Review

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Cowpea: an overview on its nutritional facts and health benefits

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Abstract

Cowpea (*Vigna unguiculata*) is a legume consumed as a high-quality plant protein source in many parts of the world. High protein and carbohydrate contents with a relatively low fat content and a complementary amino acid pattern to that of cereal grains make cowpea an important nutritional food in the human diet. Cowpea has gained more attention recently from consumers and researchers worldwide as a result of its exerted health beneficial properties, including anti-diabetic, anti-cancer, anti-hyperlipidemic, anti-inflammatory and anti-hypertensive properties. Among the mechanisms that have been proposed in the prevention of chronic diseases, the most proven are attributed to the presence of compounds such as soluble and insoluble dietary fiber, phytochemicals, and proteins and peptides in cowpea. However, studies on the anti-cancer and anti-inflammatory properties of cowpea have produced conflicting results. Some studies support a protective effect of cowpea on the progression of cancer and inflammation, whereas others did not reveal any. Because there are only a few studies addressing health-related effects of cowpea consumption, further studies in this area are suggested. In addition, despite the reported favorable effects of cowpea on diabetes, hyperlipidemia and hypertension, a long-term epidemiological study investigating the association between cowpea consumption and diabetes, cardiovascular disease and cancer is also recommended.

Keywords: cowpea; nutritional properties; functional compounds; processing; health benefits

INTRODUCTION

Cowpea, is a food legume of the family Fabaceae/Papilionaceae.[1] All cultivated cowpeas are grouped under the species *Vigna unguiculata*, which is subdivided into four cultivar groups: *Unguiculata, Biflora, Sesquipedalis* and *Textilis*. They can be distinguished from one another by different physiological factors, such as seed size and color, taste, yield and maturity time.[2] The plant is an herbaceous legume showing considerable adaptation to warm climates with adequate rainfall and is cultivated across Southeast Asia, Africa, Southern United States and Latin America. Cowpea is also traditionally cultivated in some Mediterranean countries,[3] although it is not widespread in Europe.[4] However, the origin of cowpea is considered to be Africa.[5–8] Cowpea provides food for millions of people, mainly in developing countries, with an annual worldwide production of about 4.5 million metric tons.[9] Although beans are the primary focus of the cowpea plant, both flowers and leaves are also considered as edibles in some parts of the world.[10,11]

According to recent estimates, malnutrition contributes to more than one-third of all child deaths worldwide. Particularly, child malnutrition was associated with 54% of deaths in children in developing countries.[12] This can be mainly a result of the consumption of high cereal-based meal, which is bulky, high energy and less nutritious.[13,14] Legumes along with cereals can be considered as main plant sources of energy and good quality proteins. Thus, cowpea has been promoted as a high-quality protein constituent of the daily diet among economically depressed communities in developing countries, with the aim of reducing the high prevalence of protein and energy malnutrition.[9,15,16] Nutritionally, cowpea grain is more or less the same as other pulses, with a relatively low-fat content and high total protein concentration. Cowpea is considered as a nutrient dense food with low energy density. An average cowpea grain contains 23–32% protein,[17] 50–60% carbohydrate[18,19] and about 1% fat[19] in dry basis. The total protein content of cowpea is approximately two- to four-fold greater than cereal and tuber crops.[20,21] Moreover, compared to cereal grains, cowpea protein is a rich source of the amino acid lysine and is used as a natural complimentary food with cereals.[5] However, it is deficient in methionine and cysteine compared to animal proteins.[22] Cowpea is considered as an incredible source of many other health-promoting components, such as soluble and insoluble dietary fiber, phenolic compounds, minerals, and many other

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functional compounds, including B group vitamins.[23,24] Thus, cowpea contributes greatly toward improving the quality of human health by offering a number of health benefits. Epidemiological evidence indicates that the consumption of cowpea exerts protective effects against the development of several chronic diseases, such as gastrointestinal disorders,[21] cardiovascular diseases, hypercholesterolemia, obesity,[25] diabetes[26,27] and several types of cancer.[18,28] In addition, the literature also reports on functional ingredients in cowpea that aid in weight loss,[29] improve digestion and strengthen blood circulation.[21] All of these beneficial effects exerted by cowpea are attributed to the presence of phytochemicals, resistant starch, dietary fiber and a low-fat content, along with beneficial unsaturated fatty acids. The low glycemic index of cowpea is attributed to the action of resistant starch and dietary fiber, which attenuate insulin responses and reduce hunger.[30] The major limiting factors of the consumption of cowpea in day-to-day diet include poor digestibility, a deficiency of sulphur-containing amino acids and the presence of anti-nutritional factors, such as trypsin inhibitors, oligosaccharides and phenolic compounds. Nevertheless, adequate processing methods can be used to destroy those anti-nutritional factors, and improve the bioavailability levels without much effort.[16] The present review aims to explore the nutritional and functional health properties of cowpea, which is under-rated as a result of insignificant and avertable limiting factors.

NUTRITIONAL AND FUNCTIONAL COMPOUNDS

Proteins and peptides

Studies reveal that the protein content of legumes (17–30%) is comparatively higher than cereals (7–13%).[31] Legumes are known as poor people's meat because the protein content is approximately equal to certain meat types (18–25%).[31] Cowpea is a legume, which is considered to be one of the main high-quality plant protein sources in the tropics. Generally, the protein content of cowpea differs with the variety.[32] Cowpea contains a complex and unique protein profile, including globulins (about 16 protein bands), albumins (about 20 protein bands), glutelins (21 protein bands) and prolamin (one protein band).[33,34] The protein of cowpea is mainly composed of globulin fraction (50–70%), which is further divided into two major groups: 11S (legumin) and 7S fraction (vicilin/β-vignina).

Albumins and globulins are considered to represent the major storage proteins in cowpea.[33] Fractions of albumin in seeds vary between 8.2% and 11.9%. They are categorized as enzymatic and metabolic proteins, such as lipoxygenase, protease inhibitors and lectins.[35,36] Glutelins are the next major protein fraction, comprising 14.4–15.6% of the total proteins.[36] The least concentrated storage protein type in cowpea is prolamins, ranging from 2.3% to 5.0%,[36] with high proline and glutamine contents.[33] The protein isolates from cowpea sprouts contain a low lysine/arginine ratio similar to that of protein isolates of soybean, making cowpea a potential functional ingredient for decreasing cholesterol.[37]

The uniqueness and quality of the protein in a food group mainly depends on its amino acid composition and on the physiological utilization of specific amino acids after digestion and absorption.[16] According to Gupta *et al.*,[36] the amino acid profile of cowpea varies depending on the genotype of cowpea (Table 1). Gupta *et al.*[36] compared the amino acid content in seven genotypes of cowpea, where the maximum and minimum total essential amino acid content was 33.43 g 100 g^{-1} and 27.50 g 100 g^{-1} protein, respectively.

