin, & Zhengyi, 2013](#)). Halal logistics as described by [Tieman \(2013\)](#) is not much different from conventional approaches such as managing procurement, movement, storage and handling of material parts, livestock, and semifinished or finished products throughout the supply chain network in compliance with *Shariah* principles. Essentially, the area of the halal logistics industry in Malaysia is still in the infancy stage so any matters concerning such as competencies, know-how, and publications are quite limited ([Tieman, 2013](#); [Zulfakar, Jie, & Chan, 2012](#); [Talib, Hamid, Zulfakar, & Jeeva, 2014](#)).

15.2 Palm oil supply chain

PO is one of the world's most widely used commodities, and its global demand is projected to grow with the world's rising population. However, the PO supply chain is surrounded by a complex social system and is not simply a commodity-handling process, which may preclude the implementation of halal traceability, sustainability as well as deforestation commitments. Therefore several certification standards have been developed to help producers and manufacturers abide by the current standard. Among those, this section describes Roundtable on Sustainable Palm oil and Halal Supply Chain certification that existed to ensure the quality and wholesome aspect of the PO processing and final products have complied.

15.3 Roundtable on Sustainable Palm oil certification

The International Organization for Standardization refers to traceability as the ability to verify the history, location, or application of an item by means of documented recorded identification. The elements of traceability include product source, for example, estate, process, and customer traceability. The PO supply chain is currently governed by Roundtable on Sustainable Palm oil (RSPO) certification that has been developed to set environmental and social criteria that the companies must comply to produce Certified Sustainable Palm Oil. When the RSPO criteria are properly applied, it helps to minimize the negative impact of PO cultivation on the environment and communities in PO-producing regions. This is to support an ever-increasing demand and growing worldwide concern for commodities to be produced with environmental concern in avoidance of any practices that might harm the environment or society. The RSPO certification also assures consumers that PO production is sustainable.

The RSPO has established two certification systems. The first system is to ensure that PO is produced sustainably, known as “Producer/grower Certification” or “Principles and Criteria Certification,” (P&C) and the second one is to ensure the integrity of the SPO trade, meaning that PO sold as SPO is indeed produced by certified plantations.

The P&C standard defines the practices for SPO production that addresses the legal, economic, environmental, and social requirements of producing SPO based on eight principles: (1) commitment to transparency; (2) compliance with applicable laws and regulations; (3) commitment to long-term economic and financial viability; (4) use of appropriate best practices by growers and millers; (5) environmental responsibility and conservation of natural resources and biodiversity; (6) responsible consideration for employees and for individuals and communities affected by growers and mills; (7) responsible development of new plantings; (8) commitment to continuous improvement in key areas of activity (Hashim, Tahiruddin, & Asis, 2012).

PO producers are certified by qualified certifying bodies after rigorous verification of the production process against the demanding RSPO P&C for SPO production (RSPO, 2018). The stringent certification procedures significantly decrease the possibility of consumers using un-SPO. Certification can be revoked at any moment if the rules and criteria are broken. To avoid overselling and mixing certified with conventional (or unsustainable) PO products, all organizations involved in the supply chain are audited. Once certified, these organizations have the right to claim the RSPO trademark label on their product packaging. The RSPO has established four supply chain models (SSCs) that are identity-preserved, segregated, mass balance, and RSPO Book/Credit and Claim models (RSPO, 2020). The RSPO SSCs are illustrated in Figs. 15.1–15.4, respectively.

15.3.1 Identity-preserved supply chain model

The identity-preserved supply chain (IPSC) model is designed for SPO that comes from a single identifiable certified source. When the model is used, the SPO products that are traded can be traced back to the specific oil mill and supply base. This ensures that the operators throughout the supply chain who buy or sell the certified SPO will be able to identify the origin of the palm products from other mills at any stage of the chain (Leegwater & van Duijn, 2012; Rival, Montet, & Pioch, 2016). Therefore the IPSC model requires the transport and storage of SPO products to be physically separated or isolated from other conventionally produced or uncertified palm products. The downside of the model is the system is costly and suitable for low demand of volumes only (RSPO, 2020).

15.3.2 Segregated supply chain model

The segregated supply chain model emphasized the need to ensure that the certified SPO products are kept separated from the noncertified source material similar to the IPSC model. However, the SPO of the SSC model may come from several identifiable certified sources of mill and base supply (RSPO, 2020). The model also allows the mixing of SPO products from multiple sources. Furthermore, within their facility, operators are not obliged to follow the material (Leegwater & van Duijn, 2012; Rival et al., 2016).

15.3.3 Mass balance supply chain model

When using the mass balance supply chain (MBSC) model, PO source may come from the certified SPO and conventional PO. The model allows the operators to purchase a set quantity of certified SPO and mix it with noncertified PO products. However, the MBSC oil distributed is either equal to or lower than the RSPO certified (RSPO, 2020). Therefore when the operator purchased SPO of the MBSC PO products, it is impossible to know whether the product received contains RSPO-certified SPO or -uncertified palm products (Leegwater & van Duijn, 2012; Rival et al., 2016).

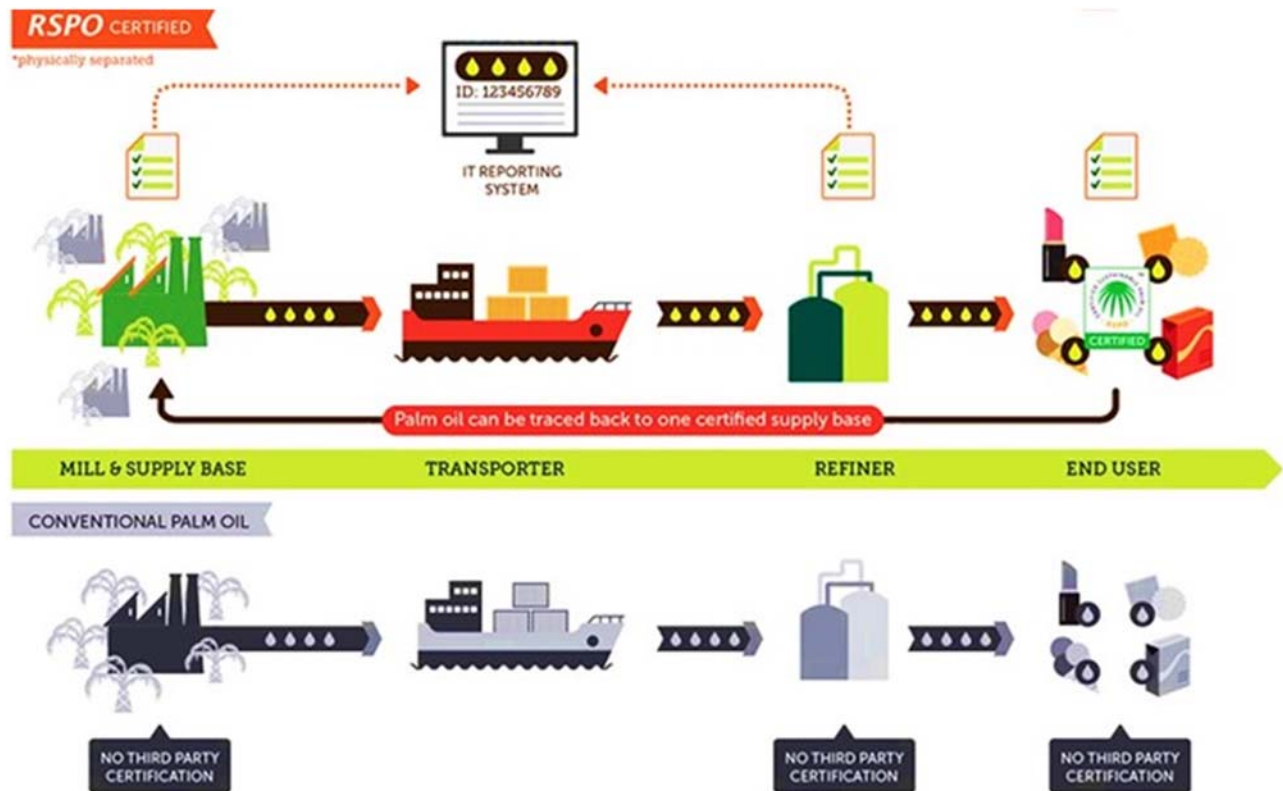


FIGURE 15.1 The RSPO identity-preserved supply chain model versus conventional supply chain (RSPO, 2020). RSPO, Roundtable on Sustainable Palm oil. Source: Reproduced with permission from, RSPO., <https://rspo.org/certification/supply-chains>.

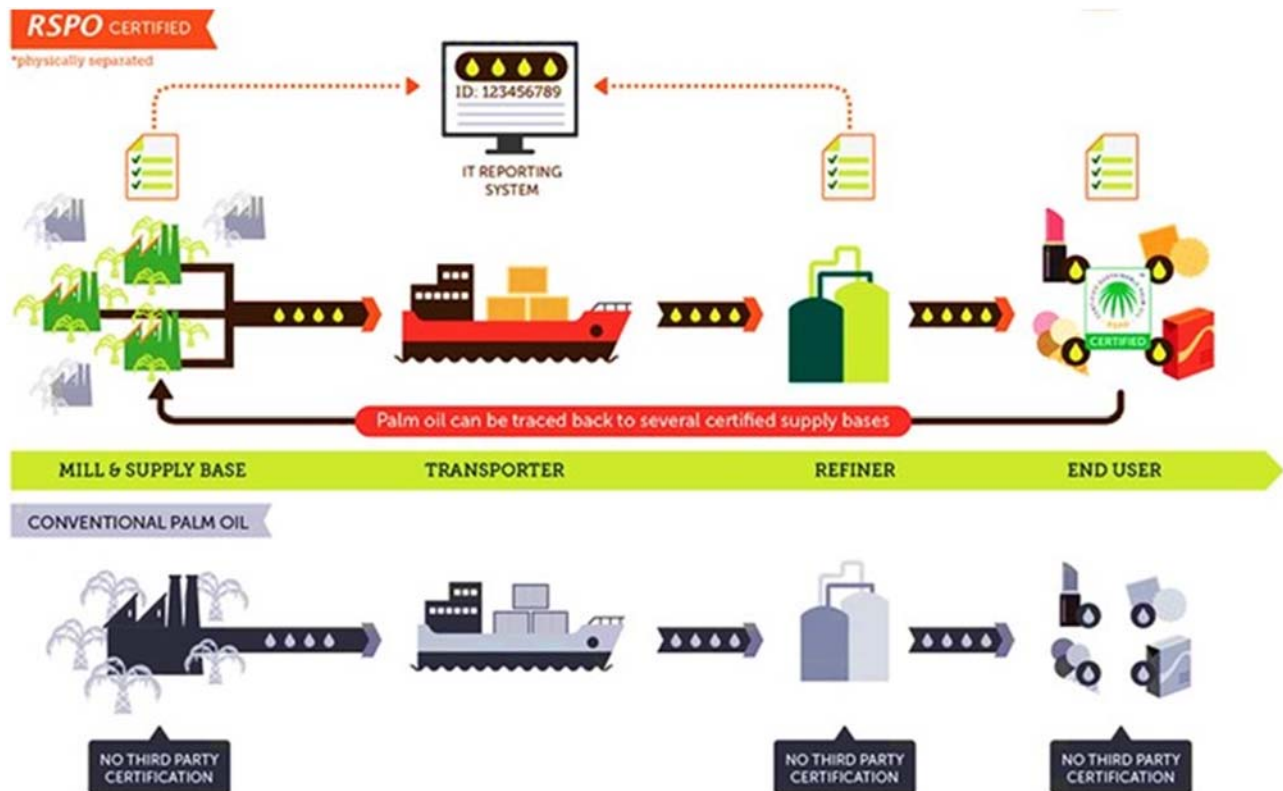


FIGURE 15.2 The RSPO segregated supply chain model versus conventional supply chain (RSPO, 2020). RSPO, Roundtable on Sustainable Palm oil. Source: Reproduced with permission from, RSPO, <https://rspo.org/certification/supply-chains>.

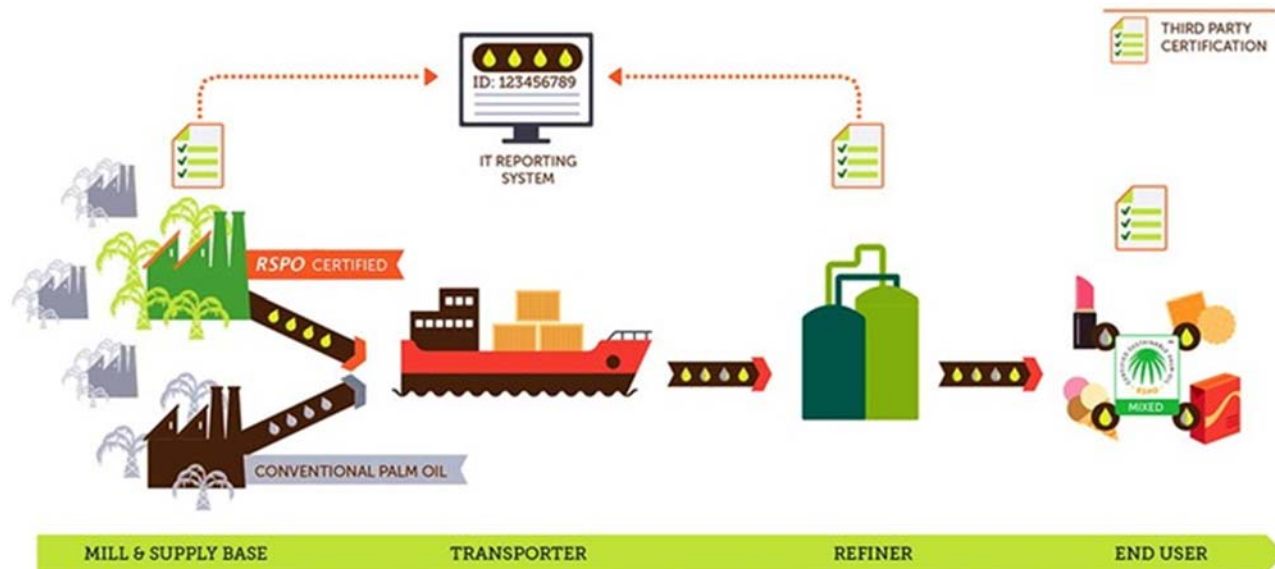


FIGURE 15.3 The RSPO mass balance supply chain model versus conventional supply chain (RSPO, 2020). RSPO, Roundtable on Sustainable Palm oil. Source: Reproduced with permission from, RSPO, <https://rspo.org/certification/supply-chains>.

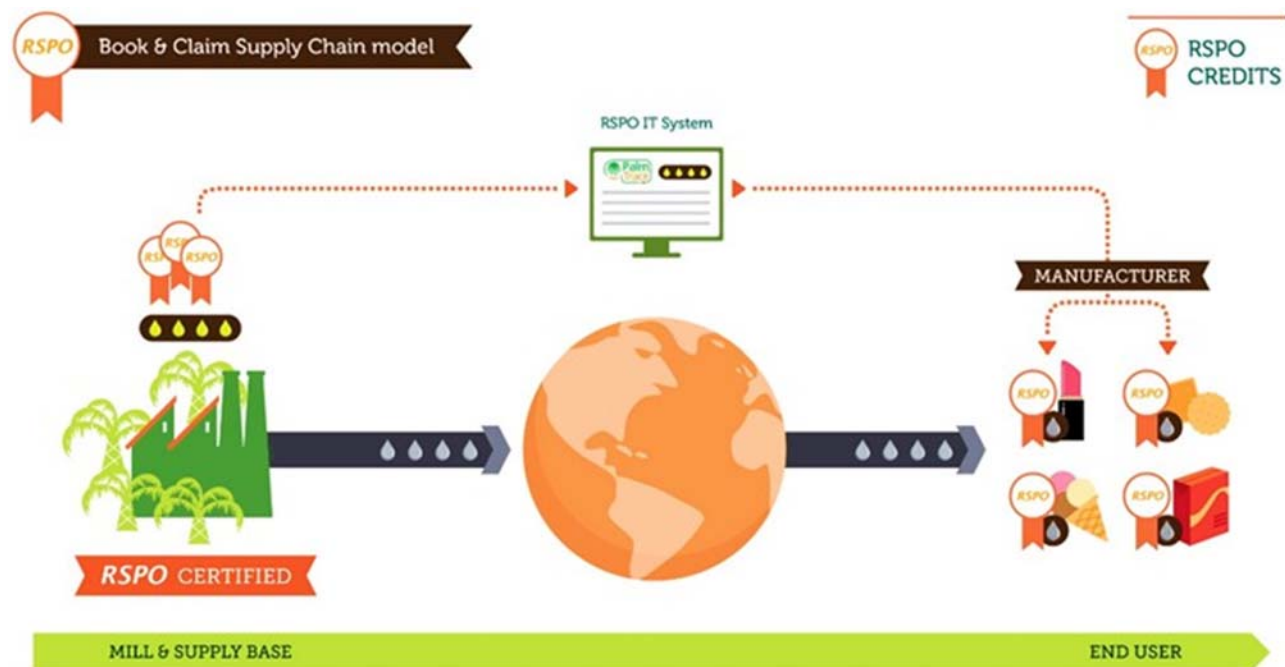


FIGURE 15.4 The RSPO Book and Claim supply chain model versus conventional supply chain model (RSPO, 2020). RSPO, Roundtable on Sustainable Palm oil. Source: Reproduced with permission from, RSPO, <https://rspo.org/certification/supply-chains>.

15.3.4 Book and Claim supply chain model

In this model the SPO products are not monitored, the end users may purchase certificates directly from producers, with no regard for the phases in the chain. The manufacturers and retailers can purchase credits from RSPO-certified growers, crushers, and independent smallholders to offset their usage of palm products. There is no link between the physical PO products utilized and the certificates that are traded. Therefore the model approach does not add to the traceability of traded PO (Leegwater & van Duijn, 2012; Rival et al., 2016).

In short, the aim of the RSPO SSCs is to stimulate and ensure the growth of SPO production. These models provide openness in the supply chain of RSPO-certified sustainable palm products while encouraging sustainable production methods.

15.4 Halal palm oil supply chain

In general, a halal supply chain incorporates a management and services system specifically for logistics service providers to maintain the halal integrity of halal-certified products as demanded by the halal manufacturers (Ngah, Zainuddin, & Thurasamy, 2014). It is assumed that the halal products cannot be truly confirmed as halal if the manufacturers are not using halal transport and logistics services. In essence, the halal supply chain focuses on preserving the halal integrity from farm to table.

There are four primary components in ensuring the halal status of the whole supply chain: procurement, production, distribution, and logistics (Rasi, Masrom, Omar, Ahmad, & Sham, 2017). Halal procurement is focused on the halal inputs or ingredients, raw materials, and other resources which must be acquired from halal permissible sources. Halal manufacturing involves identifying the possible biological, chemical, and physical hazards that may occur during processing through allocations of halal control points (HCPs). For distribution and warehousing of goods, the use of a dedicated or segregated compartment in transport and warehouse or a third-party halal-certified logistics provider is another necessary halal element. Additionally, packaging, containers, and logistics are concerned with the process activities, such as organizing, and supplies prior to their delivery to customers (Aziz & Zailani, 2017).

In a review article on the concept of halal supply chain management by Khan, Haleem, and Khan (2018), the authors stated that current definitions of halal supply chain management tend to encompass all components of halal in the complete supply chain as well as conventional supply chain management. They asserted, however, that the halal supply chain differs from the normal supply chain in terms of material, information, and capital flow, allowing halal and *Thayyib* (cleanliness) to be extended to the point of consumption.

Fig. 15.5 shows a simplified supply chain of PO. The chain begins with the cultivation, agricultural practices of PO trees, and harvesting of PO fruits, then the extraction process of the oil to obtain crude PO (CPO) or crude palm olein, followed by the refining and fractionation processes to obtain a clear PO suitable for cooking purposes or palm-based products, finally the products are package, transport, and distribution to the end users.

In most countries, the halal implementation and certification were mostly practiced by food and beverages manufacturers only, in which most of the stakeholders do not realize that product contamination may also occur during the handling, transportation, and storing activities along the supply chain. The port is one of the main players that handle

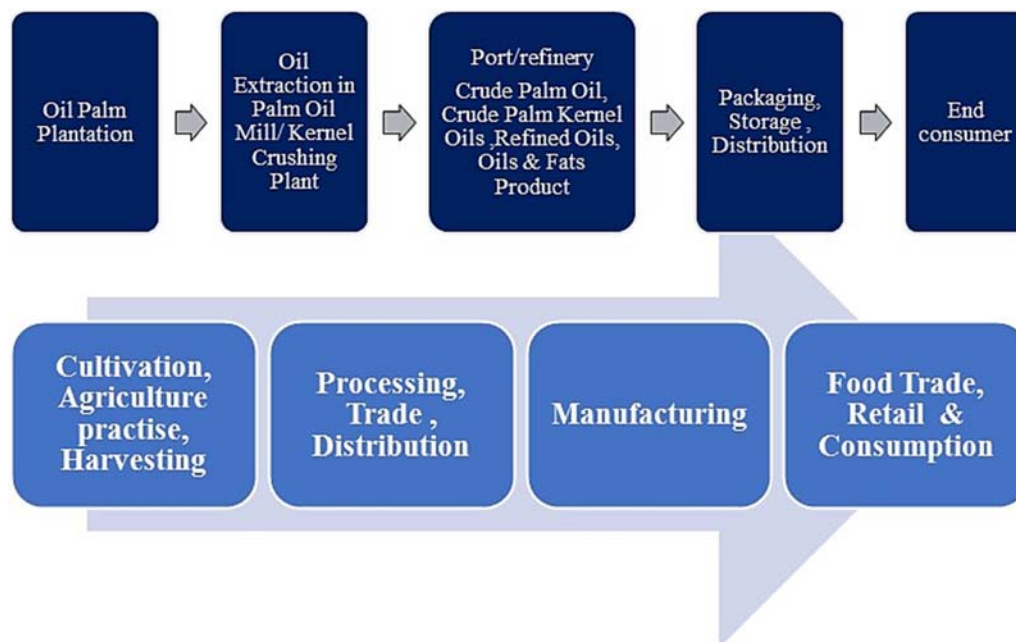


FIGURE 15.5 A simplified palm oil supply chain.

numerous activities that may expose halal products to contamination in the context of international halal trade; therefore, they must adhere to halal regulations. Jaafar, Abd Aziz, Ahmad, and Faisol (2021) reported that dedication and segregation practices, sanitation practices in the port operations, determination of HCPs, and traceability of the halal are four that are vital components of the establishment of a halal-friendly sustainable port model.

Yaacob, Jaafar, and Rahman (2016) mentioned in their paper that in halal logistics, sanitation standards involving premises, infrastructure, facilities, and employees are an important aspect. Their study indicated that the suggestions on sanitary procedures in port regions, particularly in warehouse areas, were favorably appreciated by the participants. The purpose of sanitation outlined in their paper include (1) reducing contamination and ensuring the safety of equipment, employees, and consumers; (2) creating a healthy and pleasant atmosphere; (3) safeguarding natural resources (such as surface water, groundwater, and soil), as well as providing individuals with safety, security, and dignity when defecating or urinating; (4) creating barriers between excreta and humans to break the chain of sickness and infection; (5) ensuring and promoting long-term sanitation, especially in underdeveloped nations; and (6) complying with the regulatory environment.

Establishing an effective traceability system was determined to be critical in ensuring the quality and safety of halal cargoes, as well as the ability to recall products in the event of contamination. Monitoring the movement and quality of halal cargoes, as well as process constraints throughout their flow in the port area, adds to the overall safety and quality of the halal supply chain. To establish a traceable supply chain, a system that able to has information on traceability of internal and external supply chains which includes product, process, genetics, inputs, disease, and pest as well as measurement traceability (Opara, 2002).

15.4.1 Halal certification for palm oil processing

Halal certification of PO as cooking oil has been obtained by many PO refinery manufacturers. Prior to the refining, crude oil is extracted from the palm fruit mesocarp (CPO) or from its endosperm (crude palm kernel oil). The extraction process is normally conducted within or close to the plantation since the fresh palm fruits are easily spoiled after harvest. After the removal of unwanted compounds such as water, gums and resins, the stabilized CPO is transported to the refinery facilities for further processing into clear and odorless cooking oil. Fig. 15.6 shows the flowchart of a PO refinery processing stages using two methods of refining (physical and chemical).

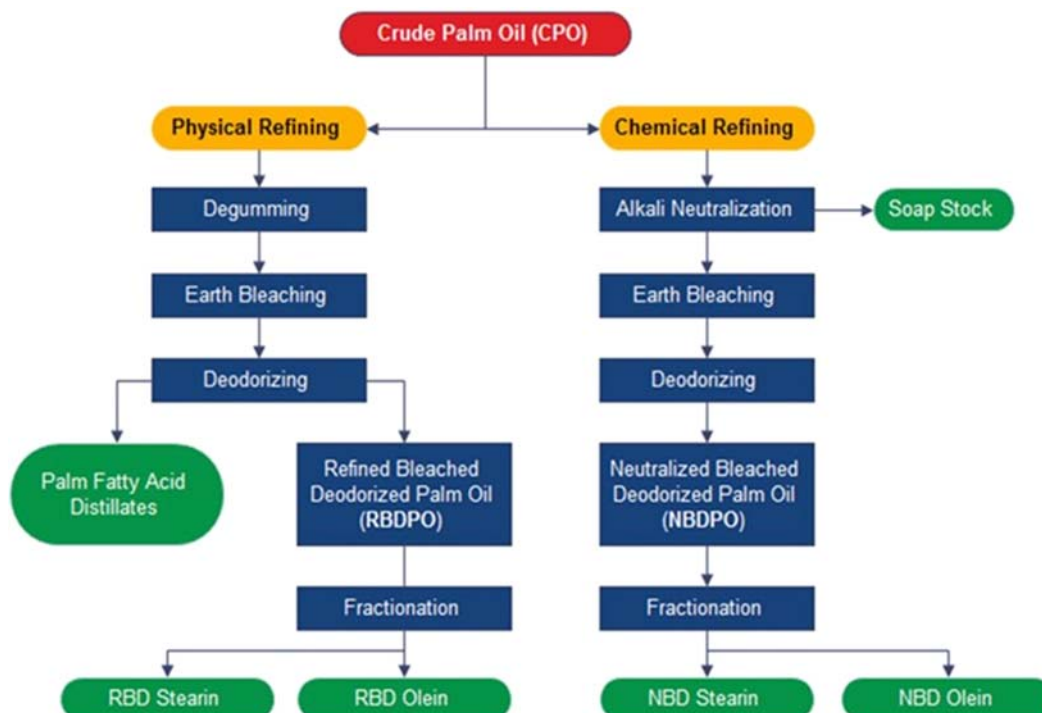


FIGURE 15.6 Flowchart in palm oil chemical and physical refining plant. Source: Based on, palmoilmillplant.com.

15.4.1.1 Halal food processing aids and lubricants

To ensure that the PO produced is free from nonhalal contaminants and hazards, it is imperative that any materials, ingredients, equipment, or machinery used during the process must follow the halal specifications. Food processing aids and lubricants in manufacturing facilities are among the main concerns. In PO refineries, all food processing aids that are found to be in direct contact with the product need to be halal certified. Similarly, lubricants used on the tools and machinery are not supposed to have direct contact with halal products at any stage of processing. However, cross-contamination of lubricant does occur in many cases, and therefore using a halal-certified lubricant would easily eliminate the problem.

There are two main chemicals used as processing aids at the degumming stage which are phosphoric and citric acids. The purpose of degumming is to remove phospholipids or gums thus increasing the stability, quality, and shelf life of the oil (Chompoo, Damrongwattanakool, & Raviyan, 2019). One of the degumming chemicals used is phosphoric acid which is a mineral acid. As a synthesized chemical, phosphoric acid is considered a halal material. The manufacturing process of phosphoric acid is also generally free from nonhalal ingredients. Another degumming agent is citric acid that is available in white crystal powder form. Similar to phosphoric acid, this synthesized chemical is generally considered a halal material. Citric acid is commonly used as a flavoring agent and preservative in various foods and beverages, especially in soft drinks and used widely as nutritional supplements or as an emulsifier, such as in ice cream.

The bleaching earth is the processing aid used to absorb unwanted color pigments and other impurities such as oxidizing compounds, soap, residual gums, and some trace metals (Ojewumi, Ezeocha, Alagbe, Obanla, & Omodara, 2021). The bleaching earth is generally composed of up to three types of clay minerals: bentonite, attapulgite, and sepiolite. The minerals act as absorbers with capacity being dependent on mineralogical structure and properties, such as surface area, particle size distribution, porosity, and surface activity (OFI, 2016). Most of the lubricants used in processing contain petroleum base stocks, which are difficult to dispose of after use and also toxic to the environment. Environmental concerns continue to increase due to the pollution exerted by the excessive lubricant use and disposal (Yahayaa, Raof, Ibrahim, Ahmad, & Gomes, C, 2018).

Moreover, machinery lubricants seeping into the world's most consumed edible oil during processing are alarming (Chu & Ananthalakshmi, 2020). The lubricant business is working to improve the environmental friendliness of its products as public awareness of environmental issues grows. Currently, there are more than 70% of commercial lubricants made from crude oil/hydrocarbon, and the utilization of vegetable base oils is gaining global interest (Owuna, 2020). Mineral oil which is commonly used as a lubricant is being replaced by H1 food-grade lubricants. In halal processing, any type of lubricant used whether it is intended or not intended for direct contact with the product must be *Syariah* and *Thayyib* compliant. A food-grade lubricant is not necessarily halal unless it is made from plant sources, does not contain additives from nonhalal sources, or is detrimental to health, and is processed hygienically. Lubricants derived from animal sources must be from halal animals that were slaughtered following the Islamic ritual method.

15.4.2 Halal control points for palm oil processing

HCPs are points, steps, or procedures that can be used to impose control and prevent or remove potential contamination. In this section, HCP determined by a PO refining company Sime Darby Oils Langat Refinery (Sime Darby Plantation Jomalina Refinery), Malaysia is used as an example. The company identified two HCPs with the potential presence of haram (unlawful) components in their manufacturing process. The first HCP is during the receiving of additives, ingredients, and processing aids while the second HCP is during the purchasing of food-grade grease. Figs. 15.7 and 15.8 show the position of HCP in the flowchart of raw material receiving stage and maintenance activity, respectively.

The receiving of additives, ingredients, and processing aids possess high risk as there might be the possibility of receiving products that are not halal-certified, or certified by foreign certification bodies that are not recognized by JAKIM, or its halal certificate has expired. The same goes for the purchasing requirement of food-grade grease. A thorough check on halal certification and expiration date should be conducted frequently.

15.4.3 Halal risk management plan for palm oil processing

A halal risk management plan should be established in a PO halal supply chain. The components plan should adhere to Islamic guidelines that determine the sources of contamination, which originated from haram and harmful sources, whereby halal products can become haram (forbidden) and hazardous, and unsafe for consumption if found exposed to any contaminants (Mohd et al., 2015). Moreover, Ackerley, Sertkaya, and Lange (2010) mentioned that the lack of

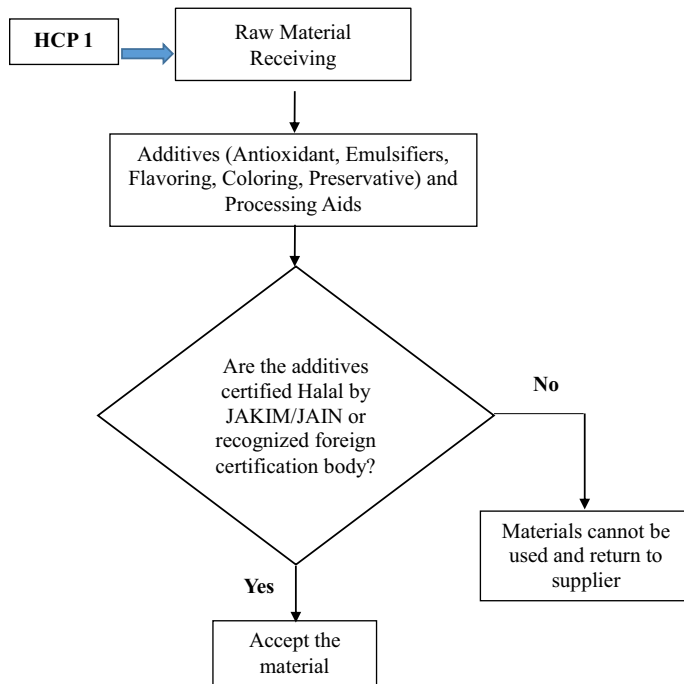


FIGURE 15.7 General process flowchart upon receiving of raw material. *Source: Based on, Sime Darby Oils Langat Refinery.*

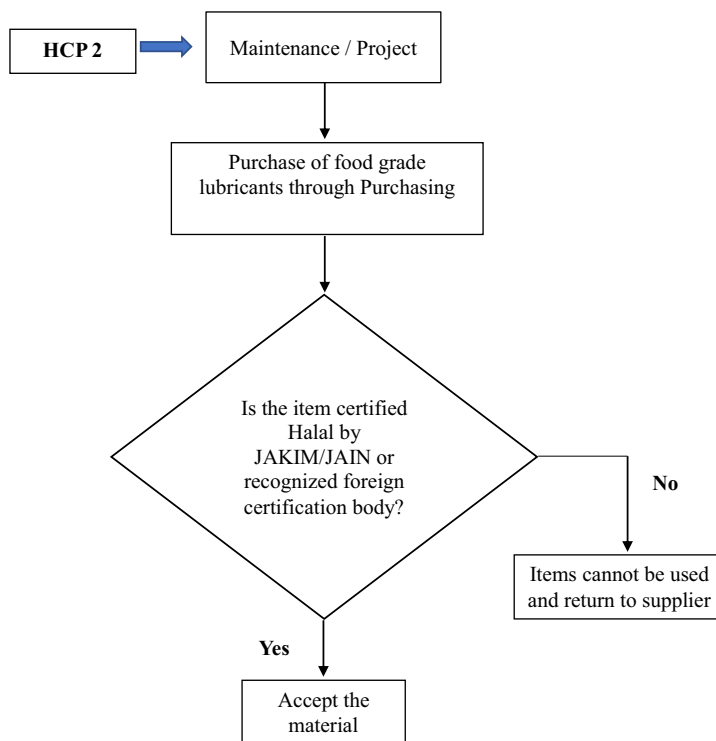


FIGURE 15.8 Process flowchart of maintenance activity (Sime Darby Oils Langat Refinery).

temperature control and sanitation practices may lead to contamination too. When processing activities are completely managed, the risks of contamination are reduced, and the number of HCPs is reduced.

An internal audit provides an overall view of an organization's whole risk management efficacy to guarantee that its halal risk management systems are correctly integrated with no flaws in controls (Jais, 2016). Table 15.1 is the general example of the halal risk management plan in the PO halal supply chain implemented at the Sime Darby Oils Langat Refinery.

TABLE 15.1 Halal risk management plan in palm oil halal supply chain.

HCP	Risk to halal	Control mechanism			Corrective action	Record
		Method	Frequency	Who		
HCP1: Receiving of additives/ ingredients/ processing aids	1. Product are not halal-certified/ certified by a foreign certification body which is not recognized by JAKIM 2. Expired halal certificate	Purchasing department to purchase only additives/ingredients/processing aids based on the list of approved halal-certified raw materials.	Every purchasing of all products being made	Purchasing department	Reject supplier which does not have a recognized halal certificate for the purchased raw materials.	Halal list material
		For new additives/ingredients/processing aids, Purchasing shall forward the halal certificate provided by suppliers to halal executives for verification prior to buying the materials.	Every purchasing of new products	Purchasing department	Reject purchase of new oil without recognized halal certificate	1. Halal certificate 2. Recognized foreign halal certification body list
		In absence of a halal certificate, purchasing need to request for process flowchart, product specification, and SDS for evaluation purpose.	Every purchasing of new products	Purchasing department	Reject purchase of oil which does not have approval from JAKIM or other approved bodies	1. Process flow chart 2. Product specification 3. Certificate of analysis 4. SDS
		Purchasing department to send reminders 3 months in advance to all suppliers for their halal certificate renewal.	3 months before halal certificate expiry	Purchasing department	Reject purchase of products whose halal certificate is expired	Halal list material
		List of latest halal-certified additives/ingredients/processing aids communicated during halal team meeting	Meeting once a year	Purchasing Department	Reject product that is not on the list of approved halal-certified materials	Halal list material

(Continued)

TABLE 15.1 (Continued)

HCP	Risk to halal	Control mechanism			Corrective action	Record
		Method	Frequency	Who		
HCP2: Purchase of food-grade grease	1. Materials are not halal-certified/ certified by foreign certification body which is not recognized by JAKIM 2. Expired halal certificate	Purchasing Department to purchase only materials based on the list of approved halal-certified materials	Every purchasing of materials being made	Purchasing Department	Reject supplier which does not have recognized halal certificate for the purchased materials.	Halal list material
		For new materials, purchasing shall forward the halal certificate provided by the suppliers to halal executives for verification prior to buying the materials.	Every purchasing of new grease or new suppliers	Purchasing department	Reject purchase of new grease without recognized halal certificate	1. Halal certificate 2. Recognized foreign
		In absence of a halal certificate, purchasing needs to request for process flowchart, product specification, and SDS for evaluation purposes. Halal executive shall send the documents to JAKIM for approval.	Every purchase of new grease or new suppliers	Purchasing department	Reject purchase grease which does not have approval from JAKIM	1. Process flow chart 2. Product specification 3. Certificate of analysis 4. SDS
		Purchasing department to send reminders 3 months in advance to all suppliers for their halal certificate renewal.	3 months before the certificate expiry date	Purchasing department	Reject purchase of grease which halal certificate is expired	Halal list material

HCP, Halal control points; *SDS*, safety data Sheet.
Source: Based on, Sime Darby Oils Langat Refinery.

15.5 Palm oil adulteration

Besides the issue of supply chain traceability of PO, the cases of PO adulterations are common in the food industry. This section would discuss the adulteration cases of PO with dyes or recycled cooking oils (RCOs) as well as adulteration of animal-based food products with PO such as butter adulterated with PO. The detection methods to identify the adulterants are also described.

The term food adulteration is defined by Choudhary, Gupta, Hameed, and Choton (2020) as a process in which the quality of food is lowered or reduced by replacing food ingredients or adding nonauthenticated substances or removal of a vital component from food for the sake of earning profit or due to other incidental reasons. In other words, food fraud or adulteration is a form of noncompliance with health, safety, economic, and ethical consequences (Ulberth & Buchgraber, 2000). Adulterants may be introduced to more expensive compounds with the objective of increasing visible amounts, lowering manufacturing costs, or for other deceptive or harmful purposes. Adulterants can also be introduced into substances unintentionally or inadvertently. Recently, owing to the increased utilization of PO, especially in ready-to-eat meals, it has become a preferential target for counterfeiting and adulteration (Andoh, Nuutinen, Mingle, & Roussey, 2019).

PO, which is currently one of the most useful edible oils on the planet, is prone to intentional adulteration. The fruit yields two types of oil: orange-red CPO derived from the flesh (mesocarp) and brownish-yellow crude palm kernel oil derived from the seeds (kernel) (Okogeri, 2013). The addition of unapproved colorants such as Sudan dyes; the high-frequency use of other waste edible oils, for instance: used frying oil or the addition of residue from the PO process are all examples of adulteration in edible PO (Andoh, 2021).

The reason for Sudan dye's adulteration in PO is to enhance the orange-red hue of the oil and the practice is found significant in West Africa, which was historically known for producing authentic PO (Andoh et al., 2019). Sudan dyes are oil-soluble azo dyes that were originally employed as an industrial dye to impart a rich-red, red-orange, or yellow-orange color to textiles and plastics. They are now forbidden for use as food colorants (Teye, Elliott, Kobina, Sam-Amoah, & Mingle, 2019). Sudan dyes have been identified in a variety of foods, according to the European Union's Rapid Alert System for Food and Feed (Rebane, Leito, Yurchenko, & Herodes, 2010). The use of these colors (Sudan I, II, III, and IV) in foods is illegal under EU and US law and is currently prohibited in practically all countries. However, adulteration of PO with Sudan dyes has been detected in Ghana (Business Day, 2015; Amoako-Mensah, 2017) and Ivory Coast (Genualdi et al., 2016). Okogeri (2013) highlighted that other adulterants used to increase the quantity of CPO include carrot, papaya, natural potash, and red dye, with potash and red dye leaf sheath of *sorghum bicolor* being the preferred and most widely used adulterants due to their abundance and low cost in Nigeria.

In Asian countries, adulteration of fresh cooking oil with used/RCO is frequent. Commonly, PO is employed as a frying medium as it has a high smoke point of 230°C and its usable life is limited to 12 days of continuous frying (Mba, Dumont, & Ngadi, 2015). According to Park and Kim (2016), the fats and oils in RCO will decompose thermally and oxidatively, increasing viscosity, darkening the color, increasing foaming, and lowering the smoking point. Furthermore, frying oil deterioration can alter the texture, taste, and overall flavor perception of the dish (Mba et al., 2015). Long-term consumption has the same negative effects as eating frequently heated cooking oil, which promotes the generation of free radicals. Higher rates of hypertension, atherosclerosis, and cancer, all of which could be linked to the intake of RCO (Lim, Abdul Mutalib, Khazaai, & Chang, 2018) or waste cooking oil (WCO).

RCO is typically reprocessed and purified through degumming, refining, bleaching, and deodorization treatments (Zhao et al., 2015). This reprocessed RCO is intended for nonfood uses such as biodiesel, soaps, and detergents. Some irresponsible traders and distributors, on the other hand, blend RCO with fresh palm olein (FPO) before repackaging and selling it to consumers as fresh palm cooking oil (Lim et al., 2018; Ghazali & Tukiran, 2021; Tan, Meriam Suhaimy, & Abd Samad, 2022). On the other hand, WCO can be easily collected from restaurants, catering services, food kiosks, and homeowners. From the halal perspective, the main concern with the use of RCO or WCO is that the oils obtained from nonhalal food establishments should not be utilized by halal food establishments (Ghazali & Tukiran, 2021). Consequently, usage of RCO or WCO in the food chain may impose food safety and quality threats other than religious and ethical impacts.

Another form of adulteration is the incorporation of PO in butter (Feizy & Jahani, 2020), PO adulteration in ghee (Ayza & Belete, 2015), coconut oil (Gamage, Rajanayake, & Mahanama, 2020), virgin coconut oil (VCO) (Marikkar, Kamil, & Raihana, 2019), mustard oil (Navya, Raju, & Sukumaran, 2017), and sunflower oil (Abhirami & Radha, 2015) among others. Adulteration indicators, including moisture content, saponification value, peroxide value, and relative density value, do not meet the standard criteria in many of these adulterated samples (Kamol, 2019). Health, economics, and

ethical impacts are at stake when consuming adulterated oil products because it is hard to differentiate between real and adulterated foods using naked eyes (Kou, Li, Liu, Zhang, & Yu, 2018).

Alongside the regulatory framework to control food adulteration, including in PO and PO-based products, the food-related organizations must develop and implement effective ways to detect adulterations. This is to ensure edible oils and fats are authentic, therefore establishing food safety. Adulteration in edible oils is being identified using advanced and appropriate procedures (Tan, Kong, Irfan, Solihin, & Pui, 2021). Adulteration of PO usually takes place during the production of CPO or PO refining process (Andoh, 2021), including the incorporation of other residues into PO (Ghazali & Tukiran, 2021). Various analytical techniques were developed to determine these adulterants and some of the techniques are discussed in the following section.

15.5.1 Detection of dyes in palm oil

The application of portable near-infrared (NIR) spectroscopy coupled with the appropriate chemometrics as a screening tool for identifying the authenticity and adulteration of PO with forbidden Sudan dyes (I, II, III, IV) was presented by Teye et al. (2019). The functional groups of the chemical structure of Sudan dyes either alone or in combination could distinguish genuine PO from adulterated PO. Detection of many types of hydrogen bond group linkages such as C–H, O–H, S–H, and N–H, which compose distinguishable overtones and a combination of fundamental vibrations in the NIRS waveband, can be used as adulterant identifier.

On the other hand, Andoh et al. (2019) used surface-enhanced Raman spectroscopy (SERS) coupled with chemometric methods to detect the presence of Sudan IV in some edible PO samples. They were able to detect quantities of Sudan IV dyes in all four of the PO samples submitted for examination by measuring and evaluating the SERS spectra of Sudan IV, prepared PO, and other PO samples. Their results support their suspicions of adulteration. The advantage of using the SERS method includes the elimination of the need for sample preparation, which is common with high-performance analytical equipment like high-performance liquid chromatography (HPLC) and others.

Okogeri. (2013) tested the possibility of adulterating CPO with natural potash (lake salt) and red dye from the leaf sheath of *sorghum bicolor*. While measurement of common quality parameters of fats and oils failed to determine the adulteration, the use of sensory qualities, particularly taste and mouthfeel, was deemed as a trustworthy method, while heating oil samples to temperatures above 100°C appear to be the easiest, rapid, and successful way for identifying red dye in CPO. However, this method, although acceptable for screening, posed some drawbacks, including bias of the evaluator and inconsistency of results.

15.5.2 Detection of recycled cooking oil

In the cases of PO adulteration, spectroscopic methods have been given wide attention. In RCO cases, for example, Ghazali and Tukiran (2021) analyzed pork adulteration in recycled frying oils by using the combination of Raman spectroscopy and principal component analysis (PCA). Raman spectroscopy and PCA were found to be able to distinguish between contaminated and unadulterated PO used as frying oils. However, it was unable to determine the percentage of pork adulteration in the spiked samples. Three major peaks could be seen from the spectra which are 1303, 1440, and 1656 cm^{-1} . The other nine minor peaks such as at 845, 870, 890, 970, 1065, 1083, 1122, 1270, and 1745 cm^{-1} could also be observed through the Raman spectra. Raman shifts or wavenumbers were assigned to specific regions based on the vibrational characteristics of chemical bonds in the sample. These spectral data then were analyzed using PCA which separates contaminated and unadulterated PO used as frying oils.

Another approach is by detecting fatty acid composition (FAC) using gas chromatography (GC)-flame ionized detector coupled with functional group spectral analyses using Fourier-transform infrared spectroscopy (FTIR), and chemometric data interpretation was demonstrated by Lim et al. (2018). When compared to FPO, there were several noticeable differences in FAC of RCO, including (1) the appearance of short-chain fatty acids (C8:0 and C10:0), trans fatty acids (trans C18:1), and other fatty acids not found in FPO (C11:0 and C20:5); (2) a significant increase in odd chain fatty acids; (3) a significant increase in MUFAs; and (4) an insignificant decrease in polyunsaturated fat (PUFA). On the other hand, the distinction of the different spectral bands between FPO and RCO can be seen at 3529 and 3442 cm^{-1} which are attributed to –OH stretching vibration. The deviation was due to the significant amounts of alcohols or secondary oxidation products formed from thermal oxidation as well as the formation of –OH functional group associated with mono- and diacylglycerols from hydrolysis, as well as the formation of hydroperoxides or primary oxidation products in RCO. The hydroperoxides could diminish over time as these unstable molecules degrade into alcohols, aldehydes, ketones, and other secondary oxidation compounds, as observed by the amplified intensity at band 3529 cm^{-1} in RCO. Qualitative discriminant analysis

of FTIR bands exhibited a score plot that successfully classified FPO and adulterated oil samples and also classified different commercial packet oils into the group of FPO. Therefore FAC measurements could be utilized in tandem with functional group determination in detecting FPO adulteration with RCO.

Using the same approach, [Tan et al. \(2022\)](#) successfully tested the efficacy of utilizing GC-mass spectrometry and attenuated total reflection-FTIR spectroscopy to detect the adulteration of FPO with inedible RCO. They also extensively explained the physical and chemical changes of RCO resulting from the reactive mechanisms of oil quality degradation.

Another study on PO adulteration with RCO using a handheld NIR spectroscopy was demonstrated by [Irfan, Pui, and Solihin \(2020\)](#). NIR spectroscopy was used to examine pure PO and RCO adulterated PO samples in the 900–1700 nm wavelength range. To distinguish between these samples, PCA was utilized, and a preprocessing approach such as Gaussian smoothing was used to examine the spectra more effectively. The NIR classification accuracy for detecting samples with less than 15% adulteration was found to be extremely low. NIR, on the other hand, efficiently differentiated between pure PO, used frying oil, RCO, and adulterated samples with concentrations of 15% and above. Therefore there are several different methods to determine PO adulteration with RCO. However, interlaboratory and intralaboratory testing needs to be done for validation purposes before setting these methods as routine or confirmatory tests.

15.5.3 Detection of palm oil as adulterant in food products

Adulteration of fats and oils is becoming more common around the world daily. As a result, various appropriate quick detection procedures for assuring food quality and safety were developed ([Huq et al., 2022](#)). This section would discuss cases of PO adulteration in three food products as summarized in [Table 15.2](#).

In the case of PO adulteration in butter, an increase in unsaturated fatty acids (C18:1 and C18:2) and a decrease in saturated fatty acids (mainly C10:0, C12:0, and C14:0) were found. The varying concentrations of cholesterol and sitosterol as marker sterols can distinguish between PO and butter. However, the final confirmation of the aptness of the approach needs further analyses for the purpose of the quantitative determination of adulteration ([Feizy & Jahani, 2020](#)).

Another researcher, [Gamage et al. \(2020\)](#) used the same technique of fatty acid analysis in coconut/PO mix in which the relationship between log area of lauric divided with area of palmitic value and percentage of PO composition was determined. Their result showed that the log value decreased as the amount of PO in the coconut/PO mix increased. Therefore the amount of PO adulteration in the coconut oil test sample can be determined using the correlation; however, the method cannot confirm the types of adulterants present ([Gamage et al., 2020](#)).

[Marikkar et al. \(2019\)](#) researched another approach to determine PO adulteration in VCO by studying the effects of heating and cooling profiles of oils using differential scanning calorimetric (DSC). Both the heating and cooling operations of VCO and PO had considerably different DSC curves. As a result, after adulteration with PO, DSC curves of VCO were shown to vary significantly. These large changes in thermal transitions and the associated DSC parameters of VCO are thought to be caused by the shifting fraction of saturated to unsaturated triacylglycerides molecular ratio. The DSC parameters were then subjected to Pearson correlational analysis and were found to have a high correlation coefficient with lower standard error values.

Generally, adulteration of food items, including PO, has a very severe impact on producers/farmers, processors or manufacturers/enterprises, consumers, and the government. Hence, food control authorities should enforce a good regulatory framework, efficient food monitoring should take place, ongoing research on the quantification of adulterants in food for routine checks, and effective analytical procedures to detect frauds are required to safeguard consumers ([Ayza & Belete, 2015](#)).

TABLE 15.2 Adulteration detection of palm oil in food products.

Food products	Detection method	Findings	References
Butter	GC-FID	Fatty acids and sterols	Feizy and Jahani (2020)
Coconut oil	GC-FID	Fatty acids	Gamage et al. (2020)
Virgin coconut oil	DSC	Heating and cooling profile	Marikkar et al. (2019)

DSC, Differential scanning calorimetric; GC-FID, gas chromatography-flame ionized detector.

15.6 Conclusion

The management of the PO supply chain is a long and complex matter, particularly when dealing with sustainability, halal aspects, and international trade and shipping. RSPO and halal certification have been deeply discussed in this chapter. While RSPO focuses on the sustainability of PO production, halal certification focuses on the Shariah-compliant aspect of the supply chain. Both RSPO-certified and halal-certified palm products are usually not continuously traceable, whereby the origin of the product could not be known at any given moment in time except for the RSPO with IPSC model. However, the high costs and low volume make the IPSC only applicable for higher segment market products. Although consumers and producers in developed countries are becoming familiar with standards which define the quality and/or geographical origin of food products, certifying and labeling products from a global and fragmented supply chain, such as PO is challenging particularly when the consumer demands clear information and traceable products. Additionally, traceability in the PO supply chain extends to the adulteration cases which are still rampant. Research is still ongoing to establish new detection methods to identify and quantify PO adulteration.

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Halal biotechnology product: halal supply chain compliance and integrity risk

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16.1 Introduction

The Islamic Shariah law defines the Muslim's dietary rules in accordance with the Quran (the holy book), Hadith (the collection of the traditions of Prophet Muhammad), and Fiqah (consensus opinions of a group of Islamic scholars) (Rahman, Razimi, & Ismail, 2020). Thus halal is a basic feature of Muslim culture and is part of Islam's belief system and moral code (Usman, Chairy, & Projo, 2021). According to the teachings of the Holy Quran, every Muslim is expected to consume only halal products. One interpretation of this clear mandate is that the 1.8 billion Muslims are on Earth now, and the estimated value of halal products is expected to reach 10 trillion USD by 2030 (Al Amin et al., 2020). Several studies have revealed halal is not just a large market, but also has bright prospects and a spirit of enthusiasm for the future expansion of products (Riaz, 2007). The rapid growth of the halal industry is due to the expansion of Muslim populations, increasing public awareness of the advantages of halal products, as well as an increase in consumption by non-Muslims (Amalia, Sosianika, & Suhartanto, 2020; Bashir, Bayat, Olutuase, & Abdul Latiff, 2019). As a result, the halal biotechnology product also has a huge amount of potential for entrepreneurs and investors. The global market for biotechnology product is vast and rapidly expanding.

For hundreds of years, Islamic religious principles have served as guiding principles and fundamental components in the design of Muslims' daily activities (Mahrnasari, Bangsawan, Rahman, & Shahril, 2020). Furthermore, religion has a long history of influencing scientific results, both directly and indirectly (Fischer, 2008). Rather than posing a direct threat to scientific knowledge, the halal biotechnology product manufacturing standards have an impact on the production of a wide variety of consumer products. Thus halal biotechnology product preparation and retailing standards are more than just a set of religious dietary or food processing requirements; it also needs to in line with the halal supply chain (HSC) compliance.

The application of biotechnology may not only dramatically alter the production of food but also cause a major change in the industrial organization of the entire HSC from producers to retailers. For instance, the current HSC approach in food industry is progressively avoiding substances such as gelatine, alcohol, glycerin, and other emulsifiers and flavoring agents that may be contaminated with porcine residues or other animal products and which can be applicable to any biotechnology-based porcine goods. Thus, to be halal-compliant, current biotechnology products must be manufactured in accordance with Islamic Shariah principles, along with the HSC compliance from farm to fork. Therefore the direction of Halal compliance of biotechnology products is the starting point of this chapter. Furthermore, HSC compliance is most crucial factor for maintaining biotechnology product as Halal. Therefore the chapter includes the description of HSC and the HSC compliance with biotechnology product context. Halal integrity assurance is a shared obligation of the various stakeholders participating in HSC. Consumers are more inclined to seek out halal products (Riaz & Chaudry, 2004). Hence, finally, the chapter describes the HSC compliance of the biotechnology product along with the integrity risk.

16.2 Biotechnology product and halal compliance

The term “biotechnology” refers to the use of biological entities in the development of products, foods, and healthcare, along with agricultural productivity, food processing, renewable energy sources, and environmental and industrial monitoring (Bakar, Hamid, Syazwan, & Talib, 2014). Biotechnology products are one of the most significant advancements in contemporary technology and food product manufacturing. It has a number of advantages, one of which is a high production yield (Karahalil, 2020). According to Islamic law, genetically modified organisms (GMOs) can be used to produce biotechnology products that are halal, depending on the species and objective of gene alteration. As a result, issues such as gene source and modification requirements are still widely debated, despite the fact that several transgenic biotechnology products have long gained halal certification. Thus biotechnology is still being debated from a halal perspective, even though biotechnology products are enhancing human well-being and eradicating undesired circumstances like disease. Many essential biotechnology products that are utilized in the pharmaceutical industry are derived from GMOs. Thus, because of biotechnology’s many advantages, some Islamic scholars believe that it is permissible to produce and utilize biotechnology products as halal under certain conditions, such as the product should not include any porcine genetic material and the objective is to benefit human health without any negative impact.

16.3 Halal supply chain

Accreditation-based management of the HSC is a growing industry that is still in its developmental stage. Each step in the HSC must be established and carried out in compliance with recognized and verified halal standards as part of a process-oriented approach. This includes product sourcing, processing, handling, warehousing, transportation, and retailing. Thus the biotechnology product manufacturing and retail must be intended to eliminate cross-contamination of halal products by chemical, physical, or biological materials with nonhalal components. Product flow, capital flow, and information flow are all significant aspects of any supply chain, whereas, in the context of the HSC, these flows must adhere to Shariah principles to ensure halal integrity to consumers (Ali, Tan, & Ismail, 2017). Thus the management of the HSC necessitates strategic coordination and collaboration across the supply chain’s stakeholders to provide halal integrity to meet customer requirements. Collaboration and coordination can be aided by resource sharing (such as ingredients, technology, information, knowledge, and skills) among the HSC participants. Operational collaboration and coordination among halal trading associates improve decision synchronization, lowering the risk associated with HSC management (Bakar et al., 2014). The HSC’s performance and efficacy are inextricably linked one to other. Improving the performance of the HSC would address issues such as process quality (Bonne & Verbeke, 2008). The improved performance of the HSC will be mirrored by a trusted Halal brand, a reliable halal certification system, and reduced customer complaints.

