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ORIGINAL ARTICLE

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Probiotic enriched fermented soy-gel as a vegan substitute for dairy yoghurt

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Abstract

The demand for vegetable protein substitutes for animal protein is growing. Soy milk, the water extract of soaked and ground soybeans is a popular alternative for bovine milk. Soy milk was tested with several non-animal derived stabilizers to produce fermented soy-gels and found agar-agar provides promising organoleptic properties in fermented soy-gels. The sensory analysis proved that Bifidobacteria (BB) used together with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* as starter culture bacteria (SCB) gives better ($p < .05$) sensory properties. Physicochemical properties were evaluated for gels fermented with SCB and BB. Results indicated, upon storage at 4°C, pH reduced ($p < .05$) and viscosity increased ($p < .05$). Both titratable acidity and syneresis were initially decreased and increased after the 10th day of storage. After 7 days, the viability of BB in fermented soy-gel was higher ($p < .05$) than in a regular bovine milk set-gel, proving the feasibility of using as a promising probiotic carrier vegan food.

Practical applications

Contemporary increase in demand for non-dairy products (fermented-gels) proved to be capable of substituting to soy milk, not only because of technological feasibility but also due to its health benefits and environmental aspects. We proposed fermented soy-gels made by incorporating agar-agar as the stabilizer is superior as a probiotic carrier food, compared to cow milk set-gels. This produces an acceptable fermented soy-gel with optimal organoleptic properties. Fermented soy-gels are likely to play a crucial role as a vegan food in the future, with huge potentials of developing as a value-added product.

1 | INTRODUCTION

Yoghurt is a semi-solid fermented dairy product, consumed in different forms according to the preferences of the consumers and availability (Yildiz, 2010). However, increasing health consciousness, changing of lifestyles and lactose intolerance have created a niche of consumers who demand lactose-free alternatives. Lactose intolerance has become a serious issue and it has been shown that in Asia up to 50% to 100% of dairy products consumers suffer from lactose intolerance (Lomer et al., 2008). Hence, there is a shift in demand for yoghurt-like products made of plant proteins. Such products are popular among vegans and lactose-intolerant consumers, as lactose intolerance can be treated with a strict lactose-free diet, a low-lactose diet, lactase supplements, or probiotic supplements (Parker & Watson, 2017).

Soy milk is the water extract of soybean (*Glycine max*) and, is used as a milk substitute for consumers with lactose intolerance, milk protein allergy, calorie concerns, and vegetarian or vegan lifestyles

(Sethi et al., 2016). Compared to cow milk, soy milk contains 300-fold less calcium and half of the phosphorus. The protein content is identical to each other and the dietary fiber can be found only in soy milk. The fat content in soy milk is two times lower than cow milk (Endres, 2001; Hajirostamloo, 2009).

Milk from plants offers various health benefits from its dietary fibers, minerals, vitamins and antioxidants (Das et al., 2012). Isoflavones in soy milk have been identified as bioactive compounds which might protect consumers against cancer, cardiovascular diseases, and osteoporosis (Nill, 2016; Omoni & Aluko, 2005). Moreover, animal model studies have shown that phytosterols found in soy milk have potentials for lowering the cholesterol levels in humans (Fukui et al., 2002). Consumption of soy milk resulted in a significant reduction in serum insulin and blood pressure, as shown from a clinical trial (Maleki et al., 2019). Besides, probiotic-fermented soy milk consumption reduced hyperlipidemia and liver injury, as reported from a mice model study (Zhang et al., 2017). Thus, foods derived from soy milk have the potential of becoming foods with various health benefits to the consumers.

Gelation, emulsification, fat, and water absorption properties of soy milk are well known. These functional properties are mainly attributed to the soybean storage proteins and the major storage proteins of soybean are β-conglycinin and glycinin (Fukushima, 2001). Considering the compositional and functional properties, soy milk is identified as a raw material, which can be developed into new food products. Yet, the use of soy milk as a food ingredient is limited due to the presence of undesirable beany aftertaste. The reason for the development of grassy beany flavor of soybean is due to the action of three classes of lipoxygenases (Fukushima, 2001). Various attempts have been made to remove or mask the off-flavors of soybean. Yet, neither of attempts could mask the off-flavors up to a satisfactory level. However, lactic acid fermentation is identified as a potential approach to reduce beany after taste.