Table 1. Amino acid profile of cowpea

Essential amino acid	Amount in cowpea (g 100 g^{-1} protein)
Histidine	1.85–2.47
Cysteine	0.84–1.08
Methionine	1.28–2.06
Isoleucine	4.17–5.46
Leucine	6.45–8.5
Threonine	3.89–5.12
Lysine	7.3–8.74
Tryptophan	1–1.33

Hussain and Basahy[38] showed that cowpea proteins are composed of at least 17 amino acids, with the majority being essential. Cowpea proteins contain amino acids such as valine, leucine, phenylalanine and lysine in slightly higher amounts than those of sulphur-containing amino acids.[16,39] However, mature seeds are reported to contain a low amount of free amino acids compared to the immature ones. This is mainly a result of the utilization of free amino acids in protein synthesis during seed development.[16,39]

In addition to the importance of essential amino acids, there is also a growing interest in protein-derived fragments called peptides, which can be used in the prevention or treatment of chronic metabolic disorders. Natural peptides are released as a result of enzymatic hydrolysis or fermentation, and are reported to act favorably inside the body.[40,41] The ability of these peptides to create favorable physiological conditions for proper bodily functions renders them 'bioactive'. For example, peptides which are labeled as functional or biologically-active are reported to show anti-hypertensive,[42] anti-dyslipidemic,[43] antioxidative,[41] anti-carcinogenic,[44] anti-diabetic[27] and anti-microbial properties.[27] Peptides usually composed of 3 to 20 amino acid residues are released as a result of enzymatic proteolysis of various animal and plant proteins.[45] The hydrolysis products exert their hypolipidemic functions by binding to bile acid, disrupting cholesterol micelles in the gastrointestinal tract, as well as by altering the hepatic and adipocytic enzyme activity and gene expression of lipogenic proteins.[41] In addition, cowpea protein isolates were found to aid in the prevention of the development of diabetes mellitus by mimicking the actions of insulin.[27] Furthermore, recent *in vitro* studies have shown that cowpea peptides act as antioxidants and aid in cancer prevention.[4,46] Bioactive peptides are also reported to possess selective cytotoxic activity against a wide range of cancer cell lines both *in vitro* and *in vivo*.[4,46]

Polyphenols and antioxidants

Polyphenols, flavonoids, anthocyanins and tannins are a diverse group of phytochemicals present in legume seeds.[23,24] These compounds can benefit human health by scavenging free radicals and preventing oxidative stress in the body.[47] They also influence a wide range of biological functions, including antioxidant,[22] anti-inflammatory, anti-cancer,[48] hypolipidemic,[25] hypoglycemic[26] and enzyme inhibitory activity.[41] The phenolic composition and their functioning mechanisms in both raw and cooked forms of cowpea seed have been reported in many studies.[23,24]

A large intervarietal difference was found among the cowpea varieties with respect to their total phenolic content.[47,49,50] Adjei-Fremah *et al.*[51] observed that the total phenolic content, condensed tannins and antioxidant capacity of seed extracts of several cowpea varieties ranged from 46.48 ± 0.78 to 119.61 ± 2.48 mg GAE 100 g^{-1}, 0.16 ± 0.005 to 0.22 ± 0.01 mg CE 100 g^{-1} and 53.20 ± 1.26 to 136.41 ± 2.11 μmol L^{-1} TE 100 g^{-1}, respectively. Furthermore, whole cowpeas contain about 70% free phenolics and 30% bound phenolics, with the seed coat containing about a 5- to 10-fold increase of the phenolic content than the seeds. The seed coat also contains approximately 10-fold more flavonoids compared to the whole seeds.[49] Coumaric acid and ferulic acid are reported to be the most abundant phenolic acids in the cowpea seed, whereas, in the seed coat, the main phenolic acid is gallic acid followed by protocatechuic, *p*-hydroxybenzoic and coumaric acids. Ferulic acid is the only free phenolic and gallic acid is the only bound phenolic present in the seed coat of cowpea. The ferulic acid content normally ranges from traces to 6.2 mg 100 g^{-1}.[49] Xu and Chang[50] reported a high positive correlation between phenolics and antioxidant activity, indicating that the overall antioxidant activity exerted by food legumes are predominated by phenolic compounds. However, only a few reports exist regarding the anthocyanins in cowpea. Ha *et al.*[48] isolated and characterized major anthocyanins in cowpea as delphinidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside. Furthermore, five minor anthocyanins were also detected and identified as delphinidin-3-*O*-galactoside, cyanidin-3-*O*-galactoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside and malvidin-3-*O*-glucoside based on the fragmentation patterns of high-performance liquid chromatography combined with a diode array detector and ion spray mass spectrometry analysis. Salawu *et al.*[52] reported that the total flavonoid content in cowpea ranged from 0.95 to 0.36 mg quercetin equivalents g^{-1}, where the cultivars with darker seed coat had higher total flavonoid content than the white cultivars. Apea-Bah *et al.*[53] indicated a high flavonoid content in cowpea flour, where the major flavonoid subclasses were found to comprise flavonols and flavan-3-ols. Similarly, the occurrence of flavonoids in cowpea has also been reported in many other studies.[54,55]

The antioxidant potential of cowpea proteins is well-known and is attributed to peptides with a molecular mass below 3 kDa.[22,41] Although the mechanisms are not well established, researchers have found a positive correlation between the antioxidative properties and hydrophobic amino acids and aromatic amino acids in cowpea peptides. Furthermore, some branched-chain amino acids, including leucine, isoleucine and phenylalanine, as well as aromatic amino acids such as tyrosine, phenylalanine, tryptophan and the sulphur-containing amino acid cysteine, are involved in antioxidative properties because of their ability to donate protons to free radicals.[22,45]

It is well-known that germination improves the nutritional quality of legume seeds. Doblado *et al.*[56] reported a 50–60% increase in the antioxidant capacity and an increase in the vitamin C content in cowpea after germination of the seeds. Luthria *et al.*[57] indicated the significance of sprouting on the nutritive value of this specific legume by observing an increase in β-carotene content, antioxidant activity, phenolic content and flavonoid content. In addition, Khang *et al.*[58] have observed that the total phenolic content of ungerminated white cowpea and germinated white cowpea changed with time, to 7.79 ± 0.02 and 19.46 ± 0.09 (mg GAE g^{-1} dry weight) in 0 to 120 h, respectively. This is approximately a 3-fold increase on the fifth day of germination compared to ungerminated seeds. Furthermore, those phenolic compounds were identified as caffeic acid, syringic acid, vanillin, ferulic acid, sinapic acid, *p*-coumaric acid, benzoic acid, ellagic acid and cinnamic acid. Although there is no sufficient information regarding the vitamin C content in dried cowpea seeds,[56,57] a high vitamin C content was reported by Doblado *et al.*[56] in germinated cowpea seeds, ranging from 23.3–25.2 mg 100 g^{-1} dry matter. Further research must be carried out on the changes in the polyphenol content during germination because there are contrasting findings reported in the literature stating germination to have an negative impact on the antioxidative properties of cowpea.[59,60]