HSC applies both the halal and the *Toyyib* characteristics and appears to be Shariah compatible with the essence of which is safe eating (Latif, Zainalabidin, Juwaidah, Abdullah, & Ismail, 2014). Furthermore, *Toyyib* qualities of halal products demonstrate that the product is clean, free of pollutants or intoxicants. The product originated from a halal source does not cause distress and suffering to those who consume/produce it and contains nutritional and beneficial components. Thus *Toyyib* is also understood as safe, nourishing, hygienic, and of high quality (Arif & Ahmad, 2011; Badrudin et al., 2012). The notion of *Toyyib* is related to food ethics, with the primary issues being product safety, animal welfare, humane handling of animals prior to slaughter, fair trade, environmental preservation, and sustainable consumption behavior. Thus the halal product manufacturing process should eliminate any materials that could be harmful to human health or the environment (Hassan, 2016). The management of the HSC necessitates an all-inclusive process that begins with manufacturing and concludes with utilization (Halaseh & Sundarakani, 2012). Table 16.1 is constructed using the HSC definitions with different criteria such as what are the major observation, what these definitions are focused on, as well as their strengths and weaknesses.

16.4 Halal supply chain compliance of the biotechnology products

16.4.1 Ex ante manufacturing

16.4.1.1 Product safety

Product safety is a critical issue since it may have a direct impact on customers’ health and may lead to death. The vulnerabilities of the halal biotechnology products HSC in terms of safety may include products that are particularly hazardous if not properly managed. Furthermore, a lengthy and complex HSC may increase the risk of biotechnology

TABLE 16.1 Examinations of halal supply chain (HSC) definitions based on distinct observation, direction, strength, and weakness criteria (Khan, Haleem, & Khan, 2018; Khan, Haleem, Khan, Abidi, & Al-Ahmari, 2018).

Observation	Directed toward	Definition	Strength	weakness	References
Supplier and buyer	Halal and nonhalal food products	"The process of managing Halal food products from different points of suppliers to different points of buyers/consumers, which involved various different parties, who are located at different places, who may at the same time, involved with managing non-Halal food products, with the purpose of satisfying the needs and requirements of both (Halal and non- Halal) customers."	A comprehensive definition that considers suppliers as well as customers in a broader Supply Chain context. A supply chain using a mixed-network approach	Toyyib and process orientation may have been discussed further.	Zulfakar, Anuar, and Talib (2014)
Halal network Halal integrity	Food products	"HSC Management can be defined as the management of a Halal network with the objective to extend the Halal integrity from source to the point of consumer purchase."	The industry may benefit from the realistic approach.	The Toyyib component is absent.	Tieman (2011), Tieman, Van Der Vorst, and Ghazali (2012)
Islamic Shariah perspective	Halal foods farm-to-fork approach	"Halal Supply Chain starting from the point of origin to the point of consumption. These activities include warehousing, sourcing, transportation, handling of products, inventory management, procurement and order management which must follow the Syariah Islamic perspectives."	The specialized definition of supply chain operations	The standpoint of Toyyib is not stated explicitly.	Omar, Jaafar, and Osman (2011)
Halal integrity	Halal products Halal network	"Halal Supply Chain is the management of a Halal network with the objective to extend the Halal integrity from source to the point of consumer purchase."	Halal products Halal network	More clarity on Toyyib perspective is required	
Inter and intraorganizational	Food products	"Process of managing the procurement, movement, storage and handling food products through the organisation and the Supply Chain in compliance with the general principles of Sharia law."	Shariah-compliant practices are prioritized in the supply chain management process.	Logistics are the primary emphasis; the Toyyib component is not properly stated.	Tieman (2009a, 2009b)
Logistics	Halal-certified product	"Process of planning, implementing and controlling the efficient flow and storage of Halal certified product from the source to the demand point."	Supply chain coverage; halal certification's significance	The Toyyib element is unclear; all supply chain operations are not being taken into account.	Che Man, Bojei, Sazili, and Abdullah (2007)

Source: Adapted from Khan, M. I., Haleem, A., & Khan, S. (2018). Defining halal supply chain management. *Supply Chain Forum: An International Journal*, 19(2), 122–131; Khan, S., Haleem, A., Khan, M., Abidi, M., & Al-Ahmari, A. (2018). Implementing traceability systems in specific supply chain management (SCM) through critical success factors (CSFs). *Sustainability*, 10(1), 204.

products' integrity and the risk of product adulteration, both intentionally and unintentionally (Ali et al., 2017; Marucheck, Greis, Mena, & Cai, 2011).

Safety concerns are more critical in biotechnology food products because of the difficulty in determining the "safety zone" for consumption besides the expiration date. Unfortunately, Voss, Closs, Calantone, Helferich, and Speier (2009) discovered that when it comes to supplier selection, food safety is ranked lower than price, delivery, and quality. Therefore the factor of biotechnology product safety is too crucial, particularly in terms of HSC compliance. It must be consistent with product integrity criteria that clearly specify safety, and rules, considering the health concerns (Regenstein, Chaudry, & Regenstein, 2003). For example, halal contains the idea of thoyyiban, which translates as "safe to consume" (Halim & Salleh, 2012). Hence the biotechnology product manufacturing with HSC should comply with product safety.

16.4.1.2 *Products purity with halal compliance*

Halal is concerned with product purity, equally materially and spiritually in addition to safety concerns. Therefore decision-making processes of halal biotechnology products' consumers are distinct from those of normal consumers. Customers of halal products focus on the product's halal status (Alam & Sayuti, 2011; Ali et al., 2017). Hence, halal biotechnology products' fundamental substance is purity. The products must be pure and free of any components that are determined as nonhalal. Therefore the purity of biotechnology products is crucial throughout the entire HSC.

16.4.1.3 *Quality of resources*

The distinction between resource quality and resource purity is based on the fact that quality often comprises product interfaces such as shelf life, freshness, size, and flavor. Only by adhering to strict halal processing rules, the biotechnology product resources can be purified. Although the border between quality and purity in the halal context is quite narrow, there are occasions where halal biotechnology items may fail to achieve quality standards. For instance, meat from nongenetically modified cattle will be regarded as pure and meets the subjective dimension of purity sufficiently, yet the microbial count of the meat may not fulfill the quality goal. Thus the quality of halal biotechnology products may depend on the firm's own product quality, as well as the quality of its suppliers. As a result, the integrity of biotechnology products might be jeopardized if the quality of the resources used is not known. Thus the notion of thoyyiban, the comprehensive perspective of halal biotechnology product quality, is linked to the safety, nutritional matter, and esthetic demand of halal products.

16.4.1.4 *Monitoring*

Traceability is a valuable tool for assisting firms in responding to food threats, documenting the chain of production practices, meeting regulatory requirements, and analyzing logistics and production costs. Thus implementing a traceability system is not required nor sufficient for attaining food integrity, especially when some types of traceability exist without the explicit adoption of a traceability system (Resende-Filho & Hurley, 2012). However, traceability and tracking capabilities demonstrate the power of HSC management (Zailani, Arrifin, Wahid, Othman, & Fernando, 2010). Therefore monitoring is essential, particularly for biotechnology-based product, because in certain cases, testing for quality is nearly impossible in postmanufacturing process. For instance, the recitation process is a crucial part of slaughtering, which needs a close monitoring process. It is quite impossible to determine by the mean of meat analysis technique, even though the meat is produced from a nongenetically modified animal. Therefore, for the biotechnology product, HSC monitoring is needed to make sure that product is produced in compliance with halal.

16.4.2 **Manufacturing in process**

16.4.2.1 *Internal structure*

Numerous studies show that following standards and rules may benefit businesses in several ways, along with economies of scale. As a result, stakeholders in biotechnology products should work to design, describe, and improve new norms and certification methods (Savov & Kouzmanov, 2009), whereas the MS1500:2009 standards are one of the world's major halal standards (Lever & Miele, 2012). MS1500:2009 is mostly viewed as a unique business strategy, and it is also a guideline for Halal food manufacturers (Daud, Din, Bakar, Kadir, & Sapuan, 2011). However, the application of MS1500:2009 is handled as a stand-alone standard and is not implemented in conjunction with other existing standards, partially because this is not specified in MS1500:2009. MS1500:2009 can be viewed as a broad consolidation

of halal food production principles in the context of today's food industry. Thus specialized biotechnology product quality standards like halal posit stricter criteria can lead to an increase in biotechnology product quality (Trienekens & Zuurbier, 2008). However, Magkos, Arvaniti, and Zampelas (2006) asserted that using a single standard is inadequate since it focuses on only one component of the product's quality.

The scientific community has highlighted concerns about genetic engineering since its commencement (Holst-Jensen et al., 2012). The creation of GMOs, and in particular their commercial application, is subject to stringent legal restrictions. The existing legal framework tackles all concerns associated with the release of biotechnology products into the environment and the consumption of GMOs for human or animal health. As a result, biotechnology products have limits such as GMO maize generated by biotechnology may be utilized as animal feed, but not for human consumption.

16.4.3 Strategy for manufacturing

Halal biotechnology product producers might concentrate on production techniques such as lean production (Cox & Chicksand, 2005), cost minimization (Ge, Gray, & Nolan, 2015), and production flexibility to achieve performance (Aramyan, Lansink, Van Der Vorst, & Kooten, 2007), whereas Aramyan et al. (2007) found that, along with food quality, responsiveness, and efficiency, flexibility was an important performance metric in assessing the agricultural food supply chain's overall performance. Restricted supply and limited number of suppliers may jeopardize product integrity, particularly when it is difficult to fulfill customer demand.

16.4.4 Involvement in joint ventures activities

In the notion of economies of scale, a manufacturer of a product agrees to provide independent operators the right to carry on business in a certain manner, at a specific location, and for a specific amount of time (Olotu & Awoseila, 2011). According to Gates (2000), a venture is not a company in and of itself, but rather a method for doing business. When it comes to perishable biotechnology goods, marketing is a powerful tool for sales and promotion, although strategic alliances may function similarly to conventional products. In the context of biotechnology-based halal products, the incorporation of special integrity criteria may be more crucial. In contrast to conventional products, halal certification necessitates the presence of certain criteria—for instance, the number of Muslim employees to maintain the HSC protocol.

16.4.5 Human resources

Previous research has claimed that the human element is the most critical and fragile component of an effective service delivery system (Murphy, DiPietro, Kock, & Lee, 2011; Wildes, 2005). This industry has a high level of employee engagement in product development and manufacturing, as well as quality control. Both short- and long-term issues develop when a firm employs too many people to meet short-term goals, and it is difficult to educate highly competent and qualified personnel in the long term. For this reason, biotechnology product manufacturers need to comply with trainee program and to help their employees move up the ladder. This would help the company get the most out of its investment in training.

16.4.6 The process of outsourcing

Strategic outsourcing, according to the literature, happens when organizations have complementary competencies (Holcomb & Hitt, 2007). Following this line of thought, outsourcing is classified as “manufacturing in progress” in this context. According to Quélin and Duhamel (2003), one of the reasons for outsourcing is to increase quality, and logistics may be the most outsourced activity. To guarantee that the biotechnology product is not contaminated with nonhalal food during shipment, the transporter must be certified as a specialized halal product shipper. Outsourcing will allow the primary biotechnology product manufacturers to concentrate on their core activities.

16.4.7 Ex postmanufacturing labeling

Labeling is associated with customer confidence and it is compulsory (Houghton et al., 2008). It is important for customers to have trust in their purchases, so labeling is necessary (Houghton et al., 2008). The halal logo produced by the

manufacturers may have a significant impact on Muslim purchase behavior for halal biotechnology products. Hence, labeling and the display of a company's logo for biotechnology products are necessary. Despite the fact that biotechnology product manufacturing met the standards' criteria for the display of the halal logo and suitable labeling, there is still the possibility of nonhalal conformance. The labeling of a halal biotechnology product should ideally include all relevant information about the product. The sources of raw materials, for example, may fluctuate from one batch of manufacture to the next. As a result, sometimes this information is critical for customers. When a biotechnology product is discovered to be nonhalal, the company's information integrity effort should address the issue as soon as possible. In this case, labeling alone is insufficient to ensure information integrity. The factor "adequacy of labeling" indicates that information transmission has been elevated to a new level. Thus the HSC in this digital era, biotechnology product manufacturers may improve their customer outreach.

16.5 Biotechnology product and halal supply chain integrity risk

This section highlights six HSC integrity risks of biotechnology products (Fig. 16.1) and describes these dimensions of product manufacturing, product certification, supplier, outsourcing practice, human resources, and halal logistics.

16.5.1 Product manufacturing

Halal biotechnology products should be produced in accordance with established guidelines and regulations. The halal biotechnological product may be exposed to a variety of risks at various points in its supply chain, and the focus industries are held accountable for these actions. Customers' satisfaction with halal biotechnology products is directly tied to the efficiency and quality of the manufacturing process, which is a critical link in the lengthy and intricate supply chain. The most significant risks during the manufacturing stage of halal biotechnology products in accordance with the HSC include contamination with nonhalal components or the use of nonhalal-certified materials derived from nonapproved altered gene products. Thus biotechnology products have a concealed halal integrity risk throughout the manufacturing stage since the ingredients' halal status is unstable.

Furthermore, when it comes to developing products manufactured from GMOs, halal certification should be a must. There is an average 6-month lead time for certification renewals (as refer to JAKIM), which are done every two years (Ali, Tan, Pawar, & Makhbul, 2014). More than likely, there will be modifications to product-related factors like suppliers' halal status, the status of ingredients, and so on; these changes are more likely to occur than those that have already been approved and certified by the appropriate authorities. A change in components might jeopardize the biotechnology product's halal certification. In addition, internal quality control systems at the companies cannot keep up with all of the ingredient adjustments automatically and need close supervision by skilled individuals. Furthermore, halal biotechnology product manufacturing is subjected to the possibility of contamination from equipment, which may have a



FIGURE 16.1 Biotechnology product's halal supply chain integrity risk.

negative impact on the halal integrity of the product. The origin of items for biotechnology product packaging, for instance, is contentious, particularly when unknown animal sources are employed, as in the oil, fats, or gelatins commonly used in the manufacturing of polyethylene, containers, and capsules.

16.5.2 Product certification

Biotechnology product is not exempt from importing raw ingredients because of the advantages of the global HSC. However, aside from Malaysia, very few countries use government interference to implement halal certification and standards. Numerous countries remain dependent on private halal organizations. As a result, despite of competent authority acknowledgment of foreign credentials, there will be uncertainty about the HSC integrity of biotechnology products. Furthermore, the lack of traceability mechanisms may lead to an overreliance on halal certification for biotechnology products. Insufficient enforcement by relevant authorities on fake halal logos and certifications may complicate their legitimacy. The compliance with halal certification procedure is only implemented at the very early stages of the supplier's appointment process (Ali et al., 2014). Due to this shortcoming, the potential risk to halal integrity grows as the visibility of the lengthy supply chain cannot be assessed, which is particularly important when there is poor traceability of genetically modified biotechnological material. It is possible that it will have an impact on the safety elements of human health and safety measures.

16.5.3 Supplier

An increase in the risk of biotechnology product integrity with HSC may be caused by rigidity in supplier selection. Such rigidity may limit the company's ability to take advantage of the market's potential sources. As a result, the local halal biotechnology sector may have to turn to the overseas market for more suppliers. Thus halal biotechnology product security can be compromised by unanticipated circumstances such as unusual or seasonal demands, product shortages, poor management, and price fluctuations of certain materials in the small local market. Despite the fact that the frequency of occurrences is ambiguous, the security risk is relevant to the integrity of biotechnology products because of the product's trusted quality and the profundity of the HSC. Furthermore, owing to the lack of standardized halal certifications globally, validating HSC integrity with certifications and suppliers' for halal biotechnology products may become challenging for the company.

16.5.4 Outsourcing practices

The biotechnology business may choose to maintain the core process in-house while contracting out the performance of smaller tasks to a third party. There may be a risk to products' HSC integrity when outsourcing procedures are implemented, even if it is widely accepted that outsourcing is beneficial. For example, biotechnology product manufacturing using HSC, the supply chain length may make it more difficult for the focal business to keep track of internal quality control processes. According to the literature, the downstream procedures were linked to product-based performance in the form of compliance with specifications and durability (Tseng, Wu, Lin, & Liao, 2008). Logistical challenges, warehousing, packing, marketing, and the recruitment of both qualified and low-skilled employees are all examples of activities that may be outsourced. Despite the fact that companies highlight the importance of agreements as a precaution for both parties, the literature used to identify contracts may violate organizational standards (Lyles, Flynn, & Frohlich, 2008), whereas it is possible that outsourcing will compromise the HSC integrity of biotechnology products because of the service provider's opaque subcontracting methods.

16.5.5 Human resource

To produce halal goods, biotechnology product manufacturers and retailers must be directly involved with the HSC. Therefore the human resource factor is the most important and critical part of an efficient service delivery system (Wildes, 2005). In the same vein, the manufacture of halal biotechnology product necessitates the use of a trained and devoted group of workers to limit the likelihood of human mistakes in the production of biotechnology-based halal products.

16.5.6 Halal logistics

Halal biotechnology products cannot be subjected to nonhalal materials in any manner, shape, or form. Therefore in the HSC-compliant for halal biotechnology products, logistics is viewed as one of the riskiest groups. Transport platforms should be ritually cleansed and shipments should be appropriately separated to guarantee that the HSC standards are met and that the logistics are committed to halal commodity deliveries (Ali et al., 2014; Jaafar, Endut, Faisol, & Omar, 2011). Unfortunately, there are just a few approved halal logistics companies to meet the demand for halal products. The integrity of halal might be questioned while logistical hazards are not reduced in this field.

16.6 Conclusion

This chapter has consolidated the literature on biotechnology products along with the description of the halal compliance. In spite of the lack of supply chain integration techniques in the related industries, it has presented a framework for HSC compliance for biotechnology product. Although supply chain integration approaches in conventional industries are indeed far from halal biotechnology product integrity risks, a conceptual understanding of how HSC may function in this context has been presented. The notion of HSC compliance in biotechnology product manufacturing companies has opened a new path in reducing integrity risks such as product manufacturing, product certification, supplier, outsourcing practice, human resources, and halal logistics. Due to the limitations of comprehensiveness, uniqueness, and novelty of the halal biotechnology industry, it has been challenging to discover further integration strategies. This means that the HSC integration theory for biotechnology products opens up a wide range of possibilities for further study. Final thoughts: we believe that this chapter content may be evaluated further for more appropriate HSC integration approach. More advanced work can offer strong direction in addressing specific concerns about the integrity of the HSC of biotechnology product with further halal industrial research.

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The development of halalan toyyiban warehouse performance measurement system model and its usability

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17.1 Introduction

Warehousing plays a vital role in streamlining the supply chain. A warehouse is a facility, building or center in the supply chain to consolidate goods to reduce transportation costs, achieve economies of scale in manufacturing or in purchasing or providing value-added processes, and shorten response times (Ramaa et al., 2012). The warehouse provider companies can offer tailored services for their customers and achieve a competitive advantage in the marketplace. The challenges are becoming harder when storing food-related products whether for dry or cold edible goods (Bond et al., 2013). Such understanding of the warehouse management and warehousing processes is limited (Vatumalae et al., 2020). Additionally, it involves the main warehouse management activities such as reducing the warehouse costs and inventory holding, increasing warehouse efficiency, increasing inventory accuracy, increasing productivity while achieving higher value for customers and higher levels of service quality (Richards, 2011). Karim et al. (2020) describe the warehouse facilities' primary function to buffer and smoothen the material flows by storing excessive flows, making reserves, and providing the goods to the market on demand. Therefore a warehouse is a vast contributor to speed up and uphold the supply chain management.

Shariah Compliance has emerged as a key issue in establishing successful halal supply chain (HSC) and halal logistics. The foundation of a halal logistics system is based on three fundamentals: uphold the halal integrity of goods across the supply chain from farm to fork, avoid cross-contamination (direct contact with haram), and ensure consumables are safe for consumption (Karia, 2019). Halal integrity is defined by Zulfakar et al. (2012) as the products remain halal from upstream to downstream supply chain, free from any activities that might breach the halal status, intentionally or unintentionally. Similarly, Mei et al. (2017) articulate halal integrity as an assurance of safe, quality, and free from malpractice food from farm to fork. They stressed that halal integrity is crucial to the success of the emerging halal market. As a key element in halal logistics, halal warehouse operators need to ensure that all the storage and handling activities adhere to Shariah Compliance.

Halal warehouse and conventional warehouse processes are almost similar, in which the activities involve receiving, putting away, storing, cross-docking, order picking, shipping preparation, and shipping. Therefore the additional purpose of a halal warehouse is to uphold halal integrity from inbound until outbound processes. The halal warehouse needs to segregate halal from nonhalal products based on the three basic principles, which are (1) to avoid cross-contamination, (2) to avoid making mistakes, and (3) to ensure warehouse operations are consistent with the expectations of the Muslim consumer (Bruil, 2010; Arshad et al., 2018).

To be certified halal in Malaysia has many benefits in terms of the higher market segment (Husain et al., 2012; Yusuf et al., 2017) and government of Malaysia has taken a significant effort to support the halal market development. Still, it has seen less interest from logistics service provider's (LSP) party. The Halal Malaysia Directory page in Halal Malaysia Official Portal, based on "logistics" category searching, accessed on January 14, 2022, found only 76 LSPs companies operating in Malaysia are currently certified halal. This number includes transportation, warehousing, and retailer service providers. This number is meager considering the high number of halal-certified food and beverage

categories, 5170 companies, and 177,518 products. This does not yet include the number of the other halal-certified categories. As such, this big gap is becoming a serious area to be studied. This aligns with [Abdul Majid et al. \(2019\)](#), which calls for more research in halal logistics among service providers.

Ensuring the integrity of halal products from receiving to shipping adds challenges to the halal warehouse providers. As one of the entities in the HSC, the halal warehouse needs to be (1) robust to minimize the impact of possible contamination, (2) effective in ensuring the integrity at each process, and (3) efficient to avoid higher prices for halal food products ([Global Islamic Finance Report, 2013](#)). These requirements present significant challenges to the halal warehouse service providers. Some LSP believes that halal logistics increases their costs ([Talib et al., 2013](#)). Proper handling and storage activities are keys to protecting halal integrity for halal products in warehouse operation. Tools and materials that are used to handle halal food products must not be reused to hold nonhalal food products as they will cause contamination. Therefore providing exceptional facilities or appropriate equipment is needed. Consequently, this will increase the cost of warehouse operations if not properly planned ([Saleheen et al., 2014](#)). According to [Bahrudin et al. \(2011\)](#), the conventional supply chain aimed to minimize cost and maximize profit, while the HSC intended to ensure halal integrity of the halal product to the end consumer. Looking at this different objective clearly shows that there are challenges that halal warehouse operators must face.

With these complexities, the halal warehouse performance model is needed for a number of reasons, including regulatory, marketing, and competitiveness reasons. Overcoming these difficulties is not a minor issue, but warehouse organizations' long-term sustainability and competitiveness may rely on the successful adoption of performance measurement. In Malaysia the concept of halal warehouses focuses on warehouse operation, including inbound, storage, and outbound processes. All these must comply with halalan toyyiban (HT) assurance pipeline Malaysia Standard MS 2400–2:2019 (warehouse), halal warehouse service providers must ensure their activities comply with the Islamic requirements and follow the requirements of this standard. According to [Husain et al. \(2012\)](#), the focus of the Malaysian halal standard is on formal documentation of the halal system. Lately, there are many instances whereby halal certification does little to prevent poor halal products from being designed, manufactured, stored, and delivered to the customers. For example, the most recent news report is the withdrawal of halal certification of a seafood-based food processing premise in Hutan Melintang by the Perak Islamic Religious Department (JAIPK) in January 2019 for failing to comply with the Malaysian Halal Certification Procedure Manual ([Vibes, 2021](#)). It is clear that relying on halal certification alone is a strategic management mistake ([Othman et al., 2016](#)). The halal warehouse providers should have a mechanism to control the process by monitoring, managing, maintaining, and improving the performance of the services through the performance measurement ([Jagtap et al., 2020](#); [Tieman, 2017](#)).

As a result of the highly competitive market environment, companies are constantly pressured to enhance their warehousing operations in system-based applications ([Frazelle, 2001](#)). Business organizations need to capitalize on supply chain capabilities and resources to bring products and services to the market faster, at the lowest possible cost, with the appropriate product and service features and the best overall value ([Gunasekaran et al., 2001](#)). Existing research has highlighted the importance of performance measurement ([Tangen, 2005](#)), whether for organization, products, or processes. According to [Tangen \(2005\)](#), the main goal of the supply chain performance measure model and framework is to support management by helping them measure business performance, analyze, and improve business operational efficiency through the better decision-making process. Therefore it is required to analyze each issue and analyze how to control and measure the warehouse performance of each process by emphasizing the halal requirements. Thus the determination of halal performance measures is needed. In this study, a framework for measuring HT warehouse performance is presented together with its prototype which is then assessed for its usability.

17.2 Development of halalan toyyiban warehouse performance measurement system model

In this study the requirement of the assurance models and the elements for performance measurement are explored and analyzed through its issues and challenges. Two groups of case studies, a case study of four HT warehouse organizations (service providers) and a case study of four selected HT assurance models, are used. Interviews and observation were conducted for the first case study, and document content analysis was conducted for the second case study ([Shadan and Arshad, 2016](#); [Shariff et al., 2020](#)). Based on the two case studies, the halal warehouse performance model is proposed by adopting the balanced scorecard (BSC) model. The performance goals and measurements are based on the strategic objectives (SOs) proposed. The HT Warehouse BSC (HTWMBSC) is developed with four business perspectives, that is, financial, customers, business process, and learning and continuous improvement as depicted in [Fig. 17.1](#).

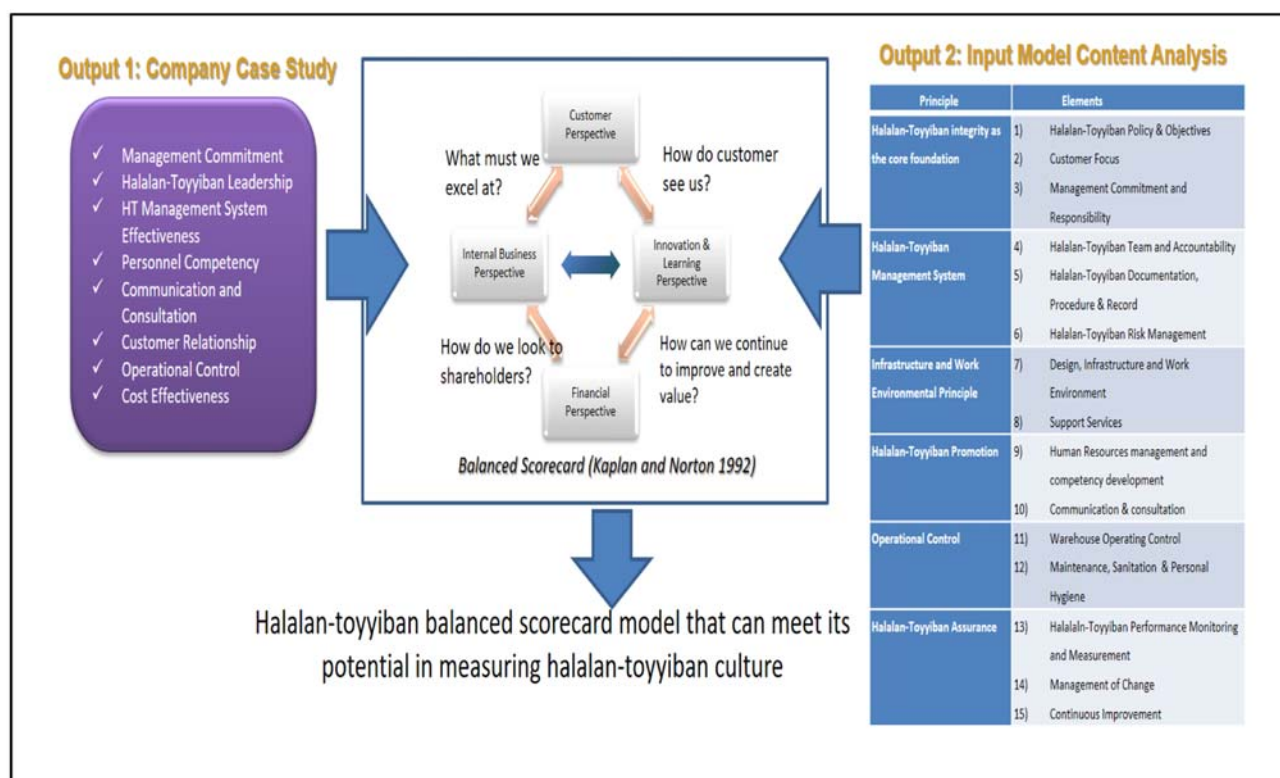


FIGURE 17.1 Model development. Adapted and adopted Shadan, H., & Arshad, N. H. (2016). *Issues in halalan toyyiban warehouse implementation*. Journal Applied Environmental Biological Science, 6(9S), 1–7 and Shariff, S. S. R., Shadan, H., Abidin, S. A. S. Z., Muhammad, A., & Nasir, S. (2020, August). *halalan toyyiban warehouse performance model: An adapted balanced scorecard approach*. In: 2020 11th IEEE control and system graduate research colloquium (ICSGRC) (pp. 357–360). IEEE.

Kaplan and Norton (1992) introduced the BSC that allows managers to look at their business performance from four essential perspectives: financial, customer, internal business, and innovation and learning. The BSC attempts to integrate all the interests of key stakeholders, that is, owners, customers, and employees, on a scorecard. The term “balanced” in the notion reflects the balance between short- and long-term objectives, lagging and leading indicators, and external and internal performance perspectives. The diverse interests and measures are categorized in the four perspectives mentioned previously of the scorecard in a slightly modified format, which can be an ideal tool for warehouse service provider’s organizations to assess and measure their HT culture and performance. Rather than measuring only the incidents and noncompliance statistics that a warehouse site might have, the BSC attempts to give a holistic and value-based balanced report (Gupta et al., 2018; Perminova and Lobanova, 2018; Arora, 2015). The HTWMBSC framework developed in this chapter focuses solely on measuring organizational (warehouse service providers) HT performance.

The BSC is a strategy execution framework. To develop the HTWMBSC, the goals of HT are formulated, how those goals fit into the organization’s overall strategy is defined, and how to track the execution of those goals is identified. Therefore the steps used in mapping the findings in previous stages are described in this section. First, we formulated the business goals, or missions mapped financial goals from the finance perspective, defined stakeholders’ needs from the customer perspective, recognized response plan from the internal perspective, and lastly identified enablers for the internal improvements from the learning and growth perspective (Shariff et al., 2020; Shadan and Arshad, 2016).

In developing the model, the following questions are answered:

1. What is the corporate strategy (mission/goal) for the context of HT warehouse?
2. Financial perspective: If the HT warehouse satisfies its customers, what outcomes will the shareholders see?
3. Customers’ perspective: To achieve the vision, how must its customers see the HT warehouse?
4. Internal business process perspective: How will the HT warehouse satisfy customer needs, meet financial goals, and keep HT integrity?
5. Innovation and learning perspective: How must the HT warehouse learn and improve to achieve the mission?

17.2.1 Halalan toyyiban warehouse performance mission

The establishment of HT policy demonstrated the commitment of the organization to enhance customer satisfaction by maintaining HT integrity as a priority. Based on the HT principle, this research formulated the inspiring mission statement for HT warehouse: *Strive to uphold Halal integrity and strengthen customer trust*. This mission incentivizes achieving the best possible performance and ensures continuous attention toward greater halal/toyyib.

17.2.1.1 Financial perspective

Based on the formulated mission, what is the financial impact of HT? In other words, *If the HT warehouse company satisfy its customer, what outcomes will the shareholders see?* From the HT aspect, this study concludes that the shareholders want to see sustainable growth through the excellent performance of upholding halal integrity (Ngah et al., 2017; Talib and Sakalayan, 2020). Therefore this study suggests mapping this under the goal: improving shareholders' value.

Failure to ensure ethical, hygienic, and immaculate food handling will impact the organization financially when this failure affects stakeholders' dissatisfaction. If halal customers are not satisfied, it will result in a reduction in customers. If the employee and halal team are not contented with HT culture, they may leave the company. The cost of recruiting and training a new employee will be increased. On the contrary, the customer's satisfaction toward HT service will increase customers' trust and continuously use the service and their products. This will also give an excellent image to the organization and attracts more new potential customers. In turn, this will increase organization's revenue.

Therefore this study proposes four SOs to measure the financial performance of the HT warehouse: (1) improve profit, (2) improve revenue, (3) improve cost structure, and (4) used of assets.

Financial sustainability is the result of operational controls and actions. Financial success is the logical consequence of doing the fundamentals well. By making fundamental improvements in their warehouse operation and productivity by reducing HT incidents, and maintaining and optimizing assets for better availability, utilization, and performance, the business goal of a profitable organization will be achieved (Haleem et al., 2020). Generally, asset utilization is a measure of how well a business is able to utilize its assets. A relatively high ratio means that the company is efficient in using its assets, whereas a low one may indicate poor asset management.

Besides, the organization should estimate all costs related to HT. The estimation includes all costs associated with problem/issues detecting and solving to understand the impact of poor HT. The cost includes corrective action costs such as Samak/Sertu costs and solution costs to prevent the repeated incident. It can also include halal brand impact costs, and even though the calculation value is difficult to quantify. Achieving high HT also has its price. However, the too costly value might not make sense for the stakeholders. The cost of high HT should be measured and evaluated. The cost-effective objective will increase more profit to the organization.

17.2.1.2 Customers perspective

To ensure the shareholder's value improves, HT needs to satisfy their clients' needs. To achieve the mission—*Strive to uphold Halal integrity and straighten customer trust*; how must the HT warehouse look to their customers? In this customer perspective, to answer this question, there is a need to answer these two questions: how are HT warehouse service providers (HTWSP) going to "satisfy customers"? And how can HTWSP ensure that they have "maintained a good HT reputation"? There are two business goals formulated from the customer perspective: customer satisfaction and HT reputation.

Two relevant SOs to HT warehouse are to improve product leadership and improve customer relationships (Tarmizi et al., 2014). In improving product leadership, BSC measures product quality. Therefore, to measure HTWMBSC in a customer perspective, the SO is to *Improve Service Leadership* by achieving the real HT of conformance. This can be indicated through the perfect order index. The perfect order index measures how many orders the warehouse ship without HT incident, where incidents are contaminated and damaged goods, inaccurate orders, or late shipments. Attaining a high perfect order rate should be the goal of every HT warehouse organization as it indicates warehouse efficiency and increased customer satisfaction. In putting together an ideal order, the warehouse service providers can encounter many complications, such as goods becoming damaged during shipment (Lester Wan, 2018; Omar et al., 2012). The key here is to understand how elements influence actual goals—HT conformance, so the organization can arrange them to prevent the noncompliance of HT incidents. This will also give an excellent image to the organization and attracts more new potential customers.

As part of ensuring the customers' perspective, the organization should also review the effectiveness of complaints and actions taken and the feedback. The organization should improve HT service related to customer requirements.

17.2.1.3 Business process perspective

From this business process perspective, halal warehouse needs to answer two questions: how will the organization "avoid contamination or HT noncompliance incidents" and "fix noncritical incidents quickly"? and how can the organization ensure that it has a "maintainable HT service"?

This research formulates these two goals: *operational excellence* and *HT management system effectiveness* (Karia and Asaari, 2016; Tieman, 2020).

The organization's commitment to ensure halal integrity is addressed through HT management. An HT management system provides a structured approach to those measures needed to achieve good HT performance within an organization. The measuring process of HT management system covers (1) adequately risk assessed and address ways of reducing the identified risks; (2) sufficient procedures well documented, easily identifiable, and easily obtainable and transmitted to employees; (3) roles and responsibilities of employees clearly described in HT-related documentation; (4) an ongoing mechanism exists for assessing compliance with the HT management system and improving HT performance; taking corrective action, when appropriate; revising the HT management system based on reviews and feedback (Shadan and Arshad, 2016).

These can be achieved through the following steps:

1. Improve external parties' HT participation and management

Having good relation with external parties, including customers (e.g., product suppliers) and subcontractors or partners, is an excellent strategy for HT performance (Mustun, 2021; Tieman, 2020). This requires commitment from management. It is recommended that an assessment or audit be made on the subcontractor or partner. Are subcontractors periodic payments reliant on outcomes of OHS audits? Do subcontractors' contracts or tenders include adherence to HT practices? When selecting subcontractors, the key personnel in charge should choose ones that he believed had the ability and commitment to meet the HT requirements of the job, instead of those with good price.

2. Improve cleanliness and equipment maintenance

Good hygienic design of equipment and facilities, specificity of the maintenance program, specificity of a sanitation program, the extent of personal hygiene requirements, specificity pesticide program, and other preventive measure design of HT control activities also need to be measured to drive HT effectiveness (Ibrahim and Othman, 2014; Zaib et al., 2015). Succeeding this objective will minimize equipment breakdown and avoiding food damage and contamination. The strategic initiative is to plan and schedule the equipment calibration, cleaning and sanitation, and pest control program. Overdue and uncompleted planned tasks may impact poor HT management. Broken equipment or tools does not necessarily mean a HT issue, but it could be a leading factor toward the noncompliance of HT.

3. Early HT problem detection

Early problem detection is one of the most crucial aspects of effective HT management. The benefits of early problem detection for a business include increased efficiency and potentially huge cost savings; the later into the operation in which problems are discovered, the more costly and time-consuming for the issues to be fixed. Early HT problem detection is a SO resulted from an effective risk management and can be achieved by measuring the proportion of issues identified through HT walks, inspections, near-miss issues reported; the proportion of identified hazards that are medium to high risk, unplanned downtime for equipment and temperature room/storage, time taken to rectify high-risk hazards, and the number of risk assessment and audit planned overdue (Zainuddin, Saifudin et al., 2020).

4. Improve HT incidents and handling issues

The ultimate goal for every HT warehouse organization is to carry out HT integrity, which means having "zero noncompliance HT incidents" (Shariff et al., 2020; Khalid et al., 2016). This goal incentivizes achieving the best possible performance and ensures continuous attention toward greater halal/toyyib. Incident management efficiency is critical in the event of any incident, problem, or issue occurs, including complaints from customers. Halal warehouses need to have an incident response team, which has qualified halal personnel as members, who are focused and able to monitor threats on an ongoing basis. Therefore they can respond promptly when incidents occur.

The team can determine what is considered a breach or threat, the roles and responsibilities of those addressing the incident, the technology available to manage the incident, and the procedures that need to be enacted to mitigate incidents, all of which speed up the response time. Everyone has the same focus and knows their role in the mitigation plan. Generally, incident management requires notification, initial review, investigation, analysis, action, and final review.

5. Improve operation and productivity

All stakeholders should have the same mission to work proactively to avoid critical HT incidents and fix noncritical HT incidents quickly. Warehouse operations will not be productive with interruptions due to incidents and time taken to solve the problem or remediation action. Improving warehouse operation and productivity is achievable when warehouse activities operate smoothly without interruption, fast service, optimized warehouse capacity, and high accuracy to avoid unnecessary cost (Shariff et al., 2020; Zainuddin, Saifudin et al., 2020). All warehousing activities from receiving to shipping need to consider critical control points and measure its cycle time, accuracy, capacity, and cost control. The number of HT incident reports indicates how well the warehouse successfully minimized error, contamination, and damages and its impact on the warehouse operation. A high frequency of equipment breakdown can also mean bad warehouse operation.

17.2.1.4 Learning and continuous improvement

How can the HT warehouse learn and improve to maintain halal integrity and straighten customers' trust? From the perspective of learning and continuous improvement, the BSC framework measures intangible assets, that is, human, information, and organization capital (Kaplan, 2009; Massingham et al., 2018). Therefore this research concludes that the HT should aim to achieve (1) having adequate right talent, (2) high-performance HT work culture, and (3) continuously making an improvement. Based on the identified output from both case studies, this research proposed SOs for HT warehouse performance learning and continuous improvement perspectives as follows:

1. Effective training and program strategy

Effective training and program strategy SO are proposed to overcome the challenge of lack of personnel awareness for both management and workers (Kaplan and Norton, 1992; Massingham et al., 2018). The organization should seek to create a thriving HT-minded organization by clearly understanding their job tasks and adequate personnel at all levels with the right competency. The human resource department should do the training needs analysis, as well as develop and conduct an appropriate training program attended by respective personnel. All employees should be trained, aware, and able to use their HT knowledge for their tasks and are competent to deal with HT emergencies. Records of qualifications and training should be maintained. Based on the records and staff performance reports, the competencies rate of the individual staff can be identified. Furthermore, based on the overall competency assessment, the organization can decide whether new talent hiring is necessary or not. When the organization routinely monitors the performance to identify potential areas of improvement, the outcomes of this training and program process lead to enhancements to the HT management.

2. Improve HT team capability

The SO to improve HT team capability will derive the effectiveness of HT performance. HT leadership is a group of credible, motivated individuals who continuously promote and motivate employees to meet and exceed the HT goals of the collective organization (Zainuddin and Shariff, 2016). HT leaders are the individuals with the most significant understanding of the importance of HT and who have the ability to inspire and communicate with their fellow employees. Therefore an HT leadership team will most effectively make up a multidisciplinary unit, touching as many departments and roles as possible. Appointment from various departments allows members of the HT team to have a clearer picture on how HT directly links every aspect of the organization and provides a comprehensive and more appropriate suggestion and view for HT guidance and improvement. This also reduces the communication gap where the information is conveyed more smoothly through the HT department representatives. Top management representative members can also enhance the capability of the HT team, or preferably led by the high-rank manager. The measure should consider qualified and appropriate HT leaders and members—with personnel who are competent, trained, and fit for their jobs. In addition, the size of the HT team should be in the manageable ratio to the organization's size.

3. Improve management commitment to HT leadership

Improve management commitment to HT SO is proposed as a leading performance measure. There must be visible evidence of HT leadership and management “walking the talk” and demonstrating by example (Shariff et al., 2020; Karim et al., 2020). Top management should demonstrate leadership and commitment to implement and improve the effectiveness of HT and demonstrated by establishing HT policy and communicating to the organization the importance of HT and meeting customer requirements. These fundamental requirements should express the organization's strategic direction and commitment to HT, and continuously review its suitability. Every personnel should be fully informed and understand the HT policy. The top management's firmness and sincerity toward HT can be shown based on the reminder and warnings issued. This management's seriousness will be realized by its

subordinate and encourages them to be more careful in the execution of operations and avoid any poor HT incidents. The high management leadership and commitment will support organizational learning and growth and continuous improvement in managing HT process.

4. Good work environment, infrastructure, and support system

The physical-social working environment provides people with the capabilities and opportunities to contribute to HT management effectively. They are actively engaged in problem-solving and decision-making and even receive education and practical training that fits their task and HT purpose (Zainuddin, Ridzwan et al., 2020).

The best achievement in the aspect of human capital is that employees hold HT integrity as a value. They actively take good care of safety, equipment, workplace cleanliness, and personal hygiene and avoid contamination.

A good working environment provides people with the capabilities and opportunities to effectively contribute to HT management as they actively engage in problem-solving and decision-making. Good two-way communication and consultation will increase employees' motivation. The organization can always publish and remind employees of HT's importance through various methods and tools such as newsletters, bulletin boards, intranet portal, email, and daily briefing. Appropriate communication processes are required to fully inform the effectiveness of the HT management system and compliances (Yahya et al., 2016). Information and communication systems are provided to support operations and to interchange information. The HT policy, goals and objectives, HT procedures of the organization can be understood and made aware by all levels through a good communication process and consultation.

5. Continuous learning

An organization's ability to innovate, improve, and learn corresponds directly to a company's value. Continuous learning processes with innovation can bring about efficiency in the operating domain of the business. How must the warehouse company learn and improve to achieve the HT mission? From the learning perspective, it is vital to map HT and customer satisfaction goals that will help to focus learning efforts (Kaplan and Norton, 1992; Rahima et al., 2016; Zaib et al., 2015). What information about the stakeholders will help to make better decisions? What IT infrastructure will support to serve stakeholders better? What changes in management can lead to reaching internal goals? Among the new things that can be obtained or improved is through the process of identifying the root cause of incidents, including consumer complaints, inspections and employee complaints, and near-missed incidents and noncompliant audits results.

6. Process improvement

This SO requires halal warehouse to review the organization's HT management's strengths and weaknesses. Does it accurately reflect how the organization wants to manage HT? When the organization routinely monitors the HT performance to identify potential areas of improvement, this process's outcomes lead to enhancements to the HT management. Achieving better process management is needed to evaluate how the organization designs, manages, and improves warehouse key processes and keeps halal integrity during the process (Kaplan and Norton, 1992; Soon et al., 2017; Mohamed et al., 2016). The process should be adequately defined, carried out under controlled conditions, with the approved new or revised documented procedure, manual, best practices, records control responsibility, a new method or others. For each process flow, it is necessary to identify whether there is a risk and what steps need to be taken for each control point. The organization should ensure the process documentation procedure is consistent with existing implementation documents. Failure to make known the process procedure may influence the performance of process tasks and the inability to perform the right action on the HT control point. A strategic initiative that can be done is identifying the number of processes and checking the processes without an appropriate number of documented procedures and related documents established. The better process management also can be indicated through the percentage of process procedure changes required due to HT problems.

17.3 Model verification result: evaluation rest of prototype (pilot model)

Based on HT warehouse performance measurement (HTWPM) model, a prototype has been constructed and proposed. The dashboard prototype system is developed as a test tool to get subject matter expert (SME) verification on the usability of the proposed model.

The usability test is carried out for SMEs to assess the developed pilot model (prototype) of the HTWPMS. (Cemellini, van Oosterom, Thompson, & de Vries, 2020) describe usability as effectiveness, efficiency, and user's satisfaction and opinion about the product/prototype.

17.3.1 Halalan toyyiban warehouse performance measurement system prototype

Aligned with BSC, this prototype employs four perspectives of performance measurement: financials, customers, business processes, and learning and growth perspectives. This prototype stimulated sample data to give a better understanding of the proposed measure. The prototype has user interface design where users can have an interaction with the system.

Fig. 17.2 is the first view of the prototype, on which one will see the instruction and the button to proceed. There is also a QR code that links to the questionnaire asking about the user opinion toward the use of the system. This section guides the user on how to properly operate the prototype by enabling the macros, including actions which users should take if they encounter problem in case of warning text appear in the Office Backstage view. The three (3) interactive buttons on the very right are available on every page if users want to leap directly to desired page.

Fig. 17.3 contains the introduction page created to provide brief overview of the HTWP model. The purpose of the model and four areas of performance measurement employed in the model were explained distinctively so user can expect the outcomes of this prototype and have better insights. There is also an interactive icon for user to proceed to next page. On the left side, the icons' images are displayed as an indicator of what the features that were used in this prototype. There is also an explanation of each level of color darkness of the icon. For the "Icon & Status" indicator, the medium dark icon means that target has been achieved by 100%, the least darkness means target achieved was between more or equal 90% and less than 100%. As for the darkest sign, meaning the target achieved was below 90%. The color and design variation are to give easy navigation for user to understand the results quickly. That goes also to the percentage-different level of color darkness implies that there are some decrements, no change and increment from the last year, respectively. The underlined words; "introduction" and "instruction" are the simple text hyperlinks for user to click if they want to go back to introduction or instruction page. This page also mentions on contribution of questionnaire that helps the research to study the important elements in HT warehouse performance. This prototype also includes invitation for user to engage with the questionnaire using QR code or hyperlink that links to the Google Form.

Fig. 17.4 is the dashboard page. This page is very crucial for the user to understand the result in general for the financial, customer, business process, and learning and continuous improvement of their organization. Line graphs were used to show the pattern of the organization's performance and its monthly fluctuations (if there is) that occurred for

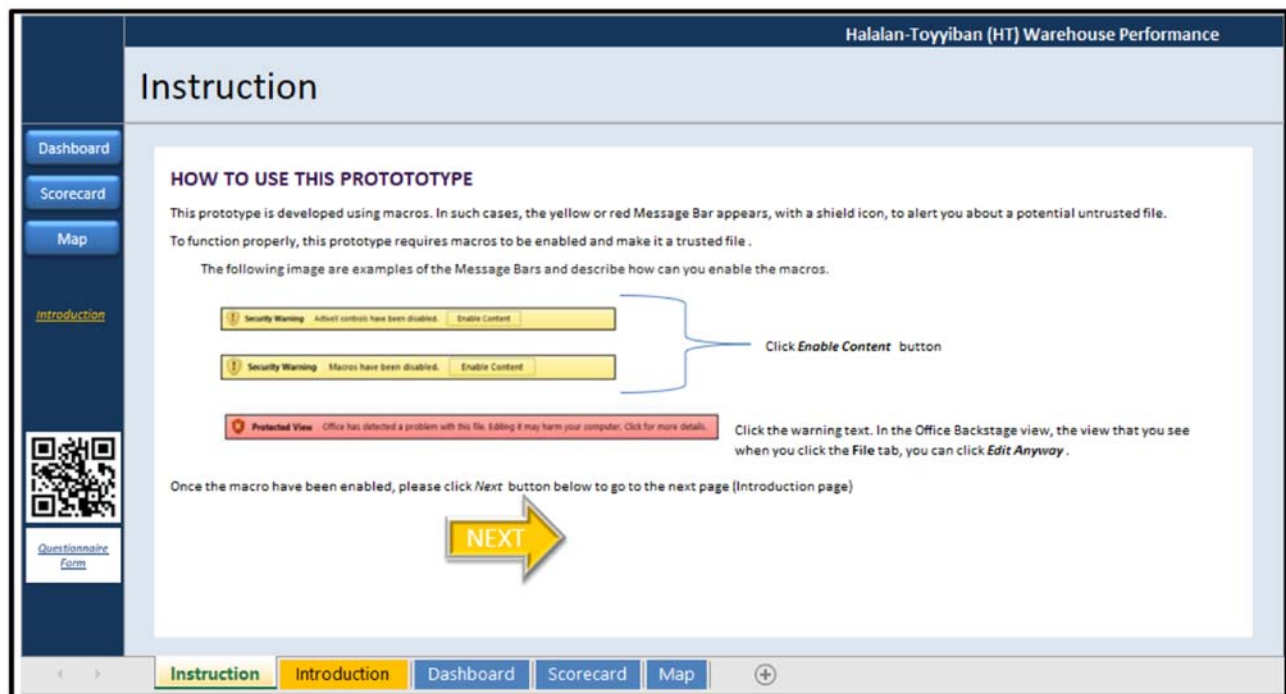


FIGURE 17.2 Prototype's instruction page.

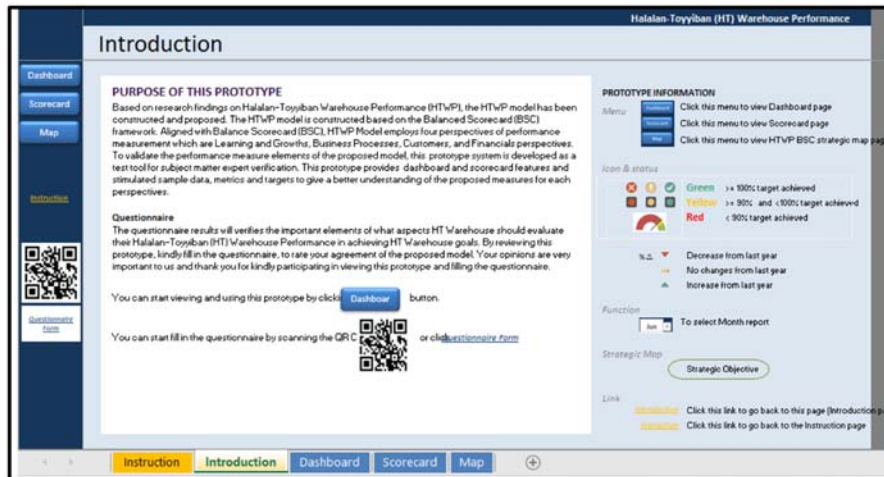


FIGURE 17.3 Prototype's introduction page.

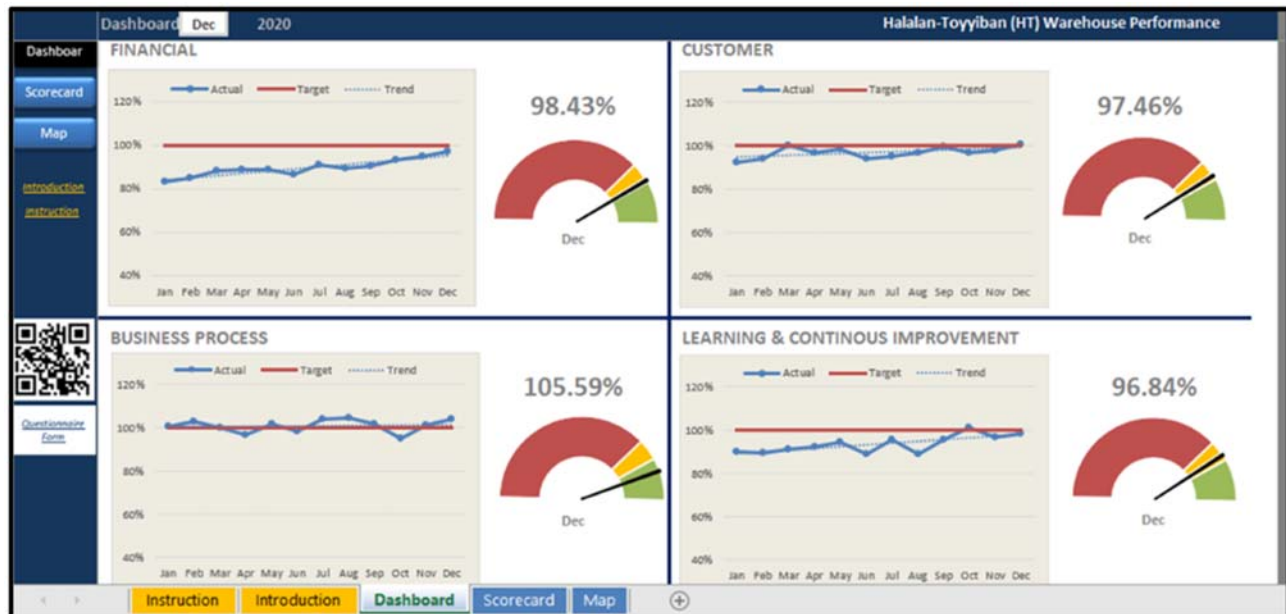


FIGURE 17.4 Prototype's dashboard page.

the whole year. The three lines on the graph represent the actual data on the organization's target and trend line. Trend line is the general direction of the company's performances that is suggested to be followed. There is also a filter component which lets user to display any filtered report.

As this research uses the BSC to measure the performance of an organization, Figs. 17.5 and 17.6 contain the pages with the four elements of the BSC. Thus a company can easily identify factors that hinder business performance and outline strategic changes tracked by the scorecards. The scorecard can provide information about the firm as a whole and linking it to the company objectives. The filter feature is also available in this page. The sign indicators are functioned to specify whether the objectives are achieved or not.

The four scorecards are lined in a specific order and contain SOs that contribute to the mission and vision of the company. The objectives are linked in causal way from bottom to the top. In Fig. 17.7 the strategy map is a tool for user to see the causal impact of one objective to another. The sign and level of color darkness still carries the same meaning and filter features are available to display specific month in the report.

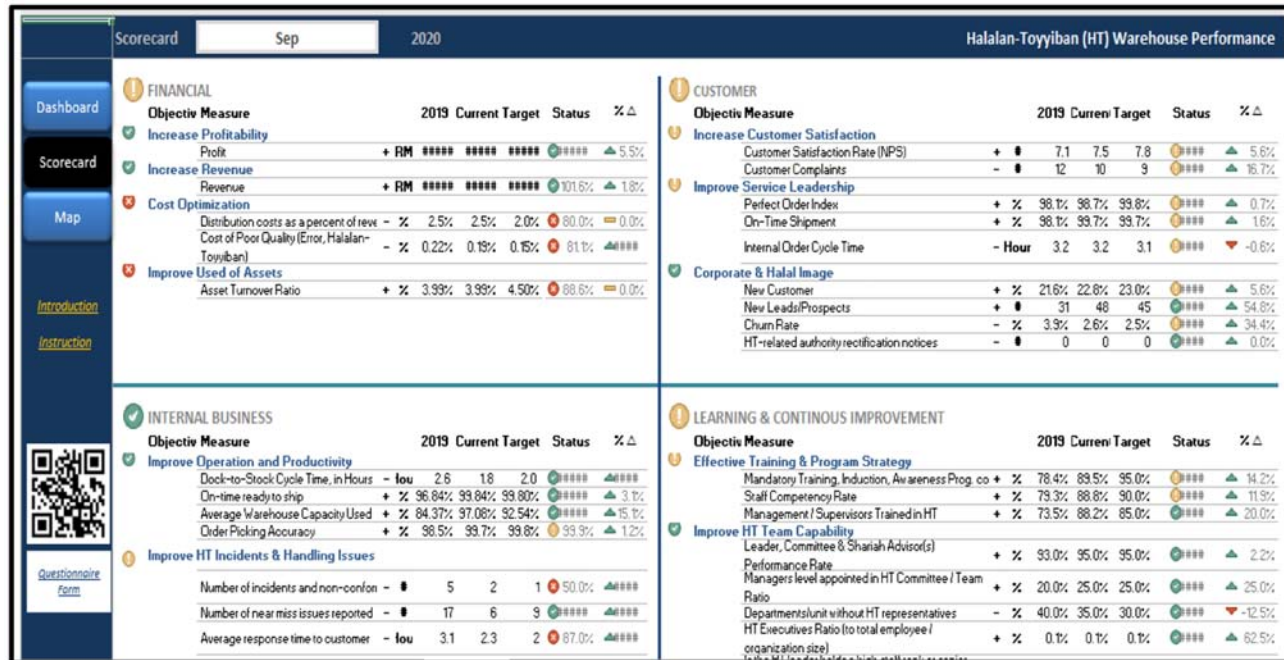


FIGURE 17.5 Prototype's balanced scorecard page (a) and (b).