Probiotics are live microbial dietary supplements that benefit the host by contributing to the microbial balance of the intestine (Rašić, 2003). Probiotics provide numerous health benefits to the host including, enhancement of the immune system, control of serum cholesterol level, and possible anticarcinogenic properties. Therefore, achieving and maintaining a higher probiotic count in dairy yoghurts through the symbiotic relationship between probiotics and prebiotics foods have been a major research focus (Hasani et al., 2016; Mousavi et al., 2019). Probiotic enrichment in soy milk yoghurt improve the physicochemical (i.e., reduce syneresis, compact network and microstructure) and microbiological properties (Cui et al., 2021).

In fact, fermented soy milk products have been shown the capability to be novel probiotic carrier foods with higher probiotic viability in food and with high faecal recovery (Shimakawa et al., 2003). Soy milk is identified as a suitable culture medium than cow milk for the simultaneous growth of bifidobacteria and probiotic lactic acid bacteria (Cui et al., 2021; Wang et al., 2002). Hence, the present study aimed to investigate the feasibility of formulating a vegan, fermented soy-gel to deliver comparable probiotic effect with acceptable sensory properties.

2 | MATERIALS AND METHODS

2.1 | Extraction of soy milk

Soybeans (sourced from a retail local supplier) with optimal physical quality without any defects were carefully selected and soaked overnight. A slurry from the de-hulled soybeans was prepared by grinding the seeds using a mixer grinder, while gradually adding hot water (100°C) up to a ratio of 1:7 on a weight basis. The mixture was simmered at 72°C for 20 min with occasional stirring. The resulting mixture was sieved through a fine piece of cloth to obtain soy milk.

2.2 | Selection of the stabilizer

Various non-animal derived stabilizers at different levels were evaluated against 0.75% (w/v) level of gelatin (standard) to select the best comparable stabilizing agent for the preparation of fermented soy-gels (Table 1). Different incorporation levels were selected based on the literature.

2.3 | Preparation of fermented soy-gels

The soy milk base was pre-heated to 60°C and, cane sugar (10% w/v) and agar-agar (0.2% w/v) were added and heat-treated at 90°C for 10 min. After cooling the mixture to 42°C,

TABLE 1 Stabilizers and their incorporation levels to prepare fermented soy-gels for the preliminary sensory studies. The different carrageenan types differ in terms of viscosity in cps (Centipoise)

Stabilizer	Level of incorporation (w/v%)
Carboxymethylcellulose	0.25
	0.75
	1.00
Isolated soy protein	2.00
	4.00
	6.00
Carrageenan	
Carrageenan (4cps)	0.40
	0.70
Carrageenan (8cps)	0.40
	0.70
Carrageenan (14cps)	0.40
	0.70
Agar-agar	0.10
	0.20
	0.40
	0.60

a representative portion of starter cultures (SCB) (0.0025% w/v) (equal amounts from *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) (Chr. Hansen, Horsholm, Denmark) and probiotic cultures (0.0025% w/v) [*Bifidobacterium animalis* subsp. *lactis* (BB) and *Lactobacillus acidophilus* (*LA*)] (Chr. Hansen, Horsholm, Denmark) were inoculated to the soy milk by introducing four different combinations. Those are SCB (Treatment 1), SCB + BB (Treatment 2), SCB + LA (Treatment 3) and SCB + mixture of BB and LA (equal amounts to give total of 0.0025% w/v) (Treatment 4). The soy milk mixture was then poured into plastic cups (80g) and incubated at 42°C. Fermentation was stopped when the gel was optimally set, identified by visual observation, approximately at 3.5 ± 1 hr. After the fermentation, the fermented gels were stored at 4°C.