Resistant starch and dietary fiber

The low digestibility of legume starch is mainly a result of the presence of resistance starch, amylose and dietary fiber in the seeds. Legume starch contains 60–70% amylopectin and 30–40% amylose, whereas other starchy food contains 70–75% amylopectin and 25–30% amylose.[61] The evidence indicates that resistant starch and amylose play a major role in overall human health. The presence of resistant starch and amylose in higher amounts reduces the rate of digestion, thereby reducing the amount of glucose released in to the system. This eventually aids in reducing glucose uptake by the intestinal cells.[61,62] In addition, because resistant starches are not completely digested by human digestive enzymes, they act as a substrate to the functional probiotics in the large intestine/colon. Fermentation of resistant starches by colonic microbes produces short chain fatty acids, such as butyrate, which provide many favorable health benefits in proper lipid function and cancer prevention.[63]

According to Eashwarage *et al.*,[64] the resistant starch content of cowpea ranged from 9.04 ± 1.26 to 9.62 ± 0.19 g 100 g^{-1} (9%), whereas the resistant starch content of raw cowpea flour reported by Chen *et al.*[65] was 12.65%. Because cowpea seeds contain a remarkably high amount of resistant starch and dietary fiber, it can be considered as a low glycemic food.[66] Cowpea can also be considered as a meal with reduced calorific value, which helps to improve glucose regulation in diabetes patients, whereas, at the same time, it facilitates better weight control for the obese.[30] Sreerama *et al.*[67] reported the predicted glycemic index (pGI) of cowpea to be 41 ± 10% and Chen *et al.*[65] found that the pGI of cowpea ranges between 33% and 50%. Furthermore, Eashwarage *et al.*[64] found an inverse relationship (negative line with −0.659 of Pearson correlation) between the resistant starch content and pGI content of legumes including cowpea.

Kirse and Karklina[19] indicated that the total dietary fiber content of cowpea ranges from 12.00 ± 0.15 to 14.80 ± 0.20 g 100 g^{-1}. Eashwarage *et al.*[64] similarly having reported total dietary fiber content to range from 13.60 ± 0.15 to 15.99 ± 0.49 g 100 g^{-1}. Furthermore, Khan *et al.*[68] reported that the crude fiber content of cowpea to be18.2%. According to that latter study, soluble and insoluble fiber ratio in cooked cowpeas was about 1:3.2. The seed coats and cell walls of legumes mainly contributed to this high content of crude fiber. The dietary fiber, both soluble and insoluble, provides plenty of benefits to human health. Soluble fiber can dissolve in water and help regulate blood cholesterol level, as well as blood glucose level. Insoluble fiber does not dissolve in water and contributes as bulk or roughage. It absorbs water in the large intestine and in the colon, thereby ensuring moist and smooth transportation of waste materials.[64] Consequently, this prevents the occurrence of haemorrhoids, constipation and many other digestive difficulties. Furthermore, the undigested bulk starches can help to protect colon cells from colon cancers. Many epidemiological studies provide evidence indicating that increased fiber consumption is inversely related to the incidence of cancer, diabetes, obesity, cardiovascular diseases and many other chronic syndromes.[69]

ANTI-NUTRITIONAL COMPOUNDS

Major limiting factors of consumption of cowpea in day today diet include poor digestibility, the deficiency of sulphur containing amino acids and the presence of anti-nutritional factors. The presence of some types of phenolic compounds, such as proanthocyanidins,[70] phytic acid,[59] tanins,[71] haemagglutinins,[72] cyanogenic glucosides, oxalic acid,[73] dihydroxyphenylalanine and saponins, might be nutritionally disadvantageous to humans. These compounds are often referred to as 'anti-nutrients', primarily because of their ability to bind proteins and chelate divalent metal ions.[70] Other than that, enzyme inhibitors in cowpea, such as protease inhibitors, are also considered as anti-nutritional compounds.[74] On the other hand, α-amylase and α-glucosidase inhibitors in cowpea can be extremely beneficial to human health because they can reduce the rate of glucose release during digestion. Ojwang *et al.*[70] reported the proanthocyanidin content of cowpea to range between 2.2–6.3 mg g^{-1}, which is similar to other legumes such as peas, lentils and faba beans.[75] Phytic acid (myoinositol 1, 2, 3, 4, 5, 6 hexakidihydrogen phosphate) is the principal storage form of phosphorus in many dry beans. Phytate is reported to decrease the bioavailability of essential minerals and proteins as a result of the formation of phytate-protein and phytate mineral–protein complexes.[76] Sinha and Kawatra[59] reported the phytic acid content of unprocessed cowpeas to be 836.0 mg 100 g^{-1}. Phytocystatins, which are involved in a variety of physiological processes, including seed development and germination in legumes, can act as protease inhibitors. They act like pseudo-substrates and bind tightly to the active site cleft of cysteine proteases and thereby inactivate their target enzymes.[74] This information is potentially useful when designing dietary guidelines for the consumption of these legumes at the same time as minimizing the potential negative effects. However, proper processing methods can be used to destroy those anti-nutritional factors, and improve the bioavailability levels, especially when it is used as a food for infants and children.

EFFECT OF PROCESSING ON FUNTIONAL COMPOUNDS IN COWPEA

Different processing methods (boiling, sprouting, steaming, frying, soaking, de-hulling and grinding) are often combined with legumes to produce different meals.[77] However, there is information available on the quality and dietary characteristics of fresh pods, which are occasionally used in folk diets in some southern European countries.[8] Cooking and sprouting of legumes greatly alters the properties and bioavailability of some nutrients.[78,79] Sprouting, the practice of germinating seeds, is one of the most adopted methods for legume processing. Several metabolic enzymes, such as proteinases, are activated during this process. This leads to the release of amino acids and peptides to synthesize new proteins.[79] Uppal and Bains[80] observed an increase in the crude protein level in cowpea from 8% to 11% after sprouting. In addition, Devi, *et al.*[79] reported that sprouting increased the total mineral content in cowpea. According to Owuamanam *et al.*,[81] the mineral composition of sprouted cowpea flour was significantly higher than the non-sprouted cowpea flour. Most importantly, Devi *et al.*[79] observed a significant decrease in fat and carbohydrate contents in sprouted cowpea compared to its non-sprouted counterparts. The decrease may be a result of the depletion of stored fat and carbohydrates that contributed to the catabolic activities of the seeds during sprouting.[82] Uppal and Bains[80] observed a 20–24% increase in crude fiber content after cowpea sprouting. Moreover, a significant reduction in anti-nutrient content was also observed after cowpea seed sprouting.[79,83] Sprouting is also known to increase the phenolics and flavonoids in a natural way.[84] Chon[28] reported that the total phenolic and total flavonoids contents, antioxidant and antioxidant enzyme activities differed based on the length of germination. However, Sinha and Kawatra[59] reported that the germination of cowpea seeds resulted in a significant decrease in the polyphenol content (32.5% loss in 72 h at 30 °C). The reduction was greater when germination was carried out for a long period of time. The reason for the losses was attributed to the presence of polyphenol oxidases and the leaching of polyphenols into the water prior to germination. Similar results were observed in a study performed by Giami,[60] where germination decreased the polyphenol content simultaneously with fat and carbohydrates.