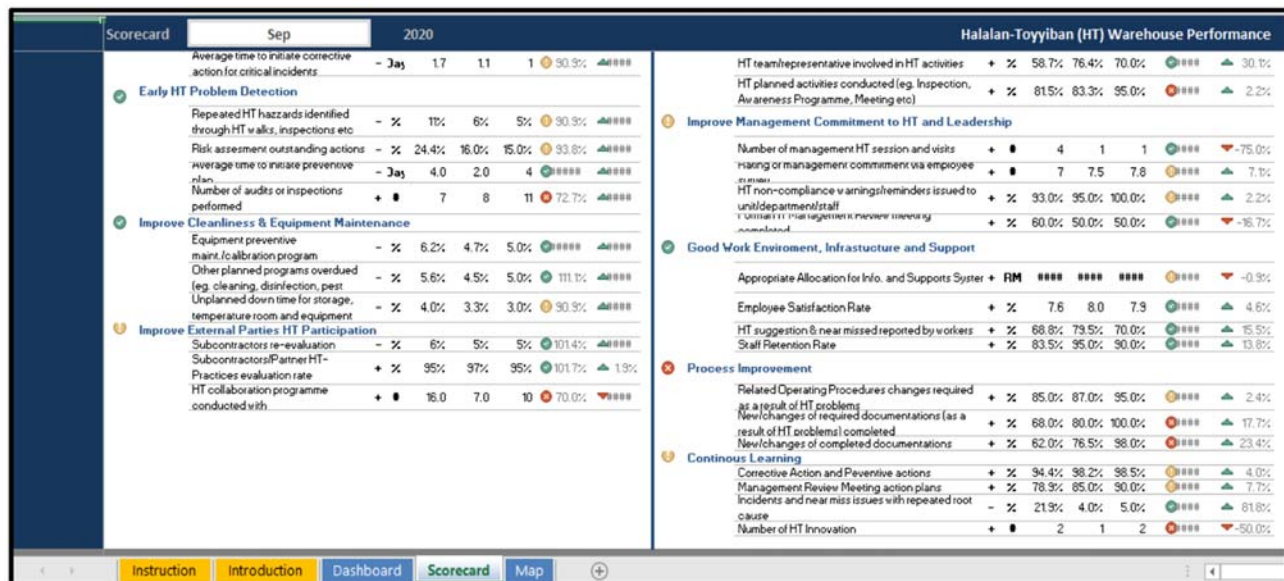


FIGURE 17.6 Prototype's balanced scorecard page (b).

17.3.2 The instrument: questionnaire

This questionnaire verifies the important measures of the aspects that need to be evaluated in achieving HT warehouse objectives. The SMEs as the respondents are requested to review the prototype and fill up the questionnaires, by rating their agreement of the proposed measures. However, note that the performance indicators (metrics) for each proposed measure and the target metrics set in this prototype are just an example and the data provided are just a sample to illustrate the performance results. The performance indicators (metrics) and target may differ according to the needs and specific objectives of the organization.

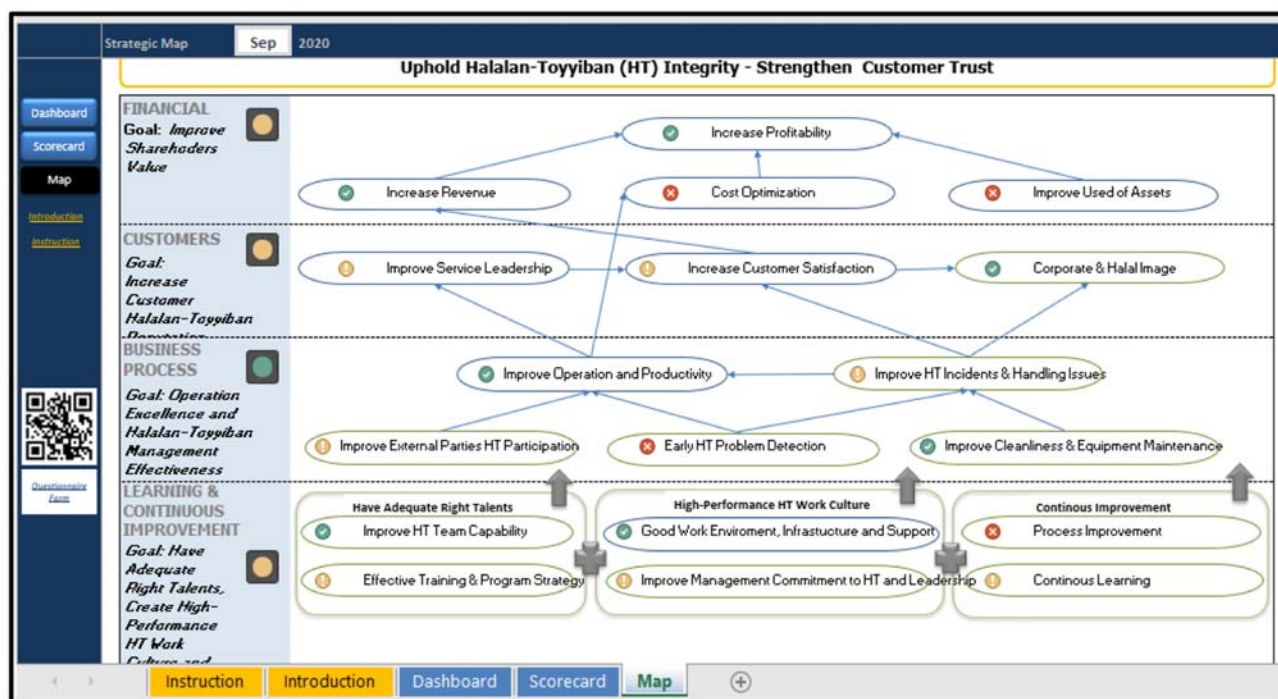


FIGURE 17.7 Prototype's strategy map page.

The questionnaire is structured by Likert scale with 10 questions relating to the organization's performance. Each question was evaluated with agreement rate from 1—strongly disagree to 5—strongly agree. There is also open-ended question where the respondent can write their perspective on organizational performances.

17.3.3 Evaluation test result

There are total 22 respondents that have responded to the questionnaire regarding the HTWPMS. Most of the respondents are the SMEs as they are warehouse manager, operation vice president, QA executives, and halal executives and have worked in related fields for more than two years. The mean score for each question is calculated and interpreted based on the agreement rate ranging from 1 to 5. The scores show that the prototype is applicable for measuring the warehouse performance in terms of assuring the HT practices in achieving the business goals. As the mean score is 4.10% or 82.0% of the overview of prototype has clear framework, meaning it is helpful for HTWSP to deliver company needs. The respondents also agreed that the measurements are relevant to most HTWSP. However, some of them have disagreed as they believe every HT warehouse providers might have different regulations to follow and different products to be stored with different procedures. They strongly agreed with mean score of 4.45% or 89.0% that the four perspectives may help practitioners to understand all the SOs' performances. Moreover, with proper implementation of the prototype, it can facilitate the HT strategy and helps company to engage staffs and its external stakeholders.

The respondents have shared several of their perspectives on HT continuous improvements which are the staffs' comprehension in halal commitments, the awareness in working environment, taken process improvements, the leadership skills and more. These statements are supported by mean score in Table 17.1 where 86% of respondents agreed that warehouse achievements need to be evaluated and with help of the HTWP model, an organization can identify the important and sufficient SOs. It is important for HT warehouse to create high-performance work culture in HT warehouse and 3.95% or 79.0% strongly agreed that the model can help to achieve it.

Excellent warehouse operation is important for the company to accomplish its business goals as the mean score is 4.36% or 87.2% agreed that effective HT system is important for internal business goals. The respondents also add that the model should include safeness protocol and dock-to-dock cycle time in internal performance measure as company can utilize the model to analyze delays and bottlenecks problem that may arise. Most respondents agreed this model can help organization to explore internal business perspectives and categorize the important and sufficient strategic business objectives.

TABLE 17.1 Mean scores of HT warehouse performance measurement system (HTWPMS).

Items	Mean	Std. deviation
Overall HTWPMS		
The HTWPMS has a clear framework that is aligned with corporate strategy and value.	4.36	0.902
The HTWPMS prototype would be useful to the HT warehouse service providers to continue to develop/ implement the system.	3.73	0.883
The measures (given in the prototype) are general and likely to apply to most HT warehouse service providers.	3.91	0.811
The HTWPMS would be easy to adapt to meet individual company needs (e.g., measure different processes/ use different measurement)?	4	0.816
Viewing HTWPMS from four perspectives (financials, customers, business processes, and learning and continuous improvement) helps practitioners to understand the performance of each strategic objective.	4.32	0.894
Properly implementing HTWPMS will facilitate the understanding of the HT strategy and helps to engage staff and external stakeholders in the delivery and review of the HT strategy.	4.45	0.51
The HTWPMS strategic objectives are sufficient to evaluate critical factors in ensuring successful HT.	3.95	0.486
Average mean score	4.10	0.76
Financial perspective		
Increasing shareholder value is an important goal and the achievement needs to be evaluated.	3.91	0.294
Strategic objective to optimize cost is an important way to increase profitability.	4.55	0.51
Average mean score	4.23	0.40
Customer perspective		
Improving customer satisfaction and good HT reputation is an important way to increase shareholder value.	4.5	0.598
Evaluating and improving service leadership is an important element to satisfy customers.	4.55	0.51
Evaluating and improving corporate and HT image is an important element to give existing and potential customers' confidence in HT services.	4.41	0.59
Average mean score	4.49	0.57
Internal business perspective		
Excellent warehouse operation is an important internal business goal for HT warehouse and the achievement needs to be evaluated.	4.5	0.598
Effective HT system in preventing, control and handling HT incidents is an important internal business goal and the achievement needs to be evaluated.	4.32	0.568
The HTWPM model categorizes important and sufficient strategic objectives to achieve business process goals.	4.27	0.703
Average mean score	4.36	0.62
Learning and growth perspective		
Having adequate right talents is an important goal for HT warehouse and the achievement needs to be evaluated.	4.18	0.588
The HTWP model identifies important and sufficient strategic objectives to measure adequate right talents (i.e., improve HT team capability, improve training; program strategy effectiveness)	4.27	0.456
Creating a high-performance HT work culture is an important goal and the achievement needs to be evaluated.	4.5	0.598
The HTWPM model identifies important and sufficient strategic objectives to measure HT work culture (i.e., improve work environment, infrastructure, and support; and improve management commitment to HT and leadership)	4.36	0.581
Continuously improve is an important goal for HT warehouse and the achievement needs to be evaluated.	4.55	0.596
The HTWPM model identifies important and sufficient strategic objectives to measure the continuous improvement of HT (i.e., process improvement and continuous learning)	4.09	0.921
Average mean score	4.33	0.62
<i>HT, halalan toyyiban.</i>		

Based on the mean score, all of the respondents agreed the HTWP model can help warehouse to improve their customer satisfactions and boost up to good HT reputations for them to increase shareholder value. For HT warehouse, in order for them to gain potential customer and maintaining a good relationship with existing customer, it is important to evaluate and improve the business. Therefore they require to improve their service leadership (mean score of 4.55% or 91.0%) to satisfy customers. Thus the measures can help corporate to learn customers' perspectives toward their service as they can develop new or greater business strategies.

For financial perspectives, the mean score of overall is 4.23% or 84.6% of respondents agreed that increasing the shareholder view is important goal and very crucial for organizations to evaluate it. Extensive training and awareness programs for staffs, careful planning in minimizing the dock-to-stock cycle time, and ensuring accuracy of order picking may incur some cost but it will positively influence the warehouse productivities as well profitability by the regulation's efficiency. Besides, almost all respondents strongly agreed that important way for corporate to increase company's profitability is by optimizing the cost.

17.4 Conclusion and discussion

HTWSP should serve an uninterrupted warehousing operation and maintain its integrity through each critical control point from receiving to shipping activities, ensuring that all customers' goods are stored and delivered fresh, safe, and halal. Customers' goods must not have been contaminated, adulterated, spoiled, denatured, or degraded. Service provided should comply with HT-wholesomeness, goodness and is a statement of quality. This means that the purpose of focusing HT can be formulated as "work proactively to prevent HT incidents and noncompliance." By achieving this mission, it will satisfy the customers with a quality service of HT, complied with related halal and toyyib standards set by regulators, and satisfied shareholders with achieving the business goal—increase revenue and profit.

This research has also validated a proposed HTWPM model to a real-world problem. The dashboard prototype system based on the HTWPM model is developed as a test tool. The verification result was gained by analyzing the Likert-scale questionnaire answered by SMEs. The evaluation proved that the prototype is applicable for the HT Warehouse Performance as a tool to improve their performance to achieve business goals and helpful for HTWSP to deliver company needs. With the proper implementation of the prototype, the system can facilitate the HT strategy and helps the company to engage staff and external stakeholders in the delivery and review of the HT strategy.

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Further reading

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Sertu and Halal critical point for managing risks in halal transportation

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18.1 Introduction

The definition of sertu in halal supply chain refers to the process of cleaning workstation, trucks, warehouse, and container to be free from dirt, stains, or any contaminants that affect the storage area of halal product. The process of cleaning activities conducted by sweeping, scrubbing, and washing depends on the degree of stains and the medium or agent of washing (Mohd Sirajuddin, Osman, Jaafar, Yusoff, & Yakub, 2016). The common agent used for washing is water as it is cheap, natural, and able to remove stains, oil, and any chemical agents. Indeed, the cleansing process is more than just a cleaning process as the process involves the elements of purification, spiritual and religious values, and faith. Ritual cleansing in sertu serves not only to remove the dirt and contaminants, but also to remove negative energy and bad spirits by practicing the religious values, faith, and beliefs with full sincerity and integrity. Sertu is considered part of the requirement in halal logistics operations that involve the process of cleansing of the body parts of the transport, trucks and containers, warehouse, surrounding areas, and infrastructure such as floors, loading bays with water according to religious motive and faith citing Islamic verses.

The requirement of the certification for halal food had been prioritized to ensure the control of authentic halal food by the regulation of the Islamic authority. Additionally, sertu is considered an important practice to be applied in halal certification to ensure the continuous monitoring of the Islamic practices in supply chain process. Apart from benefiting the food industries, halal certification helps to promote the demand and sales of the halal food products in the market. The integration of Hazard Analysis Critical Control Point (HACCP) and Sertu practices in the Quality Assurance Program is growing rapidly with the introduction of halal critical point (HCP) procedure in the company food processing and business operation. The HCP procedure will guarantee the quality, safety, and halal status of the food products from the point of production to the point of consumption. HACCP is known as a food safety management tool that is internationally recognized to address food safety problems through critical analysis and biological, chemical, and physical controls. The hazard and critical analysis will identify the contamination factors from the points of receiving the raw materials, production, processing, distribution, storage, until to the final consumption (Stanley, Knight, & Bodnar, 2011).

The implementation of HCP and halal sertu is the most secure and cost-effective method for controlling hazards during food supply chain. The implementation of HCP will enhance the food safety system and business operations and promotes the international trade by increasing food safety and overall quality assurance management program (Salleh, Halim, Abd Aziz, & Said, 2020). Past study indicated that the major cost of implementing HCP is to manage the staff awareness and operational enhancement in halal food safety practices and overall business process. The costs of capital investment and technical expertise are considered less important compared to the important benefits that the HACCP and sertu provide in protecting public health and sustaining consumer confidence nationally and internationally through the risk monitoring and proper control process (Azhari, Rahman, & Salleh, 2020).

18.2 Halal control point and sertu in halal supply chain operation

A study by Nakyinsige, Che Man, Sazili, Zulkifli, & Fatimah, (2012) defined the HCP as a point, step, or procedure in halal food supply chain at which control can be applied. Halal food cross-contamination hazard can be prevented or

eliminated by the proper control of HCP practices and sertu as part of risk control process. As stated by [Kohilavani, Abdullah, and Tajul \(2015\)](#), HCP referred as any identifiable points within the operation of halal food that might otherwise lead to a religiously unacceptable situation termed “Najasah” which led to the condemnation of the food products and thus control must be in place. If an identified HCP were not observed and controlled within the chain, it would result in the contamination of halal product as the status would become nonhalal because of its nature that may contain unlawful ingredients along the supply chain process ([Farouk et al., 2014](#)). This study by [Ali, Tan, Pawar, and Makhbul \(2014\)](#) provided resolution on the issues of HCP and sertu application in the halal food supply chain. Based on their studies, the halal control point must be identified and controlled for each operation in processing plant, trucks, and storage area. After the HCP has been identified, sertu process will be implemented to clean and remove any hazardous element that will affect the specific contaminants area. To ensure the authenticity of the process, sertu cleansing process to the trucks and storage area must be fully supervised by the halal committee of the company.

The importance of HCP has been addressed by [Zulfakar, Anuar, and Ab Talib \(2014\)](#) that emphasized on the role of the stakeholders to identify and address the HCP in their operations. It was mandatory for a halal executive to be appointed on a permanent basis in their premises to fully supervised the production of halal food business ([Demirci, Soon, & Wallace, 2016](#); [Ngah, Zainuddin, & Thurasamy, 2014](#); [Van der Spiegel et al., 2012](#)). By referring to the above requirement, sertu process must also being observed by the appointed halal committee of the company. Based on the study by [Ali et al. \(2014\)](#) and [Kassim, Hashim, Mat Hashim, and Jol \(2014\)](#), halal integrity practices and quality conformance among the logistics partners are crucial to ensure the connectivity of the HCP and sertu process throughout the risk control in the entire supply chain process. The stakeholders in the halal supply chain are responsible to ensure the implementation of HCP and sertu process based on their own business scope following the Shariah compliance. The management of the halal food establishments must identify the CCPs within their system with the necessary advisory assistance from the local halal authority. The requirement of HCP is aligned with practice of sertu whereby every truck and storage area will be gone through the process of hazard identification and risk control through the samak and cleansing process ([Mahidin, Mohd Saifudin, & Othman, 2017](#)).

The requirement of the HCP and sertu process in the halal supply chain operation was also based on the main requirement in the Malaysia Standard of MS 2400: 2019 Part 1 (Transportation), Part 2 (Warehousing), and Part 3 (Retailing). The implementation of sertu in halal supply chain operation helps to identify and eliminate potential presence of haram element which must be zero tolerance based on Syariah compliance. The practices of sertu in halal transportation can reduce the vulnerability and doubt on the safety, quality of the processing area, storage, and any halal transportation mode. Sertu process can also reduce the uncertainty among the halal consumers on the safety and quality of halal goods transported by the respective trucks. [Lodhi, Kassem, and Rautenstrauch \(2009\)](#) through his finding had highlighted that, every critical control point was important and existed at every individual stage of halal food production. All operational activities within the supply chain operation such as preparation, processing, and transportation must be fully supervised by the halal committee and any relevant party in the chain. Sertu in halal transportation operation has been developed based on the requirement of the quality assurance model and conformance to the food safety management system in accordance with HACCP, halal critical control point (HCCP), and prerequisite program in halal food system and requirement.

18.3 Sertu in halal supply chain operation

Sertu cleansing or Shariah ritual cleansing or purification cleansing involves the highest level of impurity or severe najis (najis mughallazah) in Islam laws that concerns the cleansing of the contaminant elements (or in physical contact) from dogs, pigs, and its related agent. For example, if Muslim were in a skin contact with a dog (its saliva) be it by accident or by intention, the Muslim is required to cleanse the part of contact by way through sertu cleansing protocol and procedures outlined in accordance with Islamic rulings stated.

18.3.1 Raw material—water

Sertu or custom purifying requires the utilization of water as the base component for washing and purifying cycle. The conditions of the water must be natural (mutlaq) and not mustakmal [pure water, but could not purify, i.e., the water has been used for ablution and must be free from najis (MS 2400: 2010)]. According to Syariah law, mutlak water is purely water in its original state with no mixture and able to clean and cleanse. Mutlaq water will be water that has not been in that frame of mind being used; be it to washing oneself or to wash away foulness and such or blend in with any cleanser or other such synthetic substances ([Ahmad, 2016a, 2016b, 2016c](#)). Absolute water is originally from rivers,

wells, rainwater, dew, seawater, melted snow, spring water, and tap water. The techniques of using water for the sertu operations are categorized in the subsequent process (procedure).

18.3.2 Product—soil or agent of cleansing

The conditions of the soils as stated in Shariah principles must be free from any *najs* (soil that had been used for dry ablution) (MS2400: 2010). Sertu or filtration with soil is a method for invalidating the microbes and diseases that such contamination holds. Past study has indicated that the best way to clean and eliminate the microorganisms and potential diseases that might be available in the toxins in *najs mughalazah* is utilizing soil (Ahmad, 2016a, 2016b, 2016c). Soil according to the Syariah principles refers to clay; however, in modern technology, our Shariah bodies have developed other agents of cleansing such as sertu clay product that can also be used as the cleansing agent (Kassim et al., 2014).

18.4 Requirement and guidelines for the implementation of halal critical control point and sertu in halal supply chain

Sertu for halal transporters requires full commitment and dedication from the logistics practitioner to ensure the requirement and guidelines for the implementation can be monitored and professionally managed. Thus an appointment of a halal committee must be formed in a company to ensure the sertu cleansing can be performed according to Shariah requirement and guidelines. Every aspect of halal cleansing requirement must be fulfilled and the specific budget from the management is required to ensure the cleansing material and the specific facilities for the cleansing procedure can be prepared. Additionally, the ethic and the integrity of the logistics practitioners are important to ensure the quality and the compliance of the cleansing process with the Shariah principles and the regulations stipulated in acts, standards, and regulations.

18.4.1 Halal integrity in halal supply chain

Integrity is defined as the quality or state of being complete; entirety, perfect; and wholeness. In view of the halal supply chain operation, integrity was represented based on the requirement that the food must remain halal from upstream to downstream in the supply chain. The halal operation must be free from any activities that might breach the halal status, intentionally or unintentionally. The halal supply chain operation involved in a series of movement activities before it reached its last point of consumption, including the halal transportation. Therefore the integrity of the halal food supply chain was becoming an increasing concern among the Muslim societies (Tieman & Ghazali, 2014). As stated by previous study, halal integrity is divided into two dimensions: physical and ethical. Physical integrity covers the aspects of the halalness, the cleanliness, safety, health, and nutritious attributes of the products. Ethical integrity refers to the proper practical conduct which represented the aspects of processing, handling, packaging, labeling, warehousing, transporting, distributing, financing, marketing, and selling activities.

The halal integrity was the key to halal industry success and halal industry players must invest to create a premium image for halal products (Tieman & Ghazali, 2014). Since halal food represented the inhibits credence quality characteristics which means the characteristics that not visible and verifiable even after the consumption (Bonne & Verbeke, 2008), the integrity of the halal foods becomes harder to detect unless revealed by the experts or professional services through auditing processes or any assessment procedure. Now, halal food buyers or consumers must rely on their trust and the credibility of the sellers that the integrity of the food they consumed was truly halal all the way from the beginning of the supply chain, including for the sertu cleansing process. However, as there was no global halal standard that can be used as a benchmark to represent halal integrity, things are getting more difficult as the level of halal integrity might be perceived differently by the individual consumers or the different segments of halal markets. Tieman and Ghazali (2014) have agreed that halal integrity assurance was the key factor in the development of a well-trusted halal food supply chain in the current complex and competitive food trade environment.

Halal integrity is considered transparency whereby the consumers have the right to know the authenticity and the cleanliness of the halal food products preparation. Jaafar, Faisol, Rahman, and Muhammad (2016) have agreed that to protect the halal integrity, a complete understanding of the whole halal food supply chain is required. Variations in the definitions of halal and the unavailability of a single, worldwide acceptable halal standard have made the process of understanding the halal food supply chain become more complicated. It is clearly understood that all the parties involved must be able to take every measure to ensure the halal food supply chain is not compromised. Previous studies have suggested that all parties or stakeholders involved in the halal food supply chain should bear the responsibilities.

Jaafar et al. (2016) argued that while an organization can practice a halal quality assurance system, the products' halal integrity was intact only if the products are still in their custody. Once the food products moved along the supply chain, the integrity of that product can only be as intact as before if the next receiving party in charge of similar understanding and operational practice has overseen the halal product. All efforts taken by the previous party in safeguarding the halal integrity status can be crumbled and thus increased the possibility of cross-contamination and eventually made the food products no longer fit for Muslim followers' consumption. Therefore, full participation of all parties or stakeholders in the halal food supply chain to achieve the supply chain integrity and halal practices, including the implementation of *sertu* cleansing must be emphasized.

18.4.2 Dedicated asset in halal operation

One of the highly discussed topics was about the dedicated assets in halal supply chain. The importance of the usage of dedicated asset highlights the needs for adequate and specific infrastructure to support the operation of halal products. The previous study on the halal dedicated asset defined that the dedicated asset in halal operation refers to any durable investments whether physical or human assets that are exclusively or dedicated to supporting a particular transaction or halal business with their partners (Zulfakar et al., 2014). From the above definition, the term "asset specificity" can be considered like the term of "appropriate storage, transit, and equipment" that has been mentioned by Lodhi et al. (2009). His study indicated on the importance of dedicated asset in practicing the procedure of halal food supply chain HCP and *sertu* cleansing. Apart from that, necessary training for organization workforce who deals directly with the halal facilities, including the requirement of *sertu* cleansing as part of halal transportation certification are also necessary.

It is indicated that before the growing awareness of Muslims toward the halal status and before the highly demand of halal products, most of the logistics service providers did not implement a proper segregation process in their transportation and storage activities. This situation happened due to the factors of economical budgeting that required the companies to reduce the operational cost to achieve the economies of scale in their operational cost. It was proven that the usage of the dedicated infrastructures such as transportation fleet, warehouses and storage places, handling equipment will avoid the possibility of halal products that are being mixed with nonhalal products. Therefore this practice can avoid the unnecessary human mistake and ensure operations are consistent with the expectations of the Muslim consumers. Simultaneously, a study conducted by Demirci et al. (2016) found that Muslim consumers preferred the separation of halal and nonhalal foods in the physical distribution activities. Hence, this finding had supported the other literature gathered in this study on the importance of dedicated asset in halal business operation.

In fact, many examples from all over the world especially from the Middle East and Southeast Asia countries showed that majority of the stakeholders in the halal industry have invested in providing dedicated assets for its halal production operations. Pursuing to that, many local companies have spent the cost enormously to provide the dedicated services and facilities to cater the demands for halal products and to meet the requirement of *sertu* cleansing for halal warehouse and transportation. By providing dedicated services and *sertu* cleansing procedure will create a sense of trust and loyalty from their customer. As food products move through the supply chain, they are in one of the following three stages: movement, transformation, and storage. Thus there is a need to ensure that the current infrastructures of these three states of the supply chain are free from any element of cross-contamination, either through providing new facilities or identifying new methods to separate the halal and nonhalal food products. This requirement is necessary for *sertu* cleansing implementation at the premises and transportation to control the risk of contamination of nonhalal and hazardous element to the halal product.

18.5 Research methodology

In this study the employment of interviews method was the critical key instrument to determine multiple perspectives among the participants. Interview methods helped to build up the understanding of the HCP and requirement of *sertu* cleansing as well as the factors and issues surrounding the operations of halal supply chain. As the interview session progressed with a specific participant, the researcher was able to ask or probe for additional information. This enabled the researcher to discover new and many aspects of the HCP and *sertu* cleansing. This is important to seek further clarification in the event of information uncertainty. The essence of flexibility of semistructured interview helped to change the interview questions' wording while maintaining the meaning of the questions asked during the interview session (Barriball & White, 1994). Since the participants of this study vary in term of their job level (top management to lower management) and job responsibility, a unique way of questioning styles and content were appropriate to gain each

TABLE 18.1 List of participants in this study.

No.	Stakeholder group	Code	Position of person interviewed	User profile	Working experience (years)
1.	Transporter/logistics services provider	Logistics operator no. 10	Assistant vice president	Multi National Corporation	15
		Logistics operator no.11	Assistant vice president	Multi National Corporation	21
		Logistics operator no.12	Operation manager	Multi Natioanal Corporation	12
		Logistics operator no.13	Compliance manager	Multi National Corporation	3

participant's unique experiences, thoughts, and stories. For example, the questions drafted for the logistics operators and retailers are different, although the actual meaning of both questions was still the same. Different phrases or words appropriate to different participants were also used to stimulate meaningful feedback to further explore relevant matters. The interviews were conducted at the participants' premises such as office, processing facilities, cold storage, warehouse, and supermarket/hypermarket (Table 18.1).

18.6 Research findings

Research findings described and discussed the findings from the interview conducted with the participant from halal logistics practitioner and retailers that implement the HCCP and sertu for halal transportation and storage. The findings summarized the result from the observation process that supported the findings from the interview. The findings from the interview revealed the requirements and practices needed for the implementation of HCCP and sertu for halal cleansing.

18.6.1 Handling process

Handling process is defined as the way of the food or products is being moved or transported from one custody to another custody in a supply chain process. Handling also referred to the process by which the food products or commodity being packaged or transported from one party to another party. Handling in halal supply chain referred to the isolation or segregation process during the movement of the halal products to ensure the halal status, quality, and the temperature controlled. This finding is relevant to a study conducted by Supian (2018) that mentioned on the importance of monitoring of handling process in HCP and sertu. It is stipulated that halal supply chain needs to be managed in a satisfactory manner and must be segregated from the hazardous element and any haram element during the distribution process. A study by Arshad, Bakar, and Ramli (2018) highlighted on the proper handling process to control the critical control point during the halal transportation. The study is similar with findings from the interview, most of the respondents highlighted that handling is the crucial process for the implementation of HCP and sertu cleansing. A specific dedicated place for the sertu cleansing must be placed; temperature management also is the main criterion that must be continuously monitored by all the relevant parties in the halal supply chain especially if involving the halal food product.

18.6.2 Hygiene practices

Food hygiene constituted a necessity of the Quality and Safety Food Management System for the development of HACCP, as well as for the entire requirement for the halal certification procedure. From the interview, all the respondents agreed that government, industry, and consumers play a key role for the safe sanitation procedure and food hygiene practices throughout the entire halal supply chain operation. The findings from the interview have shown that the increase of percentage of the food product recall cases had been attributed due to the poor sanitation and food hygiene practices in the company daily operation. As stated by Raheem and Demirci (2018), poor hygiene practices happened due to poor personal hygiene, lack of proper documentation of hygiene procedure, and contamination of the

operational equipment and environments during the supply chain activity and process. The findings of the study are relevant due to poor hygiene factor will increase the risks of HCP that happened in the supply chain process. It was indicated that the issues related to hygiene and sanitation practices were particularly prominent in the overall finding of this study. Hygiene practices were considered the part of requirement in *sertu* cleansing for the purpose to clean the contaminated and the affected area. Hygiene practices also were stipulated in the requirement in MS 2400: 2019 Part 1, 2, and 3 and need to be practiced ensuring the implementation of risk control and risk monitoring in halal supply chain practices.

18.6.3 Maintenance of equipment

Maintenance of equipment is particularly important to preserve the halal quality, safety, and halal status and determine the practices of HCP and *sertu* in halal transportation. The equipment and halal supply chain are considered dedicated assets that are solely being used only for operation in halal supply chain and compliance to the requirement for HCP and *sertu* cleansing practices. As stated by [Vanany, Tan, Siswanto, Arvitrida, and Pahlawan \(2020\)](#), the maintenance of equipment is particularly importance to maintain the overall production process in long term based on six sigma concepts for quality control. The examples of equipment and asset being used in the halal supply chain operation are containers, tools, forklift, trucks, and cold room storage and the necessary equipment for *sertu* cleansing. All this equipment and asset need to be maintained and monitored regularly to ease the operation and maintained the food products quality and safety. Risks of contamination to the halal product can be spread through the usage of the equipment that are not properly monitored and maintained through every stage in halal supply chain operation. As indicated by [Sulaiman, Noordin, Noor, Suhaimi, and Isa \(2019\)](#), the other crucial factors that contributed to the contamination risks in the halal supply chain operations are the usage of equipment in the food production and other handling procedure. This finding is relevant with the current finding in this study which emphasize proper maintenance in halal practices. By having the proper maintenance can help to reduce the risk of contamination and reduce the frequency of the requirement of *sertu* since the trucks and the other facilities are not being exposed to najas and other hazardous material.

18.6.4 Manpower in halal supply chain

Halal personnel in halal supply chain operation are particularly important to ensure the safe operation and halal compliance of the process involved in halal supply chain operation. Manpower in halal supply chain operation is defined as any workforce, staff, or workers that involved in every operation of halal supply chain. It was stipulated in the standard and regulation that every process in halal supply chain must be managed by the Muslim staff that also practices Islamic practices in their daily life. The finding of the study is similar with a study conducted by [Faradina et al. \(2018\)](#) that highlighted on the integrity and legal enforcement for halal supply chain practices. The establishment of halal committee in the organization had shown that the company had a community of Muslim workers with Islamic background and competencies that contribute to the halal management system and policies in the company. As such, the halal committee is required to make sure the implementation of the halal policy and practices in the company operation. In addition, it is the requirement of committee to provide training on halal procedure to the operational workers and to update on any information regarding the halal policy and compliance. If the process in halal supply chain operation was not being managed by the Muslim workers, the halal product will be exposed to the risk of contaminants of haram and hazardous element. In fact, it was against the rules of in halal policy if any of the organization appointed a non-Muslim worker as their employees. Halal personnel and the committee will be responsible to lead the task for the *sertu* cleansing in the company premises and required to follow all the guidelines stipulated in the act, standard, and regulation. This finding on the requirement of workforce for halal personnel in *sertu* and HCP practices is equivalent to the study conducted by [Ahmad \(2016a, 2016b, 2016c\)](#).

18.6.5 Dedicated asset

Dedicated asset in halal supply chain was considered any asset owned by the organization or outsourced from the other party that was solely being used only for halal supply chain activities and operation. The categories of dedicated asset in halal supply chain operation are considered the trucks, fleet, storage spaces and capacity, equipment used in the daily operation and any other category of asset that used only in halal supply chain operation. Dedicated asset such as transportation fleet, warehouses and storage places, handling equipment will help to avoid any possibilities of halal product being mixed with nonhalal products during the transportation. The finding by this study on the importance of dedicated

asset was like the study by Mohd Sirajuddin et al. (2016) that mentioned on the usage of dedicated asset for the risk control process. In addition, the usage of the dedicated asset in halal supply chain operation will avoid any probability of unnecessary human mistake that will lead to the risks of contaminant. It has been stated by the respondents from all the categories of stakeholders in halal supply chain operation on the importance to preserve the organization-owned dedicated asset in halal operation. The finding in this study is the study conducted by Ahmad (2016a, 2016b, 2016c) that emphasize on the usage of dedicated asset for halal practices, including the implementation HCP and sertu cleansing for risk control. A halal-certified company is required to ensure that the dedicated asset used for sertu cleansing are being effectively managed and restored.

18.7 Conclusion

The findings of the study emphasized that halal supply chain operation must be confined to nonhalal products or substances until it arrives at the destination. HCP and sertu cleansing are considered the best practices to ensure the conformance to the halal practices and operation. The supply chain partners in the industry must have the competencies and halal knowledge to maintain the integrity and knowledge of the requirement of HCP and sertu cleansing process. While transporting halal products, halal and nonhalal goods are not intermingled on a load carter (like trolley or pallet) or in a container/ common transportation vehicle (in case of bulk shipments). There is also an apparent requirement for special transportation for the ambient or reefer (chilled or frozen). If any contaminants happen, sertu cleansing must be applied to ensure the safety, cleanliness, and halal operation after the cleansing process. Indeed, to maintain the halal status, the specific halal product must be managed by a right person and with the right procedure based on Shariah principles. Therefore it is prohibited to put together all the products in the same transport without taking into consideration on the halal status of the products. It was a strict process for halal supply chain operations whereby the transportation of the halal supply chain must follow the requirement of halal handling process. The most important thing is, the halal logistics practitioner must comply with all the halal requirement regulated in halal standard and regulations. This is to ensure the implementation of HCCP and sertu for halal transporter can be managed based on Shariah principles, act, standards, and regulation stipulated for halal business. Therefore it is mandated that the requirement of handling, dedicated asset, workforce, hygiene practices, and maintenance of the halal equipment especially the facilities and material for sertu must be provided accordingly and well preserved.

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Section 4

Innovation in Halal Food Monitoring

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Sample preparation strategies for the analysis of contaminants in foods

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19.1 Introduction

The establishment of food safety standards and certification is critical to ensure food safety and is important for trade and consumer trust (ISO, 2021). Halal foods are foods that are free from any components that Muslims are prohibited from consuming (Riaz & Chaudry, 2003). Just like contaminated food, food believed to be halal, but not so, can spread and fit its way to Muslims around the world, which is a reason why halal standards and certification are crucial in halal assurance. The more complicated question and importance lie in the food safety expectation of halal food and how halal is interpreted in regards to food safety (Neio Demirci, Soon, & Wallace, 2016). According to Codex Standard 193–1995, the contaminant is defined as any substance not intentionally added to food, which is present in such food as a result of the production (including operations carried out in crop husbandry, animal husbandry, and veterinary medicine), manufacture, processing, preparation, treatment, packing, packaging, transport, or holding of such food or as a result of environmental contamination (CODEX STAN 193–1995, 2010).

Food safety is connected directly to human health and nutritional quality (Huang et al., 1657). Based on the perspective of food safety, it is important to accurately detect harmful substances in food in harsh environments, such as the freshness of raw materials and the nutritive values of processed food, food additives, microbial toxins, antibiotic residues, and artificial hormones (Alshannaq & Yu, 2017). For example, herbicides and insecticides are commonly used in agricultural products such as fruits, vegetables, and cereals, which can contribute to bioaccumulation. In addition, the increasing demands and regulations from authorities in more areas, such as the European Union, the United States, and China, together with the improved public understanding, are incentives for better control of food produced and especially toward monitoring of food processes. However, this task is challenging since foodstuffs mostly contain a broad range of components (Song, Xu, Chen, Wei, & Xiong, 2014). Hence, the demand is inevitable for the development and establishment of a food safety guarantee system in terms of inexpensive methods that combine one-step extraction, powerful preconcentration, simple, rapid, reliable, and accurate quantitative determination of analytes that usually present at minute levels of concentration in complex matrices. Despite the technological advances in instrumentation development, most analytical instruments cannot handle complex matrices directly due to high matrix interferences and turbidity of the food samples.

The sample preparation step has always been indispensable in any analytical procedure. Several sample preparation techniques should be performed with the following aims: (1) to convert sample or analyte into a form that is compatible with the analytical measurement; (2) to simplify the matrix, and (3) to preconcentrate the analytes to improve the sensitivity (Kanu, 2021). A clean extract produced after the sample preparation step will minimize instrument maintenance and improve trace detection and sensitivity while discarding the background interferences from matrices. The preferable and ideal sample preparation approach should demonstrate good and reliable analytical performance in the aspects of high extraction efficiency and sample clean-up, minimum solvent consumption, cost-effective, high sensitivity and selectivity can be reusable, high accuracy, and less toxic. However, the classical liquid–liquid extraction (LLE) fails to meet these requirements being a time-consuming procedure, tedious operation, consuming a large amount of toxic organic solvents, relatively expensive, and generating a huge amount of wastes. To overcome these shortcomings, new sample preparation strategies have been developed rapidly, namely, liquid-phase microextraction (LPME), dispersive

liquid–liquid microextraction (DLLME), solid-phase microextraction (SPME), quick, easy, cheap, effective, rugged, safe (QuEChERS), dispersive solid-phase extraction (DSPE), and magnetic solid-phase extraction (MSPE). The developed techniques have emerged into greener and environmental friendly techniques, which required less adsorbent and solvent usage and using a smaller sample size. Moreover, the operation is simpler than the conventional LLE with minimum extraction time. Thus this chapter will later discuss the principles/concepts, analytical merits, advantages and disadvantages, and application for each of these sample preparation techniques. It is worth mentioning that choosing a correct sample preparation technique solely depends on the specific aim or problem to be solved. Thus knowledge on the variety of sample preparation helps the food technologist or analyst reach the quantification's targeted goal. The information from this chapter will provide insights to halal scientists, analytical chemists, and food technologists in deciding the best sample preparation method, especially toward the application for food analysis to achieve efficient analysis of targeted food contaminants.

19.2 Sample preparation techniques

Today, various sample preparation techniques have been developed for the detection of contaminants in foods. The most common are LPME, DLLME, SPME, DSPE, QuEChERS, and MSPE. Each of these methods is discussed next, along with its salient features, applications, and analytical merits. Several recent comprehensive reviews have been documented on the sample preparation techniques for food contaminant analysis (Hernández-Hernández, Álvarez-Romero, Contreras-López, Aguilar-Arteaga, & Castañeda-Ovando, 2017; Jiang, Li, Cui, Wang, & Zhao, 2019; Li & Shi, 2020; Lu, Liu, & Wu, 2020; Moyo, Gitari, & Tavengwa, 2020; Ouyang, Lu, Hu, Xie, & Li, 2021; Soares Maciel, de Toffoli, & Lanças, 2018; Speltini, Scalabrini, Maraschi, Sturini, & Profumo, 2017). This chapter, therefore, aims to compile a few selected sample preparation techniques to demonstrate the capability of sample preparation method development and validation approaches toward isolation, extraction, preconcentration, identification, determination, and quantification of targeted contaminants in food matrices.

19.2.1 Liquid-phase microextraction

Over time, LPME has gone through many developments, from basic procedures to more advanced modifications. The LPME process involves a very small amount (in the microliter range) of a water-immiscible solvent (acceptor phase). A drop of acceptor phase is held at the tip of a microsyringe needle that can be directly immersed into the sample solution of suspended above the sample surface [headspace (HS) extraction]. In addition, the microliter solvent may be placed within the hydrophobic membrane's pores that are separated from the donor phase through a membrane interface. Based on these extraction mechanisms, the LPME can be categorized into three kinds of techniques that were (1) single-drop microextraction (SDME), (2) hollow-fiber LPME (HF-LPME), and (3) DLLME (Sarafraz-Yazdi & Amiri, 2010). Nowadays, miniaturized formats are gaining interest to overcome the limitation of long-established methods and toxic solvent consumption. Therefore many works have been published as evidence of a growing trend toward using faster, cheaper, easier, and more environmental friendly sample preparation methods. Table 19.1 summarizes the most recent previous studies on the application of LPME for food samples.

Soylak, Agirbas, and Yilmaz (2021) developed a new strategy by employing supramolecular solvents (SUPRASs) in LPME to extract manganese ethylene-bisdithiocarbamate pesticide (maneb) in food samples (Soylak et al., 2021). The schematic LPME extraction was shown in Fig. 19.1. In this study, a new SUPRAS was developed by combining 1-decanol and tetrahydrofuran as an extraction solvent toward maneb. The developed method was optimized by several parameters, including pH, volume of the 1-(2-pyridylazo) 2-naphthol as ligand, volume of 1-decanol and tetrahydrofuran, sample volume, and matrix component. The optimum conditions were validated and applied to determine maneb residual in rice, cracked wheat, and potato. The proposed method was proven an effective strategy for green, simple, and cheap preconcentration technique which can analyze maneb in about 15 minutes with ultraviolet visible. Seebunrueng, Phosiri, Apitanagotinon, and Srijaranai (2020) developed a method of LPME assisted with ex situ SUPRASs for the extraction of six phenoxy acid herbicides, including 2,4-dichlorophenoxyacetic acid, 2-methyl-4-chlorophenoxyacetic acid, 2-(2,4-dichlorophenoxy)propanoic acid, 2-(4-chloro-2-methylphenoxy)propanoic acid, 4-(2,4-dichlorophenoxy)butanoic acid, and 4-(4-chloro-2-methylphenoxy)butanoic acid before high-performance liquid chromatography diode array detector (HPLC-DAD) analysis. The SUPRASs were developed by combining sodium dodecyl sulfate and tetrabutylammonium bromide (Seebunrueng et al., 2020). In this work, the detection sensitivity was increased from 37 to 149 times compared to direct HPLC-DAD analysis with provides the low limits of detection (LODs) which are lower

TABLE 19.1 Previously reported works on the applications of liquid-phase microextraction for food samples.

Quantitation instruments	Analytes	Food matrices	Concentration of analytes found in sample	Linearity	LOD	Relative recovery/recovery, %	References
UV-vis	Maneb	Rice, cracked wheat, Potato	Udl	0.067–1.067 µg/L	2.25 µg/L	89–103	Soylak et al. (2021)
HPLC-DAD	Phenoxy acid herbicides	Rice samples	n.d.	10–100 µg/L	1–2 µg/L	81–108	Hsu and Ding (2022)
UHPLC-ESI(+)-QTOF-MS	Benzotriazole and benzothiazole	Brew/ beverage leaves	13.6 ng/mL	5–1000 ng/mL	0.5–4 ng/mL	65–107	Taşpınar et al. (2021)
UV-vis	Patulin	Fruit juice, dried fruit	43 µg/kg	10–750 µg/L	3.5 µg/L	94–104	Deng et al. (2021)
HPLC-FLD	PAHs	Ginseng, milkvetch, Maojian tea, Honeysuckle, Anji white tea	1.24–17.33 ng/g (ginseng), 1.02–49.87 ng/g (milkvetch), 3.81–45.02 ng/g (Maojian tea), 234.02–11.64 ng/g (honeysuckle), 11.15–541.96 ng/g (Anji white tea)	0.3–1400 ng/g	0.05–0.35 ng/g	88–109.9	Santiago et al. (2021)
GC-MS	Multiresidues	Red wine, rose wine	0.7 µg/L (Demeton-O); 2.80 µg/L (Methyl parathion); 0.565 µg/L (malathion); 24.8 µg/L (Dursban)	0.1–22 µg/L	58–121	9.8–29.1	Seebunrueng et al. (2020)
GC-µECD	Polychlorinated biphenyls	Human breast milk	n.a.	20–1400 ng/L	7–14 ng/L	70–117	Villegas-Álvarez et al. (2020)
Microspectrophotometry	Ammonia	Milk, yoghurt, cheese, beer	2.51–510 mg/L (milk), 109.5–112.8 mg/L (yoghurt), 279.5–305.8 mg/L (cheese), 4.68–12.09 mg/L (beer)	0.2–3 mg/kg	0.14 mg/kg	92.4–98.4	Jain et al. (2021)

GC-µECD, Gas chromatography with electron capture detector; *GC-MS*, gas chromatography–mass spectrometry; *HPLC-DAD*, high-performance liquid chromatography diode array detector; *HPLC-FLD*, high-performance liquid chromatography with fluorescence detector; *LOD*, limit of detection; *PAHs*, polycyclic aromatic hydrocarbons; *UHPLC-ESI(+)-QTOF-MS*, ultrahigh-performance liquid chromatography Q-Exactive high-resolution mass spectrometry; *UV-vis*, ultraviolet visible.

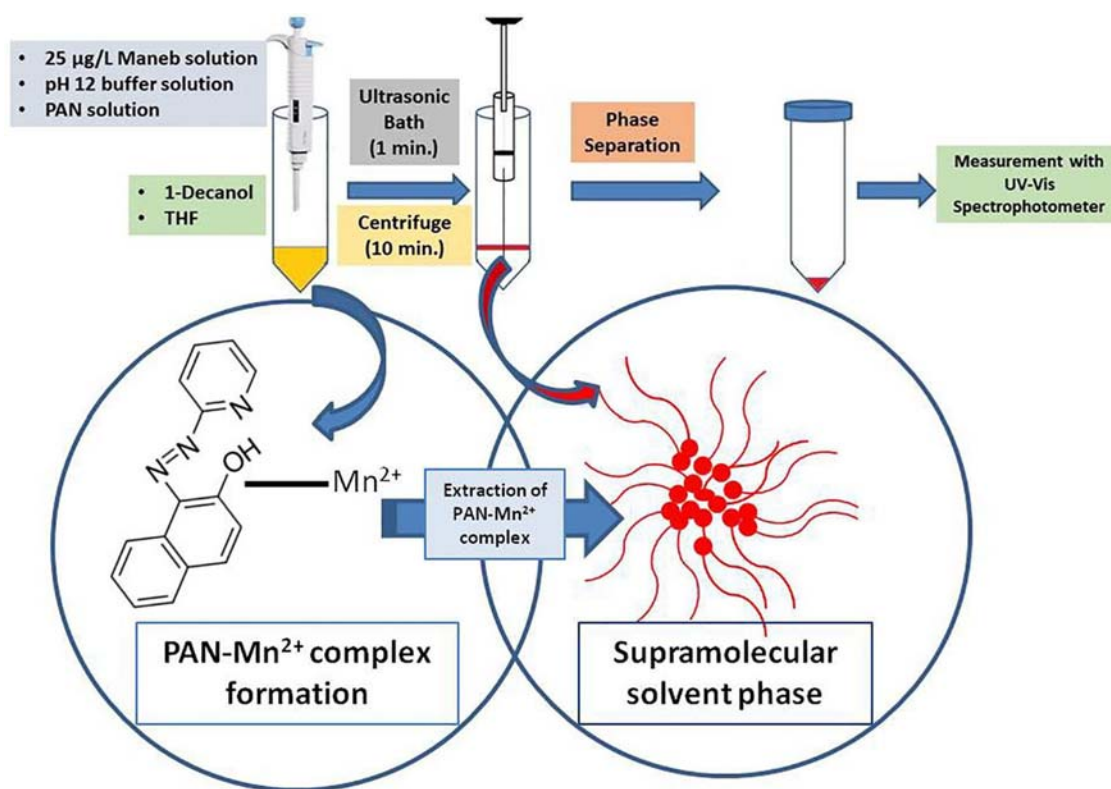


FIGURE 19.1 Schematic presentation of the presented supramolecular solvent liquid-phase microextraction study. *Reproduced with permission from Soylak, M., Agirbas, M., Yilmaz, E. (2021) A new strategy for the combination of supramolecular liquid phase microextraction and UV–Vis spectrophotometric determination for traces of maneb in food and water samples. Food Chemistry, 338. Copyright 2021 Elsevier.*

than the regulatory limit for herbicide residues. All the rice-studied samples were not detected with phenoxy acid herbicides.

Hsu et al. (2022) developed a simple method using a deep eutectic solvent (DES)-based ultrasound-assisted LPME to determine benzotriazole (BTR) and benzothiazole (BTH) derivatives in tea beverages by ultra-HPLC-electrospray ionization (+)-quadrupole time-of-flight mass spectrometry (UHPLC-ESI(+)-QTOF-MS) (Hsu & Ding, 2022). In this study, the optimization of the method was achieved using dual multivariate experimental by minimizing the number of experiments required and reducing overall cost. Based on the findings, the occurrence and concentrations of BTH and BTR residues in tea beverage matrices ranged from n.d. to 424 ng/g. Taşpınar, Elik, Kaya, and Altunay (2021) also developed a new natural DES (NADES) for the extraction of patulin in fruit juice and dried fruit samples (Taşpınar et al., 2021). In this study, the important innovation was achieved by replacing NADES as an extraction solvent instead of toxic solvents. NADES was synthesized accordingly to the target compound and the best extraction performance was achieved by choline chloride/tartaric acid and L-proline/glycerol. Using the developed method, the positive results in the selected samples were detected within the permitted limit established by the European regulation (50 µg/kg).

Santiago, dos Anjos, Nascimento, da Rocha, and de Andrade (2021) developed a simple and effective LPME based on the binary solvent (BS-LPME) for the determination of seventeen multiresidues in red and rose wines (Santiago et al., 2021). The BS-LPME is another relevant variation of LPME which combines the use of two or more extraction solvents that tend to improve the extraction performance. In the present work, the extraction device was designed to focus on minimum consumption of solvents (<200 µL), low sample volume, and low quantity of reagents. Several factors (string speed, extraction solvent volume, sample volume, ionic strength, and extraction time) that affect the extraction efficiency were optimized and applied to real sample analysis. The reported sample analysis of pesticides detected up to 24.8 µg/L. Deng et al. (2021) developed a density-tunable LPME using high-density DES (L-proline: glycerol) as extractant and low-density organic solvents (acetonitrile) as an emulsifier for the extraction of polycyclic aromatic hydrocarbons (PAHs) in tea, medicinal herbs, and liquid foods (Deng et al., 2021). In the extraction process, the emulsifier amount was adjusted to induce phase separation; either the DES-rich phase will be formed in the bottom or in the top phases (easy to collect for quantification). The developed method was applied and there were no target PAHs

detected in the tea beverage samples. However, the PAH concentration residues were detected in honey (37.86–304.29 ng/L), ginseng (1.24–17.33 ng/g), milkvetch (1.02–49.87 ng/g), Maojian tea (3.81–45.02 ng/g), honey-suckle (11.64–234.02 ng/g), and Anji white tea (11.15–236.02 ng/g).

Jain et al. (2021) developed a combination of LPME and fiber optics–based cuvetteless microspectrophotometry for sensitive determination of ammonia in food samples by the indophenol reaction (Jain, Soni, & Verma, 2021). In the present work, two processes were involved which were HS-SDME for attaining selectivity and enrichment of ammonia in the indophenol method. Then, the final extract (in microliter volume) has proceeded to the second process of LPME with fiber optics–based cuvetteless microspectrophotometry for the analysis. The developed method was applied for milk, yoghurt, cheese, and beer samples. Villegas-Álvarez et al. (2020) developed a sensitive and reliable HF-LPME for polychlorinated biphenyl in human breast milk (Villegas-Álvarez et al., 2020). Several parameters (i.e., selection of the extraction phase, pH, and salt addition) were optimized using multivariate optimization and applied for breast milk analysis by gas chromatography (GC)-MS and GC with electron capture detector. The findings revealed that the developed method is rapid, accurate, and simple for the sensitive determination of polychlorinated biphenyl in human breast milk samples.

19.2.2 Dispersive liquid–liquid microextraction

Recently, DLLME has become one of the attractive sample preparation techniques in its original and modified approaches due to the low consumption of extraction solvents. DLLME is simple and easy to be performed, relatively expensive, environmental friendly and exhibits high preconcentration factors for a wide variety of targeted analytes. DLLME is performed based on the injection of extraction and dispersive solvents into the aqueous sample to form a cloudy solution. In DLLME, equilibrium is rapidly achieved due to maximum surface contact between the microdroplets of extraction solvent and sample solution. After extraction, the sample vial or tube is centrifuged, and extraction solvent is collected with a microsyringe before analytical determination. The most critical variables of DLLME are the selection of extraction conditions and the choice of disperser solvent for the extraction of analytes. The criteria for disperser solvent include the miscibility with both extraction and aqueous sample for the formation of the cloudy solution that increases the interaction between two phases, which consequently produced high extraction efficiency (Herrera-Herrera, Asensio-Ramos, Hernández-Borges, & Rodríguez-Delgado, 2010). Other important variables in DLLME include the types and volumes of extraction solvent and disperser solvents, sample volume, ionic strength, and pH. DLLME has been widely applied in many applications, including for food analysis (Table 19.2).

DLLME was used for the extraction of tetracyclines from beef (Mookantsa, Dube, & Nindi, 2016). The LODs yielded under the optimized experimental conditions ranged between 2.2 and 3.6 $\mu\text{g/kg}$ with relative standard deviation (RSD) ($n = 21$) varying from 4.3% to 12.5%. Chloroform gave the best extraction efficiency of tetracyclines; however, dichloromethane was chosen as the extraction solvent because it is less toxic than chloroform. The dispersive solvent should be miscible with both water and the extraction solvent and should effectively dissolve the tetracyclines. Methanol was selected as the best disperser solvent in this study. According to the beef samples analyses, chlortetracycline and oxytetracycline were detected in the range of 39–73.4 $\mu\text{g/kg}$ and 38.4–82.3 $\mu\text{g/kg}$. The detected concentrations were lower than the maximum residue level established by the European Union at 100 $\mu\text{g/kg}$. Hence, the meat was fit for human consumption. A relatively similar strategy was also developed by other groups for the determination of preservatives in vinegar and cookie (Ding, Liu, Peng, Liu, & Tang, 2018) and ofloxacin in chicken meat (Timofeeva, Timofeev, Moskvina, & Bulatov, 2017).