2.4 | Sensory analysis

Sensory evaluation was done on the following day of production. The fermented soy-gels were evaluated based on the overall flavor and texture (measured as spoonability) of the resulted gels (Treatment 1–4). All samples were given a three-digit code and tested against untrained 35 sensory panelists (aged 20–45 years) in a randomized design. Panellists evaluated the texture (spoonability) and overall flavor of undisturbed soy-gel samples offered at 8°C in 80 g plastic retail containers, by ranking the preferences among four different treatments in a simplified and structured sensory evaluation form. Ranks were assigned from 1: most preferred sample to 4: least preferred sample. The same rank was not given for two or more samples.

2.5 | Determination of physicochemical parameters

Following physicochemical parameters were measured in three replicates for the soy-gel samples on every third day for a period of 14 days upon storage at 4°C.

2.5.1 | pH

The pH of the soy-gel samples was measured using the Hanna microprocessor pH meter (HannaNorden AB, Kungsbacka, Sweden) in triplicates.

2.5.2 | Titratable acidity

Titratable acidity was measured according to the method described by Noh et al. (2013), with the following modifications. Soy-gel (9 ml) was mixed with an equal volume of distilled water in a 250 ml conical flask. Phenolphthalein two to three drops were used as an indicator. The slurry was then titrated with 0.1 N NaOH until the solution become faint pink in color. Titratable acidity was measured according to the following formula.

$$\text{Lactic acid}\,\% = \frac{9\,(\text{The volume of 0.1 N NaOH} \times \text{Normality of standard NaOH})}{\text{Volume in ml of yoghurt taken for the test}}$$

2.5.3 | Viscosity

The viscosity of fermented soy-gels was measured with slight modifications as explained by (Madhubasani et al., 2020). Measurements were taken at 4°C with a B-type Viscometer, Model BL (Tokimec Inc., Tokyo, Japan) at 5 Hz with rotating spindle no. 3 at 12 rpm for 5 min.

2.5.4 | Syneresis

Syneresis was measured according to the method described by Wu et al. (2000). In brief, 20 g of fermented soy-gel sample was spread in a thin layer to cover the surface of a Whatman filter paper number 2 over a Buchner funnel. The funnel was placed over a top of an Erlenmeyer flask. Erlenmeyer flask was connected to a vacuum pump. After setting the system, the sample was sucked for 10 min. The weight of the collected liquid was measured and syneresis was calculated using the following equation.

$$\text{Syneresis}\,\% = \frac{\text{Weight of the liquid}}{\text{Initial sample weight}} \times 100.$$

2.6 | Microbiological analysis

The viable counts of BB and total lactic acid bacteria (LAB) of the fermented soy-gel was measured in duplicates on 1st, 7th, and 14th days at 4°C storage. For comparison, viable counts of BB and total LAB of a cow milk yoghurt were also measured on first and seventh day after production and stored at 4°C. Different organisms were enumerated using the spread plate method according to the method described by Prasanna et al. (2013) with different media as described below. HI media Bifidobacterium agar (HIMedia, India) was used as the growth medium for BB. MRS agar (Oxoid, England) was the medium used for enumerating the total LAB. All the plates were incubated anaerobically in anaerobic jars at 37°C for 72 hr.

2.7 | Statistical analysis

The sensory data were analyzed using the Friedman non-parametric procedure using the MINITAB (Minitab, Ltd, UK) software. All physicochemical and microbiological properties were statistically analyzed using completely randomized design using SAS software package (SAS Inc, North Carolina, USA), where means were compared using the Duncan's Multiple Range Test (DMRT).

3 | RESULTS AND DISCUSSION

3.1 | Selection of the stabilizer for the fermented soy-gels

To choose the best non-animal derived stabilizer and the best incorporation level, a series of preliminary experiments were conducted, as the type of stabilizers and its concentration in fermented gels are crucial in optimizing the texture and minimizing the syneresis (Lucey, 2004). All the stabilizers and their different levels were compared with 0.75% level (w/v) of gelatin since fermented soy-gels produced with 0.75% level (w/v) of gelatin had a highly acceptable appearance and texture without syneresis (data not shown). Similarly, Jimoh and Kolapo (2007) reported that fermented gels made with gelatin had the highest sensorial rating in soy-gels as compared to cassava starch or corn starch.