Other than sprouting, traditional preparation methods of cowpea are primarily aimed at making them more palatable. These processes may also influence the nutritional value of cowpea. Interestingly, cooking legumes in water, with or without pressure, increases the insoluble fiber content,[85] protein quality and digestibility,[86] and also inactivates protease and amylase inhibitors as well as many other anti-nutrients.[86–89] However, a marked reduction in the content of vitamins and minerals was observed during cooking because of leaching or heat destruction.[78]

A synergetic effect on the reduction of anti-nutrients such as trypsin inhibitor, hemagglutinin, cyanogenic glycoside and tannins in cowpea was observed by Onwuka[90] during soaking followed by cooking. At the same time, Omenna *et al.*[91] indicated that boiling drastically reduced the anti-nutrient factors, such as phytate, tannin and trypsin inhibitors in cowpeas. Deol and Bains[86] reported that there was a significant decrease in the phytate content of cowpea pods as a result of pressure cooking and boiling. The reduction was higher as a result of pressure-cooking. The increase in cooking time in both methods resulted in a greater decrease in phytate level. Sinha and Kawatra[59] reported that pressure cooking was the most effective method for reducing trypsin inhibitors in cowpea. Wang *et al.*[92] reported that steam blanching of cowpea greatly reduced the trypsin inhibitory activity.

Although processing methods such as sprouting and steaming increase the available nutrients in cowpea, certain studies have indicated that boiling cowpea in water retains the least amount of water soluble nutrients.[86,91] However, boiling and germination resulted in carbohydrate values that were significantly lower than that of raw and pressure cooked samples.[91] Longer cooking durations of cowpea in water tend to reduce minerals and phytochemicals, such as ascorbic acid and β-carotene, levels significantly.[86] According to Sinha and Kawatra[59], soaking of cowpeas for varying periods of time markedly reduced the polyphenol content. The loss increased with soaking time, with losses of 12.9% and 17.6% for 12 and 18 h respectively, compared to raw samples. According to the research finding, traditional cooking of unsoaked seeds also resulted in a significant reduction of polyphenols. However, the losses were comparatively lower than when soaked seeds were cooked. Barbosa *et al.*[93] attributed these losses to the water solubility of the phenolic compounds. According to that study, the total phenols were reported as 295.23 ± 24.80 and 131.59 ± 22.29 mg

GAE 100 g^{-1} in raw and cooked forms respectively, whereas the cooking broth contained 274.28 ± 21.18 mg GAE 100 g^{-1}. However Sinha and Kawatra[59] indicated that pressure cooking of unsoaked, soaked and de-hulled seeds had less of a reduction in polyphenolic content compared with other cooking methods. Similarly, according to Pereira *et al.*[94] the retention of minerals, such as zinc, was also higher when cowpeas were prepared in the pressure cooker compared to regular cooking methods.

It is well known that thermal processes promote the disruption of cell membranes of legumes and release phospholipids, which eventually increase the bioavailability of lipids.[95] Marques *et al.*[96] observed the effect of cooking on the fatty acid composition of cowpea oil extracted from raw and cooked beans. Their study demonstrated that cowpea oil is composed of high percentages of polyunsaturated fatty acids (the majority C18:2 *n*6 and C18:3 *n*3), followed by saturated fatty acids (the majority C16:0). However, according to the results, the cooking process employed was not significantly sufficient to alter the composition of fatty acids from raw samples.

The chemical structure and physicochemical properties of starch and fiber present in cowpea seeds are important determinants of their glycemic properties.[97] However, with accurate processing methods, cowpea can be considered as a low glycemic food with a low glycemic index (GI). The results obtained by Oboh and Agu[30] showed that processed cowpea (boiling: soaking, de-hulling; steaming: soaking, de-hulling and frying) had both low GI and GL (glycemic load) values (i.e. below 55). Studies have observed the GI values for the above 3 processing methods to be 46.64, 50.98 and 53.42, respectively, whereas the GL was 5.51, 6.92 and 4.49, respectively. Thus, the study reveals that the method of preparation of legume seeds, before and during cooking, may alter the GI of the food and, consequently, the responses in the body.

FUNCTIONAL PROPERTIES

ACE inhibitory activity

Angiotensin-converting enzyme (ACE, peptidyldipeptidase, EC 3.4.15.1) is a central component of the renin-angiotensin system, which controls blood pressure by regulating the volume of fluids in the body via the activation of angiotensin II (a vasoconstrictor and aldosterone-stimulating peptide) and the inactivation of the vasodilator peptide bradykinin.[98] ACE inhibitors exert their anti-hypertensive properties by decreasing the production of angiotensin II, with a corresponding increase in the level of bradykinin.[99] Thus, ACE-I inhibition is considered as an important therapeutic approach in the treatment of high blood pressure. Although only a few studies have been carried out on cowpea with respect to its anti-hypertensive properties, so far, the ACE-I inhibitory potential of cowpea has been attributed to the presence of phenolic compounds and bioactive peptides[100] (Table 2). According to Sreerama *et al.*,[67] the phenolic extracts of cowpea showed a significant and dose-dependent inhibition of ACE-I, with an IC_{50} value of 89.1 μg mL^{-1}. The activity was significantly lower than chickpea and horse gram, and was higher than that of phenolic compounds extracted from extra virgin olive oils.[118] ACE is a Zn metalloproteinase enzyme, which depends on the presence of Zn ions for its catalytic activity. Thus, the presence of metal chelating agents aids in the inhibition of this enzyme.[119] Wagner *et al.*[120] studied the structure–activity relationship of ACE-I and found that phenolic compounds, especially flavonoids, chelate a zinc ion within the active site of ACE, thereby disrupting its function. The free hydroxyl groups of phenolics are the main structural moiety found to be involved in chelating the zinc ion. However, in a study perfomed by Guerrero *et al.*,[121] a combination of sub-structures on the flavonoid skeleton, namely the (i) the catechol group in the B-ring; (ii) the double bond between C2 and C3 at the C-ring; and (iii) the cetone group in C4 at the C-ring, were found to increase ACE-I inhibitory activity. Thus, the flavonoid content and the functional groups present play an important role in the ACE-I inhibitory activity of cowpea.