A precolumn derivatization HPLC method based on DLLME was developed for the determination of six steroidal and phenolic endocrine disruption chemicals (EDCs) in chicken and fish (Wu et al., 2016). The precolumn step is necessary to convert EDCs into their derivatives with fluorescent properties to improve sensitivity. Ultrasonic radiation was employed to assist the dispersion of extraction solvent and speed up the mass transfer to the organic phase. In this study, chloroform and acetone were selected as extraction and disperser solvents, respectively, with an optimum pH of 10. Based on the analytical determination by HPLC with fluorescence detector, the LODs were measured ranged from 0.02 to 0.07 $\mu\text{g/L}$. In the analysis of food samples, DLLME was considered a reliable technique as it demonstrated good recovery between 88% and 106%. A microwave-assisted extraction (MAE) combined with the DLLME method was used for the extraction of nitrosamines in sausage and salami samples (Ramezani, Hosseini, Kamankesh, Ghasemzadeh-Mohammadi, & Mohammadi, 2015). This method coupled to GC-MS has resulted in a sensitive determination of nitrosamines with LODs in the range of 0.11–0.48 ng/g. Nitrosamines were found in the concentration range from 0.65 to 8.57 ng/g in the studied samples. MAE-DLLME combination was also used for the extraction of PAHs from grilled meat samples (Kamankesh, Mohammadi, Hosseini, & Modarres, 2015), extraction of polar heterocyclic

TABLE 19.2 Previously reported works on the applications of dispersive liquid–liquid microextraction for food samples.

Quantitation instruments	Analytes	Food matrices	Concentration of analytes found in sample (ng/mL)	Linearity (ng/mL)	LOD (ng/mL)	Relative recovery/recovery, %	References
GC-MS	PAHs	Grilled meat	0.4–15.5 ng/g	1–200 ng/g	0.15–0.3 ng/g	85–104	Kamankesh et al. (2015)
GC-MS	Nitrosamines	Sausage and salami	0.65–8.57 ng/g	0.1–200 ng/mL	0.11–0.48 ng/g	83.9–109.4	Ramezani et al. (2015)
LC-MS/MS	Tetracyclines	Beef	38.4–82.3 µg/kg	25–200 µg/kg	2.2–3.6 µg/kg	80–105	Mookantsa et al. (2016)
HPLC-UV	Polar heterocyclic aromatic amines	Hamburger patties	10.2–20.8 ng/g	1–200 ng/g	0.06–0.21 ng/g	90–105	Aeenehvand et al. (2016)
HPLC-DAD	Aryloxyphenoxy-propionate herbicides	Soy-based foods	–	0.36–9.26 mg/L	0.12–0.34 mg/L	25–66	Lubomirsky et al. (2016)
HPLC-UV	Polyamines	Turkey breast meat samples	3.15–38.03 ng/g	20–200 ng/g	0.8–1.4 ng/g	95–105	Bashiry et al. (2016)
HPLC-FLD	Steroidal and phenolic endocrine-disrupting chemicals	Chicken and fish	–	–	0.02–0.07 µg/L	88–106	Wu et al. (2016)
HPLC-FLD	Ofloxacin	Chicken meat	< LOD	6×10^{-9} – 5×10^{-7} mol/L	2×10^{-9} mol/L	90.4–102.1	Timofeeva et al. (2017)
GC-MS	BA, SA, dehydroacetic acid, and four parabens	Vinegar and cookie	30.9–71.6 mg/kg	0.02–10 mg/L	0.15–0.50 mg/kg	88.7–110.5	Ding et al. (2018)
HPLC-UV	Synthetic red dyes (amaranth, Ponceau 4R, Allura red, Azorubine, and Erythrosine)	Cherry jelly, carbonated beverages, chocolate dragee	3.2–121.1 µg/L	0.5–1000 µg/L	0.01–0.08 µg/L	90–97	Faraji (2019)
GC-MS	Biogenic amines	Meat	0.3124–22.9 mg/kg	0.05–10 µg/L	0.003–0.009 µg/g	79–101	Wojnowski et al. (2019)
ETAAS	Molybdenum	Bovine meat	8.7–14.2 µg/kg	Up to 1.5 µg/L	0.03 µg/kg	98	Machado and Tissot (2020)
HPLC-PDA	Tartrazine, quinoline yellow, sunset yellow, brilliant blue, Ponceau 4R, indigo carmine, Allura red, and carmoisine	Jellies and drinks	3.28–110.4 mg/L	5–1000 µg/L	0.02–0.05 µg/L	87.2–107.4	Gholami et al. (2021)

BA, Benzoic acid; ETAAS, electrothermal atomic absorption spectrometry; GC-MS, gas chromatography–mass spectrometry; HPLC-DAD, high-performance liquid chromatography diode array detector; HPLC-FLD, high-performance liquid chromatography with fluorescence detector; HPLC-PDA, high-performance liquid chromatography photodiode array detector; HPLC-UV, high-performance liquid chromatography ultraviolet detector; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LOD, limit of detection; PAHs, polycyclic aromatic hydrocarbons; SA, sorbic acid.

amines from hamburger patties (Aeenehvand et al., 2016), and determination of polyamines in Turkey breast meat samples (Bashiry et al., 2016). The contaminants were found in the range of 0.4–15.5 ng/g of PAHs in grilled meat, 10.2–20.8 ng/g of polar heterocyclic aromatic amines in hamburger patties, and 3.15–38.03 ng/g of polyamines in Turkey breast meat samples, respectively. MAE combined with DLLME has increased recovery yield, reduced extraction time, and increased the mass transfer of analytes from the food samples to the liquid extraction phase. The developed MAE-DLLME method required low consumption of solvent and a simple experimental setup with good precision and high sensitivity.

On the other hand, Faraji (2019) and Gholami, Marhamatizadeh, Yousefinejad, Rashedinia, and Mazloomi (2021) developed ultrasound-assisted-DLLME for the determination of synthetic dyes in jelly, beverages, and chocolates. The combination of ultrasound assisted with DLLME improves the sensitivity of the targeted analytes. The analytical performance merits further indicated good precision and extraction recoveries for the two studies with RSD < 8% and 87.2%–107.4%, respectively. The determination of all analytes generated very low LODs, 0.01–0.08 µg/L. Based on the sample analysis, synthetic dyes were detected in samples in the concentration ranged from 3.2 to 121.1 µg/L. The application of green solvent termed DES in the DLLME in work (Gholami et al., 2021) contributed to the development of a greener DLLME method. The simplified schematic diagram on the preparation of the DES and DLLME process is shown in Fig. 19.2. The ultrasound-assisted DLLME mode requires an application of ultrasound to facilitate the dispersion of the extraction solvent into the aqueous solution and to ensure the adequate formation of microdroplets. The combination of a vortex, microwave, or ultrasound is accompanied by an increase in the temperature in the extraction tube. In some studies, a dispersive solvent is needed to improve the kinetics, and it can be an organic solvent, a surfactant, or a hydrophilic ionic liquid (IL) (Rykowska, Ziemblińska, & Nowak, 2018). Another DLLME method was also developed using a green solvent ionic liquid as extraction solvent for the determination aryloxyphenoxy-propionate herbicides in soy-based foods (Lubomirsky, Padró, & Reta, 2016).

Machado and Tissot (2020) developed the DLLME method prior to electrothermal atomic absorption spectrometry (ETAAS) to determine the presence of molybdenum in bovine meat. The authors selected carbon tetrachloride as the extraction solvent and acetonitrile as the disperser solvent, whereby the selection indicated excellent preconcentration of molybdenum (EF = 200). The procedure was perceived to be promising due to outstanding analytical performance supported by ETAAS, with the precision of 4.1%, acceptable recoveries at 98% together with LOD of 0.03 µg/kg. Molybdenum was detected in the range of 8.7–14.2 µg/kg in the bovine meat. Another interesting work was developed by Wojnowski et al. (Wojnowski, Namieśnik, & Płotka-Wasyłka, 2019) on the application of DLLME to determine biogenic amines in meat for the estimation of meat's freshness. A mixture of methanol as dispersive solvent, chloroform as extraction solvent, and isobutyl chloroformate as derivatizing reagent was used in the extraction process together with an admixture of pyridine and HCl to eliminate the by-products. The straightforward methodology exhibits good linearity (0.05–10 µg/L), precision (RSD < 7%), and recoveries (79%–101%). LOD as low as 0.003 µg/g was measured by the developed method. From their findings, the authors concluded tryptamine, cadaverine, 2-phenylethylamine, and putrescine could be considered potential meat freshness indicators. In a nutshell, the DLLME approach to simplify the extraction process reduces the loss of analytes during extraction and offers high preconcentration factors with rapid extraction time prior to various instrumental quantitation.

19.2.3 Solid-phase microextraction

SPME was reported by Arthur & Pawliszyn (1990) as the innovation of SPE (Arthur & Pawliszyn, 1990). SPME requires a small dosage of sorbent dispersed typically on the surface of small fibers, to isolate and concentrate analytes from the sample matrix. Analytes are absorbed or adsorbed by the fiber phase (depending on the nature of the coating) until an equilibrium is reached in the system. The adsorbed analyte is determined by the magnitude of the partition coefficient of the analyte and the coating material fiber. Then, the fibers are transferred using the aid of a syringe-like handling device to an analytical instrument for the qualification and quantification of target analytes. There are a few extraction modes for fiber SPME, for example, direct immersion-SPME (DI-SPME), HS-SPME, and membrane-protected SPME. Overall, the SPME method involves integrates sampling, extraction, and sample introduction into one single step, providing outstanding superiority such as convenience, solventless, high extraction efficiency, and facile coupling with detection system (Kataoka, Lord, & Pawliszyn, 2000). Therefore several works have been published as proof of a growing development of the miniaturized method. In recent years, several SPME works have been developed with innovation and improvement. Table 19.3 summarizes the most recent previous studies on the application of SPME for food samples.

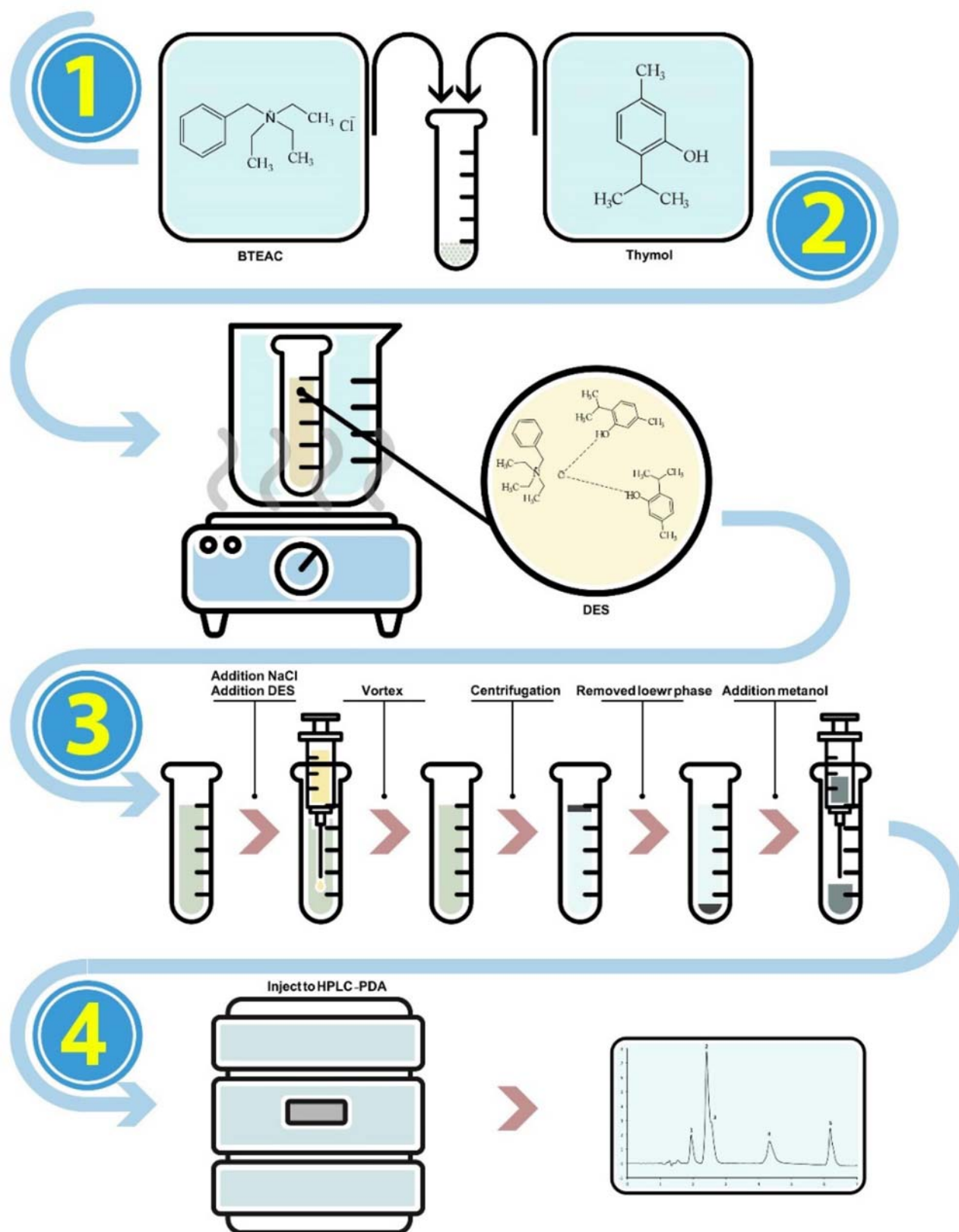


FIGURE 19.2 Schematic diagram of a synthesis of DES and representation of the steps of proposed microextraction technique, from optimization to analysis with HPLC. DES, Deep eutectic solvent; HPLC, high-performance liquid chromatography. Reproduced with permission from Gholami, Z., Marhamatizadeh, M. H., Yousefinejad, S., Rashedinia, M., Mazloomi, S. M. (2021). Vortex-assisted dispersive liquid-liquid microextraction based on hydrophobic deep eutectic solvent for the simultaneous identification of eight synthetic dyes in jellies and drinks using HPLC-PDA. *Microchemical Journal*, Devoted to the Application of Microtechniques in all Branches of Science, 170, 106671. Copyright 2021 Elsevier.

TABLE 19.3 Previously reported works on the applications of solid-phase microextraction for food samples.

Quantitation instruments	Analytes	Food matrices	Concentration of analytes found in sample	Linearity	LOD	Relative recovery/recovery, %	References
GC-FID	Furanic compounds	<i>Mopane worms</i> , peanuts, corn food	95 µg/kg	0.17–2279 µg/kg	0.54–3.50 µg/kg	67–106	Masite, Ncube, Mtunzi, Madikizela, and Pakade (2022b)
GC-MS	PAHs	Beer samples (dark-roasted, dark-caramel, and Pilsen)	19.06 µg/L	2.61–22.0 µg/L	0.003–0.128 µg/L	80.1–100.3	Resende dos Santos et al. (2021)
GC-FID	Diazinon, chlorpyrifos	Wheat	n.a.	20–4000 µg/Kg (diazinon) 200–4000 µg/Kg (chlorpyrifos)	4.00 µg/Kg (diazinon) 20.0 µg/Kg (chlorpyrifos)	84.1–107 (diazinon) 90.0–99.6 (chlorpyrifos)	Alizadeh et al. (2021)
GC-MS	Pyrethroids	Honey	n.a.	0.1–2000 ng/g	0.10–10 ng/g	75–120	Belinato et al. (2021)
HPLC-DAD	Acetamiprid, dimethipin, and amitrole	Rice apple	1.36 ng/g	5.0–1000 ng/g	0.86–1.38 ng/g	79.3–106.8	Li et al. (2022)
GC-MS	Polychlorinated <i>n</i> -alkanes	Cod liver oil	0.52 µg/g	0.075–0.75 µg/g	0.1–1.2 µg/g	n.a.	Gruszecka et al. (2021)
GC-MS	PAHs	Honey	lower than LOD	0.10–100 ng/g	0.03–0.19 ng/g	82.0–116.8	Wang et al. (2020)
GC-FID	Benzoic acid, sorbic acid, and propionic acid	Fermented food (Thai shrimp paste, pickled vegetables, soy sauce, and fish sauce)	308 mg/kg	5–150 mg/L	1.1–1.7 mg/L	83–109	Tungkijanansin et al. (2020)
HPLC-FLD	Aflatoxins	Agricultural products (ground nut and chili pepper)	8.71 µg/kg	0.1–100 µg/kg	0.01–0.07 µg/kg	88.6–99.8	Amde et al. (2020)

GC-MS, Gas chromatography–mass spectrometry; HPLC-DAD, high-performance liquid chromatography diode array detector; HPLC-FLD, high-performance liquid chromatography with fluorescence detector; LOD, limit of detection; n.a., not applicable; n.d., not detected; PAHs, polycyclic aromatic hydrocarbons.

Masite et al. (2022) developed a direct HS-SPME technique for the extraction of volatile furanic compounds (furan, 2-methylfuran, and 2-furaldehyde) in selected South African traditional food by gas chromatography with flame ionization detector (GC-FID) analysis (Masite, Ncube, Mtunzi, Madikizela, & Pakade, 2022a). In this work, divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber was used for the extraction of furan compounds in heating samples of *Mopane worms*, corn, and peanuts. The reported recoveries, LODs, and limits of quantification (LOQs) were 67%–106%, 0.54–3.5, and 1.8–12 $\mu\text{g/kg}$, respectively. The two most abundant of 2-methylfuran and furan were detected in heat-processed samples. Tungkijanansin, Alahmad, Nhujak, and Varanusupakul (2020) developed an HS-SPME followed by GC-FID for the determination of benzoic acid, sorbic acid, and propionic acid in fermented food (Tungkijanansin et al., 2020). In this work, a good linear range (5–150 mg/L), acceptable LOD values (1.1–1.7 mg/L), and high accuracy of recoveries (83%–109%) were obtained. The proposed method provides a simple preparation that combined clean-up and sample enrichment in one step extraction technique in fermented food.

Resende dos Santos et al. (2021) proposed a new cold fiber-SPME (CF-SPME) system using thermoelectric cooling coupled to GC-MS for monitoring PAH in light and dark beers (Resende dos Santos, Orlando, de Lourdes Cardeal, & Menezes, 2021). The thermoelectric coolers (Peltier) as shown in Fig. 19.3 keep the SPME fiber during the extraction process which improves the extraction efficiency. The validation results reported that the acceptable range of LODs (0.003–0.128 $\mu\text{g/L}$) and LOQ (0.011–0.427 $\mu\text{g/L}$) and good recovery values (80.1%–100.3%) were obtained. As a result, all samples analyzed were detected with benzo[*b*]fluoranthene. Gruszecka, Grandy, Gionfriddo, Singh, and Pawliszyn (2021) developed a DI thin-film SPME (TF-SPME) of polychlorinated *n*-alkanes (PCAs) in cod liver oil (Gruszecka et al., 2021). The TF-SPME was accomplished by aluminum-supported based hydrophilic–lipophilic balance/PDMS (HLB/PDMS) for the extraction of five selected PCAs from fish oil. The validation results reported a

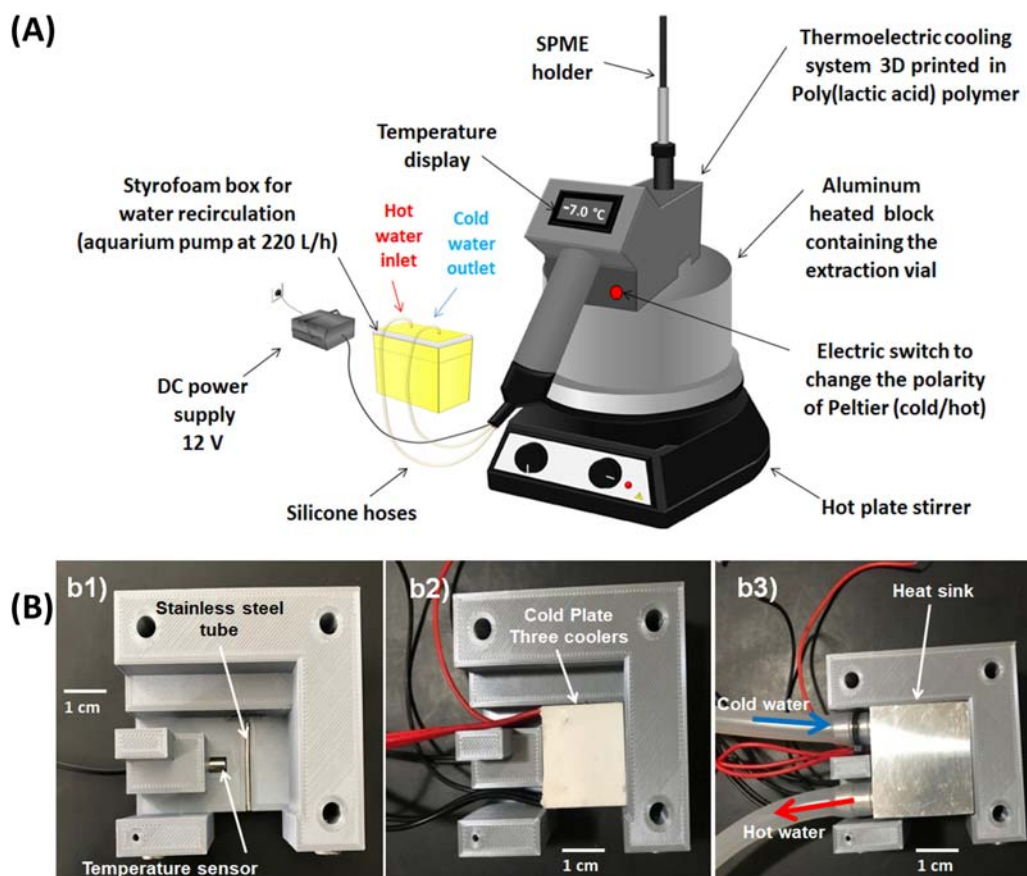


FIGURE 19.3 Design of the thermoelectric cooling system for CF-SPME: (A) schematic illustration; (B) picture of the disassembled internal parts: (b1) tube for SPME fiber and temperature sensor, (b2) cold/hot Peltier plate, and (b3) heat sink; 1-cm scale bar indicated. *CF-SPME*, Cold fiber solid-phase microextraction. Reproduced with permission from Resende dos Santos, R., Orlando, R. M., de Lourdes Cardeal, Z., Menezes, H. C. (2021). Assessment of polycyclic aromatic hydrocarbons and derivatives in beer using a new cold fiber-solid phase microextraction system. Food Control, 126, 108104. Copyright 2021 Elsevier.

linearity range from 0.075 to 0.75 $\mu\text{g/g}$ with the LODs ranging from 0.07 to 0.217 $\mu\text{g/g}$ in oil samples. Four fish oil brands were analyzed and all the samples were detected above the LODs.

Belinato, Grandy, Khaled, Suarez, and Pawliszyn (2021) developed a fully automated DI-SPME method using a matrix-compatible fiber (PDMS/DVB/PDMS) to overcome matrix influences in the analysis of pyrethroids in honey by GC-MS (Belinato et al., 2021). The optimization was performed using a multivariate approach which provides low LOQ values with an acceptable range of accuracy from 75% to 120%. Five brands of real honey were tested using the validated method. As a result, the cyfluthrin and bioallethrin were detected in the honey samples but below the maximum residue limits (MRLs). Overall, the proposed method provides a more simplified approach with minimum sample handling and demonstrating the developed automated DI-SPME method. Li et al. (2022) developed a method for the determination of pesticides in foods via covalent organic framework (COF) reinforced HF-SPME combined with HPLC-DAD (Li, Xue, Fu, Ma, & Feng, 2022). In this study, the wall pores of HF were constructed by immobilizing a three-dimensional hydroxyl-functionalized COF. Acetamiprid, dimethipin, and amitrole were simultaneously determined in rice and apple samples. Based on the reported study, the values detected in samples were lower than the LOQs and MRLs.

Alizadeh, Arbandi, Kashefolgheta, and Seidi (2021) developed a smart SPME (SSPME) with flexible sorbent is introduced on breathing of metal organic framework (MOF) nanorods flexible zinc(II)-based MOFs, namely, MOF-508, for the determination of diazinon and chlorpyrifos in wheat samples by GC-FID. The optimization was achieved using an experimental design and response surface methodology. Based on the investigation, the SSPME indicated high extraction efficiency by opening the micropores of MOF-508 during the extraction process, and the porosity of SSPME decreased during the desorption of target analytes, which reduced the desorption time and low LOD. However, the reduction of extraction efficiency was observed on the high chemical and thermal stability of developed fiber after 70 consecutive experiments. As compared to the commercial SPME fiber method, the results of the developed method were comparable. Wang et al. (2020) developed three different covalent triazine-based frameworks (CTFs) in SPME which were cyanuric chloride-biphenyl, CC-*p*-terphenyl, and CC-*p*-quaterphenyl system. The prepared CTFs were explored and investigated as the SPME coating for the extraction of some PAHs from various honey samples by GC-MS. Finally, eight honey samples were tested, including *Wolfberry*, *Robinia*, *Codonopsis*, and *Jujube honey*. The level of PAHs detected in the sample was lower than the LOQs. Amde, Temsgen, and Dechassa (2020) developed a nanorods-based ionic liquid-functionalized zinc oxide for SPME of Aflatoxins (AFB₁, AFB₂, AFG₁, and AFG₂) in food products. In this work, several extraction parameters of the SPME based on IL-functionalized ZnO-nanorods were investigated using one variable at a time. Real food samples were analyzed on two chili pepper and processed groundnut samples collected from the local market. Based on reported findings, the AFB₁ and AFB₂ were detected in all samples, while AFG₁ was detected in the groundnut sample and AFG₂ was not detected in all samples.

19.2.4 Quick, easy, cheap, effective, rugged, safe

QuEChERS was initially developed for the analysis and clean-up of pesticide residues in vegetables and fruits with high water content. QuEChERS is carried out through several simple steps: (1) acetonitrile is added in the sample solution for the extraction, (2) salting out of the sample using anhydrous magnesium sulfate and sodium chloride layer to eliminate the moisture in the sample, and (3) N-propylethylenediamine (PSA) is used as a DSPE agent as clean-up material. In recent years, QuEChERS has become increasingly attractive in the analysis of targeted molecules in various types of matrices. This is probably due to several advantages of this approach, listed as following: (1) QuEChERS as a multiresidues analysis method that is suitable for samples with high moisture content and reduce the interference of sample matrices; (2) high reproducibility and stability; (3) a wide range of residues can be extracted, including polar and nonpolar compounds; (4) simple apparatus, low cost of purchase, and maintenance; (5) fast and easy operation; and (6) safe and low pollution for the environment (Zhang et al., 2019). QuEChERS is widely combined with various chromatographic techniques such as GC and LC to provide clean-up matrix interference depending on the selection of optimized extraction and separation variables. Table 19.4 summarizes the QuEChERS applications in food analysis.

Because of the excellent performance of QuEChERS in the extraction of fruits and vegetables, many researchers aroused their interest in this technology. Dong, Xiao, Xian, Wu, and Zhu (2019) developed a QuEChERS method coupled to ultraperformance LC (UPLC)-MS/MS for the extraction and preconcentration of perchlorate and bromate ions in fruits and vegetables. The LODs yielded under the optimized conditions were ranged between 0.1 and 0.5 $\mu\text{g/L}$. Based on the optimized experimental conditions, 100 mg of C18 and 40 mg of graphitized carbon black (GCB) were used as clean-up adsorbents. GCB is mainly used to remove nonpolar compounds such as pigments, carotenoids, and sterols, which is appropriate for the purification of vegetable matrix (Dong & Xiao, 2017), while C18 can adsorb

TABLE 19.4 Previously reported works on the applications of quick, easy, cheap, effective, rugged, safe for food samples.

Quantitation instruments	Analytes	Food matrices	Concentration of analytes found in sample (ng/mL)	Linearity (ng/mL)	LOD (ng/mL)	Relative recovery/recovery, %	References
LC-MS/MS	β_2 -agonist	Meat	—	0.01–12.8 $\mu\text{g/kg}$	0.2–0.9 $\mu\text{g/kg}$	73.7–103.5	Xiong et al. (2015)
LC-MS/MS	Ractopamine	Meat and bone meal	3.87–81.25	0–250 $\mu\text{g/kg}$	1.91 $\mu\text{g/kg}$	96.3–107	Gressler et al. (2016)
LC-MS/MS	Parabens and bisphenols (A, F, and S)	Human breast milk	0.11–7.0 ng/mL	0.1–50 ng/mL	LOQ: 0.1–0.25 ng/mL	83–115	Dualde et al. (2019)
UPLC-MS/MS	Perchlorate and bromate	Fruits and vegetables	21–162 $\mu\text{g/kg}$	0.2–200 $\mu\text{g/L}$	0.1–0.5 $\mu\text{g/L}$	82–99.5	Dong et al. (2019)
HPLC-MS/MS	102 pesticides	Green tea	0.03–2.61 mg/kg	0.2–500 ng/mL	0.03–15 $\mu\text{g/kg}$	62–125	Huang et al. (2019)
LC-MS/MS	60 pesticides	Hen eggs	5–10 $\mu\text{g/kg}$	0.005–0.1 mg/kg	0.001–0.005 mg/kg	70–120	Song et al. (2019)
LC-MS/MS and GC-MS/MS	Neonicotinoids and 199 other pesticides	Honey	0.002–0.061 mg/kg	0.001–0.1 mg/kg	LOQ: 0.1–0.5 mg/kg	70–120	Gawel et al. (2019)
UHPLC-MS/MS	Heterocyclic aromatic amines	Cooked beef	0.52–94 ng/g	1–1000 pg/ μL	1–5 pg/ μL	61–108	Chevolleau et al. (2020)
UPLC-MS/MS	Vancomycin and norvancomycin	Fish meat	5.34–28.16 $\mu\text{g/kg}$	0.5–100 $\mu\text{g/L}$	0.51 $\mu\text{g/kg}$	86.7–98.6	Shen et al. (2021)
GC-MS	Pesticides	Fish feed, fish, and vegetables	ND-1.535 mg/kg	10–200 ng/mL	0.01–0.04 mg/kg	80–120	Rahman et al. (2021)
LC-MS/MS	Histamine	Canned fish	<LOD–354.05 mg/kg	1–500 mg/kg	0.3 mg/kg	85.5–105.5	Harmoko et al. (2021)
UPLC-MS/MS	19 quinolone veterinary drugs	Goat's milk	—	12–100 ppb	LOQ: 5 ppb	73.4–114.21	Bang et al. (2022)

GC-MS, Gas chromatography–mass spectrometry; HPLC-DAD, high-performance liquid chromatography diode array detector; LC-MS/MS, liquid chromatography–tandem mass spectrometry; UPLC-MS/MS, ultraperformance liquid chromatography–tandem mass spectrometry; UHPLC-ESI(+)-QTOF-MS, ultrahigh-performance liquid chromatography Q-Exactive high-resolution mass spectrometry; LOD, limit of detection; LOQ, limit of quantification.

pigments, fat-soluble impurities, and nonpolar impurities. According to the fruits and vegetable analyses, perchlorate was detected in 12 samples including in potato, lettuce, and pakchoi with concentrations ranging from 21 to 162 $\mu\text{g/kg}$. The ubiquitous occurrence of perchlorate contamination in vegetables confirms its urgency for monitoring the studied inorganic anion in commonly consumed vegetables. Xiong, Gao, Li, Yang, and Shimo (2015) performed a comparison of mixed-mode SPE and QuEChERS for the determination of β_2 -agonist in meat by using LC-MS/MS. According to Xiong and co-workers, the results obtained for the QuEChERS method were demonstrated to be more efficient according to the simplicity of operation (procedure of QuEChERS is simpler), the time required for sample preparation (20 hours per sample for SPE and 8 hours per sample for QuEChERS) and solvent consumption. The applicability of the developed QuEChERS and LC-MS/MS method was evaluated by analyzing more than 50 samples of cattle muscle. No residues of the β_2 -agonist were detected in all samples. LOD as low as 0.2 $\mu\text{g/kg}$ was obtained using the developed method. The similar approach was also developed by Gressler et al. (2016) for quantification of ractopamine, also a β -agonist in meat and bone meal. Based on the LC-MS/MS, the LOD and LOQ of the QuEChERS method exhibited good analytical capability of 1.91 and 6.36 ppb, respectively, with reproducibility of less than <6% (RSDs) and recoveries between 96.3% and 107%.

Gawel et al. (2019) evaluated the modified QuEChERS method for simultaneous analysis of 207 different classes of pesticides in honey by LC-MS/MS and GC-MS/MS. Based on the optimization of clean-up methodology in QuEChERS, a mixture of PSA, Z-Sep + (silica gel modified with C18 and zirconium dioxide group), and MgSO_4 and acetate buffered extraction was found to give the best recovery for the studied analytes. The recoveries obtained from this study were between 70% and 120% with LODs measured ranging from 0.1 to 0.5 mg/kg. Interestingly, neonicotinoids (acetamiprid and thiacloprid) were detected in 77% of samples and were the most frequently detected pesticides. Acetamiprid and thiacloprid were used for plant protection, which indirectly through contaminated nectar enter honey. The authors concluded that indirect honey contamination by systemic plant protection products is the main source of honey pollution. Other applications of QuEChERS and modified QuEChERS in the determination of pesticides in hen eggs (Song et al., 2019), green tea (Huang et al., 2019), and fish feed, fish, and vegetables (Rahman et al., 2021) were also reported.

In another study, Dualde, Pardo, Fernández, Pastor, and Yusà (2019) evaluated the QuEChERS method before quantification via LC-MS/MS for a trace amount of parabens and bisphenols in 10 samples of human breast milk. The authors used isotopically labeled internal standards and matrix-matched calibration to correct the matrix effects in this study. The analytical figures of merit further indicated good extraction recoveries within 83% and 115% with RSD <20%. The combination of QuEChERS and LC-MS/MS allowed achieving LODs ranging from 0.1 to 0.25 ng/mL. Methyl paraben presented the highest occurrence frequency (80%) with a concentration range of 0.11–7.00 ng/mL while bisphenol A was detected frequently in 80% of samples with concentrations ranging from 0.13 to 1.62 ng/mL. Conventional sample preparation approaches are often ineffective for extracting residual antibiotics, especially from the complex food matrices. Thus Ye et al. (Bang, Huang, & Lin, 2022) developed a QuEChERS method prior to UPLC-MS/MS analysis to quantify the presence of 19 quinolone antibiotics in goat's milk. Ye and co-workers studied different parameters which can influence the extraction efficiency, such as salt content, extraction adsorbent, clean-up adsorbent, and extraction time. By using a Plackett–Burman experimental design, it was possible to estimate the most effective parameters for the efficient extraction. Central composite design was then used to test the conditions. The most important factors affecting the extraction efficiency of fluoroquinolones were sodium citrate and disodium hydrogen citrate, volume of extraction solution, and low-temperature purification. The other variables such as anhydrous sodium sulfate, sodium chloride, ultrasonic extraction, and C18 adsorbent as purification powder were not significant factors in the ranges studied. The optimized QuEChERS combined with UPLC-MS/MS conditions allowed to achieve LOQ of 5 ppb for the studied quinolones. This scheme was also employed by Shen et al. (2021) which developed a sensitive method by combining QuEChERS and 96-well plate SPE for the pretreatment of vancomycin and norvancomycin antibiotics in fish meat by UPLC-MS/MS. The 96-well plate SPE was performed by using cation-exchange resin and 15% aqueous acetonitrile was used as the optimum extraction solvent. The optimized QuEChERS-SPE method showed detection limits of 0.51 $\mu\text{g/kg}$. Vancomycin and norvancomycin were detected at a concentration ranging from 5.34 to 28.16 $\mu\text{g/kg}$. Histamine contamination was also found in canned fish through the QuEChERS method developed by Harmoko, Kartasasmita, Munawar, Rakhmawati, and Budiawan (2021). The LODs and LOQs were 0.30 and 1.00 mg/kg, respectively. The recoveries were between 85.5% and 105.5%. Concentrations of histamine detected in canned fish samples in Indonesia were varied from <LOD to 354.05 mg/kg. A similar approach using QuEChERS method combined with UHPLC-MS/MS was also developed by Chevolleau, Bouville, and Debrauwer (2020) for the determination of 16 heterocyclic aromatic amines in cooked beef. LODs as low as 1–5 pg/ μL have demonstrated the sensitive determination of the targeted analytes by the developed method.

19.2.5 Dispersive solid-phase extraction and magnetic solid-phase extraction

SPE is a method that is widely used in the analytical field with good repeatability. However, this method is still considered time-consuming. Therefore MSPE is a fascinating SPE innovation that has been introduced and explored as a fast efficient pretreatment method. MSPE receives considerable attention because of its advantages such as simplicity, high efficiency, and environmental protection. In the MSPE process, a magnetic adsorbent involved will disperse in the sample solution and lead to the effective interaction area between the adsorbent and target analytes being largely improved (Jiang et al., 2019). Nowadays, magnetic adsorbents show as a good candidate for fast extraction and preconcentration. Up to date, many kinds of nanoscale MSPE adsorbents have been developed and have attracted much interest. Table 19.5 summarizes the most recent previous studies on the application of MSPE combined with detection instruments for food analysis.

There are new types of crystalline porous materials with a hierarchical porosity and high crystallinity which exhibit wide prospects in sample pretreatment consisting of a COF ($\text{Fe}_3\text{O}_4@\text{TAPB-COF}$) and MOFs (ZIF-8). The MOF-COF has been explored in the MSPE field even though there are substantial advancements in sample pretreatment with porous materials such as MOFs and COFs. The first report by Jiang, Fu, Wang, Lin, and Zhao (2021) developed a magnetic SPE with the combination of MOFs and COFs to design the MOF-COC ($\text{Fe}_3\text{O}_4@\text{TAPB-COF}@\text{ZIF-8}$) composite for the determination of trace bisphenols in functional beverages (Jiang et al., 2021). Several parameters were investigated to obtain the best extraction conditions such as extraction time, solution pH, amount of adsorbent, and ionic strength. Four plastic-packaged functional beverages were analyzed under optimal extraction conditions.

COFs have displayed outstanding performance in sample preparation because it offers good features of low density, fine structures, moderate surface area, tunable pore size and structure, diverse building units and synthetic methods, and easy functional modification. Even though COFs have shown a great performance in sample preparation, but the recycling process is a big challenge and limits their potential to some extent application. This problem can be solved by introducing magnetism into COFs to synthesize magnetic COFs. Guo and co-workers developed a core-shell structured magnetic COFs adsorbent in MSPE coupled to HPLC-UV for phenylurea herbicides, including monuron, chlortoluron, isoproturon, monolinuron, and buturon in a tea drink sample (Guo et al., 2021). The COF was designed with 3,3'-diaminobenzidine (DAB) and 1,3,5-triformylphloroglucinol (Tp) as building units, name M-TpDAB. Several crucial parameters, including the dosage of M-TpDAB, sample volume, extraction time, ion strength, sample pH, and desorption conditions, were investigated. Overall, the developed method MSPE-HPLC-UV with $\text{Fe}_3\text{O}_4@\text{TAPB-COF}@\text{ZIF-8}$ adsorbents was successfully applied. Senosy et al. (2020) developed MSPE based on nano-zeolite imidazolate framework-8-functionalized magnetic graphene oxide ($\text{Fe}_3\text{O}_4@\text{APTES-GO}@\text{ZIF-8}$) to determine the triazole fungicides residues in honey and fruit juices (Senosy et al., 2020). In this work, the employing of graphene oxide sheets could improve the dispersion of the adsorbent, while ZIF-8 would ensure a high enough surface area and active sites. In the application of analysis, all the target fungicides were found to be below the LODs.

Chinese herbal medicines (CHMs) have become popular in recent decades and are widely used in China to cure and prevent human diseases. Even though CHMs are considered safe due to the low side effect of CHMs, they might be contaminated by PAHs during the growth process and drying process. Zhou et al. (2020) developed magnetic C_{60} nanosphere-based SPE coupled with isotope dilution GC-MS method for the determination of sixteen PAHs in CHMs (Zhou et al., 2020). In the present work, the new adsorbent was prepared by modifying Fe_3O_4 nanospheres with silica and 3-aminopropyltriethoxysilane, and then finally reacts with C_{60} to extract PAHs in CHM samples. To obtain the maximum extraction performance of MSPE, several conditions were optimized by assessing extraction solvent, sorbent amount, and extraction time. The nine CHMs samples were detected with PAHs in the range of 73.6 $\mu\text{g/kg}$ (*Fructus lycii*) to 2172.6 $\mu\text{g/kg}$ (*Astragalus root*).

Zhao et al. (2020b) developed green DES and efficient media combined with functionalized magnetic multiwalled carbon nanotubes (MWCNTs) employed in MSPE for determination pesticide residue in food products by HPLC-UV (Zhao et al., 2020a,b). Variety types of DES were investigated for high extraction efficiency and consisted of proline and propylene glycol at a 1.3 M ratio. The optimized method was optimized statistically by response surface methodology using a BOX-Behnken design. Four kinds of the real sample (apple, pear, carrot, and cucumber) were applied to analyze pesticides by HPLC-UV. The results show that the pesticides were detected in pear, cucumber, and carrot.

Zhao et al. (2020b) developed a combination of MSPE with ultrahigh-performance liquid chromatography Q-Exactive high-resolution mass spectrometry (UHPLC-Q-Exactive HRMS) to analyze multimycotoxins analysis in liquid milk by UHPLC-Q-Exactive HRMS (Zhao et al., 2020b). The polyethylene glycol (PEG) ylated MWCNT, namely, PEG-MWCNTs-MNP, was used as adsorbent for isolation and enrichment of aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), aflatoxin M_1 (AFM_1), aflatoxin M_2 (AFM_2), ochratoxin A (OTA),

TABLE 19.5 Previously reported works on the applications of dispersive solid-phase extraction and magnetic solid-phase extraction for food samples.

Quantitation instruments	Analytes	Food matrices	Concentration of analytes found in sample (ng/mL)	Linearity (ng/mL)	LOD (ng/mL)	Relative recovery/recovery, %	References
HPLC-DAD	Bisphenols	Beverages	n.d.	0.25–1000 ng/mL	0.04–0.05 ng/mL	66.2–116.6	Jiang et al. (2021)
HPLC-UV	Phenylurea herbicides	Tea drink	0.8 ng/mL	1.0–100.0 ng/mL	0.30–0.50 ng/mL	82.1–98.8	Guo et al. (2021)
HPLC-DAD	Triazole fungicides	Honey fruit juice	n.d.	1–1000 µg/L	0.014–0.109 (µg/L)	71.2–110.9	Senosy et al. (2020)
GC-MS	PAHs	Chinese herbal medicine	32.5 µg/kg	5–1000 µg/L	0.02–0.11 (µg/kg)	84.7–107.2	Zhou et al. (2020)
HPLC-UV	Pesticides	Fruit vegetable	n.a.	0.1–50 µg/mL	0.02–0.05 µg/mL	76.09–97.96	Zhao et al. (2020a)
UHPLC-Q-Exactive HRMS	Multimycotoxins	Milk	n.a.	0.15–100 ng/mL	0.005–0.050 µg/kg	106.4	Zhao et al. (2020b)

GC-MS, Gas chromatography–mass spectrometry; HPLC-DAD, high-performance liquid chromatography diode array detector; HPLC-FLD, high-performance liquid chromatography with fluorescence detector; HPLC-PDA, high-performance liquid chromatography photodiode array detector; HPLC-UV, high-performance liquid chromatography ultraviolet detector; UHPLC-Q-Exactive HRMS, ultrahigh-performance liquid chromatography Q-Exactive high-resolution mass spectrometry; LOD, limit of detection; n.a., not applicable; n.d., not detected.

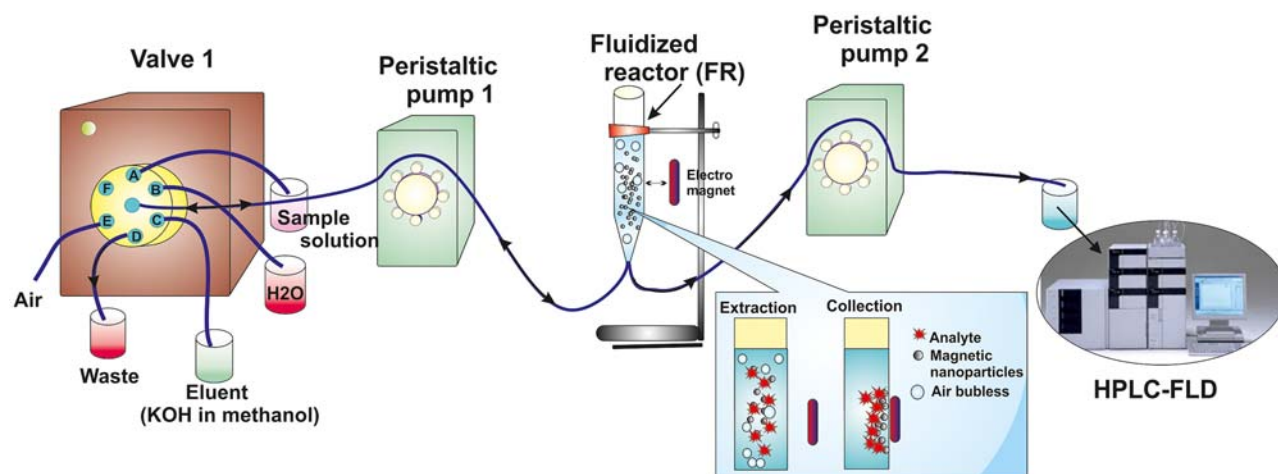


FIGURE 19.4 Manifold for automation of magnetic dispersive micro-SPE for the determination of fluoroquinolones in baby food. SPE, Solid-phase extraction. Reproduced with permission from Vakh, C., Alaboud, M., Lebedinets, S., Korolev, D., Postnov, V., Moskvina, L., ... Bulatov, A. (2018). An automated magnetic dispersive micro-solid phase extraction in a fluidized reactor for the determination of fluoroquinolones in baby food samples. *Analytica Chimica Acta*, 1001, 59–69. Copyright 2018 Elsevier.

zearalenone (ZEA), zearalanone (ZAN), α -zearalanol (α -ZAL), β -zearalanol (β -ZAL), α -zearalanol (α -ZOL), and β -zearalanol (β -ZOL) from liquid milk. The proposed method proved that the matrix effects were greatly minimized with provided lower organic solvent consumption which less waste generation. By coupling with UHPLC-Q-Exactive HRMS, 13 mycotoxins in liquid milk were quantified simultaneously. The four samples were detected with AFM₁ which is below than MRLs set by authorities of China and the United States. Vakh et al. (2018) developed an automated procedure that involves the dispersive MSPE in the liquid sample phase by air-bubbling followed by a collection of magnetic adsorbents that contain analytes in the fluidized reactor in a magnetic field, elution, and analyte detection (Fig. 19.4) (Vakh et al., 2018). By using the automated system, the proposed method allows to reduce the sample volume and magnetic adsorbent dosage due to a miniaturized fluidized reactor was implemented instead of dispersion by a magnetic bar stirring in a syringe. The automated proposed method was applied for fluoroquinolones determination in baby food.

19.3 Conclusion and future perspectives

The development of a powerful method for the detection of contaminants in food products is very important to create a useful tool for quality assurance systems and to ensure the safety of halal foods. In this chapter the advances in sample preparation strategies and their application in food analysis in recent years are summarized by highlighting the various types of sample preparation, including LPME, DLLME, SPME, QuEChERS, DSPE, and MSPE. The investigation of these sample preparation methods shows that they are well combined, by chromatographic and MS methods and other detections. The use of sample preparation has eliminated major interferences in the food matrix and allows the preconcentration of the targeted analytes before its determination by analytical methods. The characteristics of an ideal sample preparation approach include simple and easy to operate, high efficiency and selectivity toward targeted analytes, low solvent usage, inexpensive, and environmental friendly. However, most of the conventional extraction methods such as LLE or SPE fail to meet these requirements due to their complicated process, required a large amount of toxic organic solvents, and are costly. The development of modern sample preparation methods offers sample clean-up and preconcentration strategies that reduce solvent consumption and waste generation, produce rapid methods for analysis, and decrease experimental errors. In line with the principle of green chemistry, newly advanced microscale sample preparation methods are expected to develop significantly. The information on the developed modern sample preparation methodologies from this chapter will provide insights to halal scientists, analytical chemists, and food technologies in designing the best methods of analysis as the tool to detect contaminants in foods, which is one of the important aspects in ensuring the safety of halal foods.

Abbreviations

LPME	liquid-phase microextraction
DLLME	dispersive liquid–liquid microextraction
SPME	solid-phase microextraction
QuEChERS	quick, easy, cheap, effective, rugged, safe
DSPE	dispersive solid-phase extraction
MSPE	magnetic solid-phase extraction
SDME	single-drop microextraction
HF-LPME	hollow fiber liquid-phase microextraction extraction
TF-SPME	thin-film SPME
SUPRASs	supramolecular solvents
UHPLC-ESI(+)-	ultrahigh-performance liquid chromatography-electrospray ionization (+)-quadrupole time-of-light mass
QTOF-MS	spectrometry
DES	deep eutectic solvent
EDCs	endocrine-disruption chemicals
HPLC	high-performance liquid chromatography
HPLC-DAD	high-performance liquid chromatography diode array detector
HPLC-FLD	high-performance liquid chromatography with fluorescence detector
ETAAS	electrothermal atomic absorption spectrometry
UHPLC-Q-Exactive	ultrahigh-performance liquid chromatography Q-Exactive high-resolution mass spectrometry
HRMS	
UV-vis	ultraviolet visible
GC-MS	gas chromatography–mass spectrometry
GC-μECD	gas chromatography with electron capture detector
LC-MS/MS	liquid chromatography–tandem mass spectrometry
HPLC-UV	high-performance liquid chromatography ultraviolet detector
HPLC-PDA	high-performance liquid chromatography photodiode array detector
UPLC-MS/MS	ultrapformance liquid chromatography–tandem mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
RSD	relative standard deviation
udl	under of the detection limit
COFs	covalent organic framework
MOF	metal organic framework
HS-SPME	headspace single-drop microextraction
MRL	maximum residual limits
GC-FID	gas chromatography with flame ionization detector
GCB	graphitized carbon black
PEG	polyethylene glycol

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Halal detection technologies: analytical method approaches, validation and verification, and multivariate data analysis for halal authentication

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20.1 Introduction

Technologies worldwide have expanded to employ scientific knowledge, including testing, validating, and verifying practical applications, especially in real-time industries. The employment of technologies covers utilizing this scientific knowledge to develop equipment. As time goes by, the technologies have improved to serve industries, including the halal industries.

The phrase “halal detection technologies” (HDTs) describes activities related to finding evidence to solve issues about contaminant from nonhalal sources, for example, porks, dogs and their derivatives, unslaughtered animals, and alcoholic beverages within the halal industries. [Department of Standards Malaysia \(2019a\)](#) highlights in the Malaysian Standard MS 1500: 2019 Halal food—General requirements (third revision) that any discharged matters from the human and animals’ orifices such as urine, blood, vomit, pus, excrement, and placenta are also deemed as nonhalal sources. These matters shall also receive concerns when applying HDT.

Furthermore, with the concept of halal and toyyiban, which the latter means wholesomeness and safe ([Sani, Jamaludin, Al-Saari, Azid, & Azri, 2020](#)), the application of HDT also identifies and quantifies contaminants that might impose health issues on consumers, that is, intoxicating and poisonous chemicals. Due to these concerns, the HDTs have also been employed as tools for halal testing with its aims to find (1) the proof of halal status of a product or (2) the proof of conformity of a halal product as it is claimed ([Ali et al., 2012](#)) and (3) identify and quantify intoxicating and poisonous contaminants. However, since aim (3) also falls under contaminant control activities, this chapter will discuss the HDT for halal-testing application, focusing on only aims (1) and (2).

The halal testing via HDT faces challenges on the methodology for different types of samples. With the increasing demand for halal products, including the complex mixture of processed products ([Sarah et al., 2016](#)), there is a need to outline the suitable HDTs entailing the approaches and equipment for halal testing. Besides food, nonfood products, that is, cosmetics, pharmaceuticals, and halal services such as logistics, including warehouse, transportation, and retailing, also receive an increasing demand on their halal status within the supply chain. The Department of Standards Malaysia has seriously taken this demand by developing new standards and reviewing published standards that cover food ([Department of Standards Malaysia, 2019a](#)), cosmetics ([Department of Standards Malaysia, 2019f](#)), pharmaceuticals ([Department of Standards Malaysia, 2019e](#)), warehouse ([Department of Standards Malaysia, 2019c](#)), transportation ([Department of Standards Malaysia, 2019b](#)), and retailing ([Department of Standards Malaysia, 2019d](#)). The Standards and Metrology Institute for Islamic Countries also corroborated the halal testing via HDTs ([SMIIC, 2021](#)). Thus the suitable approaches and equipment to cater for this demand will be discussed in this chapter.

The HDT approaches are employed in all chemical measurements. The halal testing using HDTs applies approaches according to sample nature, such as protein-based (Nur Azira, Che Man, Raja Mohd Hafidz, Aina, & Amin, 2014), fat-based (Idris et al., 2021; Nurrulhidayah et al., 2013), and alcohol-containing (Jamaludin et al., 2017) samples. The approaches for protein- and fat-based samples are associated with nonhalal ingredients prone to dissolve into water or oil. The Malaysian Halal Management System 2020 supported these approaches with additional testing for hair, skin, and meat speciation via deoxyribonucleic acid (DNA) testing (Department of Islamic Development Malaysia, 2020). The hair and skin testings are categorized as profiling physical testings (Zulkarnail, Tukiran, & Sani, 2021), while the meat speciation test is a targeted testing (Abdullah Amqizal, Al-Kahtani, Ismail, Hayat, & Jaswir, 2017).

Ideally, each of these different approaches could only identify the nonhalal contaminants by using specific analytical equipment to provide a reliable result. The most common analytical equipment for halal testing is real-time polymerase-chain reaction (RT-PCR) for identifying the presence of porcine-DNA and high-performance liquid chromatography (HPLC) for amino acid (AA) profiling via the protein-based approach (Ismail et al., 2021). An analyst may employ the Fourier-transform infrared spectrometer (FTIR) and gas chromatography–mass spectrometer (GC/MS) to record the fat profiles of nonhalal contaminants that are miscible with fat and oil matrices. The latter needs assistance tools such as multivariate data analysis (MDA) for halal testing (Sani, Yuswan, Desa, & Azid, 2020), by which establishing a comprehensive guideline on the MDA shall assist analysts worldwide, especially those involved in halal-testing activities.

The MDA has assisted in profiling tests and, hence, provided alternative approaches other than targeted testing, for example, DNA testing due to inhibitors in the matrices reduces DNA testing sensitivity. Nonetheless, the MDA application has caused confusion among analysts due to their limited knowledge of the principle and application of the individual MDA. Moreover, most profiling tests incorporating the MDA with the analytical equipment are not validated and verified (Sani, Yuswan, et al., 2020) since their applications focus only on research. Thus this chapter provides a comprehensive review of the principle and application of MDA in HDTs. Post identifying the biomarkers via targeted testing and profiling testing via incorporation of analytical equipment and MDA, fulfilling the requirements of ISO 17025 for testing laboratories is a must to ensure the targeted and profiling tests fit their purposes (Department of Standards Malaysia, 2018).

The targeted and profiling tests shall fulfill the halal-testing requirements to ensure the HDTs are effective for halal testing. Generally, for a testing laboratory to issue a testing result, the targeted and profiling tests should undergo validation and verification processes to fulfill ISO 17025 requirements (Department of Standards Malaysia, 2018), and no exception for a halal-testing laboratory. References to achieve this requirement have indicated that targeted and profiling tests should establish performance characteristic (PC) before sample analysis (Yuswan, Sani, Manaf, & Desa, 2020).

Although the HDTs have advanced in determination of the halal status, the development of new or revised targeted and profiling tests still faces challenges. Most of the tests could validate the detection of any nonhalal contaminants in analytical equipment; nonetheless, the test fails to verify the presence of that compound in the actual sample due to complex sample matrix (Martín et al., 2009), cross-contamination from other samples (Amaral, Santos, Oliveira, & Mafra, 2017), denaturation of the compound as affected by heat (Sarah et al., 2016), etc. Halal testing, which is not required during the certification procedure, has also become one of the challenges (Ali et al., 2012) since this activity retards the development of new targeted and profiling tests.

Overall, this HDTs chapter will discuss (1) the suitable approaches on sample handling and appropriate targeted and profiling tests, (2) the principle and application of MDA in profiling tests, (3) the PC of targeted and profiling tests, and (4) challenges in HDTs.

20.2 Approaches of halal detection technologies

Since the demands of halal products have expanded to nonfood products, the development of analytical methods (AMs) via HDTs is not focused on DNA testing only. The HDTs using HPLC and GC/MS have also yielded successful results in halal testing (Azilawati, Hashim, Jamilah, & Amin, 2014; Azir et al., 2017; Ismail et al., 2021). Other HDTs have also been sought for, such as FTIR (Hashim et al., 2010) and differential scanning calorimetry (DSC) (Naquiah, Marikkar, Mirghani, Nurrulhidayah, & Yanty, 2017), but a guideline of suitable approach and AM of HDTs are absent. In this chapter the author proposes suitable AM via three approaches according to sample type: protein-, fat-, and alcohol-based approaches, where these approaches could be divided into targeted and profiling tests. Subsequently, the summary of this guideline is shown in Table 20.1.

TABLE 20.1 Approaches of halal detection technologies.