Carboxymethylcellulose (CMC) at 0.25% (Figure 1a), 0.75%, and 1.00% levels (w/v) were compared with 0.75% level (w/v) of gelatin and, at the 0.75% and 1.00% levels (w/v) of CMC incorporations resulted in powdery flavor. High syneresis was also observed at all the levels of CMC, where the highest level of syneresis was observed in the gels containing CMC at 0.25% level (w/v). Therefore, the production of fermented soy-gels using CMS was discontinued. Second, fermented soy-gels were formulated using isolated soy protein (ISP) at 2% (Figure 1b), 4%, and 6% levels (w/v). Syneresis and the powdery flavor was prominent at all the ISP incorporation levels. Our results agree with Zhang and Zhao (2013), who reported that the yoghurts made with only ISP showed low water holding capacity, and eventually increased the syneresis. Thus, the usage of ISP in fermented soy-gel production was discontinued. Third, carrageenan of three different viscosities (4, 8, and 14 centipoise) was evaluated at 0.4% (Figure 1c), and 0.7% incorporation levels (w/v) and found that the appearance of the fermented gels was not at an acceptable level for all the viscosity levels at every incorporation level. Therefore, carrageenan was excluded from further testing procedures in producing the vegan soy fermented gel. The possible reason for unacceptable appearance may be due to clot formation of carrageenan during cooling (Jianming et al., 2013). Syneresis was observed in all of the samples tested and this is in agreement with Molina Ortiz et al. (2004). Gels produced at all levels of carrageenan resulted in salty flavor because mostly commercial carrageenans are available as stable sodium, potassium, and calcium salts or most commonly as a mixture of these (Michel et al., 1997). However, the production of soy yoghurt-like gel has been a success, using carrageenan with BB cultured in reconstituted skimmed milk (Kamaly, 1997).

Finally, agar was tested as the stabilizer at the levels (w/v) of 0.1%, 0.2% (Figure 1d), 0.4% and 0.6% and compared against 0.75% (w/v) gelatin. Reduction in syneresis was observed when agar-agar was used as the stabilizer at all the levels. This is likely because polysaccharides addition to soy-gels may increase the water-holding capacity by increasing the density of the network, with smaller pores and greater capillary forces (Hua et al., 1996; Maltais et al., 2005; Yamamoto & Cunha, 2007) while binding water with their hydroxyl groups (Uresti et al., 2003). At 0.4% and 0.6% levels (w/v), the rubbery texture was observed in the gel structure, whereas 0.1% level (w/v) resulted in weaker gel with less firmness. In the present study, 0.2% level (w/v) (Figure 1d) resulted in fermented gels comparable

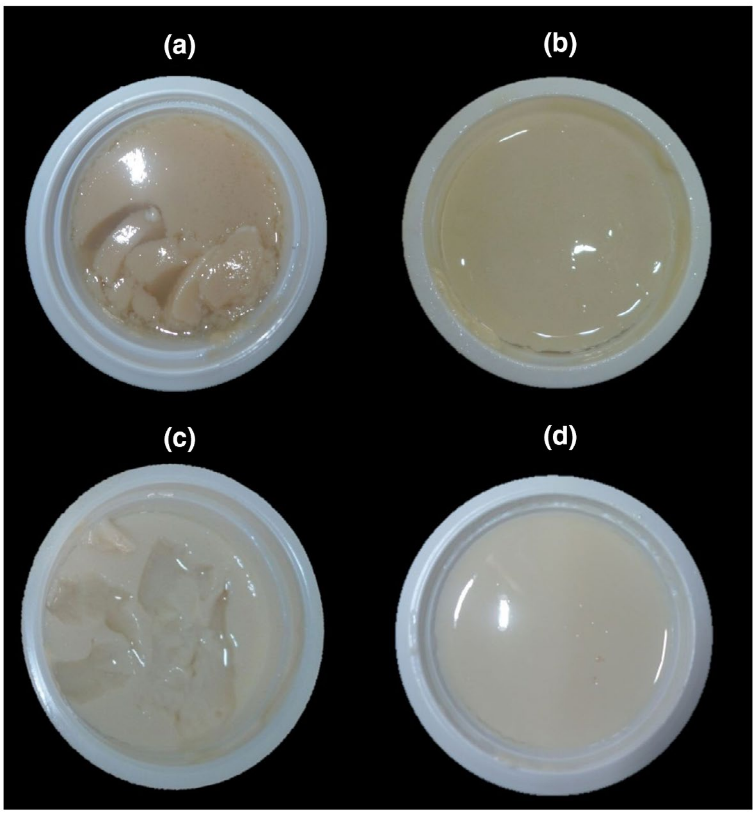

FIGURE 1 Effect of various stabilizers on appearance and syneresis of fermented soy gels illustrated at the lowest level of incorporation (as per Table 1). (a) Carboxymethylcellulose, (b) Isolated soy protein, (c) Carageenan and (d) Agar-agar