In addition, legume proteins are reported to be a rich source of hypotensive peptides with ACE-I inhibitory activity.[122] Compared to phenolic compounds, proteins and peptides are the main group of compounds shown to be implicated in the ACE-I inhibitory activity of several sources, including sea food,[123,124] meat and poultry,[125–127] egg,[128,129] milk and milk products,[130] potato,[131–133] legumes[134,135] and cereal grains.[130,136,137] Several studies have reported that protein hydrolysates exert a more potent ACE-I inhibitory activity than the unhydrolyzed protein.[112,122,134,135,138] In studies carried out by Guang and Phillips[113] and Leon *et al.*,[112] hydrolysates of cowpea protein (peptides) were reported to effectively inhibit the ACE-I activity compared to the unhydrolyzed protein. Campos *et al.*[114] reported the ACE-I inhibitory activity to be significantly influenced by the molecular weight of the peptide, where the lowest activity was observed in the > 10 kDa fractions and the highest in the < 1 kDa hydrolysate fractions. The same phenomena has been observed in research carried out on protein hydrolysates of other commodities.[130,139,140] This may be a result of the configuration of the active site, which is reported to accommodate smaller peptides more efficiently than the larger molecules.[141] ACE-I is reported to exert a higher preference for substrates, or competitive inhibitors, containing hydrophobic amino acid (aromatic or branched lateral chain) residues in the C-terminal tri-peptide.[142,143] Segura-Campos *et al.*[100] isolated peptides with ACE-I inhibitory activity and found the highest active peptide fraction to have an amino acid composition enriched with hydrophobic residues (high levels of methionine, valine, leucine, isoleucine and phenylalanine, as well as lower levels of proline, arginine and glutamine), which enhances the inhibitory potency of the peptides. However, in the study by Guang and Phillips,[113] the active peptide found in cowpea hydrolysate did not satisfy the structural requirements proposed for ACE-I inhibitory peptides, supporting the need for further studies aiming to clarify the amino acid profile of these ACE-I inhibitors and their inhibition mechanism. Although there are several sources of evidence for the *in vitro* ACE-I inhibitory activity of cowpea proteins, further investigations of the *in vivo* and clinical antihypertensive effects of cowpea peptides are necessary to confirm the effect.

Besides anti-hypertensive properties, ACE-I inhibition is also reported to have a favorable effect on other regulatory systems involved in modulating blood pressure, immune defense, diabetes and nervous system activities.[144] ACE-I inhibitors with a sulphydryl group are reported to show improved insulin sensitivity *in vivo* by modifying the actions of bradykinin.[145] Bradykinin has been reported to enhance glucose uptake *in vivo* and *in vitro* by enhancing insulin receptor phosphorylation.[145–147] In a study conducted by Motoshima *et al.*,[147] bradykinin was found to improve insulin sensitivity by enhancing the insulin-stimulated tyrosine kinase activity of the insulin receptor and down-streaming the insulin signal cascade via the bradykinin B2 receptor mediated signaling pathway. Thus, because ACE-I could be the connecting link between hypertension and diabetes, the inhibition of ACE-I may have implications other than just antihypertensive effects and can

Table 2. Functional properties and pharmacological effects of cowpea

Active ingredient	Functional properties	Physiological effects	Reference
Phenolic compounds	Antidiabetic	Inhibit α-amylase and α-glucosidsae activity	63
	Antihypertensive	ACE inhibitory activity	63
	Hypocholesterolemic	Inhibit oxidation of lipids. Decreasing of blood triglyceride, cholesterol total, LDL, and increasing of blood HDL	81,101,102
	Anti-inflammatory	Suppress expression of pro-inflammatory genes	103
	Anticancer	Antioxidants protects DNA from oxidative damage, inhibit cancer cell proliferation	47,49,63,81,82,101,104
Resistant starches and Dietary fiber	Antidiabetic	Slow release of glucose in to the system (low glycemic index)	105
	Hypocholesterolemic	Depletion of bile acids from the circulation, promotes cholesterol conversion into additional bile acids, increase excretion of fecal fat	25,29,106
Cowpea proteins, Isolates and peptides	Anticancer	Selective cytotoxic activity against a wide range of cancer cell lines	107,108
	Antidiabetic	Cowpea protein isolates mimic the action of insulin, inhibit dipeptidyl peptidase IV activity	27,109–111
	Antihypertensive	Inhibition of ACE	112–114
	Hypocholesterolemic	Functions via bile acid-binding and disruption of cholesterol micelles in the gastrointestinal tract, as well as by altering hepatic and adipocytic enzyme activity and gene expression of lipogenic proteins, which can modulate the lipid profiles. Inhibits HMG-CoA reductase activity; inhibits the expression of NPC1L1 and transcription factor SREBP2	25,40,41,115–117
		The lysine/arginine ratio has been directly associated with lowering serum cholesterol levels	

be considered as a key strategy in controlling hyperglycemia and related hypertension.[67]

Hypocholesterolemic activity

Cardiovascular diseases have become a major cause of mortality and morbidity in both the developed and developing world, and dyslipidemia is considered to be a main reason for the present condition.[41,115,148] Cholesterol metabolism is a complex scenario and, even after years of research, there is still a gap in the clear understanding of this process.[116,148] So far, treatment of hypercholesterolemia mainly focuses on modulating the intestinal absorption of fat and the endogenous biosynthesis of cholesterol.[41,148]

Observational studies throughout the world indicate that increased consumption of legumes is a promising route for reducing cardiovascular disease risk factors, such as elevated blood cholesterol, blood glucose, blood pressure and body weight.[149,150] The exerted effect is attributed to the presence of compounds, such as phenolics, phytic acid, dietary fiber, saponins, phytosterols, proteins and peptides, and their amino acid profiles in legumes.[25,101] Cowpea and cowpea isolates are reported to show hypocholesterolemic activity *in vitro*[56,101,102] and *in vivo*[25,117] (Table 2). Chui *et al.*,[102] Hachibamba *et al.*[101] and Salawu *et al.*[52] reported the phenolics present in cowpea to protect human low-density lipoprotein (LDL) from copper-induced peroxidation. Oxidized LDL plays an important role in the development of coronary heart disease by triggering the formation of fatty streaks within blood vessels, which leads to the development of atherosclerotic lesions.[102,151] Thus, the prevention of LDL oxidation can aid in the protection against cardiovascular diseases. Both the cell wall preparations and the whole seeds of cowpea varieties, Betchuana white and black-eyed pea exhibited excellent protective effects against copper-induced oxidation of human LDL.[52] In a study carried out by Hachibamba *et al.*,[101] extracts of both cooked and digested (simulated *in vitro* gastrointestinal digestion) cowpea were found to effectively inhibit LDL oxidation. Cui *et al.*[102] studied the effect of flavonoid glycosides, isolated from cowpea, on LDL oxidation. They found that the glycosides of quercetin (quercetin 3-*O*-β-D-sophoroside, quercetin 3-*O*-β-D-glucopyranosyl-(16)-*O*-β-D-galactopyranoside) and catechin (catechin 7-*O*-β-D-glucopyranoside) exhibited significant inhibitory activity compared to the activity of butylated hydroxytoluene against LDL oxidation. According to the outcome of their study, a free hydroxyl group at the C-3′ position in flavonols was suggested to be one of the key factors determining their inhibitory effect on LDL oxidation. In addition, in a previous study, a catechol group in ring B was reported to be responsible for the higher antioxidant activity of flavonoids.[152]