Approach	Approach type	Analytical method of halal detection technologies	Principle of sample analysis
Protein-based sample	Targeted	DNA species-specific testing	The sample analysis entails (1) DNA extraction: lyse, bind DNA, wash and elute; (2) screening: DNA quantification or purity, using agarose gel electrophoresis; and (3) detection using PCR.
	Profiling	Amino acid testing ^a	The sample analysis involves (1) sample hydrolysis using acid and (2) derivatization using fluorescence derivatizing agent, and (3) amino acid analysis using high-performance liquid chromatography equipped with a fluorescence detector (Abdullah Sani, Ismail, Azid, & Samsudin, 2021).
	Targeted	Polypeptides biomarkers	The sample analysis entails (1) heat and trypsin treatment on the sample, (2) polypeptide analysis using liquid chromatography–time-of-flight mass spectrometer, and (3) comparison of the molecular mass of the detected polypeptide with the mass of porcine polypeptide.
	Profiling	Protein biomarkers ^a	The sample analysis involves (1) treatment of the sample with heat and sodium dodecyl sulfate, (2) protein separation based on molecular weight, and (3) comparison of the separated protein with the porcine protein.
Oil-based sample	Profiling	Functional and fingerprinting groups ^a	Sample analysis is initiated by extracting samples using solvent and analyzed using FTIR. The analysis focuses on the functional group and fingerprinting of the spectra (Zulkarnail et al., 2021).
	Profiling	FAMEs ^a	Sample analysis measures the thermal properties of a sample extract, and the sample thermogram is compared with the thermogram of porcine extract (Mualim, Tukiran, Sani, & Fadzliillah, 2018).
	Profiling	FAMEs ^a	The sample undergoes extraction and derivatization to produce FAMEs, subjected to analysis using GC/MS (Idris et al., 2021).
Alcohol testing	Targeted	Headspace ethanol analysis	The sample is subjected to heating in a headspace bottle, and the vapor is siphoned to GC/MS to measure alcohol content (Jamaludin et al., 2017).

DNA, Deoxyribonucleic acid; FTIR, Fourier-transform infrared; FAMEs, fatty acid methyl esters; GC/MS, gas chromatography–mass spectrometer; PCR, polymerase-chain reaction.

^aResult from this HDTs can be subjected to multivariate data analysis to facilitate the halal authentication.

20.2.1 Protein-based approach

The protein-based approach involves identifying DNA, AAs, polypeptide, and even protein itself, which both targeted and profiling types apply to this approach.

20.2.1.1 Identification of targeted deoxyribonucleic acid

The DNA is a double-helix polynucleotide that encodes genetic information in the species-specific cell (Department of Standards Malaysia, 2017). The nucleotides are made up of three components where the arrangement of the component binding is as follows: a nitrogen base, deoxyribose sugar, and a phosphate group.

Since the DNA is a double-helix polynucleotide, this double-helix structure could be separated or denatured into two individual strands where each strand consists of its polynucleotides. The polynucleotides are bonded by oxygen from the deoxyribose sugar of nucleotide and phosphorus of phosphate group from another nucleotide. Since the phosphorus has two oxygens attached to it, it is acknowledged as a phosphodiester bond. Also, since the individual oxygen attached to phosphorous is connected to two carbons of deoxyribose sugar from two nucleotides, analysts refer to the phosphodiester bond as the connector between the 3-prime (3') carbon of a nucleotide and 5-prime (5') carbon of another nucleotide. In other words, the phosphodiester bond is the connector between nucleotides that make up each strand of the DNA molecule. The phosphate group, deoxyribose sugar, and phosphodiester bond become the backbone

of the DNA molecule with the 3-prime (3') carbon to 5-prime (5') carbon arrangement. Each DNA strand has a reverse 3-prime (3') carbon to 5-prime (5') carbon arrangement to each other. The connection between two DNA strands is called hybridization or annealing in which the nitrogen base plays its role (Amid, 2021).

The nitrogen base is represented by four types of base, that is, adenine, guanine, thymine, and cytosine, which attaches to the 1-prime (1') carbon of deoxyribose sugar. The adenine and guanine are of the purine (2 rings) group, while the thymine and cytosine are of the pyrimidine (1 ring) group. In the DNA molecular arrangement, the purine and pyrimidine groups pair together via hydrogen bonding, for example, adenine pairs up with thymine via two hydrogen bondings while guanine with cytosine via three hydrogen bondings (Fig. 20.1). The HDTs denaturize the two DNA strands into a single strand by breaking the hydrogen bonds and utilizing the sequence of three nucleotides from the single DNA strand to identify the species-specific cells for halal testing.

Analysts have adopted DNA testing to authenticate meat and meat products (Abbas et al., 2018; Zia, Alawami, Mokhtar, Nhari, & Hanish, 2020) and identify nonhalal gelatines in food (Abdullah Amqizal et al., 2017; Jannat et al., 2018; Rohman, Windarsih, Erwanto, & Zakaria, 2020) and cosmetics (Kim, Yu, Lee, & Hong, 2018). Application of DNA testing entails various methods related to PCR, such as singleplex PCR or conventional PCR (Ha et al., 2017), duplex PCR (Khatun, Hossain, Hossain, Munshi, & Huque, 2021), multiplex PCR (Izadpanah et al., 2018), and RT-PCR or quantitative PCR (Jannat et al., 2018; Kim et al., 2018; Rohman et al., 2020; Zia et al., 2020).

20.2.1.1.1 Principles of identification of targetted deoxyribonucleic acid

The DNA testing using PCR involves these principles: (1) extracts and purifies DNA from raw materials, processed, and highly processed products using kits; (2) estimates the DNA concentration by ultraviolet (UV) spectrophotometry; (3) amplifies the targetted DNA; that is, porcine, fish, and bovine by utilizing universal, animal, and species-specific primer sets via PCR as the HDT; and (4) confirms of the presence of targetted DNA by gel electrophoresis (GE), RT-PCR or DNA sequencing (Department of Standards Malaysia, 2017). Fig. 20.2 summarizes the process flow on the identification of targeted DNA.

20.2.1.1.2 Extraction and purification of targetted deoxyribonucleic acid

Principle (1) involves extracts, isolates, purifies, and concentrates DNA from raw materials, processed and highly processed products using kits. Before these activities, preparation of chemicals and apparatus is needed, including utilization of analytical grade chemicals. Another requirement prior to undergoing principle (1) is the utilization of sterile chemicals, apparatus, and working bench. For instance, deionized water or double-distilled water, prepared solutions, and buffers are sterilized by filtration and autoclave. However, heat-sensitive chemicals such as GE buffer and

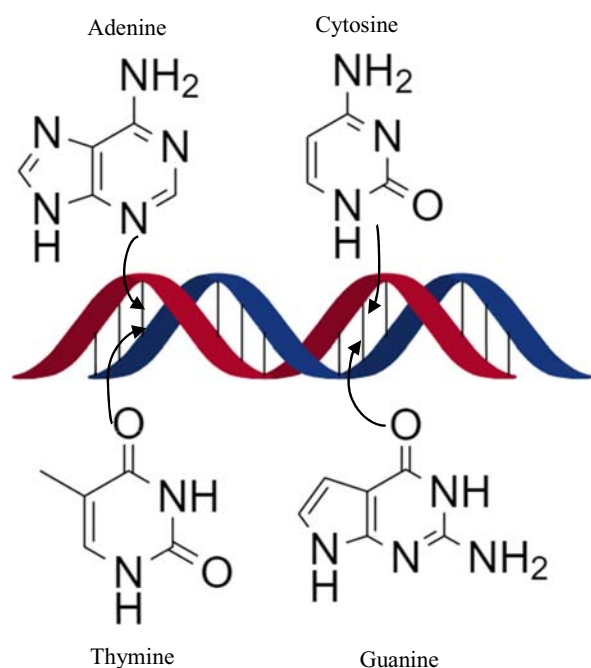


FIGURE 20.1 The double-helix DNA with its nitrogen bases, that is, adenine, thymine, cytosine and guanine (The structures were drawn via <https://chem-drawdirect.perkinelmer.cloud/js/sample/index.html#>). DNA, Deoxyribonucleic acid.

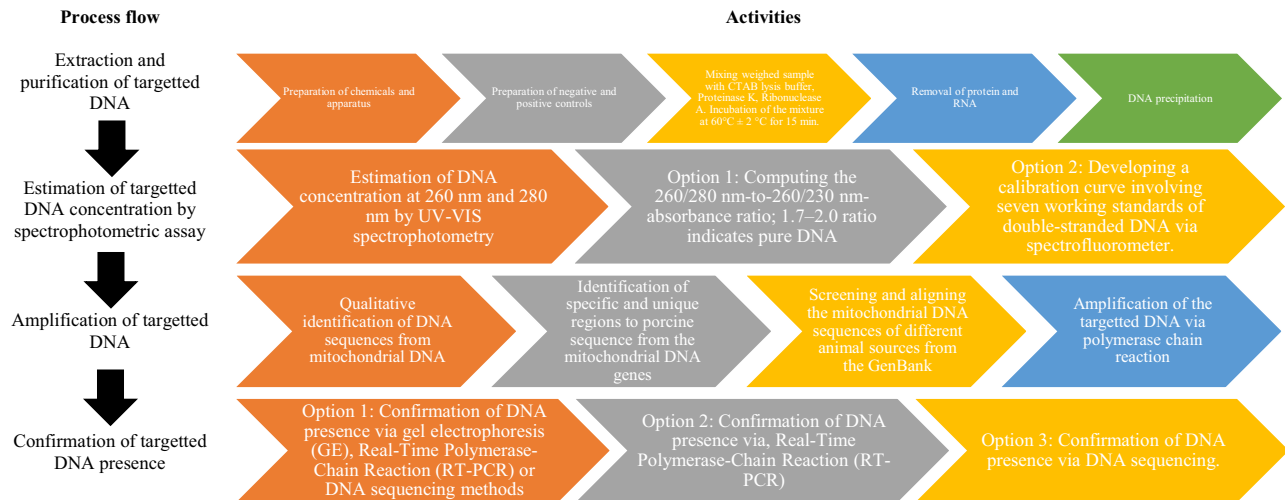


FIGURE 20.2 Summary of process flow on the identification of targeted DNA. DNA, Deoxyribonucleic acid.

enzymatic solutions shall not be autoclaved. All apparatus shall be DNA free, and to avoid cross-contamination between samples, wearing a glove is compulsory. Putting on UV light and applying 13% sodium hypochlorite and 70% ethanol solutions on working bench surfaces between series of DNA extraction may also reduce potential cross-contamination. Furthermore, to ensure reproducible results when referring to establishing a DNA testing method, maintain the same chemical concentration and proportion of the reagents (Department of Standards Malaysia, 2017).

Analyst prepares negative and positive controls, cetyltrimethylammonium bromide (CTAB) lysis buffer, and certified reference material or standard before DNA extraction. Department of Standards Malaysia (2017) also recommends the usage of routine samples that have been positively identified containing porcine DNA as the alternative positive control. The DNA extraction begins by weighing a standardized sample weight to ensure repeatability of detection and quantitation of porcine DNA. In this case, too low sample weight may cause low detection of porcine, especially when analyzing processed products, which is very low level of porcine DNA present. The weighed sample is then mixed with CTAB lysis buffer to lyse the sample (Zdeňková et al., 2018) and proteinase K (Pro K), incubated at 60°C for a few hours and added with ribonuclease A (RNase A), and incubated in a thermomixer (rotary incubator) at $60^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 15 minutes. The Pro K digests proteins in the sample and inactivates nucleases from degrading the nucleic acids of DNA while the RNase A specifically hydrolyzes RNA 3' of pyrimidine residues and cleaves the phosphodiester bond. This cleavage cut the RNA into smaller components (Messmore, Fuchs, & Raines, 1995). The 60°C incubation ensures complete lysis of the sample cells.

Post extracting the DNA, the removal of protein and RNA follows suit. The extracted DNA is cooled to 30°C for 5 minutes, centrifuged at $13,000\times g$ for 10 minutes, transferred 600 μL supernatant into a new 1.5 mL tube, added with 600 μL of 3 M sodium acetate, vortexed and centrifuged at $8000\times g$ for 15 minutes (Dalsecco et al., 2018). Repetition of these steps is imperative to remove protein and RNA in a pellet form. Centrifugation separates the extracted DNA from the protein and RNA. Since the pellet contains protein and DNA, the pellets are discarded while the supernatants are subjected to DNA precipitation.

Next, the DNA precipitation follows suit by adding 70% ethanol to the dissolving supernatant in chloroform. Then, the mixture is centrifuged at $8000\times g$ for 5 minutes. Post centrifugation, the supernatant is discarded while the pellet is left in the centrifuge tube. Due to a lower dielectric constant than water, the ethanol shields the charge of the phosphate group of the DNA and causes the DNA to be less hydrophobic; hence, the DNA precipitates (McKiernan & Danielson, 2017). The ethanol is evaporated to allow the pellet to dry for 20 minutes. If the pellet is too dry, resuspends the pellet with DNA-free water.

20.2.1.1.3 Estimation of targeted deoxyribonucleic acid concentration by spectrophotometric assay

Principle (2) estimates the DNA concentration at 260 and 280 nm by UV spectrophotometry. The absorbance of 50 $\mu\text{g}/\text{mL}$ of double-stranded DNA (dsDNA) corresponds to 1 unit at 260 nm within 1-cm path length. By employing similar UV spectrophotometry, the analyst may identify the DNA purity by computing the 260/280 nm absorbance ratio, where 1.7–2.0 ratio indicates pure DNA (Ahn, Costa, & Emanuel, 1996). Another approach to estimating the DNA

concentration is developing a calibration curve using seven working standards of dsDNA assay kit using spectrofluorometric methods. This estimation of DNA concentration employs the dsDNA than single-stranded DNA (ssDNA) because the latter produces a high fluorescence background compared to the dsDNA. This high intensity of fluorescence background leads to sensitivity reduction (Armbricht, Gloe, & Goemann, 2013). Hence, estimation of DNA concentration by measuring the dsDNA is preferable.

This dsDNA quantification involves staining the seven working standards at concentrations of 1000, 500, 250, 100, 50, 10 ng/mL of dsDNA from calf thymus (Wolf & Lüthy, 2001), herring sperm (Zhao, Wang, Pan, Hu, & Ding, 2010) or salmon sperm (Mohamadi, Afzali, Esmaili-Mahani, Mostafavi, & Torkzadeh-Mahani, 2015) with any fluorochromes, namely, PicoGreen, SYBR-Green I, or ethidium bromide dyes. These fluorochromes bind to dsDNA in the working standards and produce fluorescence at excitation and emission wavelengths of 480 and 520 nm, respectively, recorded by the spectrofluorometer. The increment of fluorescence intensities is subjected to a standard curve. The dsDNA extracted from samples was diluted at 1:100 in Tris-EDTA (TE) buffer to a final volume of 1 mL. This dsDNA dilution was mixed with 1 mL of fluorochromes in cuvettes, incubated for 5 minutes at 30°C and subjected to fluorescence measurement. The DNA concentration of a sample is determined from the prepared standard curve (Ahn et al., 1996; Kang & Tanaka, 2018).

20.2.1.1.4 Amplification of targetted deoxyribonucleic acid

Principle (3) involves identifying DNA sequences from nuclear or mitochondrial DNAs species-specific to cells before designing primers for DNA amplification. Although the nuclear and mitochondrial DNAs are the ones that possess the genetic information, the latter has been utilized for HDTs for qualitative halal testing due to approximate 1000 copies per cell (Zia et al., 2020). Naturally, mammals are among the organisms that receive genes from their ancestors via mitochondrial DNA (Alikord, Momtaz, keramat, Kadivar, & Rad, 2018). Also, mitochondrial DNA evolves at a higher rate than nuclear DNA and is very likely to possess higher sequence diversity which assists the identification of phylogenetically related species (Alikord et al., 2018; Zia et al., 2020). The mitochondrial DNA has stable nucleotide sequences against environmental stresses such as pressure, salt, and heat (Kim et al., 2018; Zia et al., 2020); therefore it is relatively present in processed products (Abbas et al., 2018; Zia et al., 2020). These advantages of the mitochondrial DNA meet the requirement of high sensitivity for qualitative halal testing (Alikord et al., 2018).

Before the selection of the species-specific primer on porcine, the researchers screen and align the mitochondrial DNA sequences of porcine, cattle, chicken, and sheep from the GenBank (GenBank, 2021) and identified the most specific and unique regions to porcine sequence from the mitochondrial DNA genes or the targetted genes, including 12S ribosomal RNA (rRNA) gene (Kang, Lee, & Kim, 2018), 16S rRNA gene (Xing et al., 2019), 18S rRNA gene (Kang & Tanaka, 2018), cytochrome b gene (Mohamad, Mustafa, Khairil Mokhtar, & El Sheikh, 2018), β -actin gene (Wu et al., 2020), adenosine triphosphate synthase protein (ATPase) 6 gene (Raharjo, Chudori, & Agustina, 2019), ATPase 8 gene (Kang et al., 2018), nicotinamide adenine dinucleotide and hydrogen (NADH) dehydrogenase subunit 2 gene (Galal-Khallaf, 2021), NADH dehydrogenase 2 (ND2) gene (Alikord et al., 2018), and the displacement strand (D-loop) of mitochondrial DNA control region (Kang et al., 2018). Among the mitochondrial DNA genes, the cytochrome b gene has been ideal for amplification since they have multiple copies and are well protected by a mitochondrial membrane (Rohman et al., 2020).

The difference between singleplex PCR, duplex PCR, and multiplex PCR lies in principle (3), in which these PCRs utilize one, two, and multiple species-specific primers, respectively. Ha et al. (2017) applied the singleplex PCR using a primer specific to the porcine mitochondrial DNA. Based on targetted D-loop gene, Ha et al. (2017) designed the GGTT CTTA CTTC AGGA CCATC (forward sequence) and GTGT ACGC ACGT GTAT GTAC (reverse sequence) according to the 5' to 3' carbon arrangement for the porcine detection. Meanwhile, Khatun et al. (2021) employed these primers, that is, a reverse sequence of both chicken (AAG ATA CAG ATG AAG AAG AAT GAG GCG) and porcine (GCTG ATAG TAGA TTG TGAT GACC GTA) according to the same carbon arrangement in their duplex PCR based on the cytochrome b targetted gene to identify the presence of porcine in processed meat products. Galal-Khallaf (2021) employed Multiplex PCR at a ratio of 1:1:1:1 by differentiating beef (GCCA TATA CTCT CCTT GGTG ACA), chicken (forward sequence—TGAG AACT ACGA GCAC AAAC), pig (forward sequence—AACC CTAT GTAC GTCG TGCAT and reverse sequence—ACCA TTGA CTGA ATAG CACCT), and donkey (forward sequence—CATC CTAC TAAC TATA GCCG TGCTA and reverse sequence—CAGT GTTG GGTT GTAC ACTA AGATG) based on ATPase6, 12S rRNA, D-loop, and ND2 targetted genes, respectively.

Nevertheless, the fivefold variability of the mitochondrial DNA copies among tissues compared to a single copy of nuclear DNA leads to an inconsistent detection limit; thus the applicability of mitochondrial DNA suits the qualitative halal testing or screening only. Alternatively, nuclear DNA is preferable for quantitative halal testing (Alikord et al., 2018; Zdeňková et al., 2018; Zia et al., 2020).

Next, amplification of the targetted DNA follows the identification of targetted DNA sequence and primer designing. In this step, the extracted and purified targetted DNA is subjected to a PCR to replicate a copy of the targetted DNA in vitro by mixing the extracted and purified DNA with primer DNA polymerase, for example, Taq polymerase and nucleotides. The PCR replicates the copy of the targetted DNA via these cyclic steps: (1) denaturation step—heating the mixture at 96°C to separate or denature the extracted and purified dsDNA and produce an ssDNA template for the next step; (2) annealing step—cooling the reaction at 55°C–65°C to allow primer binding to its complementary sequence on the ssDNA template; and (3) extension step—raising the reaction temperatures up to 65°C and allow the DNA polymerase and nucleotides extend the primers and synthesizing new dsDNA. This cycle which repeats 25–40 times, may take 2–4 hours, depending on the length of targetted DNA region being copied. An efficient PCR reaction produces a billion copies since both the original dsDNA and newly synthesized dsDNA serve as dsDNA templates in the subsequent PCR cycle. Also, abundant DNA polymerase and nucleotides in the mixture facilitate the dsDNA doubling in each PCR cycle. After the PCR, the synthesized dsDNAs are subjected to confirmation of the targetted DNA presence via GE, RT-PCR, or DNA sequencing methods.

20.2.1.1.5 Confirmation of targetted deoxyribonucleic acid presence

Post carrying out the amplification of targetted DNA, the confirmation of DNA presence in principle (4) is achieved by employing GE, RT-PCR, or DNA sequencing methods.

The GE separates the synthesized DNA from the PCR based on its size and charges when the developed gel is applied with an electrical current. The GE can be divided into conventional GE and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), where the former is used to separate RNAs, DNAs as well as proteins while the SDS-PAGE applies on separation of proteins only.

For the conventional GE, the analyst prepares the gels from the polysaccharide agarose by mixing it with a buffer solution, heating the mixture, pouring the melted mixture into a gel box, and allowing it to form a gel. The gel box is hooked with positive and negative electrodes to allow current conduction where the DNA separation moves toward the positive electrode. Since the end of the gel box contains pocket-like indentations called wells, the analyst shall ensure to cover these wells during the pouring activity of the melted mixture.

The analyst places the synthesized DNA mixed with a negatively charged loading dye on these wells. One well is reserved for a DNA standard ladder containing a known length of DNA fragments. Since various commercial DNA ladders are available commercially, choosing the most suitable size range that covers the expected fragments is recommended, for example, 100-base pair of DNA ladder is frequently sought for porcine detection (Izadpanah et al., 2018). By switching the power, the current flows through the gel and activates the movement of the negatively charged synthesized DNA through the gel matrix. Since hydrogen bond holds the agarose molecules and forms tiny pores in the gel, it allows shorter DNA fragments while holding the longer ones. Hence, the shorter DNA fragments reach the positive end of the gel while the longer ones stay nearer to the negative end.

20.2.1.2 Profiling of amino acids

AAs are the products from the translated DNA sequences (Zhang et al., 2018) that become proteins' building block (Mohanty et al., 2014). Due to their major component in protein, they could be one of the analytes or compounds for authenticating the halal sources. Nevertheless, AAs are abundant with many types, that is, 17 AAs as detected by Ismail et al. (2021); hence, the profiling approach of AAs for authentication purposes is very likely than the targetted approach. Azilawati, Hashim, Jamilah, and Amin (2015) identified and quantified 17 AAs using HPLC attached with a fluorescence detector (HPLC-FLD).

Before the analysis of AAs, the samples are subjected to freeze-drying activity to remove the moisture content, where Abdullah Sani et al. (2021) proposed to ensure the moisture content <10% to reduce interference from the sample matrix, followed by acid hydrolysis and incubation at 110°C for 24 hours.

Before optimizing amino analysis, setting up a calibration curve for each AA is required. A series of working standards entailing a minimum of seven standards is spiked with internal standards. Abdullah Sani et al. (2021) spiked L-aminobutyric acid (AABA) as the internal standard to each working standard and proceeded with injecting the standards to the ultra-HPLC diode-array detector (UHPLC-DAD). The spiking of AABA aims to reduce the effect of the sample matrix; therefore the AABA spiking shall be carried out to the acid-hydrolyzed samples. Sani, Yuswan, et al. (2020) recommended avoiding serial dilution in preparing the working standard since serial dilution would cause a systematic error.

To note, the working standards and samples undergo derivatization based on the instrumental analysis, for example, fluorescence derivatization for analysis with HPLC-FLD. Henderson and Brooks (2010) employed ortho-phthalaldehyde (OPA)

and 9-fluorenyl-methyl chloroformate (FMOC) to derivatize AAs prior to analysis with HPLC-DAD. The OPA and FMOC react with primary and secondary AAs, respectively (Woodward & Henderson, 2007). However, Ismail et al. (2021) derivatized the working standards and samples with fluorescence derivatizing agent prior to injection to UHPLC-DAD, indicating that fluorescence derivatization applies to FLD and DAD.

The analysis of AAs via UHPLC or HPLC involves the elution of two eluents or mobile phases that flow through a membrane filter to a C18 column. Abdullah Sani et al. (2021) employed an aqueous solution of AccQ.Tag concentrate (WAT052890) (1:10) as eluent A and a mixture of acetonitrile: deionized water as eluent B. The eluents gradient is set up accordingly to ensure that the separation of individual AAs occurs successfully and that the resolution between the two adjacent peaks is adequate for identification and quantification. To achieve satisfactory separation of the AAs, 10 minutes are needed for the gradient elution of eluent A and B (Ismail et al., 2021). The individual peak of AA elutes at a specific retention time. Principally, as the concentration of the working standard increase, the peak area also increases; hence a calibration curve with satisfactory linearity is achieved. This calibration curve holds the linearity equation of:

$$\frac{A_s}{A_{is}} = \frac{mC_s}{C_{is}} + c$$

where A_s is peak area of working standard, A_{is} is peak area of internal standard, m is the slope of the calibration curve, C_s is a concentration of the working standard, C_{is} is a concentration of internal standard, and c is the intercept of the calibration curve on the A_s/A_{is} axis or the y-axis. To note, the C_s/C_{is} is the x-axis (Sani, Yuswan, et al., 2020).

The linearity of calibration curve could be achieved from (1) high correlation coefficient (R) that is near to 1, (2) an insignificant difference of variance between the F value of experimental and theoretical measurement signals of working standards, (3) lower and upper bounds of the c depicts exhibit positive and negative values, respectively, and (4) residual plot with random distribution (Thompson, Ellison, & Wood, 2002). After identifying and quantifying the AAs, it is recommended to carry out MDA, such as principal component analysis (PCA), discriminant analysis (DA), partial least square (PLS)-DA, etc., to authenticate protein-based products (Zulkarnail, Tukiran, Sani, & Ismail, 2020). The MDA will be discussed in the next section.

20.2.1.3 Targeted polypeptide analysis

Besides analysis of AAs, the analysis of polypeptide takes place by incorporating the HPLC and MS (HPLC-MS), while this incorporation is also denoted as a LC-MS in general terms.

The samples are initially prepared by mixing them with ammonium bicarbonate, left to 30°C and subjected to alkylation. The alkylated samples are further digested with trypsin at 1:100 trypsin-to-protein ratio (w/w) in 25 mM ammonium bicarbonate and dried in a vacuum centrifuge (Sarah et al., 2016). The digested samples are subjected to biomarker separations by LC-MS.

The digested proteins are acidified using 0.1% formic acid or 0.1% acetic acid and subjected to desalting to remove small molecules from the polypeptides using reverse-phase C18 filtration before introducing the LC-MS analysis (Ferraro et al., 2017). A volume of 5 μ L is injected into the separation unit. For polypeptide separation, analysts could adopt the similar gradient elution used in HPLC analysis since its separation is a commonly similar unit to LC-MS. The polypeptide separation occurs in a peptide C18 column with 2.1×100 mm containing 2.7 μ m particles at 4°C via elution by 0.1% formic acid in water and 0.1% formic acid in 9:1 acetonitrile:water at 0.3 mL/min flow rate.

The MS conditions are set at 300°C drying gas temperature, 8 L/min drying gas flow, 35 psi nebulizer pressure, 10 nA corona current, and 4000 V capillary voltage. To serve the purpose of getting the targeted peptide, the analyst applies multiple reaction monitoring mode between 10.1 and 30.1 collision energy and sets porcine-specific peptide sequences, and precursor and product ions information (1) LVVITAGAR, 450.2873 precursor ion, and 786.4932, 687.4048, 588.3364, and 475.2523 product ions; (2) EVTEFAK, 412.2134 precursor ion, and 595.3286, 494.2509, and 365.2083 product ions; (3) FVIEIR, 388.7369 precursor ion, and 629.3881, 530.3197, 417.2356, and 288.213 product ions; and (4) TVLGNFAAFVQK, 647.8613 precursor ion, and 592.3553 and 521.3382 product ions (Sarah et al., 2016).

20.2.1.4 Profiling of protein

Protein profiling has been utilized to assist the authentication of halal sources. This method employs the SDS-PAGE technique incorporated with the MDA since the proteins are separated according to masses. Nur Azira et al. (2014) incorporated SDS-PAGE and PCA to classify the gelatine sources.

Protein cold extraction using acetone or chloroform and methanol mixture have been proven to successfully extract protein where the latter is preferable for extracting hydrophobic protein (Novák & Havlíček, 2016). The extract is

vortexed, centrifuged at 14,000 rpm for 10 minutes and kept for 24 hours and 20°C. The supernatant is discarded, and the lower layer of the extract is air-dried to obtain a protein pellet.

The pellet is weighed and dissolved in a buffer solution containing SDS, β -mercaptoethanol, and bromophenol blue prior to SDS–PAGE. Also, standard markers entailing myosin (220 kDa), α 2-Macroglobulin (170 kDa), b-Galactosidase (116 kDa), transferrin (76 kDa) and glutamic dehydrogenase (53 kDa) are prepared. Analyst prepares gel consisting of 4% and 6% stacking and resolving gels, respectively, stained with silver stain solution. Post these preparations, electrophoresis occurs at 80 V for 2 hours and produces polypeptide bands for the pellet and standard markers. These bands are visualized under a densitometer. To estimate the molecular weight of the bands of pellets, the analyst compares the pellet bands with the band of standard markers.

Since this is a profiling approach, executing the MDA may assist in (1) identifying the significant markers that discriminate the porcine and nonporcine proteins and (2) grouping the samples that share similar markers. The multitude of MDA types for authentication is discussed in the next section. Nur Azira et al. (2014) identified 160, 145, 135, 125, 120, 114, 106, 96, 87, 83, 76, 70, 64, 61, 58, and 53 kDa as significant bands that authenticated the halal sources.

20.2.2 Oil-based approach

As a protein-based approach is employed to determine the sources of water-dissolving materials and products, this approach is ineffective to authenticate the halal sources of oil-based materials and products. Therefore analysts experiment and seek other suitable HDTs for this purpose.

20.2.2.1 Profiling of oil spectra

A popular and one of the most sought HDT is FTIR for authentication oil-based samples such as butter (Nurrulhidayah et al., 2013), plant products (Yang & Irudayaraj, 2001), and even moisturizer, lotion, and other cosmetic products (Rohman et al., 2014).

These samples are extracted via uniformed parameters of Soxhlet, maceration, and other extraction methods using nonpolar solvents, for example, hexane, dichloromethane, ethyl acetate, and diethyl ether. Post extraction, the nonpolar solvents are removed by rotary evaporator at 40°C, kept in -20°C and subjected to FTIR analysis.

The FTIR is a technique used by emitting infrared light at various wavelengths to solid, liquid, or gas samples and collecting the infrared spectrum of the absorption activity by the sample molecules. Sample molecules absorb the infrared and react by stretching and wagging vibrations and emitting the unabsorbed infrared to the spectrometer. The spectrometer then detects the infrared and records the spectrum. Since various oil samples are infrared-active, they produce spectra by FTIR measurement and are suitable for authentication of halal sources.

The oil sample's measurement of absorbed infrared is initiated by measuring the blank using FTIR equipped with attenuated total reflection followed by measuring the extracted oil. The actual spectrum is obtained by deducting the recorded spectrum of oil from the blank. Although infrared could be divided into near ($12500\text{--}4000\text{ cm}^{-1}$), mid ($4000\text{--}650\text{ cm}^{-1}$), and far-infrared ($650\text{--}200\text{ cm}^{-1}$), measurement of mid-infrared spectra receives interest among analysts for halal authentication.

The FTIR spectrum entails two wavenumber ranges, that is, the functional range at $4000\text{--}1700\text{ cm}^{-1}$ and fingerprinting range at $1700\text{--}650\text{ cm}^{-1}$. The recorded spectra are subjected to MDA by analyzing (1) the whole spectra wavenumber range ($4000\text{--}650\text{ cm}^{-1}$), (2) functional range at $4000\text{--}1700\text{ cm}^{-1}$ or (3) to fingerprinting range at $1700\text{--}650\text{ cm}^{-1}$ to identify the cluster of oil sources and significant wavenumbers contributing to each oil source. Of these ranges, the fingerprinting range at $1700\text{--}650\text{ cm}^{-1}$ could be utilized to discriminate the halal and nonhalal sources. The lard adulteration in some vegetable oils was determined via conducting PLS and discriminant tests on $1500\text{--}1000\text{ cm}^{-1}$ spectra (Rohman, Che Man, Hashim, & Ismail, 2011). Rohman et al. (2014) chose $1200\text{--}1000\text{ cm}^{-1}$ to authenticate lard in cosmetic cream. Analysts may use the built-in spectral library to determine the functional groups characterizing the significant wavenumbers upon identifying these significant wavenumbers.

20.2.2.2 Profiling of fatty acid methyl esters

Oils consist of an abundance of fatty acids in both free and ester forms. Fatty acid methyl ester (FAME) profiling brings an alternative AM to authenticate oil samples over the targetted DNA method. Oil samples often have other constituents that render an inhibitory effect when using the targetted DNA method. Hence, the analyst identifies and profiles FAME using a GC/MS and subjected to MDA. Compared to the profiling of oil spectra using FTIR, the GC/MS analysis of

FAME is preferable since the HDT could identify the specific FAMEs contributing to the discrimination of the halal and nonhalal sources.

Following the similar oil extraction method for FTIR analysis, the extracted oil is esterified by 1 M sodium methoxide and hexane to produce FAMEs. The nonpolar phase (upper layer) consisting FAMEs is injected with an internal standard such as methyl tridecanoate (C13:0) or methyl heptadecanoate (C17:0) to reduce the matrix effect of the sample and subjected to GC/MS analysis. The GC/MS analysis involves separating the FAMEs using polar-capillary (88% cyanopropyl)aryl-polysiloxane (HP 88) column with 100 m length $\times$ 0.25 mm internal diameter $\times$ 0.2 μ m particle size. Retention time and mass spectra of the separated FAMEs are confirmed against certified reference standards (Idris et al., 2021). Upon identifying these FAMEs, the analyst quantifies the concentration of each FAME by constructing a calibration curve at $R^2 > 0.98$ and subjecting the FAMEs profile to MDA.

Chin, Che Man, Tan, and Hashim (2009) successfully identified FAME biomarkers by employing a two-dimensional GC time-of-flight MS (GC $\times$ GC-TOF/MS) without MDA. A group of FAMEs entailing 6,9,12,15-heneicosatetraenoate (C21:4n6), methyl 11,14-eicosadienoate (C20:2n6), trans-9,12-methyl octadecadienoate (C18:2n6t), trans-9-methyl octadecenoate (C18:1n9t), and methyl hexadecanoate (C16:0) were the biomarkers that discriminate the lard, chicken fat, beef tallow, mutton tallow, and cod liver oil. On the other hand, Idris et al. (2021) utilized PCA, orthogonal PLS-DA (OPLS-DA), and orthogonal PLS-regression (PLSR), and identified methyl myristate (C14:0), methyl stearate (C18:0), methyl linoleate (C18:2), methyl linolenate (C18:3), methyl arachidate (C20:0), and second stereospecific (sn-2) of C16:0, C18:0, methyl oleate (C18:1), and methyl linoleate (C18:2) as discriminant FAMEs on palm oil and lard containing fish feed.

20.2.3 Alcohol testing

Besides the absence of porcine and its derivatives in the halal product, another requirement for any product to be claimed as halal is the absence of alcoholic beverages. The presence of alcoholic beverages in halal products is determined via ethanol detection, which is prepared via fermentation. The aim of prohibiting alcoholic beverages consumption is to protect the human body from diseases and misjudgments (Nurdeng, 2009). Complete intoxication occurs when the blood alcohol content is $>0.08\%$ – 0.10% and thus affects respiration heart rate and may lead to fatality. Significantly, this range depends on the individual body acceptance of ethanol toxicity (Alzeer & Abou Hadeed, 2016).

Ethanol could be detected via GC flame-ion detection (GC/FID) (Tiscione, Alford, Yeatman, & Shan, 2011), GC/MS (Park, Kim, Lee, Jeong, & Shim, 2016), or GC-TOF/MS (Jamaludin et al., 2017) with or without headspace attachment with automatic injection. Analyst opts for headspace attachment with automatic injection over manual injection to ensure injection consistency throughout the ethanol analysis. Before injecting the sample, a calibration curve of ethanol is prepared where each working standard is spiked with constant concentration of internal standard, that is, isopropanol or acetonitrile to reduce the effect of sample matrix.

Approximately sample containing ethanol is weighed or pipetted into a headspace bottle and prespiked with the internal standard. The sample in the headspace bottle was heated at 70°C for 10 minutes and injected in split mode into a 90°C injector with a 1 mL/min constant flow rate of helium gas and eluted to the GC system. The ethanol is separated by DB-WAX polar fused silica capillary column (30 m $\times$ 0.25 mm, 0.85 μ m film thickness). The temperature programming is initiated at 35°C for 2 minutes, followed by heating to 90°C at $25^\circ\text{C}/\text{min}$, and held for 5 minutes. The detection is carried out by the FID, MS, or TOF/MS.

The presence of HDT such as GC/FID, GC/MS, and GC-TOF/MS has facilitated the authority to monitor the ethanol content in food and beverages. Naturally occurring ethanol occurs during food processing, especially by fermentation food such as *tapai*, *cencaluk* (Ahmad, Yang, & Hani, 2014), *gochujang*, *ganjang*, and *kimchi* is allowed with conditions: (1) the intention of fermenting the food is not to produce ethanol beverages and (2) the concentration of ethanol is below the allowable limit. The Malaysia National Fatwa Committee sets the allowable limit for ethanol content in fermented food and drinks to be $<1\%$ (Jamaludin et al., 2016).

20.3 Method verification and validation

Post determining the suitable approach of HDTs, the analysts should verify and validate the AM to ensure the AM fits for its purpose prior to the issuance of the testing report. The process of verification and validation of an AM entails: (1) understanding the reason to verify and validate the AM, (2) identifying the requirements of the verification and validation of the AM, (3) preparing the procedure to verify and validate the AM (Rambla-Alegre, Esteve-Romero, & Carda-Broch, 2012). The analyst should establish the PCs of the AM (Department of Standards Malaysia, 2018) that

covers specificity, calibration linearity, trueness, precision, sensitivity, ruggedness, accuracy, the limit of detection (LOD), and limit of quantitation (LOQ) that suit the AM (Lauwaars, 1998). Generally, an AM is verified and validated according to the rules and regulations of governmental bodies. In the halal certification realm, the application of HDTs depends on the procedure set by the certification body (CB). Halal CB of Thailand, namely, the Central Islamic Committee of Thailand (CICOT), has made obligatory halal testing for halal certification application prior to judgment by Islamic scholars (Nawawi et al., 2017; van der Spiegel et al., 2012). The AM shall undergo validation and verification (Sani, Jamaludin, Sowhini, & Asri, 2020) to fulfill the requirement for halal certification application by CICOT. In contrast, the Department of Islamic Development Malaysia (JAKIM) does not make halal testing compulsory for the application of halal certification, provided that the halal declaration is available.

20.4 Multivariate data analysis

Most of the HDTs employ MDA for research purposes only where these researches did not investigate the sources of halal products using validated and verified AM. The commonly used software to carry out MDA are Unscrambler X (Azilawati et al., 2015), SIMCA (Idris et al., 2021), XLSTAT (Ismail et al., 2021), etc.

The HDTs also only depend on the DNA testing method due to (1) its specificity towards porcine, bovine, and fish DNAs (Sultana, Hossain, Zaidul, & Ali, 2018); (2) DNA stability against pressure and heat treatments (Lubis, Salihah, Hossain, & Ahmed, 2017); and (3) capability of this method to detect porcine DNA in raw and processed meat (Abdullah Amqizal et al., 2017). With these advantages, the DNA testing method does not require a supporting tool such as MDA for halal authentication, provided the DNA methods complied with various acceptability ranges for method validation performance criteria (U.S. Food and Drug Administration, 2020). Due to the dependence on the DNA testing method, the other analyte measurements and MDA do not receive much attention.

As the demand for halal products expands from food to cosmetics, pharmaceuticals, etc., other AM on analytes such as FAMES and AAs has shown positive results. Analysts develop new AMs to provide alternative methods when the DNA testing method is unable to determine porcine DNA in a sample (1) containing high oil (Costa, Mafrá, & Oliveira, 2012) and (2) containing inhibitors such as polyphenols, polysaccharides, metal ions, and detergents (Kim et al., 2018).

The HDTs incorporate with the MDA to simultaneously analyze analytes (Hair, Black, Babin, & Anderson, 2014) in various sources, including animal, plant, and bacteria, where these sources have different distributions of the analytes. Infrared-active functional groups (Esteki & Shahsavari, 2018), FAMES (Azir et al., 2017), AAs (Azilawati et al., 2014), etc. are the commonly measured analytes and subjected to MDA such as PCA, cluster analysis (CA), and DA. To avoid false-negative or -positive results, the usage of MDA should also follow the requirements: (1) the AM has been validated and verified in actual samples (Department of Standards Malaysia, 2018), (2) the dataset is adequate and has variation, and (3) the collected dataset has undergone data preprocessing step, including evaluation of missing data, outlier removal, and fulfilling MDA assumption prior to the MDA application (Hair et al., 2014).

20.4.1 Collecting adequate dataset for multivariate data analysis

The samples from various sources, for example, fish, bovine and porcine, are analyzed with repetition. Although there is a common rule of collecting 30 data to establish a dataset, it is recommended to increase the number of repetitions followed by a sampling adequacy test, that is, Kaiser–Meyer–Olkin (KMO) test at a significant level (α) of 0.01. The determined KMO value is ranked as: $KMO < 0.5$ = inadequate, $0.5 < KMO < 0.7$ = mediocre, $0.7 < KMO < 0.8$ = good, $0.8 < KMO < 0.9$ = very good, and $KMO > 0.9$ excellent, and only $KMO > 0.5$ is deemed as adequate for MDA (Williams & Brown, 2012). However, achieving $KMO > 0.9$ provides an adequate dataset, especially when outliers are removed prior to the MDA.

20.4.2 Data preprocessing

Prior to the MDA, the dataset undergoes preprocessing steps to obtain essential data, avoid losing or ignoring the essential data, and discriminate sample sources by the variability of the biomarkers (Jančić-Stojanović & Rakić, 2015). These purposes can be achieved by determining missing data, detecting, and eliminating outliers (Pollet & Meij, 2017) and fulfilling the assumptions underpinning the MDA (Komsta, Heyden, Vander, & Sherma, 2018).

20.4.2.1 Evaluation of missing data

The missing data occur due to an undetected biomarker or detected biomarker below the LOD. The common practice is by converting the undetected value to a zero value and the value $< \text{LOD}$ to 0.5LOD or 0.75LOD (Martín-Fernández, Barceló-Vidal, & Pawlowsky-Glahn, 2003). Nonetheless, the conversion LOD value to 0.65LOD is recommended since fewer distortions are reported when this step is tested on the MDA (Palarea-Albaladejo & Martín-Fernández, 2013).

20.4.2.2 Identification and removal of outliers

Before conducting the MDA, the dataset is subjected to outlier detection and removal. The outlier is defined as a biomarker value that exhibits an extreme value from the majority values (Pollet & Meij, 2017). For outlier detection of the multivariate dataset, the analyst simultaneously assesses distance among the data values by determining the Mahalanobis distance measurement (MDM). The MDM measures the value distance from the mean center for each sample source and converts the distance values into a single value. The calculated MDM value identifies the biomarkers values that fall farther away from the mean center, indicating potential outliers. It is also compulsory to identify outlier present between two sample sources by calculating the MDM/df value, where df is defined as the number of biomarkers involved. The MDM/df value exceeding 2.5 indicates possible outliers. Then, the potential outliers in the group are confirmed via box and whisker plot, Grubbs or Dixon tests at α of 0.01 (Saiful et al., 2019).

20.4.2.3 Fulfilling multivariate data analysis assumption

After identifying and removing outliers, the analyst shall also review the dataset to ensure that the testing assumptions are met. This assumption involves normalization (Rani et al., 2019), homoscedasticity, and linearity of the datasets. To establish dataset normality, the dataset shall be subjected to normality tests, for example, Shapiro–Wilk test at α of 0.01, and non-normal biomarker values shall be transformed to ensure normal distribution. To establish dataset homoscedasticity, the analyst shall ensure the variance dispersion is equal within a sample source by performing a Levene test at α of 0.01. Else, the dataset shall be subjected to transformation. To establish the dataset linearity, the analyst shall ensure the R of the model is close to 1 or transform one or all biomarkers. Various transformation methods are applicable to MDA such as standardize $n - 1$, standardize (n), center, standard deviation⁻¹ ($n - 1$), standard deviation⁻¹ (n), rescale from 0 to 1, rescale from 0 to 100, Pareto, log transformation, etc.

20.4.3 Dataset exploratory by principal component analysis

Incorporating MDA to authenticate halal products has beneficially impacted research in this field. The MDA method, such as PCA, has brought detailed insight on the significant biomarkers in the halal authentication study, determined a correlation between the biomarkers, and proposed the best biomarkers that significantly contribute to the sample sources (Abdullah Sani et al., 2021). To cater to this purpose, the analyst shall perform the PCA, choose the best number of PCs with cumulative variability $> 75\%$, examine a correlation between the biomarkers, develop the groupings of sample sources, and select the biomarkers with high factor loadings ($\text{FL} > 0.75$) that significantly contribute to the groupings. High cumulative variability denotes a high percentage of the data is explained in the PCA. Upon identifying the significant biomarkers, it is recommended to ensure the new dataset consisting of only these significant biomarkers has $\text{KMO} > 0.5$. Else, the analyst shall collect more data to fulfill the requirement of KMO value before further dataset analysis. The increased value of KMO, cumulative variability, and FL of the biomarkers in this new dataset (Fig. 20.3B) as compared to the original dataset (Fig. 20.3A) indicates the PCA has successfully identified the significant biomarkers and developed a distinct grouping of sample sources. For instance, the S2 and S3 samples are mixed in Fig. 20.3A, while S1, S2, and S3 samples are clustered in their respective groupings in Fig. 20.3B. Additionally, B1–B3, B6–B11, B13, B16, and B17 are the significant biomarkers as depicted in Fig. 20.3B.

20.4.4 Validation of groupings of sample source by cluster analysis

Validation of the developed groupings of sample sources in PCA is needful via CA. The CA computes the similarity or dissimilarity between sample sources based on agglomeration criterion and clusters the similar sample sources into groupings by constructing a dendrogram. The dendrogram draws a dotted horizontal line across the vertical line of the

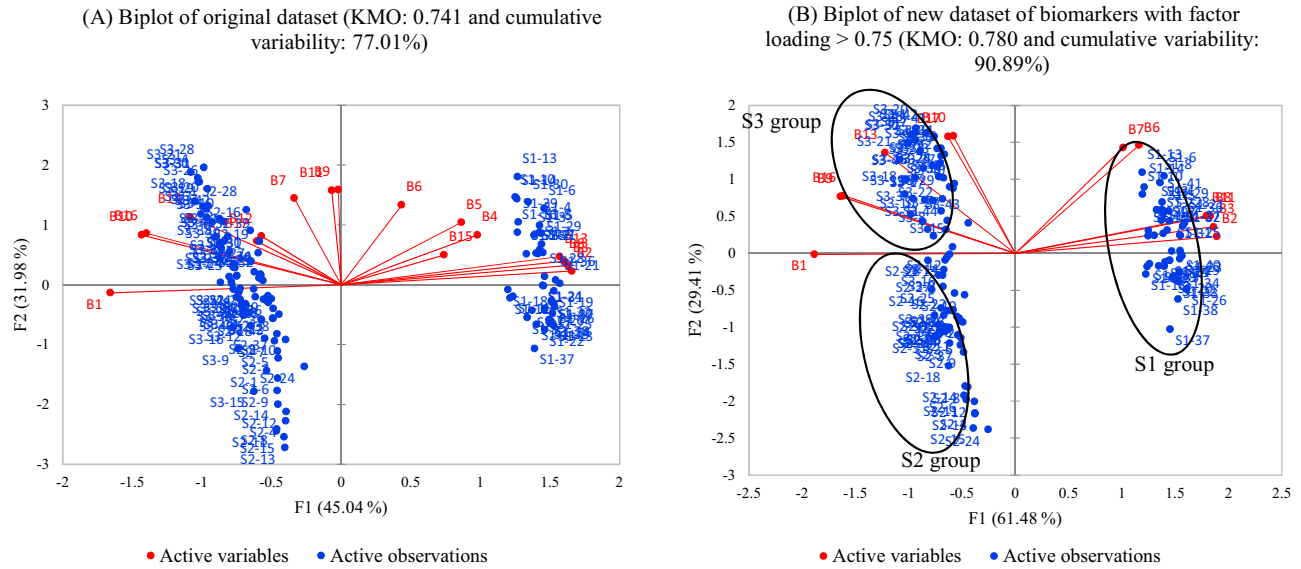


FIGURE 20.3 Biplot of (A) original dataset and (B) new dataset of biomarkers with factor loading > 0.75.

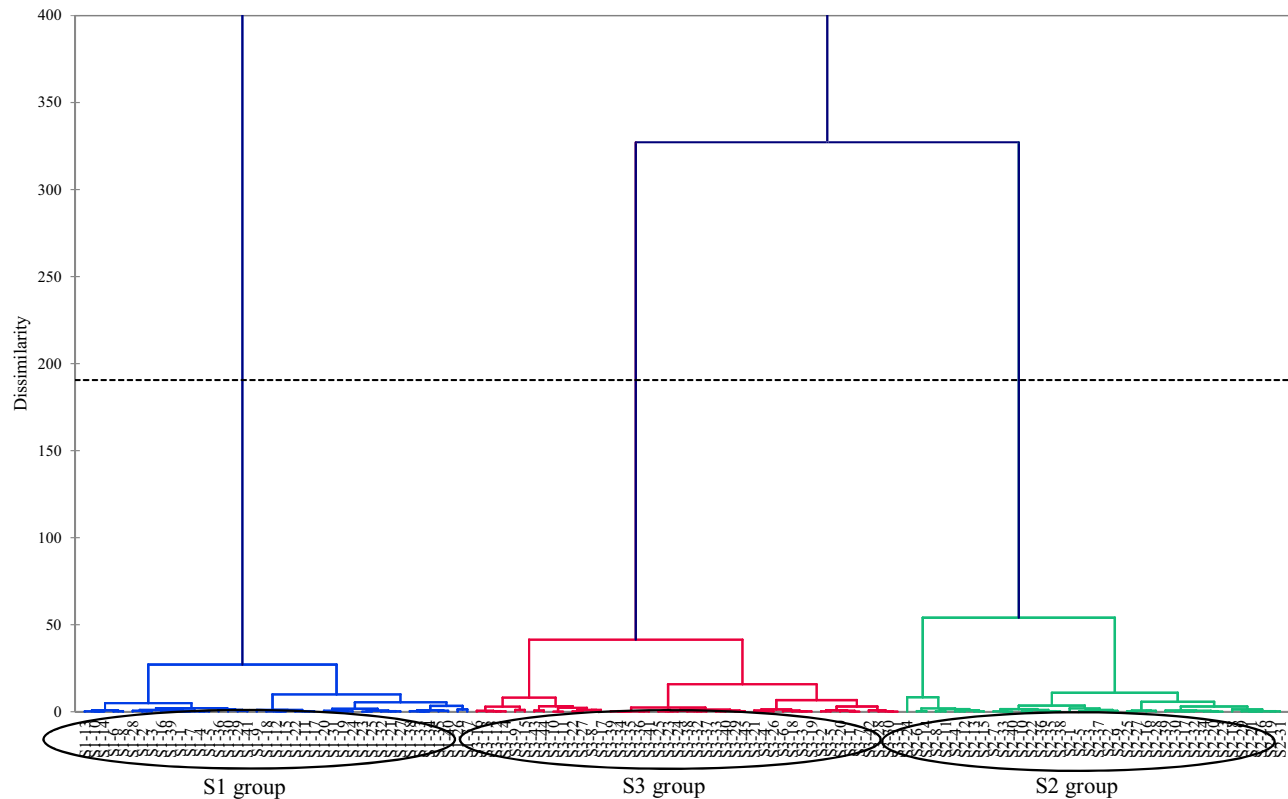


FIGURE 20.4 Dendrogram of three groupings of sample sources.

dendrogram and exhibits several groupings via several intercepts between the dotted horizontal line and the vertical line of the dendrogram (Sani, Yuswan, et al., 2020). Fig. 20.4 shows three intercepts that denote three groupings of sample sources and confirm the PCA's developed groupings. For instance, in halal authentication, the CA confirms the PCA's developed fish, bovine, and porcine groupings.

20.4.5 Authentication of sample sources by discriminant analysis

DA is an explanatory and predictive technique that verifies the validated groupings of sample sources by CA, confirms the significant biomarkers that contribute to groupings of sample sources, and authenticates the sample source for unknown samples (Zulkarnail et al., 2020). This step needs training and validation datasets that only includes the significant biomarkers determined in PCA and confirmed by CA.

Using the newly established dataset from the PCA and CA and 20 data for validation dataset, the analyst shall confirm 100% correct classification of the groupings of the sample sources, and Fisher distances test at α of 0.01 between the groupings are significantly different. The P value $< .01$ indicates the groupings are distinct and confirms the significant biomarkers contributing to the groupings (Table 20.2). Hence, the DA has successfully developed an authentication model. After these confirmatory tests, the unknown samples introduced as a testing dataset could be authenticated using this DA-developed authentication model, and only the lard-adulterated samples are subjected to regression analysis (RA).

20.4.6 Determination of adulteration level by regression analysis

Regression analyses such as PLSR, PLS-DA, principal component regression, OPLS-DA, and multiple linear regression are employed to develop the regression model for the determination of adulteration level in the unknown samples. This step also needs training, validation, and testing datasets that only includes the significant biomarkers determined in PCA and confirmed by CA and DA. The training and validation datasets consist of groupings of sample sources with a determined percentage of lard adulteration, while the testing dataset comprises unknown samples. Before the RA, these datasets must also fulfill the KMO > 0.5 requirement. Post fulfilling this KMO requirement, the RA is executed at α of 0.01 (Alexandre, Goraieb, Bueno, & Wiley, 2010).

If different types of RA are executed, the determination coefficient (R^2), mean square error (MSE) and root MSE (RMSE) of each regression model shall be compared. The model with the closest R^2 to 1 and the lowest MSE and

TABLE 20.2 Classification matrix of training, validation, and testing datasets by discriminant analysis.

Dataset	Correct classification, %	Number of samples and <i>P</i> values of Fisher distance ^{a,b}			Total samples
		S1 group	S2 group	S3 group	
Training dataset					
S1 group	100.00	41 (1)	0 (<.0001)	0 (<.0001)	41
S2 group	100.00	0 (<.0001)	40 (1)	0 (<.0001)	40
S3 group	100.00	0 (<.0001)	0 (<.0001)	45 (1)	45
Total	100.00				126
Validation dataset					
S1 group	100.00	20 (1)	0 (<.0001)	0 (<.0001)	20
S2 group	100.00	0 (<.0001)	20 (1)	0 (<.0001)	20
S3 group	100.00	0 (<.0001)	0 (<.0001)	20 (1)	20
Total	100.00				60
Testing dataset/unknown sample					
S1 group	100.00	20 (1)	0 (<.0001)	0 (<.0001)	20
S2 group	100.00	0 (<.0001)	20 (1)	0 (<.0001)	20
S3 group	100.00	0 (<.0001)	0 (<.0001)	20 (1)	20
Total	100.00				60

^aA total of 12 (B1–B3, B6–B11, B13, B16, and B17) out of 17 biomarkers are confirmed as the significant biomarkers ($P < .01$).

^bCalculated P value of Fisher distance $< .01$ indicated two clusters are significantly different.

RMSE is selected because these characteristics indicate the selected model has the lowest probability ($\leq 1\%$) of incorrect determination of adulteration level. Determination of Z-test or T-test value of the predicted and actual adulteration levels should also be evaluated to assist the selection of the best regression model. The P value > 0.01 of the Z-test or T-test denotes that the null hypothesis is accepted; the predicted and actual adulteration levels are not significantly different and proves that the selected regression model is a useful halal authentication tool.

20.5 Challenges

The advancement of HDTs still faces challenges in developing new and suitable AM. Although the validation of AM has successfully fulfilled the PC, most of AM could not determine the halal status of an actual sample or halal products due to complex sample matrix (Martín et al., 2009) and denaturation of the analytes during heat treatment on the sample (Sarah et al., 2016). It is recommended to analyze various actual samples to ensure the scope of the validated AM and increase the LOQ value of the AM to differentiate the blank baseline and the actual sample. Cross-contamination from the supply chain of the actual sample also contributes to the false-positive result (Amaral et al., 2017). For halal testing, sampling should be performed at the manufacturing site instead of at the retailing store for this purpose. Since the CB has the authority to make the halal testing a voluntary measure, the development, validation, and verification of AM of HDTs may halt when the CB ignores this measure. For instance, JAKIM accepts halal certification applications without requesting halal testing, with its built in halal system. Hence, the development of new AM using HDTs has declined in Malaysia. On the contrary, the CICOT has made the incorporation of halal testing and traceability evidence as a compulsory measure for halal certification in Thailand; thus there is an increment of new AMs in Thailand Halal Science Center (van der Spiegel et al., 2012) which supports the halal declaration made by the manufacturers.

20.6 Conclusion

The development and utilization of AM using HDTs are critical to assure the halal status of products as claimed by the manufacturers, and the implementation of halal testing creates trust for consumers locally and abroad. The three approaches suggested in this chapter help the analysts to identify the suitable approach, treat the samples accordingly, and perform proper halal testing using the AM of HDTs. Each AM should be validated and verified using actual samples before the AM is used to test client's samples and issuance of the testing report. It is also compulsory for the analysts to ensure the dataset collected from the measurement using validated and verified AM has fulfilled the prerequisite requirements of MDA to support the HF testing result. Appropriate control actions should also be taken attentively on challenges during the analysis of samples. The fulfilment of these requirements by ISO17025 accredited halal-testing laboratories provides and maintains a reliable and quality halal testing result to the consumers.