to the 0.75% level (w/v) of gelatin and therefore, opted to continue in the further experiment as the best non-animal derived stabilizing agent in fermented soy-gel production.

3.2 | Sensory evaluation

Sensory evaluation was carried out to select the most consumer-preferred combination of bacterial cultures regarding the texture (measured as spoonability) and overall flavor score of the soy-gel made with agar. Sensory properties were varied between different treatments as shown in Figure 2, in agreement with (Liu et al., 2019; Priyashantha et al., 2019), who showed that bacterial cultures influenced the flavor in soy-yoghurts as well as sensorial properties of bovine fermented milk, respectively.

According to the Friedman test, T2 and T3 showed the lowest and highest median value for texture (spoonability), respectively, followed by T4 and T1. For overall flavor, T2 and T4 resulted in the lowest and highest median value, respectively, followed by T1 and T3. Since T2 resulted in with lowest median values for both texture and overall flavor and therefore, selected as the best sample. Thus, the results indicated that the inclusion of probiotic bacteria has altered the sensory properties of soy fermented gels compared to the gels without adding probiotics (T1). The difference in gel properties is likely to result due to the varied abilities in organic acid production and proteolytic activities of specific probiotics (Donkor et al., 2006). BB has proven to enhance the taste of the soy products (Murti et al., 1993) and in the present study, the enhanced flavor was observed when only BB was added together with SCB (T2) to soy-gels. The choice of SCB is an important consideration in fermented milk production in the dairy industry (Priyashantha et al., 2019) and in agreement, the present study suggests that the combination of bacterial cultures influence the sensorial properties of resulting soy-gels. In the current study, fermented gels made with T2 was decided to evaluated for physicochemical properties and microbiological analysis during the storage, as other gels are less likely to be successful in producing an optimal gel, from the sensory perspectives and possibly consumer acceptances.

3.3 | Physicochemical properties of soy-gels fermented with the combination of the starter culture and bifidobacteria (T2) and stabilized with agar-agar

3.3.1 | pH

Changes in pH of fermented soy-gels over the storage period are shown in Figure 3. pH of the sample significantly reduced during the storage period at 4°C, in agreement with the Murti et al. (1993) and Ghorbani et al. (2012). (Shahbandari et al., 2016) reported a significant decrease in the pH of stirred soy yoghurt during storage at 4°C for 14 days. Farnworth et al. (2007) reported that the initial drop in pH is faster in soy milk than in cow milk. Similarly, the initial pH (Day 1) 4.65 dropped faster up to the Day seventh and then, gradually reduced up to the final pH (Day 13) of 4.38. This decline in pH was likely to occur due to the continuation of fermentation by the LAB even during the refrigerated storage (4°C). Toward the end of storage, drop in pH was not significant, probably due to the buffering effect arising from increased dry matter content in soy-yoghurt (Ghorbani et al., 2012). The pH values reported in the present study