Peptide fragments isolated from plant proteins are reported to exhibit their cholesterol-lowering properties by perturbing the intestinal absorption of dietary cholesterol and the enterohepatic bile acid circulation. Accordingly, this either disrupts cholesterol micelle formation, interferes with the cellular cholesterol carrier, or inhibits the lipogenic enzymatic activities and gene expression in hepatocytes and adipocytes[43,133,153,154] (Table 2). Prior to intestinal absorption, it is necessary for the dietary cholesterol to be incorporated into micelles along with phospholipids and bile salts.[43] This micellization is needed to facilitate its absorption into intestinal mucosal cells via Niemann–Pick C1-Like 1 (NPC1L1) receptors.[40] Cowpea proteins and peptides are reported to exert their hypocholesterolemic effect by both disrupting the incorporation of cholesterol into simulated micelles, thereby reducing the intestinal absorption of fat, and by inhibiting the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR); the rate-determining factor in hepatic cholesterol production that is needed for the biosynthesis of cholesterol.[40,41] The peptides from raw and cooked cowpea beans, subjected to *in vitro* simulated human digestion, were reported to reduce cholesterol micellar solubilization and inhibit HMGCR.[41] Cooking facilitated the release of peptides capable of reducing micellar solubilization of cholesterol and negatively affected the HMGCR inhibitory activity compared to the raw cowpea peptide. The ability to reduce cholesterol solubility was attributed to the presence of peptides rich in hydrophobic amino acids.

Similar to the *in vitro* studies, cowpea has been shown to reduce serum lipids in both experimental animals[25,29,37] and humans.[117] Studies conducted by Frota *et al.*[25,115,117] demonstrated that the protein isolated from cowpea to modulate lipid homeostasis, leading to a significant reduction in total and non-high-density lipoprotein (HDL) cholesterol in both hypercholesterolemic hamsters[25] and humans.[117] The whole cowpea seed diet exerted its hypocholesterolemic activity in hamsters by increasing the fecal excretion of cholesterol compared to the cowpea protein isolate and casein diets.[25] The whole cowpea seed diet increased the excretion of fecal cholesterol by 3.5-fold and bile acids by 1.5-fold compared to the casein diet (Control group). The difference observed in the whole cowpea and protein isolate fed groups was justified by the presence of other compounds, such as tannins, phytic acids, plant sterols, saponins, resistant starches and soluble fiber in the whole cowpea diet. These compounds are also reported to aid in the decreased absorption of cholesterol from the gut.[115,155,156] In another study conducted by Perera *et al.*[29] on rats fed with a high-fat diet, all four cowpea varieties (Bombay, Waruni, Dawala and MI 35) tested were found to improve the lipid profile of rats compared to the control group. One of the mechanisms found was to occur via the increased excretion of fecal fat, which perfectly correlated with the soluble fiber present in the test samples. The literature with respect to legume foods shows that most of the hypocholesterolemic effect exerted was via fecal elimination of cholesterol by binding on to insoluble fibers.[25,29,156,157] The ability of dietary fibers to bind on to bile acids is well documented and various studies have been carried out specifically regarding cowpea.[106] The depletion of bile acids from the system, via binding on to soluble fiber fractions followed by their removal with fecal matter, promotes the conversion of endogenous cholesterol into additional bile acids. This eventually results in significant reductions in liver and serum cholesterol levels.[106,158]

So far, there is only one study reporting the hypocholesterolemic effect of cowpea protein isolate on humans.[117] Frota *et al.*[117] investigated the effect of cowpea protein on the lipid profile and biomarkers of inflammation and endothelial dysfunction in adults with moderate hypercholesterolemia. Cowpea protein isolate was found to significantly reduce the total cholesterol, including LDL, non-LDL cholesterol and apo-lipoprotein B, and to increase HDL cholesterol level in adults with moderate hypercholesterolemia. However, the consumption of cowpea protein did not modulate the serum inflammatory or endothelial dysfunction biomarkers [C-reactive protein (CRP), soluble intercellular cell adhesion molecule-1 (sICAM1) and soluble vascular cell adhesion molecule-1 (sVCAM1)] compared to the control group. The same finding was observed in a study conducted on soy, where supplementation with soy or soy protein isolate did not alter CRP concentrations in humans.[159] Despite the already reported literature on the favorable effects of cowpea with respect to modulating the level of serum lipid, further investigations should be carried out to confirm its effects on the blood lipid profile of humans, specifically employing longer follow-up studies in different ethnic groups with different degrees of dyslipidemia. In addition, the molecular mechanisms involved in the reduction of hyperlipidemia by compounds present in cowpea are not yet completely understood. A recent study reported that cowpea peptides interfere with the intestinal absorption of cholesterol by inhibiting the expression of NPC1L1 and reducing cholesterol synthesis via a lowered expression of the transcription factor SREBP2 (consequently HMGCR and LDLR).[116] However, there is still a huge gap to be filled in the literature regarding the actual mechanism by which cowpea components exert their hypocholesterolemic activity. Thus, further studies should be carried out to enable a clear view with respect to the mechanistic approach of cowpea on hypocholesterolemia.

Hypoglycemic activity

The treatment for diabetes mainly focuses on controlling the blood glucose level by stimulating insulin secretion from the β-cells of pancreatic islets, inhibiting the insulin degradation process, repairing or regenerating pancreatic β-cells, and inhibiting the starch hydrolases, α-amylase and α-glucosidase.[160] Legumes have been reported to be a rich source of both soluble and insoluble dietary fiber, as well as phytochemicals and proteins with enzyme inhibitory activities. Several studies have reported the beneficial effects of cowpea in the dietary management of elevated blood glucose and dyslipidemia, as associated with diabetes[26] (Table 2).