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Conflict of interest statement

There is no conflict of interest.

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Section 5

Sustainability in Halal Supply Chain

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Healthy aquatic ecosystem, towards sustainable food supply

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21.1 Introduction

Due to the stagnation of catch fisheries, aquaculture production is under severe pressure to expand to fulfill the rising food needs of world population. Food supply sustainability is a global problem, but there are several approaches to achieve it. The four key themes that are emerging are quality, consumer reduction, aquatic resources, and food supply chain reform. Aquaculture, on the other hand, has resulted in a host of environmental difficulties, which would be exacerbated if aquaculture output were increased. Climate change will put the world's aquaculture productivity in jeopardy much more. Aquaculture is endangered by cyclones, droughts, and floods, as well as global warming, ocean acidity, unpredictability of rainfall, salinity, and sea level rise. Aquaculture must significantly reduce its environmental effect to remain sustainable. Climate change adaptation is also critical for increasing fish production while minimizing environmental impact. Integrated aquaculture, recirculating aquaculture systems (RAS), and expanding seafood farming are just a few of the adaptation options that might assist boost aquaculture output, environmental sustainability, and flexibility to climate change. Furthermore, the agricultural industry produces a great deal of waste, which is generally discarded, presenting a danger to global health and food security. The synthesis of these biomolecules has received a lot of attention in recent years because of the potential of agroresidues application in a variety of industries, including aquaculture, agriculture, food processing, and medicine, enabling the production of new sustainable commodities. Aquatic food on a global scale contributes greatly to human nutrition and health, as well as being sought for and appreciated for cultural and culinary reasons. The supply and availability of such food in the foreseeable future, including wild-caught and farmed, is a vital and continuous challenge for humanity. Production should be sufficient, hygienic, and healthy to meet present needs and desires, but it must also be environmentally, socially, and economically sustainable. Sustainably production, as well as ensuring that food production does not jeopardize other ecosystem processes, is required to retain the productivity and diversity of the food supply and the ecosystems that support it. Economically and socially sustainable supply is required to guarantee that food-producing communities, industries, and supply chains continue to operate and workers are treated with respect.

Aquatic food has a wide range of socioeconomic significance across the world, as well as a wide range of food production methods. Aquatic food may either play a significant role in daily nutrition or supplement a healthy and well-balanced diet with diversity and a few vital components. Fishing and aquaculture are pursued for a number of reasons, from meeting immediate subsistence requirements to generating large cash for multinational corporations selling in international markets. As a consequence, the worldwide implications of increased food demand will vary according to the dependence on and availability of aquatic food by nations and people within those countries. In certain nations, food security will be achieved, where everyone has access to adequate, secure, and nutritious food that satisfies their dietary requirements at all times, whereas in others, it will not be possible to achieve this goal (FAO, 1996). Individual countries' prospects are dictated by the relative significance of aquatic food in present and future diets, as well as the sustainability, kind, safety, and appropriateness of domestic production, import and export capacity, supply chain function, and food distribution equity. Due to fewer emissions and a high feed conversion rate, fish and poultry are a more sustainable alternative to beef and swine, and fish are without comparison the most efficient protein-converting higher animals ever farmed by man

(Nijdam, Rood, & Westhoek, 2012). However, there are a few other factors to consider. One is the topic of climate change, its anthropogenic implications, and strategies for achieving sustainable aquaculture. Additionally, the development of fish farming is expected to imply not just an increase in the number of facilities but also an intensification of farming practices, which has implications for farmer working and living conditions, as well as fish welfare and the environment.

Given that aquaculture is expected to expand, a wide range of factors needs to be considered in ensuring that more sustainable practices can be used to combat the current reduce of fish stock through consistent propagations of aquaculture. Climate change and anthropogenic issues impacted the current aquatic ecosystem that plays role as critical food supply. This review highlights the ecological functions of aquatic ecosystems with plankton as the basal point of primary producer to most pelagic fishes and crustaceans, as well as the disturbances to the aquatic community, and how climate change and anthropogenic affects the way organisms in the aquatic ecosystem and how aquaculture activities adapted to these two factors.

21.2 Ecological-network functions in stability of aquatic food web

Ecosystems are made up of a variety of species that interact with one another either directly or indirectly via their shared environment, such as nutrients, light, or space (Douglas et al., 2018). A holistic view of ecological responses to global change is usually the most effective approach (Boyd et al., 2018). Trophic interactions are recognized as significant regulators of community form and function (Thakur & Geisen, 2019). Food webs, which are composed of linked food chains, aid in our understanding of how ecosystems change. Interpreting the planktonic food web structure is crucial to understand the ecological system, material cycling, and energy transfer. Primary producer size structure is strongly linked to the hydrological variables that govern nutrient availability and stratification in food webs (Gao et al., 2021).

Phytoplankton is the primary producer in the aquatic food chain and is devoured by primary consumers such as zooplankton, small fish, and crabs (McMahon et al., 2021). In a food web, primary consumers were consumed by the secondary consumer, such as small sharks, octopuses, baleen whales, and median sizes fish. After that, this secondary consumer also will be consumed by the tertiary consumer or well-known top ocean predators such as huge sharks, toothed whales, billfish, and large seals. Then, aquatic species from all of the food web levels were consumed by humans to fulfill human requirements for growth and health. Understanding the food web's structure, particularly species diversity and interactions, is required to define population function, energy and substance flow (Bauer & Hoyer, 2014). Furthermore, the food web provides a comprehensive tool for discussing issues at all levels of ecological organization, enables us to assess the possible implications of natural and anthropogenic disturbances, and predicts future improvements from individuals to ecosystems (Seibold, Cadotte, MacIvor, Thorn, & Müller, 2018). A comprehensive review of trophic interactions is essential for predicting the adaptive implications of habitat destruction, since anthropic stresses may alter food webs through bottom-up climate impacts or top-down cascading after the loss of high trophic level organisms.

Complex food webs provide sustenance for a variety of ecosystems (Bieg et al., 2018). If a predator's preferred prey becomes limited, it may change to consume more any other species. Though a single change in the component of the food web can trigger a trophic cascade, affecting organisms at numerous trophic levels especially when the food resources are limited. Small alterations may have many implications, reverberating throughout vast ecosystems via direct and indirect interactions. Contamination, overexploitation of natural resources, habitat alteration, and climate change are just a few of the difficulties affecting our biosphere (Laghari, Ghaffar, & Mubeen, 2022). Our lives are largely reliant on natural ecosystems, thus the responses to global change in our natural features are of significant relevance to policymakers.

21.3 Plankton as primary producer; diversity and abundance of plankton distribution

Primary producers, such as bacteria and phytoplankton, are responsible for forming the lowest trophic level of the aquatic food web (Wilken et al., 2018). Photosynthetic organisms produce their own energy and therefore do not require food (Enamala et al., 2020). Numerous organisms photosynthesize, utilizing the energy of the sun to produce carbohydrates. Certain primary producers, on the other hand, may create energy without sunlight by metabolizing chemicals produced by hydrothermal vents, methane leaks, and other geological events. Marine and freshwater biological productivity is determined by the number of phytoplankton produced and its trophic transmission within food webs. The arrangement and function of the aquatic food chain in marine ecosystems may have a significant impact on energy movement and carbon regulation (Armengol, Calbet, Franchy, Rodríguez-Santos, & Hernández-León, 2019). Furthermore, seasonal variations in environmental circumstances, such as nutrient availability, light, and hydrological conditions, also could impact plankton community abundance, size structure, and composition, throughout the planktonic ecology, as well as trophodynamics (Okazaki, Tadokoro, Kubota, Kamimura, & Hidaka, 2019).

Phytoplankton blooms triggered by increased light in the stratification of the water column are dominated by micro-sized algae such as diatoms (Biggs, Huisman, & Brussaard, 2021). Herbivorous zooplankton such as copepods easily transfer this high production to the top levels of trophic or transport it to deeper strata (Schukat et al., 2021). The continuous water column stability depletes the surface water nutrient over the summer, resulting in a decline in production and a change in primary producers, with domination of small and various sizes of algae, bacteria, and detritus (Burger, Moloney, Walker, Parrott, & Fawcett, 2020). In the microbial food chain, microbivores zooplankton such as heterotrophic nanoflagellates and microzooplankton devour this production and transport it to higher consumers. In the autumn, storms may weaken water column consistency and alter nutrients in surface water, leading to a transient phytoplankton bloom that disappears swiftly when light levels fall (Gu, Cheng, Qi, & Wang, 2020). The multivarious food web is created by microbial activities and feeding on larger phytoplankton. When the water is mixed in the winter, the food web may usually act as a microbiological pathway.

21.4 A source of plankton to support secondary production

Plankton distribution and abundance in an aquatic ecosystem are important because of their role as a sensitive indicator of climate condition changes (McQuatters-Gollop et al., 2019). A plankton lifeforms indicator is policy approved to measure pelagic biodiversity. In marine environment, the flow of energy in the base of marine food web drives the function of the ecosystem, therefore the relationship between basal energy and fisheries production was not easy (Cresson et al., 2020). Trophodynamics, or the transfer of nutrients and energy between different levels of the trophic, significantly influences the form of ecosystems and their services in the water (Trebilco, Melbourne-Thomas, & Constable, 2020). The loss of energy across trophic levels from a single large basal source, such as pelagic primary producers in seawater, is often shown as an undersea heavy base, resulting in low productivity at various trophic levels. Both biotic and abiotic factors may have a favorable or negative impact on the phytoplankton community in permanently open and temporarily open-close estuaries, which regulate primary production, respiration, and phytoplankton biomass standing stocks (Tagliarolo & Scharler, 2018). The cumulative effects of these ecosystem functions on phytoplankton cycles are probably system dependent, resulting in a significant degree of variability in the pelagic community's response to changes in river water flow and estuary mouth state.

On the other hand, the subsidization of organic matter or the entrance of migratory aquatic animals enhances the quantity of nutrient and energy availability in the ecosystem; linked systems have larger biomass of high trophic level species, resulting in a top-heavier representation (Cresson et al., 2020). This hypothesis highlights the critical significance of reflecting the ecological connection. The critical role of connection between pelagic and benthic structures in marine systems has been thoroughly studied. Downward coupling, such as benthic environments being powered by pelagic primary production, is common in the majority of marine ecosystems globally and has attracted considerable attention as a result (Cresson et al., 2019). Nonetheless, upward fluxes such as the integration of benthic primary or secondary production into pelagic systems may play a role and need more exploration. Additionally, comparisons of systems with varying environmental characteristics are regarded as a useful pattern for identifying critical environmental and anthropogenic drivers of trophic functioning.

The trends identified for common species, as well as the substantial variances in pelagic input, suggest differences in baseline food supplies across the ecosystems. The pelagic contribution for the zooplanktivorous species was very comparable among ecosystems, although the contribution for pelagic piscivores was substantially different, and the contribution for benthic species was much higher (Tilves Matheu, 2019). Benthic species are regarded as actual opportunists because of their greater trophic flexibility and potential detritivore, whereas pelagic species may be more specialized and hence limited in their feeding options (Lennox, Crook, Moyle, Struthers, & Cooke, 2019). A recent study found that both benthic and opportunistic populations responded to eutrophication-induced increases in pelagic primary production by integrating larger amounts of pelagic-derived material, but pelagic organisms remained pelagic. Thus the current global change in marine ecosystems shows how important it is to have an integrated view of how they work, both to understand basic mechanisms and to maintain the sustainability of ecosystems that are being used.

21.5 Disturbance to aquatic community

Table 21.1 discusses impacts of climate change on aquatic community.

21.5.1 Climate change effects

Presently, the consequences of climate change have been documented across a range of critical economic sectors and services on a worldwide scale (Godde, Mason-D'Croz, Mayberry, Thornton, & Herrero, 2021; Johnson et al., 2021; Mirtl et al., 2018). The major sector that impacts global food security, nutrition, and livelihoods has heightened concern

TABLE 21.1 Impacts of climate change on aquatic community.

Impacts	Description	Examples
Rising temperature	• Temperature fluctuations may alter the pattern and composition of nutrients in the water. • Phytoplankton biomass production is dependent on temperature and nutrients. As temperature increases, zooplankton productivity also increases (Marañón et al., 2018; Spear et al., 2019; Hayashida et al., 2020; Zhang et al., 2021).	• Increasing temperature may cause temporary imbalances between predators and preys. Consequently, zooplankton is functioning at the boundary between primary and planktivorous production. (Rodríguez-Malagón et al., 2021). • A decrease in zooplankton survival, growth, and reproduction could be caused by competition with harmful phytoplankton which grows as a result of climate change (Thorson et al., 2021).
	• Chronic stress due to rising temperature may have an effect on the neuroendocrine and osmoregulatory systems, impairing cardiorespiratory function and aerobic range, as well as immunological responses of aquatic species.	• Mortality of the cold-water species, e.g., Atlantic halibut, salmon, and cod, is expected to increase due to the projected 1.5°C increment of global mean temperature (Afonso, 2020; Whitney et al., 2016).
Ocean acidification	• Ocean acidification occurs when pH of seawater decreases as a consequence of atmospheric carbon dioxide, CO₂ accumulation (Basu and Mackey, 2018). • A rise of CO₂ in water will result in a drop of pH, causing water quality to deteriorate and productivity to decline (Ninawe et al., 2018).	
	• When ocean acidity rises, the supply of carbonate essential for the formation of coral skeletons in shell-forming animals, including shrimp, mussels, oysters, and corals, decreases (Barbosa, 2020).	• Production of wild spat oysters decreased due to high predation rate among juveniles as a result of failed coral skeleton growth (Barbosa, 2020). • The occurrence of calcified otoliths in finfish makes them susceptible to the effects of ocean acidification, potentially impairing growth and development, RNA viability, tissue damage, and respiration impairment (Clements and Chopin, 2017; Thiessen, 2019).
Harmful algal blooms and disease outbreak	• Temperature fluctuations are likely to have an impact on diseases such as bacterial, parasitic, viral, and fungal infections. • The reproduction rate, pathogenicity, life cycle duration, and transmission of infections among finfish and shellfish species are predicted to increase when temperature increase.	• Warm water diseases, such as sea lice, are expected to remain a challenge in salmon production due to the increased temperature.
	• Changes in climatic circumstances have also been linked to the outbreak of dangerous algal blooms. Algal blooms are a severe challenge to the long-term viability of fisheries and aquaculture in terms of environmental sustainability (Brown et al., 2020). • The poisonous algal species may cause stress or death in finfish and shellfish. Additionally, symptoms such as irritation, atrophy, and necrosis in organs of mollusk have been reported as a consequence of hazardous algal blooms.	• The outbreak of <i>Pseudochattonella cf. verruculosa</i> and <i>Alexandrium catenella</i> species, linked to climate-induced changes in water column stratification, caused an unparalleled loss of fish in Chilean (Maulu et al., 2021).
Variability of rainfall	• Changes in precipitation will cause either higher rainfall (flooding) or periods of low/no rainfall (drought). • Drought risks are anticipated to be greater at 2°C of global warming in a particular location than at 1.5°C, (Gay-Antaki & Liverman, 2018).	• Heavier precipitation has been indicated to alter macroalgal productivity, such as seaweed production (Rankin & Jones, 2021). • Variation in nutrient loading due to varied precipitation may favor short-lived algae species over the longer-lived seaweed species which is favorable for production.

(Continued)

TABLE 21.1 (Continued)

Impacts	Description	Examples
	Increased rainfall, especially if it comes in the form of heavier storms, will exacerbate the production risks in lowland regions (Binder & Selim Suner, 2021).	Flooding may cause the introduction of invasive species and deterioration of water quality especially in pond production.
Sea level rise and changes in water salinity	Water salinity and accessible foods in the environment influence the quantity and variety of zooplankton and phytoplankton. Thus changes in salinity may affect taxonomic composition, decomposition, nutrient cycles, and food web efficiency of the ecosystem (Yuslan et al., 2021).	Increased salinity in freshwater may diminish zooplankton diversity, particularly the cladoceran, hence shift the species' adaption to a more salt-tolerant species (Yuslan et al., 2021).
	Increasing sea levels not only affect coastal habitats that sustain wild fish stocks, but also the production of inland and marine aquaculture.	Seawater infiltration may cause increased mortality of the cultured species, especially in coastal areas. On the plus side, sea level rise may expand regions suited for brackish water farming of valuable species such as shrimp and mud crab (Du et al., 2019).
Extreme climate phenomena	- Extreme phenomena, such as cyclones, waves, and storms, are predicted to have a significant impact on fishery resources (Pryor et al., 2020; Toussaint, 2021). - Major phenomena like storms are indicated to have a substantial role in integrating water and nutrients that were formerly limited to certain areas (Calderó-Pascual et al., 2020), potentially promoting the production of aquatic organisms.	Phytoplankton is affected by extreme wind occurrences which increase nutrient loading and deepen the mixed layer, while phytoplankton biomass was increased by medium-intensity wind than high-intensity wind. (Mesman et al., 2021).
Anthropogenic impacts	- Human-induced disturbances, like the release of pollutants, have harmed aquatic ecosystems and resulted in significant reduction of their functions (Vörösmarty et al., 2010). - Flowing areas like rivers are the most threatened habitats because of the considerable anthropogenic contaminants such as run-off from agriculture and industrial sectors, and domestic sewage (Xiong et al., 2019). - Regardless of whether the contaminants are chemical or solid, aquatic ecosystems are sponges for anthropogenic contaminants (Bhat et al., 2017).	Aquatic ecosystems are reported to contain a wide range of pollutants, e.g., radioactive elements (strontium, cesium, radon), metals (cadmium, mercury), organic compounds (chlorofluorocarbons, trichloroethylene, tetrachloroethylene, xylenes, formaldehyde, benzene), waste from agriculture activities (fertilizers, pesticides), household products (detergents, cleaning products), and fuel substances (nitrogen oxides, sulfur oxides, carbon monoxide) (Hampel et al. 2015; Bashir et al., 2020).
	- Recently, microplastics have been found to be harmful to aquatic animals, thus highly concerning in regard to human health. - The term "microplastics" refers to plastic particles of a diameter less than or equal to 5 µm (GESAMP, 2016). - Packaging, beverage bottles, synthetic textiles, vehicle tires, personal care products, cosmetics products, and electronic equipment are all examples of microplastics that were found to pollute the aquatic environment (Fendall & Sewell, 2009; Andrady, 2011).	A wide range of commercial species, including fish (Atlantic cod, horse mackerel, European pilchard, red mullet, and European sea bass), bivalves (mussels and oysters), and crustaceans (shrimps), have been found to ingest microplastics (Bellas et al., 2016, Güven et al., 2017; Bessa et al., 2018).

about climate change consequences (Godde et al., 2021; Macdiarmid & Whybrow, 2019). Various reports reveal that the effects of climate change differ according to geographical regions, economies, climatic zones, production systems, and cultured species (Cissé, 2019; Galappaththi et al., 2021; Godde et al., 2021; Macdiarmid & Whybrow, 2019). It is expected that the changing climate will have a negative impact on output and sustainability because of increasing temperatures, ocean acidification, and disease outbreaks, as well as alterations in rainfall and sea level rise and extreme weather occurrences (Azani et al., 2021; Galappaththi et al., 2021; Mukhopadhyay, Karisiddaiah, & Mukhopadhyay, 2018)

21.5.2 Rising temperature

Temperature has a significant impact on the growth and development of aquatic organisms (Yang et al., 2018b; Yang et al., 2018a). A sufficient supply of nutrients could result in significant increases in biomass production, but a deficiency in nutrient supply could have a negative impact on temperature (Rehman, Farooq, Asif, & Ozturk, 2019). According to Hayashida, Matear, and Strutton (2020) and Mara  n, Lorenzo, Cerme  o, and Mour  no-Carballido (2018), phytoplankton biomass is dependent on temperature and nutrients. Spear et al. (2019) and Zhang et al. (2021) also claimed that as temperature increased, zooplankton productivity increases. The risk of harmful phytoplankton production in the ocean has grown as a result of climate change and eutrophication relationships. A decrease in zooplankton survival, growth, and reproduction could be caused by competition with phytoplankton exploitation (Thorson et al., 2021). Due to increasing temperatures and the occurrence of temporary imbalances between predators and prey, the coastal ecology alters plankton species (Rod  r  ez-Malag  n, Speakman, Sutton, Angel, & Arnould, 2021). Consequently, zooplankton is the major secondary producer in the oceans, functioning at the boundary between primary and planktivorous production. Thus nutrient transport to higher trophic levels is vital. Plankton physiology and production may be seriously affected as a result of the increasing temperature of the aquatic environment. This demonstrates how temperature changes might shift species toward those more adapted to warmer waters, potentially disrupting the food web.

Poikilothermic fish may be particularly sensitive to climate-related temperature changes (Campana, Stef  nsd  ttir, Jakobsd  ttir, & S  lmundsson, 2020). The mortality of the majority of fish, particularly cold-water species such as Atlantic halibut, salmon, and cod, as well as intertidal shellfish, is likely to increase as a consequence of the projected 1.5  C increment in global mean temperature (Afonso, 2020; Whitney et al., 2016). For example, chronic stress may affect the neuroendocrine and osmoregulatory systems of various commercially significant species, impairing cardiorespiratory function and aerobic range, as well as immunological responses. As a consequence of temperature fluctuations, environmental sustainability is significantly affected by deep-water thermal gradient; it has the potential to alter the pattern and richness of nutrients in the water, and upwelling has the latent to result in significant economic damage for accessible producers.

21.5.3 Ocean acidification

Ocean acidification happens when the pH of ocean water decreases over time as a consequence of atmospheric CO₂ accumulation. Approximately 50 times more CO₂ is accumulating in the oceans than in the atmosphere (Basu & Mackey, 2018). Global warming of 1.5  C or higher would negatively affect the growth, reproduction, calcification, survival, and population of various aquatic species (Parra & Espinoza-Villalobos, 2020). A rise in the concentration of CO₂ in water might result in a drop in the pH of the water, which would endanger the ecological sustainability of aquatic production procedures by causing water quality to deteriorate and productivity to decline (Ninawe, Indulkar, & Amin, 2018). Furthermore, when ocean acidity raises, the supply of carbonate essential for the formation of coral skeletons (Calcification) in shell-forming animals including shrimp, mussels, oysters, and corals decreases, posing a danger to ocean aquaculture productivity. For instance, the productivity of wild spat oysters may decrease because of higher predation rates among juveniles as a result of failed coral skeleton growth, which reduces harvesting rates (Barbosa, 2020).

Increasing acidity levels in saltwater might have a substantial impact on aquatic animals' physiology and metabolism by altering intercellular transport pathways. The capability of organisms to adjust to fluctuations in ocean acidity will rely on their adaptive capability, rate of development, and various biophysical feedbacks. Ocean acidification may potentially alter macroalgal (seaweed) production; however, this will rely on species' acquisition kinetics of inorganic carbon (Ho, McBroom, Bergstrom, & Diaz-Pulido, 2021). Calcifying organisms, for example, are expected to be the most impacted in areas where CO₂ is not the primary cause of acidification. Although seaweed is a significant carbon sink, few studies have examined the implications of climate alteration on seaweed supply, mainly because seaweed production is confined to certain places. According to Rastrick et al. (2018), the implications of unexpected conditions in ocean carbonate chemistry are difficult to predict because of the difficulties in tracking long-term biological responses to very small changes detected in laboratory conditions.

Acidification affects zooplankton in several ways. Changes in the quantity, content, and quality of their food supply, although more complex to evaluate, could significantly alter organism function. During the early phases of development, lower survival, hatching success, and inconsistent larval growth have all been seen in a variety of zooplankton species. Therefore, acidification may affect zooplankton development, growth, spawning, and population size, both directly and indirectly (Campoy et al., 2020). Even though the consequences of ocean acidification on finfish are incompletely defined, the occurrence of calcified otoliths in finfish, particularly marine species, makes them susceptible

to the effects of ocean acidification, potentially impairing growth and development, RNA viability, tissue damage, and respiration impairment (Clements & Chopin, 2017; Thiessen, 2019).

21.5.4 Harmful algal blooms and disease outbreak

Unexpected changes in the temperature range are likely to have an impact on diseases such as bacterial, parasitic, viral, and fungal infections. Pathogen susceptibility in fish and shellfish is a crucial factor in disease transmission, and it is anticipated that both direct and indirect temperature stresses have an impact. The reproduction rate, pathogenicity, life cycle duration, and transmission of infections among numerous finfish and shellfish species are predicted to increase when the temperature rises. Epizootic disease outbreaks are already one of the most significant challenges limiting the effectiveness of aquatic animal production systems in many nations across the globe. Warm water diseases, such as sea lice, are expected to remain an issue in salmon production, and increased warming is anticipated to exacerbate infections in cold temperate environments, necessitating more treatments and hence increasing costs. Equally, cold water illnesses that afflict Atlantic salmon, such as vibriosis and winter ulcer, may go extinct as a result of the growing adverse circumstances that may encourage the development of this fish species.

Changes in climatic circumstances have also been linked to the outbreak of certain dangerous algal blooms, according to many studies. Algal blooms are a severe challenge to the long-term viability of fisheries and aquaculture in terms of environmental sustainability (Brown et al., 2020). For example, it has been observed that the taxonomic groupings flagellates and dinoflagellates, as well as other dangerous species, include potentially poisonous or nuisance species that may cause stress or death in finfish and shellfish. The outbreak of *Pseudochattonella cf. verruculosa* and *Alexandrium catenella* species, linked to climate-induced changes in water column stratification, caused an unparalleled loss of fish in Chilean (Maulu et al., 2021). Additionally, other research has shown diseases such as irritation, atrophy, and necrosis in a variety of bivalve mollusk organs as a consequence of hazardous algal blooms. The methods by which climate change affects hazardous chemicals in aquaculture are still unclear, however Glibert (2020) found that temperature fluctuation affects the metabolism of the most prevalent harmful algae.

21.5.5 Variability of rainfall

Changes in precipitation will have two distinct effects on aquatic productivity and sustainability: higher rainfall (flooding) and periods of low or no rainfall (drought). Drought risks are anticipated to be greater at 2°C of global warming in a particular location than at 1.5°C (Gay-Antaki & Liverman, 2018), whereas flooding occurrence patterns are impossible to forecast with confidence. Increased rainfall, especially if it comes in the form of heavier storms, will exacerbate the production risks in lowland regions (Binder & Selim Suner, 2021). The entrance of invasive fish species and the deterioration of water quality may have a negative influence on the ecological sustainability of supplies when pond water and fish are mixed with those found in the wild.

Heavier precipitation, which might result in varied coastal waters, has also been found to alter macroalgal production, such as seaweed productivity (Rankin & Jones, 2021). Variations in nutrient loading due to varied precipitation may also favor invasive short-lived algae species over the longer-lived seaweed species that are generally regarded acceptable for production. Climate change-induced water scarcity would increase competition for water among users such as aquaculture, agriculture, household, and industrial (Urama & Ozor, 2010). However, further research is required to determine how various fish species and life stages react to variations in precipitation patterns.

21.5.6 Sea level rise and changes in sea surface salinity

Salt concentration levels have risen dramatically as a result of rising seawater levels and coastal erosion, impacting aquatic habitats and biodiversity (Jevrejeva, Jackson, Grinstead, Lincke, & Marzeion, 2018). Changes in water quality affect aquatic life. In aquatic ecology, zooplankton is a key population for energy transmission from primary producers to fish. Small fish nutrition is greatly influenced by zooplankton and ecosystem species. The health of the environment influences both foraging efficiency and predation risk in fish. Environmental variables such as water clarity, climatic change, and nutritional food content impact the distribution and abundance of zooplankton species. Salinity and accessible foods in the environment influence the quantity and variety of zooplankton and phytoplankton. Thus changes in water salinity may affect the taxonomic composition, decomposition, nutrient cycles, and food web efficiency. Increased salinity in freshwater may diminish zooplankton diversity, particularly in the cladoceran community, and hence shift the species' adaption to a more salt-tolerant species (Yuslan et al., 2021).

In addition, increasing sea levels may affect coastal habitats that sustain wild fish stocks. Modifications in species composition, richness, distribution, ecosystem productivity, and phenological variations will affect inland and marine aquaculture. Salinity is recognized as a variable feature that represents freshwater input from rainfall, glacier melt, river flow, water loss due to evaporation, and the mixing and exchange of ocean surface water with subsurface water (Johnson et al., 2021). Furthermore, coastal aquaculture operations provide social and environmental advantages which may be impacted both directly and indirectly by rising oceans, hurting the sector's productivity and viability. On the plus side, sea level rise may expand regions suited for brackish water farming of valuable species such as shrimp and mud crabs. Sea salinity variations can be caused by higher evaporation from rising temperatures and changing ocean circulation, or by direct climate change (Du, Zhang, & Shi, 2019), which may impact ocean circulation, carbon and nitrogen cycling, and heat storage. The majority of aquatic organisms have certain salinity needs that must be satisfied to prevent death and loss of production. A changing climate is anticipated to increase abalone farm mortality, especially in coastal locations, due to salty water infiltration from marine habitats. In general, changes in water salinity would raise mortality rates for various species, affecting the sector's economic and social viability.

21.5.7 Extreme climatic phenomena

Extreme climatic phenomena, such as cyclones, waves, and storms, are predicted to have an impact on fishery resources (Pryor, Barthelmie, Bukovsky, Leung, & Sakaguchi, 2020; Toussaint, 2021). According to Dube, Nhamo, and Chikodzi (2021), extreme climatic occurrences have grown in recent years in various locations and now account for at least 80% of all climate-related disasters. It is expected that these phenomena will occur more frequently across Africa, mainly in East and Southern Africa (Dube et al., 2021; Fanzo, 2021). However, major climatic occurrences such as storms are anticipated to have a substantial role in integrating water and nutrients that were formerly limited to certain temperature columns (Calderó-Pascual et al., 2020), potentially promoting the sustainable development of aquatic organism yield.

Additionally, storms may play a critical role in lowering water temperatures and associated dangers to both cultured and wild organisms (Kelly et al., 2020). Phytoplankton is affected by extreme wind occurrences by increasing internal nutrient loading and deepening the mixed layer. Phytoplankton productivity was unaffected by storms if the mixed layer was deep. After storms, increased incoming shortwave radiation and hypolimnetic nutrient concentration stimulated growth, whereas increased surface water temperatures lowered phytoplankton concentration. More phytoplankton biomass was produced by medium-intensity wind than by high-intensity wind. Mesman et al. (2021) demonstrate that the effects of storms on phytoplankton are complex and are likely to vary according to local environmental variables.

21.5.8 Anthropogenic impacts

Aquatic ecosystems occupy more than 70% of the earth's surface area, providing not only fundamental requirements like food, energy, and medicine but also regulatory and ecological support services that are critical to the world's ecological processes and functions. Thus ecosystem health is an important factor in the health and survival of humans. The fisheries and aquaculture industries continue to play a significant role in ensuring the world population's food security, mostly through the production of fish and shellfish. This aquatic ecosystem—aquatic food connection is vulnerable to unsustainable human activities, climate change, and unexpected disasters like the COVID-19 pandemic (Yusoff, Abdullah, Aris, & Umi, 2021). According to Vörösmarty et al., (2010), human-induced disturbances, such as the release of chemical pollutants, have harmed aquatic ecosystems around the world and resulted in habitat degradation as well as a considerable reduction in aquatic ecosystems' function and service. Biodiversity is well-known in flowing water settings, such as rivers and streams (Vörösmarty et al., 2010). Unfortunately, flowing areas such as rivers are the most threatened habitats because of the considerable anthropogenic disturbances they get in their large basins, such as the run-off from agriculture activity, and industrial and domestic sewage (Xiong et al., 2019). There have been numerous routine monitoring programs to determine the variation of physicochemical parameters effect caused by chemical pollution in numerous rivers, streams, coastal areas, open oceans, and affected locations (Xiong et al., 2019).

Regardless of whether the contaminants are chemical or solid, aquatic areas are sponges for anthropogenic contamination such as industrial sewage and leakage (Bhat et al., 2017). For example, they can contain a wide range of pollutants like a toxin, such as lead and mercury, as well as plastic and nonplastic debris (Hampel, Blasco, & Segner, 2015). Aquatic habitats have been targeted by a wide range of toxins and their devastating effects of strontium, cesium, and radon are among the radioactive elements that reach aquatic ecosystems, as well as metals, which are cadmium and mercury; organic compound waste from industrial activities, such as chlorofluorocarbons, trichloroethylene and tetrachloroethylene, xylenes, formaldehyde, and benzene; waste from agriculture activity such as fertilizers and pesticides;

household products such as wash detergents and cleaning products; and fuel substances such as nitrogen oxides, sulfur oxides, hydrocarbons, carbon dioxide (CO₂), and carbon monoxide (Bashir et al., 2020).

In recent studies, microplastics have been found to be harmful to wildlife in the environment. In light of the high consumption of seafood and the high incidence of microplastics in marine animals that humans eat, studies on effects of microplastics on human health were concerned. The term “microplastics” refers to plastic particles of a diameter less than or equal to 5 µm, with no maximum limit (Gesamp, 2016). Two types of plastics pollution were found in marine environment, which are pieces of larger plastic debris or preexisting particles that are as small as micro- or nanometers in size. According to Fendall and Sewell (2009) and Andrady (2011), packaging and beverage bottles; synthetic textiles; vehicle tires; personal care and cosmetics product, such as facial cleansers, bath gels, and toothpaste; and electronic equipment are all examples of microplastics that were found polluted the aquatic environment. Thus microplastics are an extremely diverse collection of small particles of plastics that have various sizes, shapes, and chemical contents (Andrady, 2017; Hidalgo-Ruz, Gutow, Thompson, & Thiel, 2012). A wide range of commercially valuable animals, including fish, such as Atlantic cod and horse mackerel, European pilchard, red mullet, and European sea bass; bivalves, such as mussels and oysters; and crustaceans, such as shrimp, have been found to have microplastic ingestion (Bellas, Martínez-Armental, Martínez-Cámara, Besada, & Martínez-Gómez, 2016; Bessa et al., 2018; Bråte, Eidsvoll, Steindal, & Thomas, 2016; Güven, Gökdağ, Jovanović, & Kideys, 2017).

In other words, despite the well-known implications of pollution from chemicals in habitat degradation, responses of biotic to water pollution in human-affected ecosystems, particularly at the community level, have rarely been studied (García-Chicote, Armengol, & Rojo, 2017; Xiong et al., 2019; Zhao et al., 2017). According to a study by Jeppesen, Nøges, and Davidson (2011), water pollution caused by human activities causes biotic reactions in aquatic ecosystems, which are essential for determining environmental degradation because rise in environmental stresses has a detrimental effect on aquatic biota, and biological integrity is an essential component of ecosystem health. Understanding how anthropogenic disturbance affects aquatic species is crucial for the success of restoration efforts and the wise management of running water ecosystems (Pander & Geist, 2013). Involvement of plankton is crucial in the food web of aquatic animals as they transfer and cycle the nutrient in the water that is important for the sustainable production of small larvae (Du et al., 2015; García-Chicote et al., 2017). It has long been assumed that it is one of the earliest and most sensitive taxonomic groups to be influenced by environmental change, such as changes in aquatic ecosystem trophic structure (Lin et al., 2017). Environmental filtering produced by local pollution restricted zooplankton populations at the river size since different environmental variation was simple to arise as a result of frequent human disturbance at fine geographic scales (Xiong et al., 2017; Yang, et al., 2018b; Yang et al., 2018a). Zooplankton community assembly processes are degraded by anthropogenic environmental perturbations, making them useful markers for tracking community responses at the community level (Ismail & Mohd Adnan, 2016; Xiong et al., 2019).

The shift in indicator species, notably from huge arthropods to tiny plankton, was a significant sign of ecosystem disruptions that connected to increased anthropogenic disturbance in flowing water habitats. Anthropogenic disruptions must thus be carefully explored at several levels, including community, population, and species. These research outcomes should be completely integrated into present control practices to improve our awareness on the impact of anthropogenic disturbance and as well as to enhance the effectiveness of flowing aquatic habitats for the restoration and protection of the aquatic ecosystem.

21.6 Adaptation in aquatic ecosystem recovery through aquaculture activities

The production of food products from aquaculture activity eventually increased from 2001 until 2018 (Food & Agriculture Organization of the United Nations, 2020). Aquaculture activity is typically conducted in three distinct locations such as freshwater area, coastal area, and marine onshore and offshore area (Food & Agriculture Organization of the United Nations, 2020). Asia catches a lot of fish, accounting for 89% of all fish captured in 2018 with China as the leading in the worlds of fish-producing country, with 58% of production followed by other countries such as Thailand, Bangladesh, India, Indonesia, and Myanmar (Food & Agriculture Organization of the United Nations, 2020). Capture fisheries and aquaculture are two industries that work together to create and deliver food. More than 3.2 billion people acquire their protein from fish. Fish consumption of ~9 kg per individual from the year 1961 grew up to ~20.3 kg per individual in the year of 2020 (Food & Agriculture Organization of the United Nations, 2020). Fisheries and aquaculture provide 35%–38% of the world’s food supply, and they produced \$152 billion in exports in 2017 (Food & Agriculture Organization of the United Nations, 2020). Most of the people in the world who work in fisheries and aquaculture reside in Asia. The rest dwell in Africa (10%), Latin America and the Caribbean (10%), and other regions of the world (4%). Aquaculture has made a lot of technical development during the previous several years. This

allows the sector to increase its existing production to satisfy the need for seafood (Kobayashi et al., 2015; Maulu et al., 2021; Morris, Backeljau, & Chapelle, 2019). Nevertheless, the changes in climate conditions have become a huge challenge for the maintenance of food production activity including from aquaculture sectors (Food & Agriculture Organization of the United Nations, 2020; IPCC, 2018).

Aquaculture operators may make a significant contribution to mitigate the climate change impact by modifying the operation procedures to lower the quantity of harmful gases such as greenhouse gases (GHGs) discharged into the atmosphere. To reduce air and water pollution to a minimal, culture methods and technologies must be more environmentally friendly. Innovative technologies such as the use of RAS and solar energy must be employed (Barange et al., 2018). Aquaculture's primary source of pollution is caused by feed production and use since the floating feeds might cause high water pollution compared to the use of sinking feeds (Maulu et al., 2021). However, that is not to claim that mitigation is a solution for climate change in the short run. The advantages of mitigation may take several years to manifest (Elum, Modise, & Marr, 2017; Maulu et al., 2021). As a result, successful mitigation efforts must be coordinated globally, as GHGs were accumulated from time to time across the globe (IPCC, 2014). Since mitigation and adaptation measures should be utilized in tandem, more effective outcomes will be obtained (IPCC, 2019).

Finally, it is becoming evident that climate change poses a threat to the entire aquaculture value chain. However, most of the literature now available discusses simply how aquatic items are manufactured and not how they are sold or utilized. Due to the restricted emphasis on scientific study, it is difficult to determine the extent to which the aquaculture business will be impacted and how we might react. As a result, future research and models should encompass the whole aquaculture value chain. As the aquaculture business expands and the consequences of climate change become more apparent, it is necessary to conduct a more comprehensive examination of how climate change will influence aquaculture and how to mitigate its effects. As a result, initiatives for mitigation and adaptation would be more effective. The summarization of explanations and examples of adaptations applied in aquaculture industries has been explained in Table 21.2.

21.6.1 Prefeed technology; enrichment and potential culture species

Feeding technology and enrichment need to be made to improve aquaculture products and to help fight climate change. This is one of the steps that can be taken to make food for humans more sustainable. The best way to improve aquaculture is to feed the animals before they are grown, especially the first starter diet, which is made up of things like zooplankton, with a good, nutritious food (Suhaimi et al., 2022). On the other hand, adding physical enrichment to a hatchery environment can make fish or crustaceans that are more adaptable to their environment (Moberg, Braithwaite, Jensen, & Salvanes, 2011). So, to improve survival after being released, one can try to make the hatchery look like a natural environment. Having a lot of different things happen in the fish's environment may help them be more flexible and adaptable (Moberg et al., 2011). It has been demonstrated that structural enrichment makes fish more adaptable in terms of feeding, aggressiveness, and stress recovery (Salvanes, Moberg, & Braithwaite, 2007; Strand et al., 2010). All of these variables appear to have a role in the boldness or caution complex. They were more adaptable when placed in a simple plain test setting; fish from enriched backgrounds could switch from shoaling to more individual behavior. Fish kept in simple tanks remained together regardless of the surroundings. Individuals who raise fish in an enriched habitat are more territorial, whereas fish bred in a simple, open-water setting remain in the same location regardless of the surroundings. Salvanes and Braithwaite (2005) revealed that supplementing cod fish diets enhanced their hostility and their use of refuge following an attack. Enriched fish were less likely to flee attacks and attacked more plain fish, although plain fish were substantially larger. This reveals that fish have both a territorial sense and the capacity to assess their opponents during a fight.

Temperature variations are quite prevalent in aquatic habitats. They might be seasonal or occur on a daily basis. Fish, which are critical to aquatic ecosystems, are unable to escape temperature fluctuations. On the other hand, fish can only survive by constantly adjusting to temperature variations. Generally, it is assumed that living things have more "adaptability and dispersal" (Rahman et al., 2021). Growing tilapia is one strategy to mitigate the climate change impact on the aquaculture sectors (Mtaki et al., 2022). According to Rahman et al. (2021), tilapia aquaculture has enormous potential because it can be produced in freshwater, brackish water, or saltwater. The aquaculture of tilapia is critical for fish production, distribution, marketing, and consumption. This increases the worth of the fish and also increases the amount of food available (Ahmed, Young, Dey, & Muir, 2012). Because tilapia contributes significantly to food production, it is frequently referred to as "aquatic chicken" (Maclean, 1984). Indeed, tilapia, like chicken, may be an excellent source of affordable animal protein. Many people refer to tilapia as the

TABLE 21.2 Adaptations in aquaculture industries.

Impacts	Description	Examples
Prefeed technology; enrichment and potential culture species	- Feeding technology and enrichment is the best way to improve aquaculture, which is to feed the animals before they are grown, especially the first starter diet, which is made up of things like zooplankton, with a good, nutritious food (Suhaimi et al., 2022). - Structural enrichment makes fish more adaptable in terms of feeding, aggressiveness, and stress recovery, especially when placed in a simple plain test setting; fish from enriched backgrounds could switch from shoaling to more individual behavior. Individuals who raise fish in an enriched habitat are more territorial, whereas fish bred in a simple, open-water setting remain in the same location regardless of the surroundings (Salvanes et al. 2007; Strand et al. 2010).	Previous study has revealed that supplementing cod fish diets enhanced their hostility and their use of refuge following an attack (Salvanes and Braithwaite, 2005).
	Fish, which are critical to aquatic ecosystems, are unable to escape temperature fluctuations. On the other hand, fish can only survive by constantly adjusting to temperature variations. Generally, it is assumed that living things have more “adaptability and dispersal” (Rahman, Shahjahan, & Ahmed, 2021).	- Growing tilapia is one strategy to mitigate the climate change impact on the aquaculture sectors (Mtaki, Limbu, Mmochi, & Mtolera, 2022). - Tilapia rearing at low temperatures (10°C), has reveals that immunology, and genes involved in nucleic acid and protein synthesis, amino acid metabolism, lipid and carbohydrate contents are activated (Yang et al., 2015). - Tilapia is a species that thrives at high temperatures, i.e., <i>T. mossambica</i> dies at least 38°C at this point (Allanson & Noble, 1964). - Tilapia can tolerate temperatures as high as 34°C without significantly affecting their development rate (Xie et al., 2011; Islam et al., 2020).
Innovative aquaculture smart technology; RAS	- Aquaculture is conducted in a range of ecosystems and climates, ranging from various types of water with different climate and weather conditions which is from river, lake, and stream to the ocean, and from Atlantic to tropical environment, comprising ~622 fish and crustacean species cultivated worldwide (FAO, 2020). - Aquaculture operations were impacted by changes in the weather, such as flooding, rainfall, drought, storms, hurricanes, typhoons, rise in temperature, fluctuations of salinity gradient, acidification of ocean, and rises of sea level (Ahmed et al., 2019). - RASs may be a feasible adaptation method in light of environmental concerns and the susceptibility of aquaculture fish production on the impact of different environmental changes and climate conditions (Bueno & Soto, 2017; Ahmed et al., 2019).	- Indoor aquaculture farms or RAS technology is innovative aquaculture technology that is used in closed rearing systems and is added with a biofiltration system to improve the water quality and eliminate harmful substances from the rearing system. These fish farms depend on confined ways of raising (Bostock et al. 2010; Espinal & Matulic, 2019). - RASs are increasingly being utilized to house young Mediterranean marine fish and salmonids before they were transferred to outdoor grow-out systems such as cages or flow-through raceways (Terjesen et al., 2013; Bostock, Lane, Hough, & Yamamoto, 2016; Clarke & Bostock, 2017). - In cage and net-pen aquaculture, RAS may be employed to create broodstock and seedstock (Malone, 2013). RASs were created in Europe and North America as an alternative to cage salmon farming (Murray, Bostock, & Fletcher, 2014). This technology was created to generate alien fish species without hurting native species or degrading the natural environment’s biodiversity.

(Continued)

TABLE 21.2 (Continued)

Impacts	Description	Examples
Probiotics assist in sustainability of aquaculture activity	- Beneficial bacterial candidates are created predominantly by terrestrial microorganisms acting as natural defensive mechanisms for cultivated species. - Probiotics have garnered substantial interest in aquaculture as a disease-preventive approach (Newaj-Fyzul & Austin, 2015). - Prebiotics, medicinal herbs, probiotics, and immunostimulants have recently gained appeal in aquaculture as functional feed additives (Adel, Yeganeh, Dadar, Sakai, & Dawood, 2016). - Probiotics may interact with or antagonize other gut microbes by resisting colonization or directly suppressing and reducing the development of opportunistic illnesses. Additionally, probiotics may benefit the host's health by regulating physiological or immunological functions (Dawood et al. 2016 c,d; Adel, Caipang, & Dawood, 2017a). - Probiotic may produce substance that function as a bactericidal agent against pathogenic bacteria in the host's gut, so serving as a barrier to the development of opportunistic infections (Marañón, Lorenzo, Cermeño, & Mouriño-Carballido. 2012).	- Antibiotics, bacteriocins, siderophores, enzymes (lysozymes, proteases), and/or hydrogen peroxide are only a few of the useful molecules produced during bactericidal action, as is the intestinal pH adjustment caused by the creation of organic acids (Verschuere, Rombaut, Sorgeloos, & Verstraete, 2000). - The inclusion of probiotics in aquafeeds reduces the prevalence of intestinal diseases in a range of cultured species (Vijayabaskar & Somasundaram, 2008; Dahiya, Sihag & Gahlawat 2012; Sihag & Sharma, 2012). - Utilization of probiotics that have bacterial cells, such as <i>Bacillus</i> sp., <i>Lactococcus</i> sp., <i>Micrococcus</i> sp., <i>Carnobacterium</i> sp., <i>Enterococcus</i> sp., <i>Lactobacillus</i> sp., <i>Streptococcus</i> sp., and <i>Weissella</i> sp., helps the aquatic animal resistance (Gatesoupe, 1999).
Agro-based resources as potential sources for aquafeed	- In response to recent climate changes, the need to produce healthy and organic food for humanity, and achieve food sustainability targets that emphasize the development of improved food sources using viable and appropriate techniques, primary protein source producers (livestock, cows, and fish farming industries) are being asked to make a massive effort to secure protein-mediated amounts at a low cost. - Mushroom farming is another lucrative endeavor that utilizes rice or wheat straw to provide a high-protein supplementary food source while also allowing ecologically friendly and sustainable agricultural waste disposal (Chang and Wasser, 2017). - Mushrooms are an essential nutrient and other essential nutrients that are low in calories, and many species have therapeutic characteristics. As a consequence, mushroom factories create huge amounts of agricultural wastes defined as spent mushroom substrate, SMS, after each harvesting session (Muchena, Pisa, Mutetwa, Govera, & Ngezimana, 2021). - SMS has already been shown in testing to be a viable source of animal food (Deblais et al., 2018). SMS may also be utilized as an aquaculture feedstock.	- Aquaculture may benefit from the usage of <i>Yucca schidigera</i> and its products, which are medicinal plants that have a variety of useful features when utilized in aquaculture (Adegbeye et al., 2019). - The usage of yucca improves protein metabolism in the fish muscle, perhaps resulting in a reduction in ammonia output (Paray et al., 2021). <i>Agaricus bisporus</i> substrate has been investigated as a food supplement for carp (<i>Cirrhinus cirrhosus</i>) (Nepomuceno, Brown, & Brown, 2021). - Due to the enormous difficulty faced by tilapia farmers in maintaining feeds (72% of total production expenses) as reported by the Asian Development Bank in 2004, employing SMS as a feed supplement is a more cost-effective solution. When SMS was utilized as a fish feed, tilapia growth and immune responses were enhanced (Van Doan, Hoseinifar, Tapingkae, Chitmanat, & Mekchay, 2017). - SMS has also been used as a component of carp diet (<i>C. mrigala</i>) (Nepomuceno et al., 2021). Leyva and colleagues in 2020 mentioned that a particular formulation containing 5% SMS and other agricultural and animal wastes combined with rice bran increased carp development.

(Continued)

TABLE 21.2 (Continued)

Impacts	Description	Examples
The use of fermentation technique in aquafeed as potential strategy for sustainable aquaculture	• Aquafeed costs have risen dramatically in recent years, increasing the entire cost of aquatic animal farming (Hodar, Vasava, Mahavadiya, & Joshi, 2020). Alternative strategies for substituting unorthodox feed items were tried (Montoya-Camacho et al., 2019). • Fermentation improves vitamin availability, protein digestibility, and amino acid levels in feedstuffs, as well as their palatability and fermentation may be used to enhance organic compounds, ammonium, protein, fibers, and calcium (Zhu et al., 2020).	• Bacterial cells, such as <i>Bacillus</i> sp., <i>Enterococcus</i> sp., and <i>Lactobacillus</i> sp. cells, yeast such as <i>Saccharomyces</i> sp., and fungal such as <i>Aspergillus</i> sp. have all been used during fermentation (Chavez-López et al., 2020).

RAS, Recirculating aquaculture system.

“everyman’s fish” (Smith & Pullin, 1984). A study of tilapia rearing at low temperatures (10°C) has revealed that immunology and genes involved in nucleic acid and protein synthesis, amino acid metabolism, lipid, and carbohydrate content are activated (Yang et al., 2015). Acutely low temperatures of 8°C can injure the kidneys of tilapia and impair the immune system in their kidneys, resulting in their death (Zhou et al., 2019). Tilapia is a species that thrives at high temperatures. The temperature at which *Tilapia mossambica* dies is at least 38°C at this point (Allanson & Noble, 1964). Tilapia can tolerate temperatures as high as 34°C without significantly affecting their development rate (Islam et al., 2020; Xie et al., 2011). Finally, tilapia is an excellent aquaculture fish for responding to current and future climate change.

21.6.2 Innovative aquaculture smart technology; recirculating aquaculture system

Aquaculture is conducted in a range of ecosystems and climates, ranging from various types of water with different climate and weather conditions which is from river, lake, stream to the ocean, and from Atlantic to tropical environment, comprises ~622 fish and crustacean species cultivated worldwide (Food & Agriculture Organization of the United Nations, 2020). Aquaculture utilizes a variety of agricultural technologies and practices and cultivates a varied array of species. By 2020, Asia will account for 89% of global aquaculture production (Food & Agriculture Organization of the United Nations, 2020). Aquaculture is crucial for increasing food production and guaranteeing a healthy source of protein for human needs (Allison, 2011; Béné et al., 2016). There is still more to be done to feed the world’s growing population, which is expected to reach 9.7 billion by 2050 (United Nations, 2019). This is a legitimate concern that has considerable weight in government and researcher debates around the world (Berners-Lee et al., 2018; Ehrlich & Harte, 2015). Global aquaculture production would be unable to meet ambitious objectives such as 109 million tons in 2030 and 140 million tons in 2050 due to the growing global population and stagnating fish populations (Food & Agriculture Organization of the United Nations, 2020).

On the other hand, environment climate change has a substantial influence and become a vital threat to the global production of aquaculture products (Brander, 2007; De Silva & Soto, 2009). Aquaculture operations were impacted by changes in the weather, such as flooding, rainfall, drought, storms, hurricanes, typhoons, rise in temperature, fluctuations of salinity gradient, acidification of the ocean, and rises in sea level (Ahmed et al., 2019). Aquaculture farmers in the coastal area of the sea are more vulnerable to the changes of climate in the surrounding compared to the land culture, and this unstable climate condition will have a detrimental effect on aquaculture production in the future (De Silva & Soto, 2009; Ahmed et al., 2019). It is vital to design and deploy adaption approaches to overcome these difficulties. RAS may be a feasible adaptation method in light of environmental concerns and the susceptibility of aquaculture fish production on the impact of different environmental changes and climate conditions (Ahmed et al., 2019; Bueno & Soto, 2017). Cultivations of fish or crustaceans through RAS definitely can improve the production since it can be done on land-based or indoor cultivation, and this adaptation of aquaculture technology can indirectly minimize the effect of natural climate conditions on aquaculture production.

Indoor aquaculture farms or RAS technology is innovative aquaculture technology that uses close rearing systems and is added with a biofiltration system to improve the water quality and eliminate harmful substances from the rearing system. These fish farms depend on confined ways of raising (Bostock et al., 2010; Espinal & Matulić, 2019). RAS, which recycle water using mechanical or biological filters, may be expensive to install and run, but they are incredibly productive and ecologically benign. The technology of RAS system has improved dramatically over the previous two decades, and this cultivation system was increasingly used among aquaculture farmers in recent years. The use of RAS is mandated by biophysical conditions, such as insufficient water supply, poor water quality, and an adverse climate (Murray et al., 2014). As Malone (2013) said, RAS may be used as a cost-effective alternative to more traditional ways of production when environmental restrictions, disease outbreaks, availability of land, salinity fluctuations, unstable temperature, and insufficient water supply impose constraints on more conventional techniques. On the other hand, RAS were developed and used for a variety of reasons. RAS are increasingly being utilized to house young Mediterranean marine fish and salmonids before they were transferred to outdoor grow-out systems such as cages or flow-through raceways (Bostock et al., 2016; Clarke & Bostock, 2017; Terjesen et al., 2013). In cage and net-pen aquaculture, RAS may be employed to create broodstock and seedstock (Malone, 2013). RAS were created in Europe and North America as an alternative to cage salmon farming (Murray et al., 2014). This technology was created to generate alien fish species without hurting native species or degrading the natural environment's biodiversity (Ahmed & Turchini, 2021).

Aquaculture solutions proposed so far will boost world fish output while also enhancing the sustainability of aquatic animal production and climate resilience. Nevertheless, the help from a researcher on technical protocols, operation cost, and cultural techniques was important to guarantee the success of these initiatives. Therefore other stakeholders from the public and private sectors, as well as the aquaculture industry, also must cooperate. Adaptation solutions and research must be founded on a thorough understanding of the underlying social, economic, and environmental challenges to improve the goods production from aquaculture industry and indirectly improve the sustainability of aquatic sources from natural environment.

21.6.3 Probiotics assist in sustainability of aquaculture activity

Accomplishment of aquaculture activity to produce food for human consumption was efficient when assisted with natural materials such as organic products compared to the use of chemical or synthetic medicine. Apart from the challenging issue of feeding and supporting the world's rapidly rising population, aquaculture is vital for ensuring that development is ecologically sustainable, notably in the production of aquafeeds. Beneficial bacterial candidates are created predominantly by terrestrial microorganisms acting as natural defensive mechanisms for cultivated species. Probiotics have lately gained popularity as helpful microorganism options for preserving aquatic animals' health and well-being in cultured organisms. The purpose of this study was to establish the critical nature of using probiotics as a long-term strategy for controlling and sustaining the growth of fish or crustaceans, consumption of food, and survivability in culture system. Additionally, an explanation of the host microbiota's ability to produce diverse probiotic strains is presented, as is the probiotics' capacity to enhance host immunity and exert interaction effects on host-derived probiotics. Aquaculture is crucial for the world's rising population's long-term livelihood prospects and food security (Wang et al., 2018).

As a consequence, aquaculture activities have been escalated for the maintenance of productivity, resulting in increased aquatic animal and environmental stress (Dossou et al., 2018a), disease outbreaks increased, eroding output, and endangering the long-term sustainability of aquaculture. To manage aquatic organism illnesses, chemical compounds and antibiotics such as sulphadiazine, chlortetracycline, sulphamethoxazole, co-trimoxazole, amoxicillin, and oxytetracycline have been utilized (Guardiola et al., 2016). Nevertheless, their use resulted in a host of problems, and their hazards may have an indirect effect on human health, and it has become vital to discover natural alternatives and growth boosters for healthy fish and crustaceans. By improving immunity resistance, helping in digestibility, and improving immunity defense system against infections, host terrestrial microbes contribute significantly to aquatic animals' health maintenance. Bacterial ability to interact with the digestive system dictates how the intestinal microbiota functions, which may benefit the host by regulating biological activity (Ramrez & Romero, 2017). Terrestrial microorganisms may aid in feed consumption by providing vitamins and digestive enzymes, as well as the capacity of nutrients to compete for adhesion sites. Additionally, it has been shown that antimicrobial chemicals produced by gut microorganisms enhance immune responses while decreasing dangerous bacteria. This community of microbes will exist in various regions of the host; hence, they were divided into two groups: "allochthonous microbiota," that attach to the digested food which moves through the intestinal tract, and "autochthonous microbiota," that reside in the tissues of host (Tarnecki, Burgos, Ray, & Arias, 2017). Due to the unique

nature of the bacteria's colonization, indigenous bacteria had a more beneficial impact in specific conditions than bacteria from other sources.