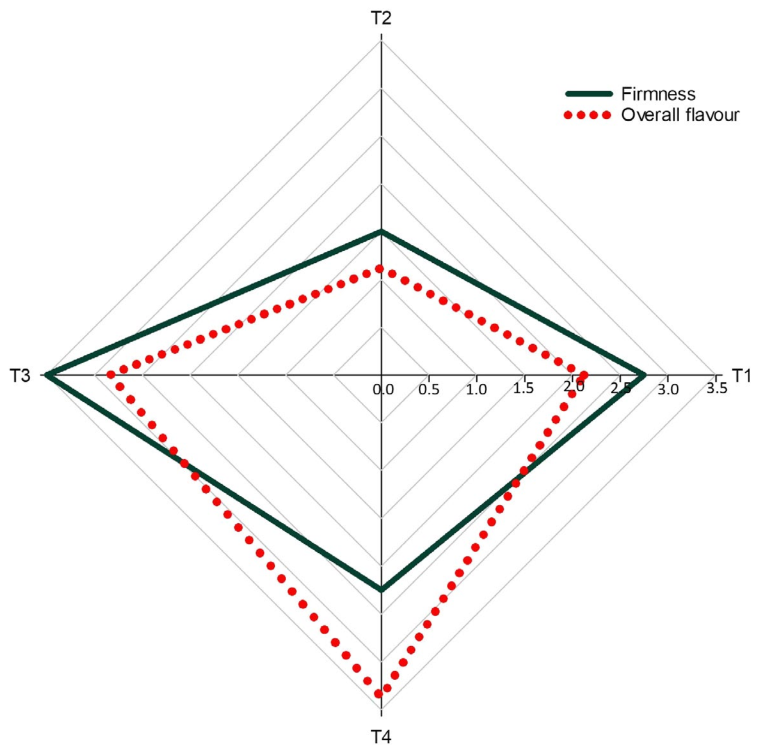

FIGURE 2 Radar plot of sensory evaluation for all treatments, T1: starter culture bacteria, T2: starter culture bacteria + Bifidobacteria, T3: starter culture bacteria + L. acidophilus and T4: starter culture bacteria + mixture of Bifidobacteria and L.acidophilus. Note: 0 and 4 in the scale, indicate the most preferred and least preferred ranking of its attribute in the sensory score sheet, respectively

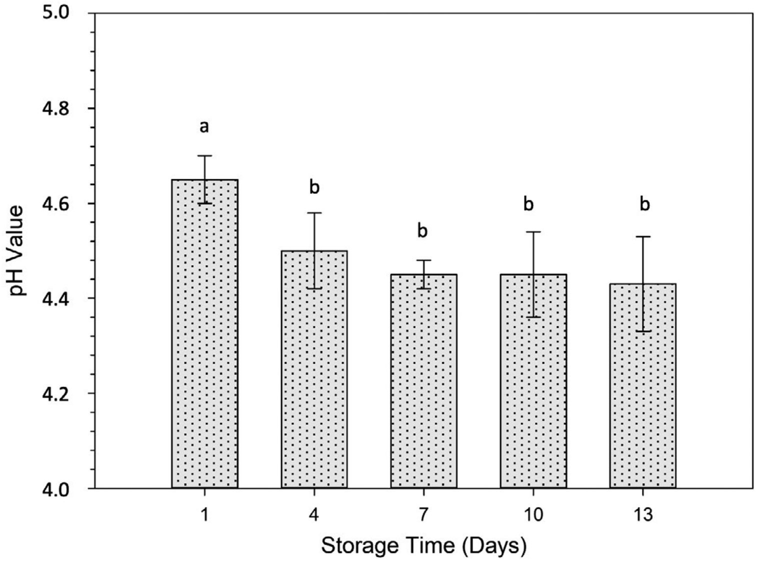

FIGURE 3 Changes of pH values during the storage of fermented soy-gels produced with T2: starter culture bacteria + Bifidobacteria. Different superscript above the bars indicate that are significantly differ from each other ($p < .05$)