The ability to inhibit the activity of starch-hydrolyzing enzymes is considered to be an indicator of exhibiting anti-diabetic properties. In a study carried out by Sreerama *et al.*,[67] the phenolic extracts of cowpea were found to inhibit the activity of α-amylase and α-glucosidase in a dose-dependent manner. Compared to the chickpea and horse gram flour extracts, the cowpea extract showed significantly higher α-glucosidase inhibitory activity with an IC_{50} value of 52.8 $\mu g\ mL^{-1}$, which was lower than that of α-amylase (159.1 $\mu g\ mL^{-1}$). The inhibition of these enzymes aids in reducing the digestion rate of consumed starches, thereby aiding in the slow release of glucose into the circulation and preventing the steep rise in postprandial blood glucose level.[26,67] The enzyme inhibitory pattern of cowpea observed can be considered beneficial in controlling hyperglycemia compared to some commercially available drugs because it can prevent the side effects of high α-amylase inhibition. Which can result in the abnormal bacterial fermentation of undigested carbohydrates in the colon, leading to flatulence, bloating and so on. In another study, consumption of cowpea was found to significantly reduce the fasting plasma glucose concentrations of diabetic rats and was also reported to equally reverse diabetes-associated dyslipidemia.[26] Furthermore, as discussed above, the antioxidant activity and inhibitory activity exerted against the enzyme ACE-I by cowpea may aid indirectly in managing glucose uptake and glucose-induced radical generation in mitochondria, which is linked to hyperglycemia and hypertension.[67]

Cowpea is considered as a low GI food and is recommended in diabetic diets.[105] The already reported GI of cowpea ranges from 29 to 61.[30,105,161] In a study carried out by Onyeka[105] the GI of cowpea was reported to differ based on the variety. Except for one cowpea variety (Patasco (GI-61.57)), the GI value of all the other five varieties fell under the low GI group (0–55). The Ife brown was found to have the lowest GI value, which was attributed to the presence of high amount of slowly digestible starch, dietary fiber and phytochemicals. The high level of dietary fiber in legumes has long been attributed to their usefulness in managing diabetes.[26] Diets high in fiber, particularly soluble fiber and insoluble fiber, aid in diabetes management by decreasing the glucose releasing rate during digestion, as a result of an increase in the viscosity of the digestate and a reduction in the gastric emptying time. Because insoluble fiber increases the non-caloric bulk in the diet, it affects the secretion of various gut hormones (peptide YY, glucagon-like peptide and ghrelin) by acting as a satiety factor.[162] In addition, the fibers also insulate the carbohydrates from the digestive enzymes, which may indirectly have an inhibitory effect on the enzymes.[30] In a relevant study, the brown cowpea variety was found to have a lower GI value than the white, as well as the white and black variety.[161] However, the GIs of cowpea were higher than pigeon pea, groundnut and African yam bean. Furthermore, the processing method is also reported to influence the GI of cowpea, where boiled cowpea was reported to have a lower GI value than mashed or fried cowpea.[30]

In another study, the blood glucose responses of individuals, with and without diabetes, fed a diet prepared with cowpea (moin–moin) and bambara groundnut (okpa), were compared.[163] The test diets improved the blood glucose responses in both diabetics and non-diabetics compared to the group receiving the white bread diet. The outcome of the study revealed that okpa prepared from Bambara groundnut (GI = 59) was a better diet for diabetics than the cowpea diet (GI = 66). However, the cowpea diet showed a very low GI (GI = 38) value on non-diabetic subjects compared to the groundnut diet (GI = 78). Although the mechanisms underlying these effects are not clear, these results were attributed to the high amount of dietary fiber present in Bambara groundnut.

Additionally, recent studies have demonstrated the potential of cowpea peptides to regulate hyperglycemia via several mechanisms[27,109–111] (Table 2). Barnes *et al.*[27] found that cowpea peptides mimic the action of insulin and induce protein kinase B (Akt) phosphorylation in the skeletal muscles of rats via activation of the insulin-signaling cascade. Akt acts as a signaling molecule in the insulin-signaling pathway and is required to induce cellular glucose transport. It also plays a key role in several other cellular processes such as apoptosis, cell proliferation, transcription and cell migration.[27] A study completed by Venancio *et al.*[110] provides evidence for the insulin-like activity of cowpea peptides, where the amino acid sequence of a protein isolated from cowpea was found to be similar to the sequence of bovine insulin. Furthermore, in a study carried out by De Souza *et al.*[111] peptides generated from sprouted cowpea subjected to enzymatic hydrolysis were reported to inhibit the enzyme dipeptidyl peptidase IV, which is responsible for degrading incretins. Because incretins play an important role in regulating the postprandial glucose levels by stimulating insulin secretion, the inhibition of dipeptidyl peptidase is considered as a promising therapeutic target in the treatment of type II diabetes.

Anticancer activity

Dietary antioxidants play an important role in protecting the cells against damage caused by free radicals.[41] Oxidative stress is reported to be an important risk factor in the development of several types of cancers. Consumption of legumes has been reported to be associated with a reduced incidence of some types of cancers in epidemiological studies.[54,164,165] The anticancer effects of legume seeds are attributed to the antioxidant activities of various components present in legumes, including hydrophilic phenolic compounds, saponins and phytates.[49,166] The phenolic compounds in legumes are considered to play a protective role in the body against oxidative stress via their antioxidant properties, such as their free radical scavenging capacity and metal-chelating properties.[54,67,167] Through these activities, legumes aid in the inhibition of lipid peroxidation, human LDL oxidation, oxidative hemolysis of human erythrocytes and DNA damage caused by free radicals.[54,168]

The crude phenolic extracts of raw and processed (boiled and micronized) cowpea, as well as the extracts subjected to simulated *in vitro* gastrointestinal digestion, have been shown to exhibit antioxidant properties via their ability to scavenge free radicals and metal chelating properties[47,53,67,101,104] (Table 2). In addition, the extracts have also demonstrated good antioxidant activity with respect to protecting erythrocytes from oxidative hemolysis[168] and LDL oxidation.[167] Apea-Bah *et al.*[53] studied the effect of sorghum–cowpea composite porridge on its antioxidant activity. The sorghum–cowpea composite porridge demonstrated a better potential to scavenge nitric oxide (NO) radical than the maize–soybean composite porridge. NO is a physiological marker of oxidative stress and the observed scavenging activity of NO by sorghum–cowpea demonstrates its potential in alleviating radical-induced oxidative stress in a physiological system. In a follow-up study, the composite porridge and its simulated digests were found to effectively inhibit human LDL oxidation and protect DNA from radical-induced oxidative damage.[167]

The ability of cowpea to prevent DNA damage caused by free radicals can be considered as a remedy to prevent the development of cancer by protection against radical-induced point mutations in DNA and the consequent carcinogenesis (Table 2). Salawu *et al.*[52] reported that both cell wall preparations and whole seeds of cowpea show potential protective effects against AAPH-induced oxidative DNA damage. According to their results, the whole grain extract showed better activity than the cell wall extract. This was attributed to the synergistic effects exerted by the flavonoids and phenolic acids present in the whole grain, unlike the extracts from the cell wall preparations that mostly comprised hydroxycinnamates. In a study carried out by Nderitu *et al.*,[54] boiled cowpea and its simulated *in vitro* gastrointestinal digests showed potent activity with respect to inhibiting oxidative DNA damage. Between the two tested varieties, the enzyme digest of the red cowpea type was three-fold more effective than the cream cowpea type in protecting DNA from oxidative damage, which was attributed to the higher phenolic content of the red cowpea cultivar.