Aquaculture is quickly becoming the most potential sector for sustaining the production of animal protein and ensuring nutrition and securing food for human consumption. Aquaculture activities have increased in intensity, creating stressful conditions for aquatic species and the environment as a whole (Dawood et al., 2016a,b; Hossain et al., 2016). As a consequence, disease outbreaks are quickly eroding the aquaculture industry's viability. Aquaculture often makes use of chemical compounds and antibiotics to treat infectious diseases (Adel et al., 2016; Dawood et al., 2017b,c). On the other hand, antibiotics may have a detrimental effect on the host and the environment. As a consequence, it is critical to discover safe and effective antibiotic alternatives to grow the aquaculture industry (Yan et al., 2017). One of the most significant advancements in sustainable aquaculture is the use of probiotics as a nontraditional method of increasing growth, the efficiency of nutrients, and the health of fish and crustaceans (Dawood et al., 2016a,b; Van Doan et al., 2017). Due to the importance and convenience of probiotics, it was essential to pay close attention to their methods of action, both in vivo and in vitro, but mainly in the gut environment.

Probiotics have garnered substantial interest in aquaculture as a disease-preventive approach (Newaj-Fyzul & Austin, 2015). In aquaculture, chemotherapeutics have been used for several years; nevertheless, the efficiency of antibiotics is restricted because of adverse reactions such as the emergence of bacteria that resist antibiotics (Dawood & Koshio, 2016b; Adel et al., 2017a; Adel, Yeganeh, Dawood, Safari, & Radhakrishnan, 2017b). Prebiotics, medicinal herbs, probiotics, and immunostimulants have recently gained appeal in aquaculture as functional feed additives (Adel et al., 2016). Probiotics may interact with or antagonize other gut microbes by resisting colonization or directly suppressing and reducing the development of opportunistic illnesses. Additionally, probiotics may benefit the host's health by regulating physiological or immunological functions (Dawood et al., 2016c,d; Adel et al., 2017a).

Probiotics may produce a substance that functions as a bactericidal agent against pathogenic bacteria in the host's gut, so serving as a barrier to the development of opportunistic infections (Martinez Cruz et al., 2012). Antibiotics, bacteriocins, siderophores, enzymes (lysozymes, proteases), and/or hydrogen peroxide are only a few of the useful molecules produced during bactericidal action, as is the intestinal pH adjustment caused by the creation of organic acids (Verschuere et al., 2000). It has been shown that the inclusion of probiotics in aquafeeds reduces the prevalence of intestinal diseases in a range of cultured species (Vijayabaskar & Somasundaram, 2008; Dahiya et al., 2012; Sihag & Sharma, 2012).

Numerous approaches have been studied in aquatic animals for manipulating the gut microbiota, which is crucial to the effectiveness of feed digestion, to improve feed consumption and host health (Burr, Gatlin, & Ricke 2007). Probiotics gained prominence as growth factors in the development of functional diets for a variety of farm and aquatic species (Ouweland, Salminen, & Isolauri, 2002; Dawood & Koshio, 2016a). The word "probiotic" was developed by Parker (1974), and it has been defined by a number of experts as "organisms and chemicals that contribute to gut microbial stability." Probiotics are now defined as a live microbial feed additive that improves the gut microbial balance of the host animal (Fuller, 1989). Probiotics are currently defined as "live microorganisms that provide a health benefit on the host when administered at the recommended doses" (FAO/WHO, 2001). Due to their shared biological system, it has been hypothesized that probiotic bacterial cells administered in an aquatic setting may influence the composition of terrestrial microorganisms (Verschuere et al., 2000). As a consequence, probiotics should be supplied to fish and shellfish larvae that are often released into the aquatic environment at an early ontogenetic stage (Kapareiko et al., 2011). Temperature fluctuations, stress density, and intensity may all have immunosuppression and physiological repercussions, while feed additives with immunomodulatory properties can aid an organism's stress response in becoming more effective (Magnadottir, 2010). Probiotics may be utilized as a microbial dietary supplement to enhance host health by changing mucosal and systemic immunity, as well as nutritional and functional behaviors and microbial balance in the gut (Villamil, Tafalla, Figueras, & Novoa, 2002). For example, utilization of probiotics that have bacterial cells, such as *Bacillus* sp., *Lactococcus* sp., *Micrococcus* sp., *Carnobacterium* sp., *Enterococcus* sp., *Lactobacillus* sp., *Streptococcus* sp., and *Weissella* sp. may help the aquatic animal resistance (Gatesoupe, 1999).

21.6.4 Agro-based resources as potential sources for aquafeed

In response to recent climate changes, the need to produce healthy and organic food for humanity, and achieve food sustainability targets which emphasize the development of improved food sources using viable and appropriate techniques, primary protein sources producers (livestock, cows, and fish farming industries) are being asked to make a massive effort to secure protein-mediated amounts at a low cost. Antibiotic and chemotherapeutic treatments have recently been encouraged to be limited in cattle, dairy, and aquaculture production because of their negative influence on climate

resistance in animals and humans, as well as the risk of pollution of the environment. As a result, natural substitutes for feed additives, immunostimulants, and antioxidants are critical.

Aquaculture may benefit from the usage of *Yucca schidigera* and its products, which are medicinal plants having a variety of useful features when utilized in aquaculture (Adegbeye et al., 2019). Various research works have shown that adding yucca to the meals of aquatic species or adding it to their water increases their performance. The usage of yucca improves protein metabolism in the fish muscle, perhaps resulting in a reduction in ammonia output (Paray et al., 2021). Protein metabolism enhances feed utilization, increasing feed intake and rate of development. As a result, feeding nutritious yucca to aquatic species increases their nutrition, immune response, and antioxidative responses. Yucca is indigenous to the desert of the Southwest United States and Mexico, which are characterized by severe heat, lack of water, and harsh conditions (Becerra-Absalón, Muñoz-Martín, Montejano, & Mateo, 2019). On the other hand, Yucca is well-known for its anti-stressor properties and positive benefits, which makes it an ideal phyto-genic supplement for fish aquaculture (Saeed et al., 2017). Yucca contains a high concentration of saponin and resveratrol, which may help remove ammonia from water and minimize its negative effect on the functioning and security of aquatic animals. As a result, yucca and saponin are two commercial aquatic items used in fishponds and intensive cultivation systems. It increases the efficiency of aquatic species' environment, feed consumption, rate of growth, and antioxidative and immunological capabilities. Additionally, yucca bolsters the immune system's defenses against pathogens and intruders.

Mushroom farming is another lucrative endeavor that utilizes rice or wheat straw to provide a high-protein supplementary food source while also allowing ecologically friendly and sustainable agricultural waste disposal (Chang & Wasser, 2017). Edible mushrooms of the division Basidiomycota exhibit a diverse variety of metabolic capacities, which has a substantial influence on the substrate needed for production. Mushrooms are an essential nutrient and other essential nutrients that are low in calories, and many species have therapeutic characteristics. As a consequence, mushroom factories create huge amounts of agricultural waste defined as spent mushroom substrate (SMS) after each harvesting session (Muchena et al., 2021). After a period of mushroom cultivation is complete, SMS refers to the composted organic growth medium that is left unused (Nepomuceno et al., 2021). One of the difficulties in dealing with SMS is a lack of trash management (Gong et al., 2019). Due to the mushroom industry's abundance, waste control is an issue (Mahari et al., 2020). SMS is mostly used as fertilizer on land, disposed of in situ, or burned (Hanafi et al., 2018). The composition of the SMS is critical in determining its fitness for usage (Hanafi et al., 2018). SMS has already been shown in testing to be a viable source of animal food (Deblais et al., 2018). SMS may also be utilized as an aquaculture feedstock. For instance, abandoned *Agaricus bisporus* substrate has been investigated as a food supplement for carp (*Cirrhina mirigala*) (Nepomuceno et al., 2021). However, various factors should be considered while developing an animal feed diet. Consideration should be given to the animal's species, mushroom strains, SMS nutritive content, cell wall component, digestibility, and voluntary intake. To ensure that SMS and its related commodities continue to be recognized in the future, it is also vital to assess and ensure consistency of SMS effectiveness (Chen, 2021). Due to the enormous difficulty faced by tilapia farmers in maintaining feeds (72% of total production expenses) as reported by the Asian Development Bank in 2004, employing SMS as a feed supplement is a more cost-effective solution. When SMS was utilized as a fish feed, tilapia growth and immune responses were enhanced (Van Doan et al., 2017). Doan and colleagues (2017) noticed a substantial increase in growth and immune response parameters when tilapia was given 1% SMS. SMS has also been used as a component of carp diet (*C. mirigala*) (Nepomuceno et al., 2021). Leyva-López, Lizárraga-Velázquez, Hernández, and Sánchez-Gutiérrez (2020) mentioned that a particular formulation containing 5% SMS and other agricultural and animal wastes combined with rice bran increased carp development. However, a higher proportion of SMS (12%) resulted in a slower rate of development. Increasing death rates are also observed during the warm cropping season leading to a shortage of dissolved oxygen, environmental pollution, and an increase in water temperatures (Roufou et al., 2021).

21.6.5 The use of fermentation technique in aquafeed as potential strategy for sustainable aquaculture

Feeding expenditures account for more than 70% of overall operational costs in aquaculture (Khan, Begum, Nielsen, & Hoff, 2021). Aquafeed costs have risen dramatically in recent years, increasing the entire cost of aquatic animal farming (Hodar et al., 2020). Alternative strategies for substituting unorthodox feed items were tried (Montoya-Camacho et al., 2019). Although uncommon components are affordable, their application in aquafeed is restricted due to their high fiber and antinutritional factor (ANF) concentration, which inhibits feed decomposition (Ghosh, Ray, & Ringø, 2019). ANFs have been eliminated or significantly reduced by the use of thermal processing and microorganisms' fermentation (Dawood & Koshio, 2020). Several ANFs, on the other hand, are resistant to heat. According to earlier studies,

fermentation increased protein content while decreasing fiber content, ANFs, and hazardous levels in feed components. Unusual fermented feedstuffs may be used instead of fish meal (FM) to reduce manufacturing costs (Kader et al., 2019). By-products or waste commodities from agribusiness, farm-made feeds, and manufacturing businesses are known for their low cost and ability to operate as a kind of management of waste in promoting superior cleanliness. For example, all kinds of animal feed ingredients; such as silkworm, maggot, insect, grub, earthworm, snail, tadpoles; and plant wastes such as jack bean, cottonseed meal, soybean meal, *Cajanus*, *chaya*, duckweed, maize bran, rice bran, palm kernel cake, groundnut cake, brewers waste; and as well as wastes from animal sources and food processing for human consumption (Banerjee, 2017). Two significant disadvantages of typical dietary feed components are their dietary fiber contents and low protein content. Fermentation improves vitamin availability, protein digestibility, and amino acid levels in feedstuffs, as well as their palatability and fermentation, may be used to enhance organic compounds, ammonium, protein, fibers, and calcium (Zhu et al., 2020). Bacterial cells, such as *Bacillus* sp., *Enterococcus* sp., and *Lactobacillus* sp. cells, yeast such as *Saccharomyces* sp., and fungal such as *Aspergillus* sp. have all been used during fermentation (Chaves-López, Rossi, Maggio, Paparella, & Serio, 2020). To ensure the global aquaculture industry's continued development, a potential alternative to FM is to use economical, readily accessible, and fermented protein sources that improve disease resistance in aquatic animals by boosting growth, intrinsic defense systems, and well-being (Dossou et al., 2018b). In contrast to poultry and cattle, there is currently a dearth of knowledge on fermented feed for aquatic animals.

21.7 Aquafeed: halal status and its contribution to aquaculture industry

Animal feed is considered critical aspects in halal supply chain and one of the critical control points in animal production. It is now necessary to establish a halal standard for feed to guarantee a halal and tayyib food supply chain from farm to fork. This is due to repeated instances of improper feeding practices in aquaculture production that have been reported in the media and the gazette of the fatwa that forbids feeding cultivated fish with nonhalal feed. The animals that live with the consumption of filth and impurities which as a result produce the unpleasant smell and changes to its meat are referred as al-jallalah (Saidin et al., 2022). In Malaysia, despite the country's established legal and regulatory framework regarding animal feed, none of the laws specifically address the challenges of halal animal feed with regard to its components, processing, manufacture, transportation, and storage (Saidin & Rahman, 2016). Some feed millers and producers are unaware of the significance of halal feeding for animal production because they lack understanding and exposure to the Shariah perspective of halal animal feed and feeding among industry stakeholders (Zailani et al., 2010). Therefore it is thought that one of the main causes of incorrect feeding practices and fraud in the manufacturing of animal feed is the lack of particular halal rules and regulations relevant to the production and use of halal animal feed (Saidin et al., 2022). In MS1500:2019, Malaysian halal food production standard, clauses 4.5.1.1.1 (h) and 4.5.1.1.2 (c) stated that farmed halal animals and aquatic animals that are intentionally and continuously fed with najis are considered to be nonhalal and should not be consumed or used as food ingredients. Additionally, the feed must be free of porcine and its derivatives as well as not contain filthy sources to qualify for Malaysian Good Agricultural Practices (MyGAP) accreditation (Department of Standards Malaysia, 2017).

Based on the viewpoints and recommendations offered by the Islamic scholars, the adoption of the al-istibra' or quarantine method is regarded as a good approach to ensure that the animal-based goods are halal. A proper quarantine process should be used as a purification method to remove contaminations from jallalah animals. Thorough evaluation of halal critical points along the feed supply chain from farm to fork, good animal feeding practices at the farm level, and good manufacturing practices during the manufacturing process and distribution of animal feed should all be implemented by those involved in the animal feed industry collectively (Saidin et al., 2018). Hence, it is wise to include a guideline that would increase the manufacturing of safer and halal aquaculture feed, given the urgent requests and needs among feed manufacturers and farmers, as well as animal feed and livestock industries as a whole.

21.8 Conclusion

The current review emphasizes the recent development and obstacles in maintaining a healthy ecosystem for the sustainable development of aquatic resources. The ecological-network functions determine the stability of aquatic food web with plankton as primary producer and the basal source of food for most organisms in the aquatic environment. The disturbance to aquatic community certainly affects the healthiness of the ecosystem especially when climate change factors such as rising temperature, ocean acidification, HABs, diseases, rainfall variability, as well as sea level rise had given a negative impact on maintaining the sustainability of the aquatic biota. Furthermore, the vast anthropogenic impacts on aquatic organisms have also come into the circumstances of damaging the overall aquatic populations. To

mitigate the current scenario by sustaining the fisheries resources through aquaculture activities, the latest development of aquaculture through systematic culture technology, feed technology, and agro resources to be applied in aquaculture activities has risen many potentials for overall resources sustainability.

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Treated waste-based planting media for sustainable agricultural activities to enhance halal food production

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22.1 *Istihalah* (agent of change) involved in treated sewage sludge for agricultural activities

Istihalah means transformation and change of a substance into another substance in terms of composition and properties (Kashim et al., 2015). The *istihalah* concept involves a complete transformation of a substance through a certain process which changes its original properties into the present properties (Fig. 22.1). The phase of using a natural agent of change involves mixing and transformation processes in scientific terminology, the *istihalah* concept involves the aspect of chemical decomposition.

22.2 *Istihalah* concept and use of animal waste as plant fertilizer

Muslim scholars unanimously agree that the *istihalah* concept is change in physical form and content (Nuryani & dan Kashim, 2014). Change in physical form and content encompasses total transformation causing the unclean source to be no longer identifiable. Al-Zuhayli (1997) held the opinion that the classic method of *istihalah* may be viewed through the example of animal waste which changed to become ash due to a burning process. The unclean source here means the waste of cows, goats, chicken, pigs, and so on, which changed physically in form and composition so that the original source is no longer identifiable.

It needs to be understood that the *istihalah* unanimously agreed upon by scholars as clean and permissible for Muslims is *istihalah tammah*. In the meantime, *istihalah naqisah* is being debated by scholars (Kashim, Alias et al., 2018; Kashim, Hasim et al., 2018). In the concept of *istihalah tammah* or known as *istihalah sahihah* process, transformation occurs when there is a change in the original nature of the unclean substance into a new clean substance. This reaction occurs due to mixing of unclean substance with a change agent from natural and chemical substances such as soil, minerals as well as clean plant waste materials resulting in a new and clean substance (Fig. 22.2), for example, fruit trees fertilized with pig waste fertilizer. After the pig waste is mixed with soil that contains microorganisms for decomposition through the process of chemical degradation, the nutrients will be absorbed by the roots of the fruit trees and finally results in quality fruits beneficial for human consumption. In this situation, the fertilizer, which was originally *haram* pig waste, plays the role of an external agent to fertilize the soil and produce high-quality fruits (Nuryani & dan Kashim, 2014).

The *istihalah* concept is said to occur when unclean animal waste such as from cows, goats, chicken, pigs, and so on is processed and changed into a beneficial end substance for human use as plant fertilizer. The animal waste has changed its properties to become a new substance which is clean and beneficial for humans to use. However, the legality depends on the transformation which occurred. The transformation must be total so that the original substance is no

ORIGINAL SUBSTANCE → AGENT OF CHANGE → FINAL SUBSTANCE

FIGURE 22.1 *Istihalah* concept (Nuryani & dan Kashim, 2014).

ORIGINAL SUBSTANCE (Haram) → AGENT OF CHANGE (Mixing Process) → FINAL SUBSTANCE (Halal)

FIGURE 22.2 Concept of *istihalah sahihah* (Nuryani & dan Kashim, 2014).

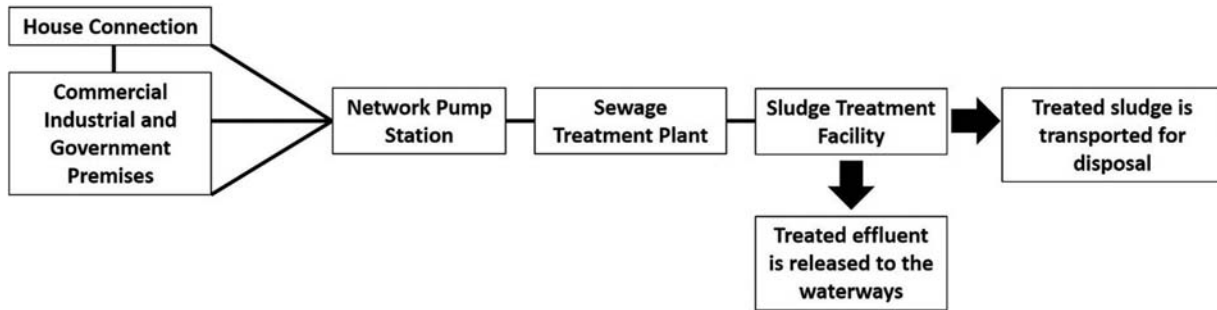


FIGURE 22.3 Process of producing treated wastewater (effluent) and treated residual sludge by sewerage company, Indah Water Konsortium Sdn. Bhd (Indah Water Konsortium Sdn, 2010).

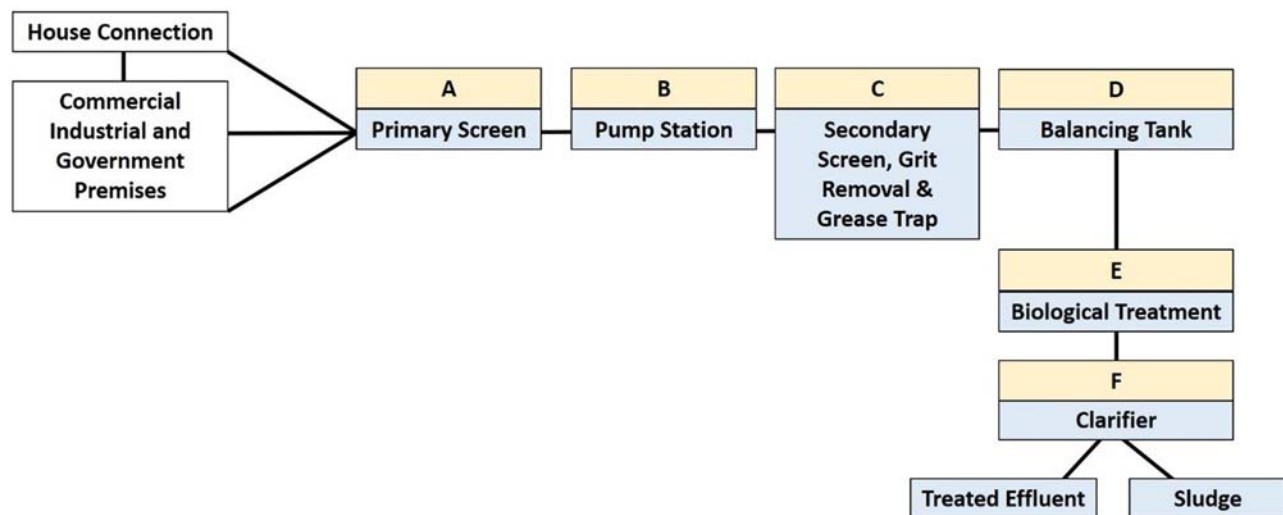


FIGURE 22.4 Wastewater goes through various processes including primary filtering process, secondary filtering, grit and grease filtering, sedimentation process, biological treatment and water purification, to produce treated wastewater (effluent) and treated residual sludge (Indah Water Konsortium Sdn, 2010).

longer identifiable whether through the methods of polymerase chain reaction (PCR) or real-time PCR (Sano, Fukushi, Yoshida dan, & Omura, 2003; Tsai, Palmer, dan, & Sangermano., 1993). If the transformation is total, then the legal rule is that it is clean and *halal* for Muslims to use (Kashim, Alias et al., 2018; Kashim, Hasim et al., 2018).

However, discussion on this issue is not yet settled as some scholars' dispute whether the legal rule on using animal waste is the same as for human waste. This is because the same *istihalah* method is applied to human waste as is done to animal waste. Human waste processed in a sewage treatment plant produces treated residual sludge used for agricultural and modern landscaping plant fertilizer in developing countries such as United States, European Union, Japan, Australia, New Zealand, and Singapore (Kirchmann, Borjesson, Katterer, & Cohen, 2017; Lamastra, Suci, & Trevisan, 2018; Stabnikova, Goh, Ding, Tay, & Wang, 2005; Christoudoulou & Stamatelatou, 2016).

22.3 Concept for using human waste (treated sewage sludge) as plant fertilizer

The sewerage company, Indah Water Konsortium Sdn. Bhd., is responsible for managing sewage treatment plants in Malaysia. After wastewater is treated at the sewage plant, the resulting effluent is released into rivers and treated residual sludge is disposed of in a landfill (Figs. 22.3 and 22.4). The treated sewage sludge goes through a thickening

process, digestion process, polymer addition process, and mechanical dewatering process to produce 20%–25% dry solids from treated sewage sludge (with 75%–80% moisture content) before being disposed off at a landfill (Fig. 22.5).

In Malaysia, 3,000,000 metric tons of treated residual sludge are produced yearly and the amount is expected to increase to 7,000,000 metric tons by the year 2020 (Indah Water Konsortium Sdn, 2010). The urbanization process and economic development, aligned with population increase are the main factors for the yearly increase in treated residual sludge. Estimated cost for disposal of treated residual sludge at the landfill is USD 0.33 billion since the year 1998 and the management cost continues to increase from year to year (Kadir & Mohd, 1998).

22.4 The chemical composition of treated residual sludge

Treated residual sludge contains organic substance based on macronutrient, micronutrient, and trace elements sources beneficial for planting (Table 22.1). The application of treated residual sludge on agricultural land or landscaping plants

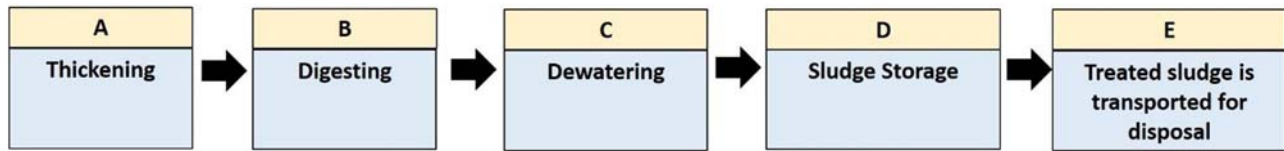


FIGURE 22.5 Treated residual sludge goes through thickening process, digestion process, polymer addition process, and mechanical dewatering process before being transported by lorry to a landfill (Indah Water Konsortium Sdn, 2010).

TABLE 22.1 Chemical properties of treated residual sludge after mechanical dewatering process (20% solid, 80% moisture).

Chemical properties	Symbol	Average value	Reference value
pH (H ₂ O)	—	6.4	3.8–6.9 ^a
EC (mS/cm)	—	4.2	—
Carbon (%) amount	C	20	5.2–51 ^a
Nitrogen amount	N	2.8	0.5–5.9 ^a
Carbon/nitrogen ratio	C:N	7.4	3.4–35.4 ^a
Phosphorus (%)	P	1.2	0.24–0.78 ^b
Potassium (%)	K	0.4	0.05–0.12 ^b
Calcium (%)	Ca	0.03	0.3–1.2 ^b
Magnesium (mg/kg)	Mg	668	112–1575 ^b
Iron (%)	Fe	2.9	1.9–4.0 ^b
Copper (mg/kg)	Cu	113	63–190 ^b
Manganese (mg/kg)	Mn	683	32–362 ^b
Sodium (mg/kg)	Na	388	—
Zinc (mg/kg)	Zn	771	153–3218 ^b
Nickel (mg/kg)	Ni	125	13–26 ^b
Aluminum (%)	Al	2.8	—
Cadmium (mg/kg)	Cd	2.3	0.5–5.8 ^b
Chromium (mg/kg)	Cr	177	—
Plumbum (mg/kg)	Pb	40	36–72 ^b

^aRosazlin, Fauziah, Rosenani, and Zauyah (2005).

^bRosenani, Kala, and Fauziah (2008).

improves the quality of land structure and organic contents in soil (Chu & Wong, 1987). Moreover, treated residual sludge contains organic substance catalysts such as amino acids, enzymes, and boosters to accelerate plant growth (Tomati, Grappelli, & Galli, 1983).

22.5 Use of treated sewage sludge in food production

Based on research sources in the agricultural sector, the nitrogen (N) content in treated residual sludge can be absorbed by the roots of corn plants by as much as 37%–76% from the overall amount of nutrient N (Fauziah, Rosenani, Zaharah, Halimi, & Ahmad, 2000; Rosenani & Fauziah, 2000). For the categories of trace elements (Fe, Cu, Mn, Na, Zn) and heavy metals in treated residual sludge, the contents do not exceed the values recommended by the European Community Standard as well as the 1983 Food Act of Malaysia and 1985 Food Regulation Act and the treated residual sludge is safe to be applied to agricultural lands.

22.6 Experiment results of laboratory analysis on coliform bacteria in treated residual sludge

Treated residual sludge goes through various processes at the sewage treatment plant. The biological treatment process shows the methods of decomposing dangerous substances such as ammonia gas, hydrogen sulfide gas, and dangerous coliform bacteria (*Salmonella* and *Escherichia coli*) in treated residual sludge through biological activity (Fig. 22.6). Biodegradation organic substances during the process of biological treatment process will be converted to gases which will evaporate into the air and dangerous tissue cells of coliform bacteria (*Salmonella* and *E. coli*) will be separated from the residual sludge by sedimentation process.

Laboratory analysis to detect the presence of dangerous coliform bacteria (*Salmonella* and *E. coli*) is conducted on a sample of treated residual sludge from sewage treatment plant after treatment in Fig. 22.4–22.6. The sample of residual sludge is sent to a laboratory certified by the Chemical Institute of Malaysia (IKM) and the panel laboratory for chemical tests of the Department of Islamic Development of Malaysia (JAKIM).

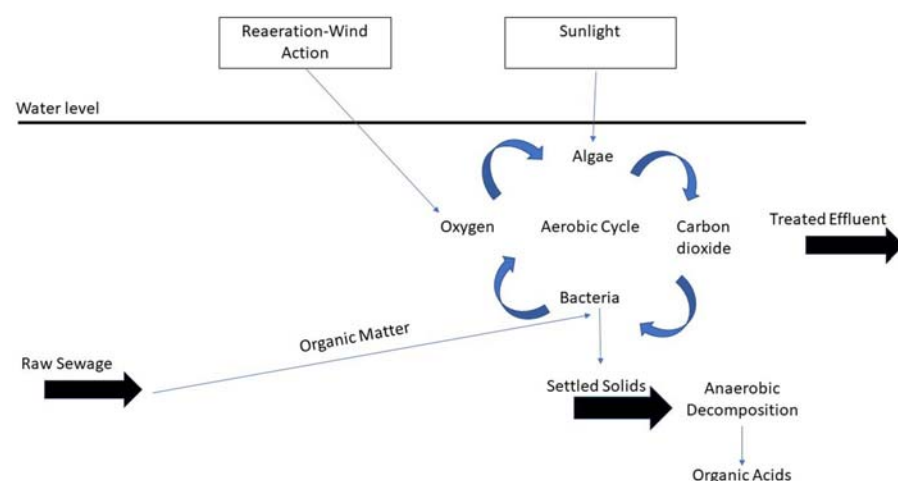


FIGURE 22.6 Biological treatment process in the oxidation pond before production of treated residual sludge (Indah Water Konsortium Sdn, 2010).

TABLE 22.2 Sample of treated residual sludge (20% solid; 80% moisture) from the sewage treatment plant.

Analysis type	Unit	Analysis result	Method
<i>Escherichia coli</i> (<i>E. coli</i>)	MPN/gram	None detected	FDA-BAM Bab 4
<i>E. coli</i>			BP 2013 (Appendix XVI B)
<i>Salmonella</i>			FDA-BAM Bab 5

Tables 22.2 and 22.3 show the test results of laboratory analysis of dangerous coliform bacteria in the sample of treated residual sludge. Further research on applying the use of treated residual sludge mixed with soil agent of change according to a specific ratio was done on spinach plants for 30 days. The results showed that there were no coliform bacteria detected in the spinach leaves and roots 30 days after the spinach was harvested (Tables 22.4 and 22.5).

The laboratory test analysis showed that no dangerous coliform bacteria (*Salmonella* and *E. coli*) were detected (Tables 22.2–22.5). In addition, the use of polymer in the processing of treated residual sludge from wastewater removes the tissue cells of dangerous bacteria during the process of sedimentation (Fig. 22.5). This is because polymer is positive and function as a coagulant which attracts the negative charge of the tissue cells of dangerous coliform bacteria in residual sludge and finally the tissue cells are expelled from the treated residual sludge during the process of sedimentation (Jiang & Zhu, 2014; Liu, Carroll, Long, Gunasekaran, & Runge, 2016).

Laboratory analysis conducted by Sukor, Azira, and Husni (2017) involved the method of *attenuated total reflectance* (ATR) using spectroscopy instrument *Fourier-transform infrared* (FTIR) for analyzing the sample of residual sludge dried at ambient temperature and the polymer sample used in the treatment process of residual sludge at the sewerage treatment plant. IR spectra are recorded with Shimadzu 8300 spectrometer instrument using the technique ATR. The sample is placed at the Crystal Window during the scanning procedure. Analysis is conducted with 32 scans and four spectrum resolutions measured in the frequency range of $4000\text{--}400\text{ cm}^{-1}$ (Sukor et al., 2017).

Frequency range of $3500\text{--}1000\text{ cm}^{-1}$ spectra FTIR provides information on the composition and function of treated residual sludge at the sewerage plant (Fig. 22.7) (Sukor et al., 2017). Frequency range of $3500\text{--}1000\text{ cm}^{-1}$ spectra FTIR provides information on the composition and function of polymer used in the treatment process of residual sludge at the treatment plant (Fig. 22.8) (Sukor et al., 2017).

Each functional group has its own role in forming the function of residual sludge and polymer molecule as change agent in the chemical reaction process. For example, functional group hydroxyl (O–H) will act as phenolic compound that chelates all types of heavy metals (iron, copper, mercury, cadmium) in the soil to be filtered by the root system so as not to endanger the process of transporting the nutrients from the roots to the leaves and fruits (Smith, Fowler, Pullket, & Graham, 2009). In addition, phenolic compound, O–H contained in residual sludge (Fig. 22.7) and polymer sample (Fig. 22.8), also causes an increase in the rate of cation exchange in the soil (Sukor et al., 2017).

TABLE 22.3 Sample of treated residual sludge (80% solid; 20% moisture) after drying in oven (temperature 60°C).

Analysis type	Unit	Analysis results	Method
<i>Escherichia coli</i> (<i>E. coli</i>)	MPN/gram	None detected	FDA-BAM Bab 4
<i>E. coli</i>			BP 2013 (Appendix XVI B)
<i>Salmonella</i>			FDA-BAM Bab 5

TABLE 22.4 Sample of spinach leaf fertilized for 30 days with treated residual sludge mixed with soil (40% soil: 60% treated residual sludge).

Analysis type	Unit	Analysis results	Method
<i>Escherichia coli</i> (<i>E. coli</i>)	MPN/gram	None detected	AOAC991.14
<i>Salmonella</i>	MPN/gram		FDA-BAM Bab 5

TABLE 22.5 Sample of Spinach root fertilized for 30 days with treated residual sludge mixed with soil (60% soil: 40% treated residual sludge).

Analysis type	Unit	Analysis results	Method
<i>Escherichia coli</i> (<i>E. coli</i>)	MPN/gram	None detected	BP 2013 (Appendix XVI B)
<i>Salmonella</i>	MPN/gram		FDA-BAM Bab 5

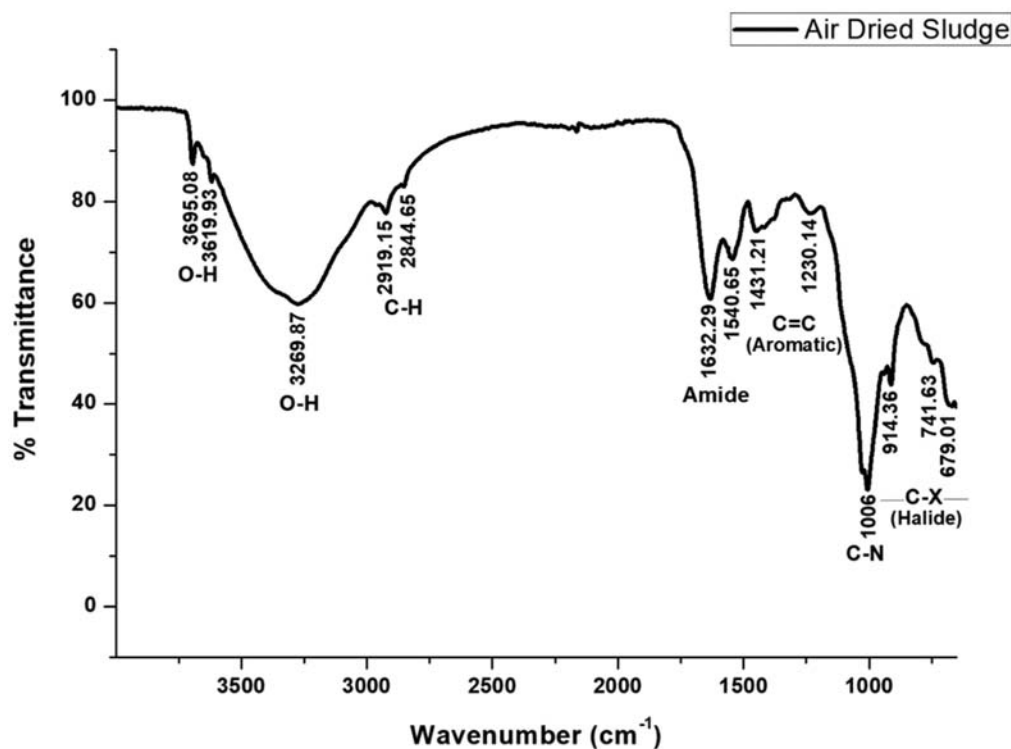


FIGURE 22.7 Functional group from the sample of residual sludge air dried at ambient temperature using spectroscopy instrument FTIR. FTIR, Fourier-transform infrared.

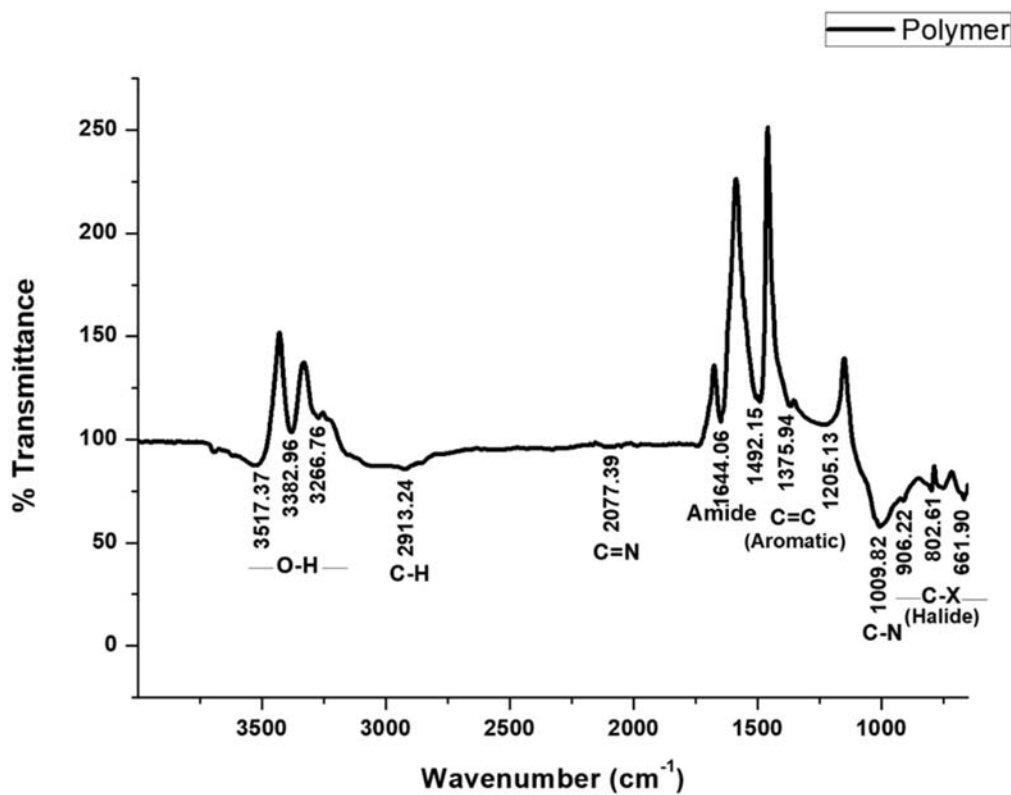


FIGURE 22.8 Functional group from sample of polymer using spectroscopy instrument FTIR. FTIR, Fourier-transform infrared.

22.7 Determining the legality of using human waste (treated residual sludge) based on *al-Istihalah al-tammah* method

The legal ruling on the use of treated residual sludge is that it is *mubah* (permitted) or allowed and if it does not give any unclean effect on the end product such as plants and fruits (Husni, Nasohah, & Kashim, 2015). Determining this ruling is based on the following factors.

22.8 Total transformation of the unclean substance

Upon examining *al-Istihalah* method in the writings of past scholars, they had explained in detail the process of *isti-halah tammah* that occurred to the food sources for humans. It requires a change in the legal ruling from *haram* to *halal* (Kashim et al., 2015). For example, conversion of blood into milk and meat for consumption, from alcohol into vinegar, wastewater that is used to water plants which bear fruits whereby the wastewater becomes soil, pig that has fallen into the sea changes into salt and *al-Jallalah* livestock (animals that feed on the waste of other animals) that are confined and well-fed until they change back to what they were before (Husni et al., 2015). All the examples given by the scholars are not different in principle from making fertilizer products from treated residual sludge used as a medium for planting and fertilizing. It has been proven that there is no negative effect on the plants fertilized by human or animal waste. This is because there has occurred total transformation to the fruits or vegetables. The chemical test results on the effect of applying treated residual sludge on the nutrients of the spinach plant showed that applying the planting medium of 40% soil and 60% treated residual sludge gives a higher nutrient content in the spinach than using only 100% soil as planting medium (Fig. 22.9).

Based on laboratory chemical test analysis, the legal ruling is that plants and fruits are *halal* (permissible) and clean to be consumed by Muslims on condition that there is no effect of uncleanness or waste in the relevant products. Ibn and Ahmad b Sa'id (1990) asserted the appropriateness of applying *al-Istihalah tammah* method in determining the legal ruling of a food resulting from unclean source. According to him:

When the characteristics of an unclean or haram substance have totally changed (to become not unclean), then the unclean nature is cancelled even though before that it was known as unclean and forbidden to be eaten. The status of a new product after having totally changed is based on the present characteristics such as of the fruits and vegetables. It is no longer an unclean or forbidden substance but instead it has changed to become another substance with a different ruling...

Ibn al-'Arabi also asserted:

Scholars differ in opinion on the legality of the resulting product from unclean source such as vegetables watered with wastewater or fertilized with animal or human waste. They held the opinion that the legal rule for all products is that they are clean because the unclean substance has changed. The properties have changed thus the legal ruling attached to it will definitely change. The same has occurred on vegetation growing on a rubbish heap for which a fatwa issued stated it to be halal to be eaten because its nature has totally changed. (Anon, 1986).

Nazih Hammad, one of the committee members of the *Akademi Fiqh Islam* (Islamic Fiqh Academy) and *Majlis Fiqh Amerika Utara* (North American Fiqh Council), held the opinion that

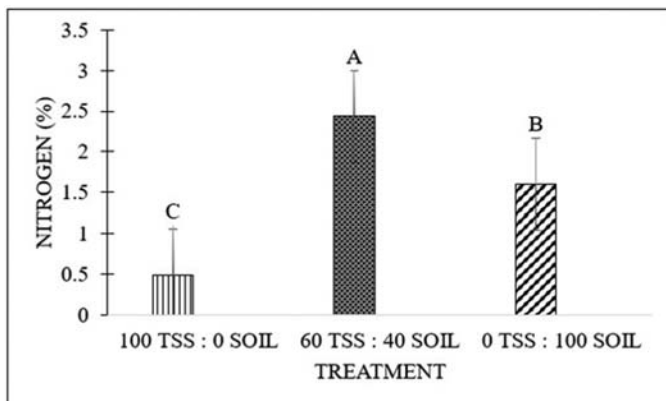


FIGURE 22.9 Nitrogen nutrient content in leaves of spinach planting medium in different ratios: 100 TSS: 0 SOIL represents 100% treated residual sludge and 0% soil, 60 TSS: 40 SOIL represents 60% treated residual sludge and 40% soil, 0 TSS: 100 SOIL represents 0% treated residual sludge and 100% soil. The different alphabetic letters in the average values of nitrogen are based on the method of mean separation using Tukey's HSD method at alpha < 0.05 (Rasyidah dan & Sukor, 2019). TSS, Total sewage sludge; SOIL, ultisols.

the issue of determining the legal rule on the use of animal or human waste for the purpose of plants has to be scrutinized. Further, he stated that the change in legal ruling must be based on the change in mass or matter. Science and technology have verified that the unclean properties no longer exist in the new product. The item has changed to become another item (clean) different from what it was before (unclean) in terms of matter, taste, smell, chemical composition, and other physical criteria. Thus, the legal ruling changes from haram to halal... (Anon, 1986; Husni et al., 2015).

Hence, in this issue, the writer is of the opinion that the method of *al-Istihalah al-tammah* is appropriate to be applied to determine the legal ruling on plants or fruits resulting from use of treated residual sludge (Fig. 22.10). The reason is that the determinant factor of the legal rule on the issue of food does not lie purely in its type or external form, but is subject to a clear or consistent legal reason (*'illa*) of prohibition. As the legal reason for the prohibition is that the unclean substance is no longer in the fruits or plant, the original ruling of *haram* becomes void as a result of the actual change in total in the substance whether in terms of taste, smell, color, and chemical nature (Husni et al., 2015). This ruling is appropriate because it fulfills the current reality, especially when part of the consumer goods or foods for Muslims are from unclean sources or waste. But after recycling and process of repurification, the goods or foods become safe for consumption. For example, tap water is clean even if it originates from wastewater. This is because its unclean property has gone and it has changed to become water purified of all types of uncleanliness in terms of smell, taste, and also color. This statement is in line with the fiqh principle related to *al-Istihalah al-tammah* as supported by Ibn al-Andalusi (1990) and Ibn, Taqi al-Din, and Abd al-Halim (1988). This method means “Indeed the unclean substance, when it changes its condition (become another substance that is clean), it becomes pure.”

In addition, there are other fiqh principles which support the abovementioned method, such as “A change in legal status depends on change in name (of a substance) and the change in name (of a substance) depends on the change (that occurs) to its properties or nature (essence of substance)” (Kashim et al., 2017).

22.9 Positive effects on management of treated residual sludge on food production and ecosystem

The use of treated residual sludge on agricultural land helps reduce farmers' dependence on commercial fertilizers. More interestingly, it is proven that it helps to reduce release of heavy metals which pollute the environment. Based on the study on spinach treated with biochar sewage sludge, sewage sludge biochar treatment, which charred at the temperature of 300°C, significantly reduced Mn concentration in spinach leaves in comparison to control. Sewage sludge charred at 300°C contained hydroxyl (O–H) functional group (Fig. 22.11) that could chelates soil heavy metals to be filtered by the root system. Hence, spinach is safe to be consumed and the soil productivity increased, whereby the rate of soil cation exchange capacity also increased (Sukor et al., 2017).

At present, the method of disposing treated sewage sludge is through disposal in landfills. If this continues, it will cause environmental pollution which endangers the universal society and involve a high management cost to the country. Thus the use of treated residual sludge by applying it to agricultural lands or landscaped plants needs to be more widely spread in supporting the policy, Malaysian Green Technology Master plan 2017–30 (Fig. 22.12).

Malaysia depends too much on landfills to manage 95% of solid waste (including residual sludge). The landfills are a main source of released methane gas (47%). Methane gas (CH₄) has a capacity of 28 times more potential to increase

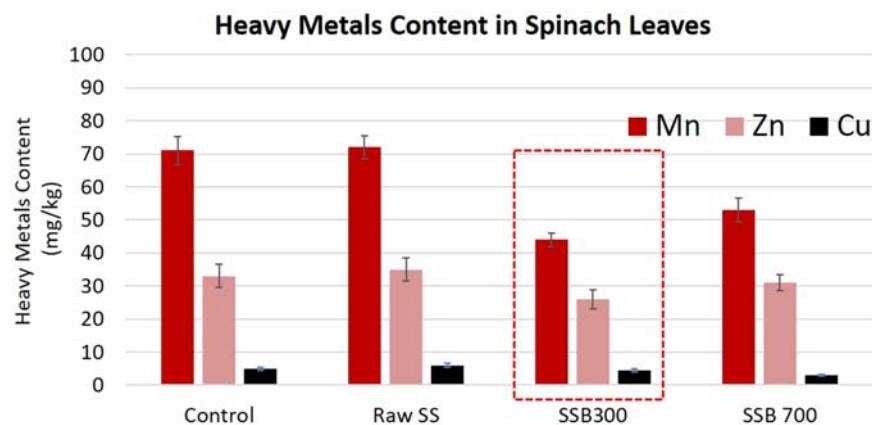


FIGURE 22.10 Heavy metals content (manganese, Mn; zinc, Zn; copper, Cu) in spinach leaves based on a dry weight basis. Raw SS, Raw sewage sludge; SSB300, sewage sludge biochar pyrolyzed at 300°C; SSB700, sewage sludge biochar pyrolyzed at 700°C.

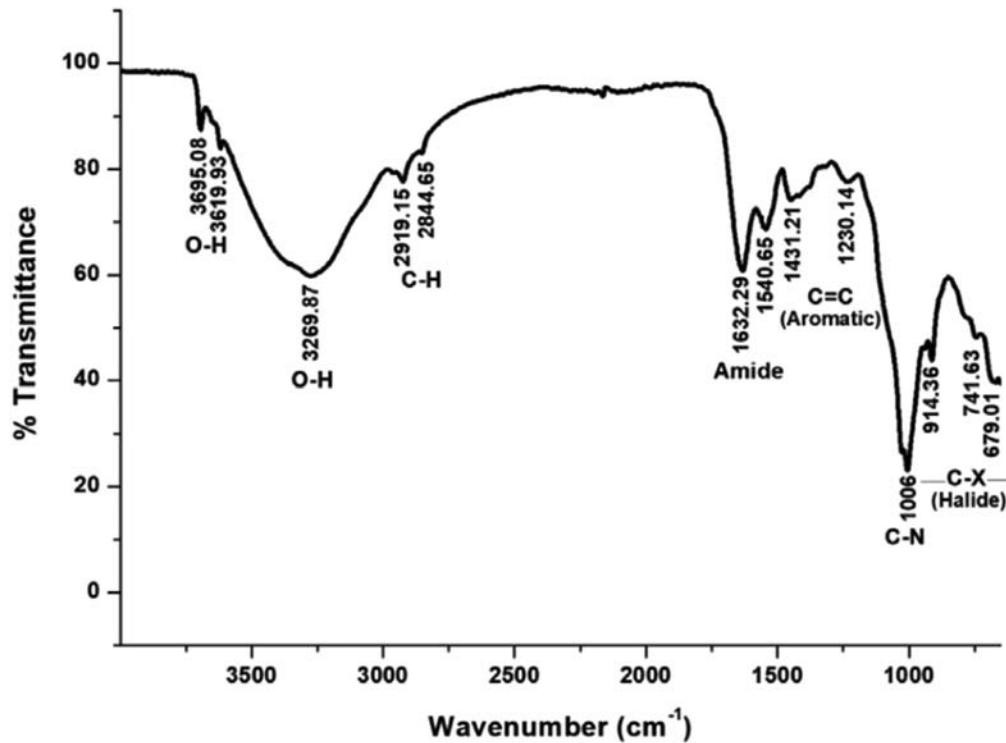


FIGURE 22.11 Hydroxyl (O–H) functional group via FTIR spectroscopy. *FTIR*, Fourier-transform infrared.

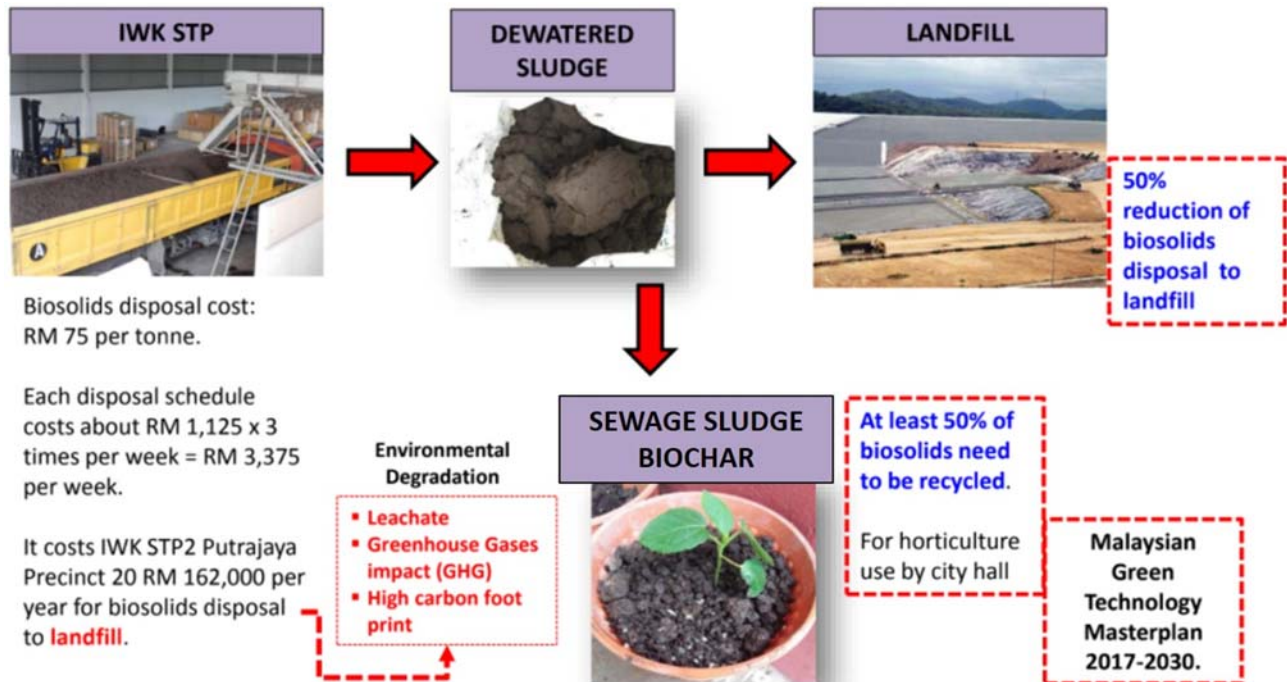


FIGURE 22.12 The business-as-usual approach of treated sewage sludge from the sewage treatment plant (STP) usually goes to the landfill. The circular economy context repurposes the treated sewage sludge in a renewable source.

greenhouse effect (Global Warming Potential) than carbon dioxide gas (CO₂) which plays a role as the main agent in causing ozone layer depletion. According to the Malaysian National Policy on Management of Policy Solid Waste (2006), Malaysia needs to achieve the target of reducing solid waste (including residual sludge) disposed at landfills by 40% and carbon emission reduction of 38% by involving more efficient and cost effective management of solid waste sources. Malaysia needs to make a paradigm shift from a linear economy system which involves creating and disposing (create and dispose) solid waste to a circular economy involving recycling activity (reuse and recover).

Efficient management of treated residual sludge helps to overcome the problem of society's perception concerning the presence of dangerous coliform bacteria and heavy metals by reusing treated residual sludge for agricultural lands or landscaped plants. The original substance (treated residual sludge) from the sewerage treatment plant is mixed with soil according to a certain ratio that is suited to the plant species. In this matter, the soil element is the agent of change. Next, the mixture is dried and change in matter occurs resulting in an end substance which can be commercialized to be utilized.

In addition to the soil element, treated residual sludge may also be mixed with plant waste, according to a certain ratio, such as zeolite, composted palm oil trunks, compost from landscape plant waste (fallen leaves, dry grass, tree branches, weeds) can also be used as change agents according to a certain ratio for landscaping use such as planting chrysanthemums (Dzulkurnain et al., 2017; Kala, Rosenani, Fauziah, & Thohirah, 2009; Sukor et al., 2017).

22.10 Conclusion

Overall, the al-Istihalah al-tammah concept on determining the status of food fertilized with treated sewage sludge has been described and deemed as halal. With increase in human population from year to year, failure to efficiently manage treated sewage sludge will put stress on the environment and threaten the sources of clean water supply. Therefore treated residual sludge requires a more organized management system. Society, regardless of religion and race, needs to be more sensitive that the use of treated sewage sludge can be applied in the agricultural sector for food production after going through the process of transformation using the *istihalah* concept.

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Brine waste management in desalination industry: a prospective wealth from waste

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23.1 Introduction

The global water demand has been increasing largely due to increase in population (Piesse, Global Food, & Water Crises Research Programme, 2020). World population is expected to reach 11 billion by 2100 according to UN Population Division (Hara, 2020). Therefore, global water need is expected to grow by 55% between 2000 and 2050 from 3500 in 2000 to 5425 km³ by 2050 (Graham et al., 2018; Islam & Karim, 2019). However, there has been increase stress on the fresh water source. So much so that, by 2025, 2.7 billion people can face water shortages according to the United Nations (Ahuja, 2021). So also, the losses due to drought is expected to be five times more than today in EU by 2100 (Roo, 2020). Therefore, there has been unstoppable drift toward desalination of seawater to meet water demands because of the decrease in freshwater sources, the increase of population, and new advancements made in desalination technology (Liu et al., 2020). Around 75% of the earth is covered with water. Interestingly, around 95% of this water are saline in form of seas, oceans, and others. Around 3% is frozen to form glaciers, therefore not available for our use (Nazir et al., 2020). Only around 1% is available in form of fresh water for our use. It may be interesting to know that 95% of the global water is salty, but only 1% of our drinking water is from desalination. Therefore, seawater is abundantly available for our use. As many nations are increasing their desalination capacities, they have to also deal with attendant waste (brine) generated from the desalination process. To put simply, brine is high concentration of common salt in water. By the way, brine is a by-product of many industrial activities such as desalinations, paper industries, cooling tower, natural gas extractions (Mavukkandy, Chabib, Mustafa, Al Ghaferi, & AlMarzooqi, 2019). By this, brine does not usually contain only common salt but also other useful chemicals based on the activities of the source industries. Similarly, brine usually contains residual pretreatment chemicals in addition to heavy metals due to pipe corrosion (Jones, Qadir, van Vliet, Smakhtin, & Kang, 2019). Thus brine is known to be corrosive and toxic to marine environment, therefore its free discharge into sea has been restricted by stronger legislation (Panagopoulos, Haralambous, & Loizidou, 2019). On the other hand, the cost of desalination is generally high thereby limiting its acceptance by many countries (Panagopoulos et al., 2019). Moreso, according to Islamic view, particularly making reference to a special fatwa [defined below] on the matter was issued by the Council of Leading Islamic Scholars (CLIS) of Saudi Arabia in 1978. The fatwa (CLIS 1978) postulated that:

Impure waste water can be considered as pure water and similar to the original pure water, if its treatment using advanced technical procedures is capable of removing its impurities with regard to taste, colour and smell, as witnessed by honest, specialized and knowledgeable experts. Then it can be used to remove body impurities and for purifying, even for drinking. If there are negative impacts from its direct use on the human health, then it is better to avoid its use, not because it is impure but to avoid harming the human beings (Osman, Kobayashi & Fujiki, 2020).

Modern filtering techniques are the best and most efficient methods for purifying water. Experts in water treatment say many chemicals are used to purify dirty water. In this case, the council considers the water is fully pure and can be used for ritual purification and drinking as long as it does not harm human health. If water is not to be drunk, it is for grounds of public health and safety, not Islamic law. The council advises against consuming treated water due to health concerns and societal disapproval. However, utilizing this water to irrigate crops or parks is legal (Osman et al., 2020).

As a result, creation of valuable products from brine seems to be a sustainable way out from this stalemate. In the one hand, extraction of valuable chemicals from brine will make the resultant solution less toxic and can be safely discharged or reused. On the other hand, it will generate more income thereby reducing the overall cost of desalination process. Brine can be managed to recover more water from it, recover chemicals and generate more energy (energy recovery) (Ogunbiyi et al., 2021).

Thus, in this chapter, we will look at different brine management from perspective of water recovery, mineral recovery, and energy recovery. The chapter will also report how different countries have feared in their brine management on the scale of water recovery, mineral recovery, and energy recovery.

23.2 Brine today

It is important to X-ray the past to appreciate today and project for the future. Brine is the by-product of several industrial process and particularly desalination process. Desalination is the process of removing salt and other minerals from the seawater or brackish water so as to get fresh water. Desalination usually has two products; one is the permeate (clean water) and the other is the concentrate known as the brine (Ogunbiyi et al., 2021). At present, no desalination technology and particularly plant achieves 100% water recovery (Bello, Zouari, Da'ana, Hahladakis, & Al-Ghouti, 2021). In fact, for every liter of fresh water produced from seawater, there is consequential production of 1.5-L brine as a by-product. Globally, we consume 10.2 billion m³ of fresh water per day. The global total install capacity for the production of freshwater from desalination stood at 99.7 million m³/day from 20,971 projects (Ihsanullah, Atieh, Sajid, & Nazal, 2021). The projects include 19,744 plants. Therefore desalination contributes 1% to the global daily water supply. Consequentially, the global generation of brine stood at 149.55 Mm³/day (Ihsanullah et al., 2021). Middle East and North African region are responsible for 48% of global rejected brine production (Gheraout & Gheraout, 2020; Jones et al., 2019). In addition to NaCl, reject brine always contains residual chemicals from pretreatment and cleaning process. The possible chemicals are NaOCl, FeCl₃, AlCl₃, H₂SO₄, HCl, NaHSO₃ together with some heavy metals such as Fe, Cr, and Cu (Al-Absi, Abu-Dieyeh, & Al-Ghouti, 2021; Mavukkandy et al., 2019). In general, reject brine can contain residual amounts of coagulants, flocculants, antiscalants, organics, microbial contaminants and any particulates rejected by the reverse osmosis (RO) membranes or thermal plants. Thus free discharge of brine into water body has been restricted by laws because of its apparent toxicity. Therefore, at present, efforts have been on the rise on how to manage brine and by extension mine it for valuable products. Coincidentally, seawater has been the traditional source of table salt (NaCl) for several millennia. So, by default, the first cheapest win (valuable) of brine mining is the table salt. In recent years, the global brine market has experienced rapid growth and it is expected to reach \$1.67 billion by 2025 (Industry Arc, 2020). Currently, it is used as a preservative for food products through the process of brining. Brine contains a very high salt content, which prevents the growth of bacteria and helps in preserving the food items. It also has industrial applications in medical and oil and gas industry (Industry Arc, 2020). However, the current brine uses cannot consume the current global production of the brine. Several brine management processes can be categorized into water recovery, mineral recovery, and energy recovery.

23.2.1 Water recovery

The journey into the brine mining in terms of history, science, and technology started with brine volume minimization. Desalination process produces a huge volume of brine waste. Therefore the starting point to manage the brine is to reduce its volume. Long before now, this was achieved by evaporating the brine residual water through thermal process without any deliberate effort to recover the water for any use. Recently, the use of membrane in the desalination process has made it easy to recover the brine residual water for meaningful use. The current water recovery technologies involve recovering the additional water from brine up to or beyond the saturation concentration (Mavukkandy et al., 2019). It is done either through minimum liquid discharge (MLD) approach or zero liquid discharge (ZLD) approach.

23.2.1.1 Zero liquid discharge

ZLD is a treatment method that removes all residual water from a rejected brine. The goal of ZLD is to produce clean water that may be reused (e.g., irrigation), saving money and benefiting the environment. ZLD is a mix of desalination technologies intended at producing high-quality fresh water from rejected brine paste (Alnouri, Linke, & El-Halwagi, 2018). The fresh water produced by ZLD is extremely pure (with a water recovery rate of 95%–99%) and can be used for a variety of purposes, including drinking water, irrigation, and process cooling water. Simultaneously, compressed

solid waste can be disposed of in an environmentally acceptable manner in the local environment or transported for further processing and usage as a useful resource (Xiong & Wei, 2017). ZLD systems are generally different in terms of design, arrangement, and functioning, and so each system is unique. As a result, establishing a single ZLD system for all desalination facilities is impossible.

A conventional ZLD system, on the other hand, has three steps: preconcentration, evaporation, and crystallization. Membrane-based technologies are used in the first stage to recover water and reduce the volume of the brine. This stage is critical for the system since it considerably reduces the size of the system's next two pricey phases. Water recovery, brine volume reduction, and solid product manufacturing are mostly accomplished in the next two steps using thermal-based technologies.

Thus, there are two types of technologies employed in brine treatment/ZLD systems: membrane-based and thermal-based technologies. The composition of the feed brine, the purity demand of fresh water, and the final concentration of the concentrated brine required for either safe disposal or other beneficial applications all influence the design of a ZLD system. As a result, a ZLD system may comprise some or even all of the therapeutic technologies (Panagopoulos et al., 2019).

23.2.1.2 Minimum liquid discharge

MLD involves various processes that enable plants to recover fresh water from the brine as much as possible so as to reduce the volume of brine to deal with subsequently (Panagopoulos, 2021). High water recovery from brine has both environment and economic benefit. The recovered water can be subsequently reused thereby reducing the overall cost of the desalination process (Ogunbiyi et al., 2021). MLD is largely membrane-based technology; therefore, it has recently gained increasing attention because of its significantly lower energy demand and cost (Panagopoulos, 2021). The membrane-based technologies that are applicable for MLD are RO, osmotically assisted reverse osmosis (OARO), high-pressure reverse osmosis (HPRO), forward osmosis (FO), membrane distillation, etc.

23.2.1.2.1 Reverse osmosis for brine management

RO is the most widely membrane system used to desalt brine waters. RO produces more fresh water from the feed brine and more concentrated brine often referred to as RO brine, reject, or concentrate. In the RO system, hydraulic pressure is applied to the feed brine to force some brine residual water to move through semipermeable membrane into another compartment. The pressure must be higher than the osmotic pressure differential between the feed brine and ejected fresh water (permeate). The conventional RO only works with feed brine with maximum total dissolved solid (TDS) of 70,000 mg/L and maximum pressure of 82 bar to give 50% water recovery (Panagopoulos et al., 2019). Water recovery of the conventional RO system reduces to 10% if the TDS increases to 85,000 mg/L (Panagopoulos et al., 2019). Membrane fouling is another dominant limitation in membrane technology as listed in Table 23.1. It blocks the membrane pores thereby reducing recovery and increasing energy demand. It must be noted that, the membrane propensity for fouling increases as the feed brine TDS increases. These limitations have activated extensive researches over the years to produce a membrane process configuration that can withstand higher pressure and work with feed brine with higher TDS with little fouling propensity and give higher water recovery. An effective membrane configuration centers on playing around water chemistry of the feed solution, improving membrane module and process engineering.

Importance of water chemistry to improve reverse osmosis Water chemistry is one of the areas researchers have worked on to improve efficiency of RO configuration. It basically involves the removal of foul-forming ions from the feed brine through chemical process before exposing the brine to membrane. It reduces foul potency of the feed brine without compromising the RO configuration efficiency. It can be achieved through chemical precipitation, seeded precipitation, and ion exchange. Chemical precipitation is the change of foul-forming ions in the feed brine into solid particles. Chemical precipitation is used to remove foul-forming ionic constituents from the brine by the addition of counter-ions to reduce their solubility. A typical precipitation sequence is as follows: reagent addition, pH adjustment, flocculation, sedimentation. Separation of the solids formed from liquid can be achieved by a cheaper filter. Seeded precipitation on the other hand is a process where precipitation of the fouling ions is initiated by crystals (seeds) by providing precipitation growth sites. The common crystal seeds are calcite, barium sulfate, gypsum, or dolomite marble powder. Basically, the seed materials do have chemical similarity with the fouling ions; therefore the fouling ions have preferential tendency to grow on the seed materials. Ronquim, Sakamoto, Mierzwa, Kulay, and Seckler (2020) added calcite seed in their bid to achieve ZLD of industrial wastewater. The secondary membrane was able to achieve 96.3% RO water recovery adopting a RO intermediate softening with CaO addition and calcite seeding, as well as MgSO₄

TABLE 23.1 Different brine minimization techniques.

	Advantages	Limitations	References
RO	It requires lesser energy. It is safe and environmental friendly. It does not require the use of any chemical. It has compact design	Membrane fouling. Pretreatment of membrane is required. It requires the use of external pressure device	Bunani et al. (2018), Ronquim et al. (2020)
HPRO	It can withstand higher pressure. It can be used for brine feed that has more than 70,000 mg/L TDS. It has higher water recovery compared to RO	Scaling is higher. It requires pretreatment. It requires the use of higher external pressure. Few membrane are available	Al-Absi et al. (2021), Zhao et al. (2019)
FO	It does not require any external hydraulic pressure. It is cheaper. Lower energy consumption	It requires external draw solution. Selection of appropriate membrane	Abdullah, Tajuddin, and Yusof (2019), Akinpelu, Ali, Johan, Saidur, Qurban, et al. (2019), Panagopoulos et al. (2019)
OARO	It has improved water recovery. It uses less energy compared to RO	It requires external hydraulic pressure. It uses draw solution. It has several stages	Bartholomew, Mey, Arena, Siefert, and Mauter (2017), Peters and Hankins (2019)

FO, Forward osmosis; HPRO, high-pressure reverse osmosis; OARO, osmotically assisted reverse osmosis; RO, reverse osmosis; TDS, total dissolved solid.

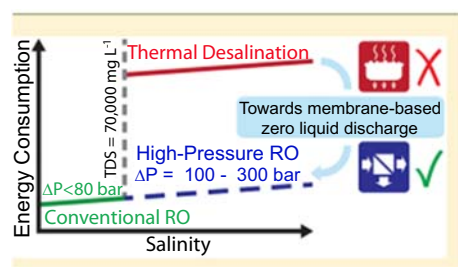


FIGURE 23.1 HPRO conceptual diagram (Davenport et al., 2018). HPRO, High-pressure reverse osmosis. Reproduced with permission from Davenport, D.M., Deshmukh, A., Werber, J.R., & Elimelech, M. (2018). High-pressure reverse osmosis for energy-efficient hypersaline brine desalination: Current status, design considerations, and research needs. *Environmental Science & Technology Letters*, 5(8), 467–475. <https://doi.org/10.1021/ACS.ESTLETT.8B00274>. Copyright ACS 2018. *Further permissions related to the material excerpted should be directed to the ACS.

dosing for additional silica removal (Ronquim et al., 2020). This configuration reduced energy used by ZLD desalting by 60% and plant installation and operation costs by 40% (Ronquim et al., 2020). Similarly, ion-exchange application in RO is a method where scale-forming ions are removed or replaced by nonobjectionable ions on the resin. Technically, scale-forming ions are removed by charged functional group on the ion-exchange through electrostatic attraction while the ion-exchange resin removes some other uncharged compounds such as natural organic matter from the brine (Liu, Haddad, Sauv  , & Barbeau, 2021). Hu, Hu, Ouyang, and Feng (2014) were able to demonstrate that ion-exchange membrane could achieve 93.3% boron reject from wastewater using RO–electrodeionization configuration. Bunani, Arda, and Kabay (2018) also applied similar configuration for the removal of boron from reject brine.

23.2.1.2.2 Improving membrane module

Over the years, researchers have been working on developing membranes that can resist higher pressures. HPRO technology is a type of RO that uses specific membranes to work at pressures higher than 82 bars (Zhao et al., 2019) as presented in Table 23.1. In terms of higher working pressure and TDS treatment, HPRO methods are thought to be an improvement over RO processes. Due to the high risk of membrane fouling and scaling, as well as low water recovery, RO processes are limited to a maximum operating pressure of 82 bar and feed solutions ranging from 50,000 to 70,000 mg/L TDS (maximum efficiency 50%) (Al-Absi et al., 2021). HPRO, on the other hand, can be used with brine feed solutions with more than 70,000 mg/L TDS. Although few membranes of this type are commercially available, certain HPRO membranes may concentrate RO brine to half its original concentration (Davenport, Deshmukh, Werber, & Elimelech, 2018). On the other hand, the average amount of freshwater produced by some of these membranes is extremely modest. Because HPRO is a developing technology, the optimization of process aspects is currently being researched (Davenport et al., 2018). Davenport et al. (2018) captured the HPRO concept in Fig. 23.1. As seen in the figure, HPRO membranes can function at pressure of 100 to 300 bar which is far more than what is obtainable with

conventional RO membrane. Pall Corporation, for example, has just released the Disc Tube HPRO module system (DT) (Al-Absi et al., 2021). This system was created specifically for the treatment of landfill leachates. There are already a variety of commercially available DT module variants that can function at various pressures (82–150 bar). However, the DT modules' freshwater recovery % is quite low (3 m³/day) (Al-Absi et al., 2021). Dow Chemical Company is also now producing improved HPRO brine treatment membranes that can function under 120 bar pressure.

However, like Pall Corporation's brine treatment systems, these HPRO brine treatment systems have an exceedingly poor water recovery rate (8%) (Al-Absi et al., 2021). Saltworks Technologies Inc. increased the working pressure of an HPRO brine treatment system in 2019. The HPRO system's operating pressure can reach 124 bars, and it can concentrate RO brine solutions to a maximum TDS of 130,000 mg/L. Saltworks Technologies Inc. revealed that their HPRO brine treatment system may reduce the volume of the initial brine solution by half Zhao et al. (2019). This has a significant benefit in terms of reducing the negative effects of brine solutions (Panagopoulos et al., 2019). Nonetheless, if sustainable and efficient brine treatment is the goal, limited freshwater recovery is a fundamental downside of HPRO technology. Furthermore, using high pressure in a brine treatment membrane increases the danger of scaling, which can result in a large number of scaling compounds that require pretreatment (Al-Absi et al., 2021).

23.2.1.2.3 Forward osmosis

FO is a water separation method that extracts freshwater from the feed brine using a semipermeable membrane and the natural energy of osmotic pressure. Water is transported through the membrane by osmotic pressure, while all dissolved solutes are retained on the opposite side (Panagopoulos et al., 2019).

FO, unlike RO and HPRO, is a membrane-based system that uses osmotic pressure gradients rather than hydraulic pressure as seen in Table 23.1. A relatively high-concentration solution (draw solution) is required in this process to generate an osmotic pressure gradient across a semipermeable membrane, forcing water molecules to be transferred from the less concentrated solution to the more concentrated solution (draw solution) (Abdullah et al., 2019).

Organic, inorganic, nanoparticle-based solutions and other draw solutions can all be employed (Cui & Chung, 2018). The common membranes in FO are cellulose acetate, cellulose triacetate (CTA), polyamide thin-film composite, and biomimetic membranes (Vrouwenveld, 2017). Companies like Fluid Technology Solutions Inc. and Oasys Water Inc. have developed FO units that can treat brine with a TDS concentration more than 70 g/L (Eyvaz et al., 2018). In addition, there are issues with polarization and fouling in this process. Nonetheless, the studies revealed much better freshwater recovery rates (up to 98%) than previously published technologies (Panagopoulos et al., 2019). The most recent research efforts have focused on surface modification of existing membranes and the development of novel membranes to improve antifouling and surface hydrophilicity (Panagopoulos et al., 2019). Materials for surface modification, such as polydopamine and nanoparticle-decorated graphene oxide nanosheets (AgNP-GO), have recently been used, with promising results (Panagopoulos et al., 2019).

23.2.1.2.4 Osmotically assisted reverse osmosis

OARO is a nonevaporative, membrane-based technique for desalination of high-salinity brines with good recovery and energy efficiency. This improvement allows water to be transported even when the feed's osmotic pressure exceeds the membrane's burst pressure. The OARO is the most recent membrane-based technology. It is a method that combines the principles of RO and FO technologies (Photiou, Koutsokeras, Constantinides, Koutinas, & Vyrides, 2021) as listed in table. A series of OARO stages and a final RO stage make up the OARO system (Peters & Hankins, 2019). In terms of the OARO concept, it uses hydraulic pressure to transport water across a semipermeable membrane against the osmotic pressure difference between feed and permeate, similar to RO. Unlike RO, which has a low permeate salinity, OARO has a permeate-side saline sweep solution (also known as a "draw solution") to reduce the osmotic pressure difference across the membrane. This modification improves water recovery and permits desalination of higher salinity water solutions without surpassing the membrane burst pressure (Bartholomew et al., 2017). The cascade osmotically mediated RO is a relevant technique to OARO, which is also known as counter-flow RO (CFRO) (COMRO). All use the same underlying technology; however, their stages/modules are organized differently (Bouma & Lienhard, 2018). The distinction is that in COMRO, the stages are connected by closed recirculating loops, but in OARO/CFRO, the stages are connected by a cascading counter-flow pattern. Because it is a dilution through series-organized steps, it is not a simple desalination technique. With up to 72% freshwater recovery, OARO can treat 100–140 g/L TDS brine effluents (Bartholomew et al., 2017). Hyrec has recently developed a commercial OARO system that has successfully concentrated seawater brine with a TDS of 250 g/L. (Panagopoulos et al., 2019). As it is obvious, OARO is confronted with both RO and FO technological challenges. Furthermore, OARO, like FO, has a more complicated operation than

RO/HPRO (Panagopoulos et al., 2019). Both the specific energy consumption (SEC) (6–19 kWh/m³) and the treatment cost (US\$2.4/m³) are greater than previous osmosis-related technologies due to OARO's immaturity; however, these values are predicted to fall dramatically as this young technology matures in the next years (Bartholomew et al., 2017).

23.2.1.3 Process engineering for improving reverse osmosis

One of the methods to avert fouling of RO membrane is to reengineer the RO configuration in a way to prevent the growth of scaling particle on the membrane. One of the such methods is vibratory shear-enhanced process. Vibratory shear-enhanced processing (VSEP) is a method that uses torsional vibration on membrane to promote separation and reduce fouling. Solids and foulants can lift off the membrane surface due to high shear rates at the membrane surface. Due to the high hydraulic pressure vertically placed on membrane surfaces in traditional spiral-wound RO modules, scale crystals and colloidal foulants in feedwater are prone to deposit on membrane surfaces and block filtering. VSEP was designed to reduce colloid and scaling deposition on membrane surfaces by imparting shear stress on membrane surfaces through vibration and rotation (Zsirai, Qiblawey, A-Marri, & Judd, 2016). VSEP uses a plate-and-frame membrane structure rather than a spiral-wrapped membrane structure. Solids and foulants are lifted from the membrane surface and pores by high shear stress and carried away with the bulk flow (Shi & Benjamin, 2011). Because of the strong shear, membrane holes remain exposed, allowing substantial permeation fluxes across the membranes.

This is in contrast to traditional cross-flow membranes, where suspended colloids at the boundary layer significantly clog membrane pores (Liu, Xu, & Das, 2019).

VSEP is not limited by the solubility of salts or the presence of suspended solids in feedwater because shear force is applied near the membrane surface. As a result, VSEP has several benefits, such as less pretreatment, membrane scaling resistance, and higher filtration flux for high-strength wastewater (Subramani, DeCarolis, Pearce, & Jacangelo, 2012). The disadvantage of VSEP is that it requires a lot of energy to keep oscillatory vibration going. High fluxes (50–100 LMH) were reached in a research using VSEP to treat primary RO concentrate (Shi & Benjamin, 2011), and deposition of colloidal silica on membranes was minimized as a result of the vibratory shear (Subramani et al., 2012). Precipitation of barium sulfate, on the other hand, was not adequately alleviated. The overall recovery of feedwater was increased from 75% to 93% by minimizing scaling potential. VSEP was utilized to treat RO concentrate from a brackish water desalting plant in the United States, and 80% of the concentrate was recovered (Delgado, 2009).

23.2.2 Resource recovery from brine

Resource recovery or mineral recovery is popularly called brine mining. Brine mining is the extraction of valuable resources (elements or compounds) that are dissolved in brine.

Brines are significant sources of common salt (NaCl), calcium, iodine, lithium, magnesium, potassium, bromine, and other elements, as well as prospective sources of a variety of others. Brine mining helps to reduce waste and recover resources. Interest in resource recovery from brine (formerly considered a waste) was first expressed in the 1970s, but little progress was made in this area until 2001. Most brine treatment techniques currently do not provide potable water for resale to offset treatment costs. RO reject brine or membrane concentrates, on the other hand, include metals and minerals that, once recovered, could provide substantial economic benefits and avoid disposal issues. To successfully extract important minerals and metals from water, several approaches have been researched at the bench and pilot scale. There is a good chance that no one technique will be able to achieve the most efficient resource recovery, and that the best results will come from combining technologies. There are various techniques to extract minerals from the brine. In Table 23.2, several mineral recovery methods are listed with their benefits and limitations.

23.2.2.1 Crystallization

Crystallization is the conversion of a chemical from a liquid solution to a solid crystalline state. Crystallization is frequently used as the final step in the treatment of RO brine to achieve zero-liquid discharge. This makes crystallization techniques effective for the recovery of a variety of useful and valuable substances (along with the recovery of water) from wastewater, such as recovering of NaCl, Na₂SO₄, Na₂CO₃, ammonium (Lu et al., 2017), as well as phosphates the reclamation of pure water, the removal of heavy metals (Pb²⁺, Mn²⁺, Ni²⁺, Cu²⁺, Ag⁺, etc.) and water softening by removing Ca²⁺, Mg²⁺ (Lu et al., 2017). Crystallization has been thoroughly investigated as a standard unit operation and has a wide range of applications in a variety of industries throughout the world. Brine crystallizers have the advantage of being able to handle brine with up to 300,000 mg/L TDS (Ogunbiyi et al., 2021). Mohammedsmaeili, Badr, Abbaszadegan, and Fox (2010) used a three-step procedure that includes lime–soda treatment, a secondary RO unit,

and evaporation/crystallization to treat RO concentrate and recover by-products. Ca and Mg were removed from the lime-soda treatment stage as CaCO_3 (95%), $\text{Mg}(\text{OH})_2$ (51%–58%), and CaSO_4 (92%) as Product I in Stage 1 (Mohammadesmaeili et al., 2010). In Stage 2, the concentrate was evaporated/crystallized to yield Na_2SO_4 (as 88% Na_2SO_4 and 8% NaCl) and potash in the form of glaserite, $\text{K}_3\text{Na}(\text{SO}_4)_2$ (as 25% $\text{K}_3\text{Na}(\text{SO}_4)_2$ and 70% NaCl). Crystallization technology has more numerous advantages over other procedures, including a high recovery rate, the capacity to recover both high-quality water and precious salts at the same time, and the absence of other supplemental materials (membrane, catalyst, adsorbent, ion-exchange resin, oxidant or reducing agent, etc.). For example, in the membrane process, which is commonly used to reduce wastewater and recover pure water, a huge amount of membrane is required and must be replaced on a regular basis due to membrane obstruction and contamination. In addition, substantial preparation is required in the membrane process, which consumes various chemical ingredients. Other important compounds, such as salts, cannot be recovered directly from highly concentrated wastewater after the membrane process, despite the fact that the volume of wastewater is reduced. To recover the salts from the wastewater, other methods are required. Furthermore, because many laws are based on concentrations rather than volume, some disposal issues will arise. Crystallization, on the other hand, does not require any additional ingredients and can recover salts as well as pure water because it is capable of producing high-purity products from impure solutions. Furthermore, in addition to good operability and stability, crystallization has a wide range of schemes to achieve energy-savings and ensure high recycling efficiency. It is widely acknowledged that if appropriate design is done, crystallization could be energy-saving, highly efficient, and easy to scale up (Al-Mutaz & Wazeer, 2014). With so many benefits as listed in Table 23.2, crystallization is seen as a viable wastewater treatment process, particularly for the recovery of valuable resources. As a result, crystallization techniques have been widely used in actual wastewater treatment applications around the world in recent years (Williams, Ahmad, & Connolly, 2013). The crystallization process is divided into two types: evaporative crystallization and freeze crystallization.

TABLE 23.2 Different mineral recovery techniques.

Methods	Minerals	Advantages	Limitations	References
Crystallization	NaCl , Na_2SO_4 , Na_2CO_3 , Pb^{2+} , Mn^{2+} , Ni^{2+} , Cu^{2+} , Ag^+ , K_3Na $(\text{SO}_4)_2$	High recovery rate capacity to recover both high-quality water and precious salts at the same time absence of other supplemental materials. Minimal maintenance cost. It increases the live span of the adjacent membrane	It only removes solutes. Process kinetics are more complex. It may need adjacent filter to remove the agglomerated particles	Al-Mutaz and Wazeer (2014), F et al. (2010), Ogunbiyi et al. (2021)
Adsorption	$\text{Mg}(\text{OH})_2$, MgCl_2 , MgSO_4 , Mg $(\text{HCO}_3)_2$, lithium	It is simple to operate. It is economical and selective. It is regenerative. It works at wide pH range. It can be adaptive to many configuration	It generates sludge. It can be affected by cocontaminants. It requires additional step for product recovery	Akinpelu, Nazal, and Abuzaid (2021), Khadhri, El Khames Saad, Ben Mosbah, and Moussaoui (2019), Vardhan, Kumar, and Panda (2019)
Precipitation	Lithium	It is easy. It can be used to recover amorphous compound. It can be specific. It gives pure products	It requires additional step for product recovery. It requires additional chemical to operate. It increases the TDS of a solution	Zhang et al. (2019)
Electrodialysis	HCl , HNO_3 , NaOH , and H_2SO_4	It requires relatively low energy consumption. It is very specific. No pressure is required	Frequent membrane replacement. Feed brine pretreatment is necessary to prevent ED stacks fouling. Elaborate controls are required, and keeping them at optimum condition can be difficult	Reig et al. (2014), Rozoy, Boudesocque, and Bazinet (2015)

ED, Electrodialysis; TDS, total dissolved solid.

23.2.2.2 Adsorption

Adsorption is a mass transfer technique that is commonly used to remove various contaminants and targeted chemicals from wastewaters and brine solutions. It is selective, economical, and efficient. With design and operation flexibility, total mineral recovery from brine can be achieved. Adsorption is a process in which a solid material called an adsorbent receives the desired material (adsorbate) on its surface (Akinpelu, Ali, Johan, Saidur, Chowdhury, et al., 2019). Furthermore, the adsorbate often forms chemical or physical connections with the adsorbent surface. When the forces of attraction between the adsorbate and the adsorbent are due to covalent bonds, chemical bonding develops. Physical bonding between the adsorbate and the adsorbent, on the other hand, occurs when van der Waals forces are dominant. It becomes easier to reverse the adsorption process and renew the solid material by a desorption process when the van der Waals attraction forces between the solid targeted materials are weaker. Scientists can reuse the adsorbent numerous times by regenerating it as mentioned in Table 23.2, which is a cost-effective and environmentally responsible strategy (Vardhan et al., 2019).

Fixed-bed and batch adsorption are technologies used to recover contaminants as well as valuable minerals and metals from brine solutions. A fixed-bed adsorption technique uses a column with a fixed adsorbent material that receives contaminated solutions on a continual basis. Two layers of glass beads and/or wool are commonly used in the design of a fixed-bed adsorption column to prevent adsorbent losses and provide mechanical support to the bed (Khadhri et al., 2019). Batch adsorption, on the other hand, entails the addition of a known amount of adsorbent to a known volume of contaminated solution. The adsorption process is then allowed to attain an equilibrium condition. Due to its efficient performance, batch adsorption technology is commonly used when the concentration of pollutants or valuable compounds is high. Batch adsorption is also less expensive than fixed-bed adsorption and requires less maintenance due to its simplicity.

Mg(OH)₂ was precipitated and then adsorbed on the surface of magnetite (Fe₃O₄) microparticles, with recovery from the solution using magnetic separation, according to Lehmann, Nir, Kuflik, and Lahav (2014). Depending on the type of acid used to re-dissolve Mg (OH)₂, three products (MgCl₂, MgSO₄, and Mg (HCO₃)₂) could be generated, all of which are >97% pure. The authors ran a basic cost study and found that MgSO₄ and Mg(HCO₃)₂ were appealing to create, while MgCl₂ could be generated at a cost comparable to other similar commercial goods.

Lithium demand is increasing at a 10% annual rate on the global market. Lithium recovery from salt-lake brine is now one of the most used methods for producing lithium salt, as salt-lake brines contain more than 60% of the world's lithium deposits (Xu et al., 2016).

However, most salt lakes have a high Mg/Li mass ratio (>40) (Wang, Zhong, Du, Zhao, & Wang, 2018), making lithium extraction from salt-lake brine a difficult process. Chen, Lin, and Yu (2020) investigated magnetic lithium aluminum-layered double hydroxides (MLDHs) for lithium adsorption from Qarhan Salt Lake brine with an Mg/Li ratio of 284. With a reduced Fe₃O₄ content (5.83 mg/g at 13.11 wt.% vs 3.46 mg/g at 30.58 wt.%), the MLDHs had a better adsorption capacity. When the solution was exposed to an external magnetic field for 10 minutes, almost 97% of the MLDHs were recovered.

23.2.2.3 Precipitation

Aqueous precipitation is a unit activity that results in the formation of a solid from a supersaturated solution, with supersaturation generating a chemical potential that drives the reaction. Removal of solvent by evaporation, addition of another solvent, change of temperature or pressure, addition of other solutes, oxidation–reduction processes, or combinations of these can all lead to a nonequilibrium, supersaturated state (Ma et al., 2020). The creation of solids with a crystalline structure from a liquid phase is known as crystallization. Precipitation, in this sense, refers to the creation of solids (not necessarily crystalline, but also amorphous) from a liquid phase under extremely high supersaturation circumstances. Processes involving chemical interactions between added reagents and/or other species in the solution, where the products have very low solubility, are commonly referred to as precipitation. Notwithstanding, the two terminologies overlap sometimes. Precipitation is a very easy process compared to the other methods outlined earlier, and it has been employed in large-scale industrial recovery of lithium from brines with low Mg/Li mass ratios (below 8.0) (Zhang et al., 2019). Precipitation techniques for extracting Li⁺ from brines with high Mg/Li ratios have been developed in recent years. Under ideal conditions, aluminum-based materials were employed to precipitate Li⁺ from brines with high Mg/Li mass ratios (20–40), resulting in a recovery of 78.3% in the precipitate with an Mg/Li mass ratio of only 0.02 (Wang et al., 2018). However, because of the substantial loss of lithium, the precipitate must be treated to recover the lithium. The new improved approach yielded a lithium recovery in solution of around 95%, and the precipitated MgAlCO₃-LDHs might be utilized as hosts for nanoscale processes, sorbents, polymeric additives, and catalysts (Zhang et al., 2019).

23.2.2.4 Electrodialysis

ED is a method of separating ions from an aqueous solution using electrically charged membranes and the driving force of an electrical potential difference. It is used to desalinate or concentrate a liquid process stream containing salts. In contrast to distillation techniques and other membrane-based processes (such as RO), dissolved species are transported away from the feed stream in ED. ED gives the practical advantage of substantially better feed recovery in many applications since the quantity of dissolved species in the feed stream is far less than that of the fluid. ED has been used to purify water and produce food-grade salt from seawater concentration for more than half a century. ED is known as an efficient concentration step for seawater desalination (SWD)—RO brine to be used as a raw material in the chloralkali industry (Reig et al., 2014). In particular, ED bipolar membrane (EDBM) technology has been used effectively in biochemical and food processing systems, and it is now being promoted for environmental applications (e.g., recovery or valorization of waste effluents for the production of acids and bases) (Rozoy et al., 2015; Ye et al., 2015). In water desalination, strong bases and acids are employed to alter the water quality in terms of organoleptic perception or pH modification, or as reagents for RO or ultrafiltration membrane cleaning processes (Birnhack, Voutchkov, & Lahav, 2011). Acid and base generation from a variety of effluents, including sludge, wastewater containing metals, dilute salt solutions, and lake brines, has recently been investigated (Tran et al., 2015). Some laboratory or pilot-scale investigations using saltwater or brines to create compounds (acid and base) with EDBM have been published (Badruzzaman, Oppenheimer, Adham, & Kumar, 2009; Venugopal & Dharmalingam, 2014). In 10 hours of operation, Badruzzaman et al. (2009) processed RO concentrate in an EDBM pilot plant with a 64 cm² active membrane area produced 0.2 M of mixed acid (HCl, HNO₃, and H₂SO₄) and base (NaOH, KOH). Ibáñez, Pérez-González, Gómez, Urtiaga, and Ortiz (2013) employed synthetic RO brines as the feed solution in an EDBM pilot plant with a membrane size of 200 cm² to produce 1 M of acid (HCl) and base (NaOH). The electricity consumption would be reduced as the brine concentration increased, according to both studies.

23.2.3 Energy recovery from brine

Energy recovery from brine has always focused on osmotic power. Osmotic power or blue power is the energy recovered from the salinity gradient between high saline solution (brine) and low saline solution which can be seawater or treated municipal wastewater.

23.2.3.1 Pressure retarded reverse osmosis

A polymeric semipermeable pressure retarded reverse osmosis (PRO) membrane lies at the heart of this process, which harvests energy from a salinity gradient. As shown in Fig. 23.3, water naturally permeates from the feed solution side to the draw solution side when the PRO membrane separates a high-salinity draw solution from a low-salinity feed solution, while solutes are selectively rejected. The trans-membrane water flux can either be used to drive hydroturbine to generate electricity or generate hydraulic pressure. The generated hydraulic pressure will then be harvested and returned back into desalination process as captured in Fig. 23.2. The effective salinity difference across the membrane selective layer determines the magnitude of the water flux. Previous research has found that the salinity in saltwater can support a commercial-scale PRO plant as long as efficient PRO membranes with a $W > 5 \text{ W/m}^2$ can be created (Song, Liu, & Sun, 2013) (Fig. 23.2).

Earlier efforts to recover energy from brine focused on generating electricity from the osmotic power. Statkraft launched the first PRO prototype plant in 2009, with the goal of generating 10 kW of energy utilizing seawater and river water as draw and feed solutions, respectively (Gerstandt, Peinemann, Skilhagen, Thorsen, & Holt, 2008; Sarp, Li, & Saththasivam, 2016). Despite the project's initial promising future, it was shelved in 2014 due to a lack of cost-effective commercial-scale PRO membranes capable of generating a minimum power density of 5 W/m², which was required for a PRO process to be economically viable (Sarp et al., 2016).

The Japanese Mega-ton Water System spearheaded another PRO program in 2011, which aimed to recover energy from brine while also reducing the environmental impact of brine discharge. The draw and feed solutions for a PRO prototype plant were brine from a saltwater desalination plant (460 m³/day) and treated municipal effluent (420 m³/day), respectively. They stated that employing 10-in. CTA hollow-fiber membrane modules, the prototype plant achieved a maximum power density of 13.3 W/m² and a potential energy-savings of 30% at a bigger scale (Kurihara & Takeuchi, 2018). In addition to the PRO, reverse ED (RED) and capacitive mixing (CAPMIX) are some of the new technologies being investigated to recover energy from brine.

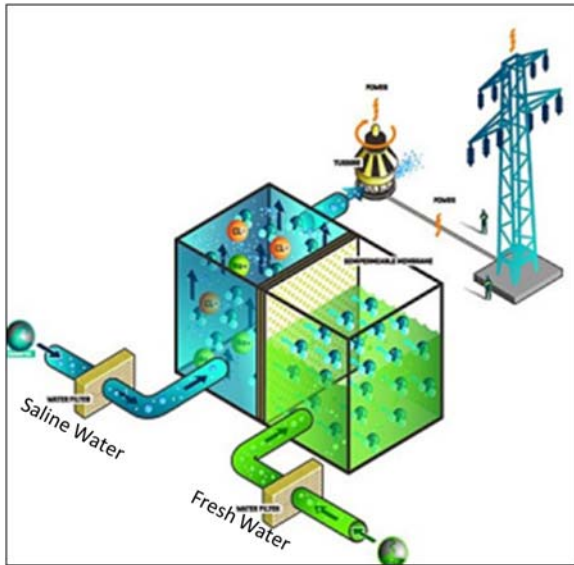


FIGURE 23.2 Pressure retarded reverse osmosis (Alsvik & Hägg, 2013).

23.2.3.2 Reverse electrodialysis

RED is a salt battery that works by transporting (salt) ions across membranes. As a result, voltage is generated across each membrane, and the system's overall voltage equals the sum of the voltages across all membranes (Ogunbiyi et al., 2021).

High power densities have been achieved in tiny lab-size devices, but the technology must be translated to large industrial-scale stacks before it can be commercialized. Furthermore, power density is a significant characteristic, and efficiency, defined as the quantity of energy harvested versus the amount of energy available in the feedwaters, is critical for commercial activities (Moreno, Grasman, Van Engelen, & Nijmeijer, 2018). Moreno et al. (2018) studied the effects of stack size and membrane type on power density, thermodynamic efficiency, and energy efficiency in a systematic way. The residency duration is a useful metric for evaluating different stack sizes and converting lab-scale experimental results to larger pilot stacks, according to their findings (Moreno et al., 2018). At the operational level, there are various prototype demonstrations of this technology and more work need to be done to enter commercial scale.

23.2.3.3 Capacitive mixing (CAPMIX)

CAPMIX refers to a variety of electrode-based methods that are being developed to generate electrical energy from salt gradients. The CAPMIX technique generates electricity by charging and discharging electrodes in a cycle. These capacitive electrodes can capture energy in two ways: through variations in membrane potentials caused by ion concentration gradients or through work performed by expanding the electric double layer. The capacitive electrodes are coated with ion-exchange polymers that allow only selective charge transfer (anions or cations) to each of the electrodes for energy recovery depending on changes in membrane potentials (also known as Donnan potentials).

This capacitive Donnan potential (CDP) technique involves four independent processes to harvest energy as seen in Fig. 23.3. Due to the production of a membrane potential at each electrode, the capacitive electrodes are initially polarized using a high-concentration solution (seawater) under open circuit circumstances (Step 1). Fig. 23.3 showed that the capacitive electrodes are connected to an external load, allowing electrical current to flow through a circuit and ionic current to flow through the electrolyte (Step 2). The circuit is reopened (no current) and a low-concentration solution (river water) is injected, inverting the polarity of each membrane potential (Step 3). The electrodes are then discharged by connecting them to an external load and causing an electrical current to flow in the opposite direction (Step 4). The voltage window formed by the membrane potential defines the net energy extracted. As a result of the four-step CDP cycle's spontaneous energy creation, the process does not require any electrical input energy (Hatzell, Cusick, & Logan, 2014). In general, the technology is still at bench scale (Ogunbiyi et al., 2021).

Recently, the recovery of hydraulic energy has become more popular than the electric energy production. Particularly as it reduces overall energy cost in an eco-friendly manner. The majority of the energy used in traditional desalination comes from fossil fuels, which contributes to greenhouse gas emissions. Desalination processes in

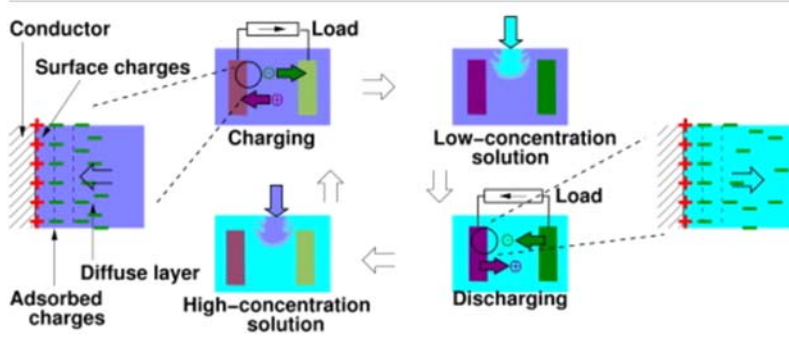


FIGURE 23.3 CAPMIX diagram (Brogioli, Ziano, Rica, Salerno, & Mantegazza, 2013). CAPMIX, Capacitive mixing.

Australia emitted 1193 kt CO₂e in 2015 (Heihsel, Lenzen, Malik, & Geschke, 2019), accounting for 1% of total power emissions. Operational adjustments and the use of energy recovery devices (Park, Kim, Yang, & Hong, 2020), advancements in membrane technology (Hailemariam et al., 2020), the use of integrated/hybrid membrane systems (Ang, Mohammad, Hilal, & Leo, 2015), and renewable energy sources have all been tried to address these challenges (Shemer & Semiat, 2017).

The SEC compares energy efficiency in seawater reverse osmosis (SWRO) plants that include (1) seawater intake, including low-pressure pumps; (2) screening and pretreatment; (3) RO system, including membranes and high-pressure pumps (HPP) (65% of SEC); (4) permeate system, including posttreatment, storage, and pumping to the distribution network; and (5) concentrate (brine) disposal. Depending on feed circumstances, product water requirements, and plant efficiency, SEC can range from 3 to 6.7 kWh/m³ (Wang, Zhou, Li, Tang, & Hu, 2019). To raise feedwater pressure above the osmotic pressure of seawater, HPP normally require between 55 and 70 bar pressure (Gude, 2018). HPP has traditionally been a significant contributor to overall energy usage and operational costs of SWRO desalination (Karabelas, Koutsou, Kostoglou, & Sioutopoulos, 2018).

23.2.4 Resourceful use of brine by different countries

The rich and water-scarce Middle East, with Saudi Arabia and the United Arab Emirates accounting for 22% and 10.1% of global desalination capacity, respectively, are the largest players in the desalination industry. Overall, the Middle East and North Africa account for 47.5% of global desalination capacity, followed by East Asia and the Pacific (18.4%), North America (11.9%), and Western Europe (9.2%). Conversely, due to the complexity and costs of desalination, Sub-Saharan Africa accounts for a surprisingly small share of global desalination capacity: only 1.9%. Kuwait, Bahrain, Saudi Arabia, Qatar, the United Arab Emirates, and Oman make up the GCC. These countries have limited access to freshwater. There are no permanent rivers in Kuwait, Saudi Arabia, Qatar, or Bahrain (except for a few small rivers and stream in the Hejaz region). Desalination provides Qatar with 99.9% of its potable water (Sezer et al., 2017).

23.2.4.1 Saudi Arabia

Saudi Arabia is the world leader in desalinated water production, with a daily capacity of 117 million cubic feet. Saudi Arabia pursued this feat because of its climate and regional realities. The kingdom is largely desert with rare surface water. It is the biggest country in the world without a river. It has irregular and scanty rain ~88 mm/year. The summer is very intense in the kingdom; therefore evaporation rate is very high. The volume of the few available fresh water is falling. However, growing population, rapid industrialization, and growing social and religious tourisms have continued to increase the demand for fresh water. Interestingly, it is only country that is blessed with Red sea in the west and Arabian/Persian gulf in the east as seen (Fig. 23.4A).

High demand for fresh water in one and availability of abundant seawater bodies, on the other hand, have made Saudi Arabia to aggressively pursue water desalination to meet up with its fresh water demand. It started as earlier as 1928 with the establishment of Kendasa (Condenser) by King Abdul Aziz ("Desalination in Saudi Arabia an Overview N. Nada General Manager Desalination Nomac," 2021). The first membrane (RO) desalination plant was commissioned in 1968. Currently, the country has 33 desalination plants scattered along its coastline, as seen in Fig. 23.4B. Notwithstanding, the kingdom has 97% water penetration and 60% of this water is coming from desalination process. Consequently, Saudi Arabia produces most amount of brine at rate of 31.53 million m³/day, equivalent to 22% of the world's total (Jones et al., 2019).

Fig. 23.5. Consequently, the huge volume of resultant brine from the kingdom's desalination plants will continue to rise. As a result, the kingdom has launched series of programs to instigate research activities and real-time business investments on how to derive different valuables from brine as seen in Fig. 23.5.

One of the easiest valuables to get from brine is the salt which can also be in form of pure NaCl. Each year, more than 280 million tons of salt are generated worldwide through solar evaporation of saltwater, traditional rock mining, and saline brine solution mining. This is made up of 39% of the total used by the chloralkali business to make chlorine and sodium hydroxide, 22% for human consumption, 22% by the soda ash industry, 9% for road deicing, and 9% for other purposes (Nayar et al., 2019). Saudi Arabia ranked 19th in 2019 among the salt producing countries globally with production of 2.239 million metric tons in 2019. In 2019 Saudi Arabia exported \$12.5 million worth of salt (including table salt and denatured salt) and pure sodium chloride, whether in aqueous solution or with added anticaking or free-flowing additives.

Therefore the local scientists in Saudi Arabia together with their international collaborators through government-run desalination research institute have been working on several solutions in which the brine volume will be minimized and useful minerals like sodium chloride salt, magnesium, and calcium are extracted from seawater alongside clean water. In Saudi Arabia, saline water conversion corporation (SWCC) through its premier research center [Desalination Technology Research Institute (DTRI)] has developed state of the earth technology which promises to collect valuable salts and minerals from brine while enhancing freshwater recovery by up to 65%. Fig. 23.6 showed the operational sketch of the new technology. The new technology allows to make commercially valuable products from the concentrate (brine) generated by desalination plants and at the same time try to achieve ZLD of the brine. By the way, the Saudi Arabia ZLD system market is forecast to grow from USD 44.72 million in 2020 to USD 73.94 million by 2026 (Water Online, n.d.) (Fig. 23.6).

The new technology (Dual Brine Concentrator) as shown in Fig. 23.6 makes it possible to harvest precious minerals from brine produced by desalination facilities at a low cost. The target elements are magnesium (Mg), calcium (Ca), sodium chloride (NaCl), bromine (Br), lithium (Li), potassium (K), and sulfate (SO_4^{2-}). The market readiness of this technology is currently demonstrated at DTRI's testing facility in Jubail which produces commercial-grade sodium chloride brine suitable for use by the chloralkali industry. The technology is a high-efficiency membrane separation and energy recovery technology. FEDCO, PACT Engineering, Hydranautics, PROTEC, Piedmont, Toyobo, Pacifica Water Technologies, and FTS are among the companies on the team. Members of the DTRI collaborative team are combining their innovative ideas and practical experience to advance desalination technology and science toward a better and

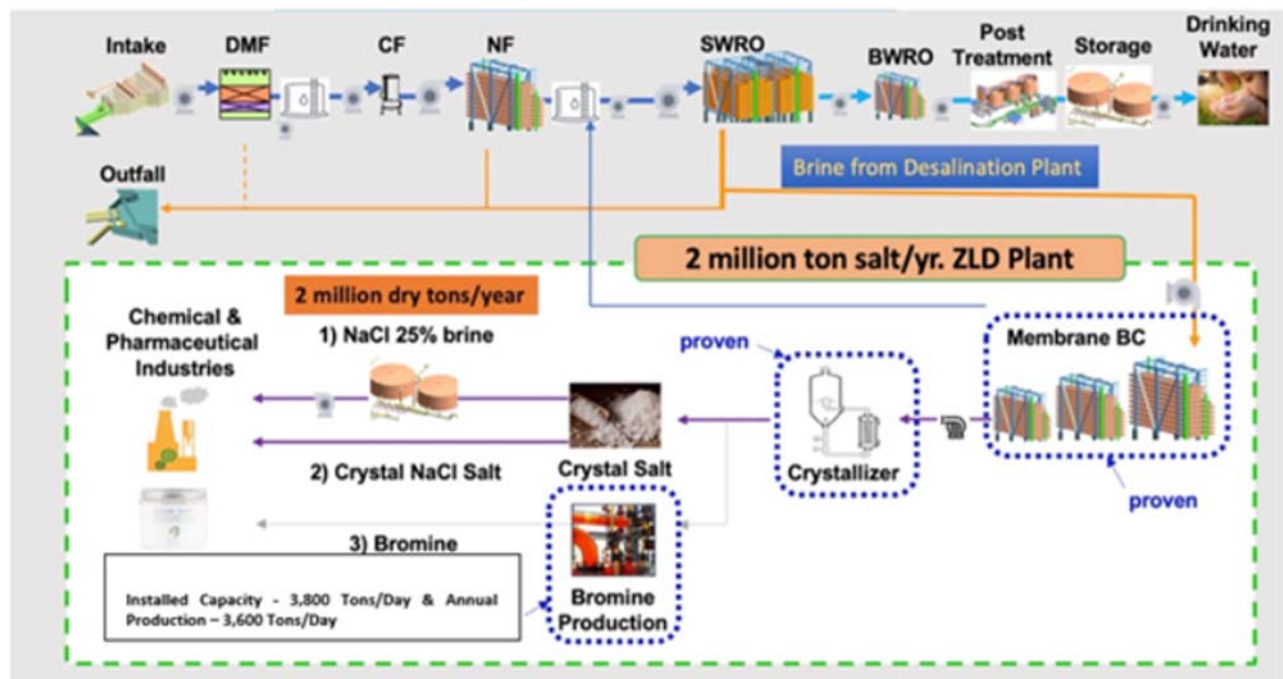


FIGURE 23.6 General schematic of the brine mining plant for NaCl and Br (Al Amoudi, Ihm, & Voutchkov, 2021). Reproduced from IDA Global Connections Fall 2021

greener world, where desalination brine is no longer considered a waste but a valuable strategic product with high economic significance. FEDCO developed and commercialized the technique, which has distinct advantages for both desalinated water generation and brine concentration. The key targets of the technology are;

- reduction of energy use for brine concentration by over 50% (7.5 vs 17.5 kWh/m³),
- production of 20% more desalinated water than conventional SWRO plants,
- brine with 10%–15% higher concentration,
- 15%–20% lower capital costs, and
- over 15% lower brine production costs (Al Amoudi et al., 2021).

In 2020 SWCC signed memorandums of understanding and began negotiations with a number of Saudi Arabian chloralkali-producing companies to supply a total of 2 million dry tons/year (2 MM tpa) of high-purity (99% or higher) sodium chloride as either a liquid solution of 25% or salt crystals. SWCC plans to design, build, and manage a 2-MM tpa brine concentration plant with a bromine manufacturing plant with an installed capacity of 3800 tons/year based on these agreements (Al Amoudi et al., 2021).

Similarly, in April 2019, Red Sea Development Company in Saudi Arabia launched the Brains for Brine Challenge, challenging academics, scientists, engineers, and the water industry to develop innovative new solutions to manage brine as a waste product of water desalination—a long-standing environmental challenge for the scientific community.

Therefore it can be seen clearly that Saudi Arabia with the highest brine production globally is making an impressive progress to recover useful product from the brine.

It can be seen from Table 23.3, that different countries are at different stage of the brine utilization. All the countries listed in the table have all adopted water recovery from brine through the use ZLD techniques. Like it was discussed in the earlier section, brine minimization is the gateway to the brine utilization. Similarly, Saudi Arabia, United States, the Netherlands, and China are adopting mineral recovery from the brine. The minerals are magnesium (Mg), calcium (Ca), sodium chloride (NaCl), bromine (Br), lithium (Li), potassium (K), sulfate (SO₄²⁻), HNO₃, H₂SO₄, etc. United States, the Netherlands, and China have made significant gains in the area of mineral recovery from brine. Saudi Arabia, the largest brine producer, is still at pilot stage for the recovery of some of the important minerals. Furthermore, Saudi

TABLE 23.3 Comparative study of use of brine by different countries.

Country	Amount of brine (m ³ /day)	Water recovery	Mineral recovery	Energy recovery
Saudi Arabia	31.55 (Jones et al., 2019)	ZLD (Al Amoudi et al., 2021; Officer, 2019)	Magnesium (Mg), calcium (Ca), sodium chloride (NaCl), bromine (Br), lithium (Li), potassium (K), and sulfate (SO ₄ ²⁻) (Al Amoudi et al., 2021)	Pressure exchanger (Magazine, 2019)
UAE	20.94 (Mogielnicki, 2020)	ZLD (“Zero Liquid Discharge Solutions,” 2017)		Pressure exchanger (Desalination, 2019)
United States	5.28 (Jones et al., 2019)	HPRO, ZLD, FO, VSEP, OARO (Panagopoulos et al., 2019)	NaCl, HCl, HNO ₃ , H ₂ SO ₄ , NaOH, KOH, Li (Badruzzaman et al., 2009; Company, 2022; Incorporation, 2022; Katal, Shen, Jafari, Masudy-Panah, & Farahani, 2020)	Pressure exchanger, CAPMIX, gas-to-liquid process by US Navy (Corporation, 2016; Hatzell et al., 2014; Tina Casey, 2015)
The Netherlands		ZLD (Smart Water Magazine, 2020)	Sulfate salts, sodium bicarbonate, NaCl (Water Technology, 2020)	Osmotic pressure, RED, CAPMIX (Elshof, 2015; Gerstandt et al., 2008)
China	1,785,000 (Gong, Wang, Zhu, Bai, & Wang, 2019)	ZLD (Asia Pacific, 2019)	Li, NaCl, magnesium (Mg), calcium (Ca), sodium chloride (Gong et al., 2019; Song, Nghiem, Li, & He, 2017)	Pressure exchanger ()

CAPMIX, Capacitive mixing; FO, forward osmosis; HPRO, high-pressure reverse osmosis; OARO, osmotically assisted reverse osmosis; RED, reverse electrodialysis; RO, reverse osmosis; TDS, total dissolved solid; ZLD, zero liquid discharge.

Arabia, UAE, United States, the Netherlands, and China have embraced pressure exchanger technology to recover energy from brine. Not only that, United States and the Netherlands have gone far ahead of other countries in the recovery of energy from brine by the use technologies such as CapMix, RED, osmotic pressure (blue energy) to generate electricity.

23.3 Conclusion

As the world's population grows, so does the demand for water. The availability of fresh water, on the other hand, has been depleted. Due to depleting freshwater resources, population increase, and significant developments in desalination technology, saltwater desalination has become unstoppable. Many countries are increasing their desalination capacity, but they must also deal with the waste that comes with it (brine). Desalination, paper making, cooling towers, and natural gas generation all produce brine. Because Brine has been prohibited from being dumped into the sea, because it is corrosive and harmful to marine life. On one hand, recovering valuable chemicals from brine reduces the toxicity of the solution, making it safer to dump or reuse. It will also raise revenue, which will cut the overall cost of desalination. More water, chemicals, and energy can be recovered from brine.

The MLD or ZLD procedures are used to recover the water. The membrane-based technologies that are relevant for MLD are RO, OARO, and HPRO. Minerals can be recovered in a variety of methods from brine. Crystallization, precipitations, adsorption, ED, and membrane distillation are examples of these techniques. PRO, RED, and CAPMIX are approaches being investigated to recover energy from brine. Various countries are at various stages of brine usage. Saudi Arabia, UAE, United States, the Netherlands, and China have all adopted water recovery from brine through the use ZLD techniques. Mineral and energy recovery from brine have made more significant progress in the United States and the Netherlands. Saudi Arabia, the world's largest producer of brine, has been investing heavily in brine recovery to recover more water, minerals, and energy.

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Innovation of Food Products in Halal Supply Chain Worldwide

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A comprehensive overview to understand concepts behind controversial halal ingredients, their purpose and available halal alternatives

Innovation of Food Products in Halal Supply Chain Worldwide is a resource of information on fundamentals and food guidelines of halal food production. Unlike other texts on the halal food market and halal certification, this book promotes halal product innovation by presenting exciting newly developed ingredients that are substitutions of non-halal ingredients with halal alternatives, such as lard substituted with modified vegetable fats, pig with halal goat/beef/camel/fish gelatine/collagen, alternative meat substitute, or even additives. Innovations in halal processing technologies cover latest techniques in halal production and authentication, and halal tracking/traceability in halal transport and logistics, a vast area at the end of a supply chain. It is a comprehensive approach to halal food production, logistics, and traceability in one book. All chapters are written by acknowledged experts in their field; thus the book has brought together the top researchers in this field, essential to a huge percentage of the world's population.

Key Features

- Understands the advancement of available halal substitutes and replacers
- Offers tool to enhances product sustainability and food security through innovation
- Fosters innovation in food science with alternative halal ingredients



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