The potential anticancer properties of cowpea can also be demonstrated by studying the ability of the extracts to inhibit cancer cell proliferation *in vitro* (Table 2). The phenolics present in whole cowpea seed, seed coat and cotyledons were reported to inhibit the proliferation of hormone-dependent mammary (MCF-7) cancer cells.[49] According to the results, free phenolics present in whole cowpea seeds were found to be more effective compared to the bound phenolics with respect to inhibiting the growth of MCF-7. The bound phenolics in seed coats were found to promote the growth of MCF-7 cells. Although the exact mechanism was not found, this was attributed to the presence of phytoestrogens such as isoflavones and oleanolic acid-derived saponins in cowpea. Extracts of seed coats and cotyledons also inhibited the proliferation of MCF-7 cells, although to a lesser extent compared to the whole cowpea seeds, indicating a synergistic effect between the phenolics and other phytochemicals present in these parts.

In addition to the phenolics, a Bowman–Birk protease inhibitor, the black-eyed pea trypsin/chymotrypsin inhibitor, isolated and purified from cowpea seeds, was found to reduce cell viability and proliferation of MCF-7 breast cancer cells in a study conducted by Joanitti *et al.*[107] In another study, a protein (isolated from small brown-eyed cowpea seeds) with a molecular weight of 36 kDa and with a high sequence similarity to polygalacturonase inhibiting proteins, was reported to inhibit the proliferation of MBL2 lymphoma and L1210 leukemia cells[108] on the contrary, in studies conducted by Lima *et al.*[166] and Xu and Chang,[50] cowpea extracts did not show any significant activity with respect to inhibiting the growth of several cancer cell lines, including MCF-7. It should be noted that only a few studies have been carried out on the anticancer properties and mechanism of action of cowpea and its compounds and further studies are recommended in this area to reach a conclusive result.

Anti-inflammatory activity

Inflammation, a biological response of body tissues to harmful stimuli, is also known to be involved in a host of diseases, such as obesity, atherosclerosis and rheumatoid arthritis, and even in certain types of cancer. Recent investigations have demonstrated that the polyphenols in plants, particularly flavonoids, exhibit anti-inflammatory activities both *in vitro* and *in vivo*.[169] Cyclooxygenase, lipoxygenase, phospholipase A2 and nitric oxide synthase are some of the enzymes involved in the inflammation process, and cytokines, such as interleukin (IL)-1b and tumor necrosis factor (TNF)-α, as well as nitric oxide, are included among the important resultant products of these reactions.[170] *In vitro* assays based on an assessment of anti-inflammatory activity measure the effect of bioactive compounds with respect to inhibition of the pro-inflammatory enzymes, production of the pro-inflammatory cytokines and their ability to scavenge nitric oxide radicals.[103,117,151,171]

Lee *et al.*[171] studied the effect of different solvent extracts of cowpea seeds with respect to inhibiting nitric oxide production, nitric oxide synthase mRNA and protein expression in lipopolysaccharide-stimulated RAW264.7 macrophage cells. According to their results, the ethyl acetate and *n*-butanol fractions exhibited promising anti-inflammatory activity. Compounds inhibiting NO production in RAW264.7 cells were isolated and identified as oleanolic acid, linolenic acid, linoleic acid, 7-ketositosterol, stigmasterol-glucose and soyasaponin-1. Among these, linolenic acid and linoleic acid were found to inhibit NO production significantly. Nitric oxide is considered as a crucial agent in the modulation of various acute and chronic inflammatory disorders.[172,173] Thus, the inhibition of NO production can be considered as a useful therapeutic strategy in the treatment of inflammatory diseases.

Ojwang *et al.*[103] evaluated four major cowpea phenotypes (black, red, light brown and white) containing different phenolic profiles for their anti-inflammatory properties on non-malignant colonic myofibroblasts (CCD18Co) cells challenged with an endotoxin [lipopolysaccharide (LPS)]. The cowpea phenolics down-regulated pro-inflammatory cytokines (IL-8, TNF-α, VCAM-1), transcription factor nuclear factor (NF)-κB and modulated microRNA-126 (specific post-transcriptional regulator of VCAM-1) (Table 2). Interestingly, all these effects were observed at a concentration as low as 2.0 μg mL^{-1}, suggesting the potential for beneficial effects with the normal consumption levels of cowpea. Another interesting finding of the study was that the flavonoid profile of cowpea was found to be an important determinant for the exerted anti-inflammatory effects rather than the phenolic content, where the red variety with the highest level of flavonols was most effective at down-regulating LPS-induced IL-8, TNF-α and NF-κB gene expression, as well as microRNA-26 protein expression. Furthermore, the white low polyphenol variety, which contained only flavonols (no anthocyanins or flavan-3-ols), was most effective at down-regulating VCAM-1 gene expression. On the other hand, the highest polyphenol containing light brown variety (high in flavan-3-ols) was found to be comparatively less effective.

In contrast, in a study conducted by Frota *et al.*,[117] cowpea extracts did not show any significant effect on the anti-inflammatory biomarkers investigated (CRP, sICAM1 and sVCAM1) compared to the control. However, it should be noted that the phenolic compounds present in cowpea, such as the flavonols (especially glycoside of quercetin and anthocyanins, as well as procyanidins), have been reported to show significant anti-inflammatory effects. For example, the flavonols, quercetin and kaempferol have been reported to inhibit cytokine-induced activation of NF-κB in parenchymal liver cells, probably by protecting cells against oxidative species, inhibiting the anti-inflammatory enzymes and down-regulating the NF-κB pathway.[174,175] Anthocyanins are also reported to inhibit TNF-α-induced endothelial leukocyte adhesion molecule-1 and ICAM-1 expression in cultured HUVEC.[176] Thus, the controversial results obtained so far require further investigation because the flavonoid profile of cowpea appears to be highly relevant to its anti-inflammatory properties.

CONCLUSIONS

Cowpea is a rich source of bioactive compounds, such as peptides, resistant starch, dietary fiber, phytochemicals and antioxidants, as well as certain types of vitamins and minerals, possessing specific properties that benefit human health in various ways. Other than the phenolic compounds, cowpea proteins, peptides and protease inhibitors in cowpea have been reported to improve the lipid profile, blood glucose level and blood pressure, and also to aid in cancer prevention by suppressing the growth of several cancer cell lines. Furthermore, being more than an individual compound, the cowpea as a whole is reported to exert its positive effects on disease prevention, indicating a likelihood of synergistic interactions between the compounds present in cowpea. However, the *in vitro* data on the anticancer and anti-inflammatory properties of cowpea are inconclusive. Thus, further studies in this area are suggested. In addition, despite the already reported favorable effects of cowpea on diabetes, hyperlipidemia and hypertension, a long-term epidemiological study investigating the association between cowpea consumption and diabetes, cardiovascular disease and cancer is recommended.

Conflict of interests statement

The authors declare that there are no conflicts of interest regarding the publication of this article. All of the authors reviewed the paper and approved the final version submitted for publication.

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