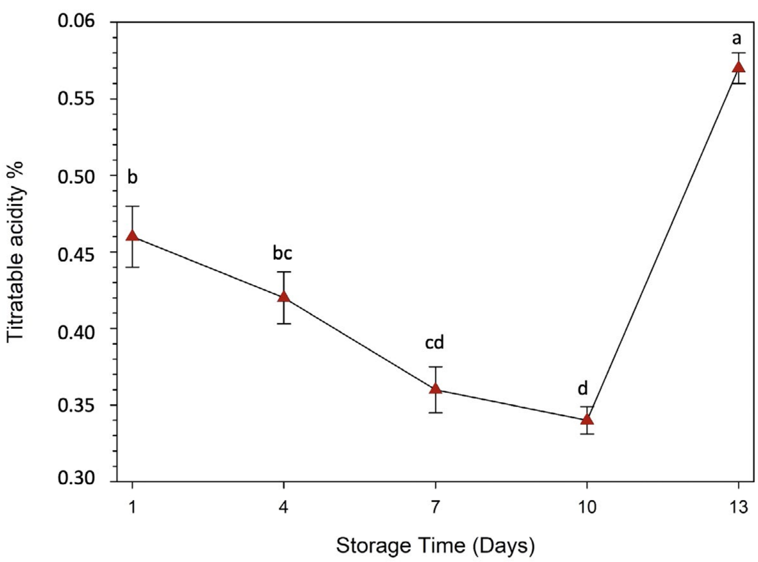

FIGURE 4 Titratable acidity of fermented soy-gel samples produced with T2: starter culture bacteria + Bifidobacteria, measured during the storage period. Different superscripts at each time point indicate that the values are significantly different from each other ($p < .05$)

is in agreement with those of literature, which considered to be optimal for aroma development (Murti et al., 1993).

3.3.2 | Titratable acidity

Effect of storage time on titratable acidity is shown in Figure 4. The titratable acidity of the soy-gel significantly reduced to 0.34% by 10 days of storage from 0.46% in the first day of storage. Subsequently, titratable acidity significantly increased up to 0.57% by the 13th day. The reduction of the titratable acidity with the storage contradicts the results of Jimoh and Kolapo (2007), who showed increasing titratable acidity upon storage. However, Ghorbani et al. (2012) reported an increase of the acidity in soy-gel during storage in agreement with the current study. Since the current research finding is agreeing as well as contradicting with previously reported observations, further research is recommended.

3.3.3 | Viscosity

Changes in viscosity in fermented soy milk during the storage is shown in Figure 5. The viscosity of the samples increased ($p < .05$) during the storage. The results are in agreement with Murti et al. (1993) and Ghorbani et al. (2012). Even though rapid pH drop forms irregular and disharmonic protein structure in fermented gels, refrigerated storage for sufficient time might rearrange its structure according to Ghorbani et al. (2012). Thus, the rearrangement of the protein structure of the yoghurt during refrigerated storage may increase the viscosity. Advancing of interactions of dry matter with each other and with water with the time, lead to increase in the volume of the dry matter by resulting a higher viscosity in the product (Shahbandari et al., 2016). However, Cavallini and Antonio Rossi (2009) have reported that the storage time has no significant effect on the viscosity of the soy-gels.

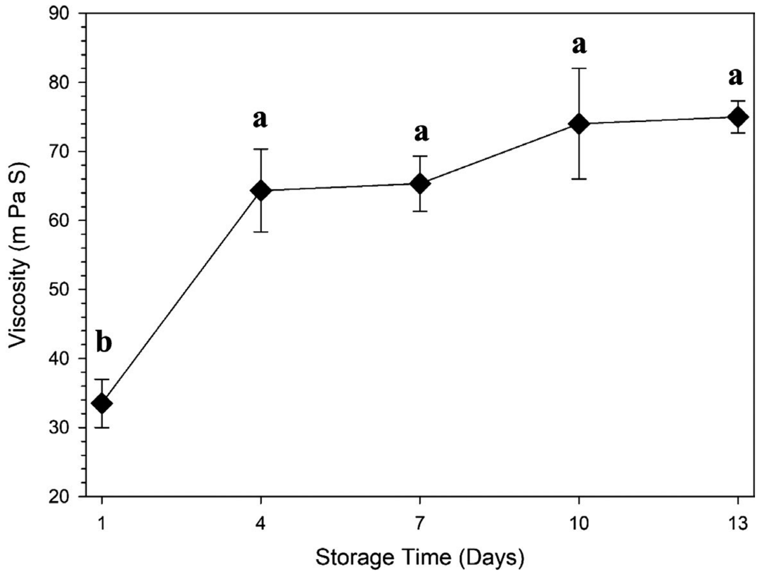

FIGURE 5 Changes in viscosity of fermented soy-gel samples produced with T2: starter culture bacteria + Bifidobacteria, measured during the storage period. Different superscripts at each time point indicate that the values are significantly different from each other ($p < .05$)

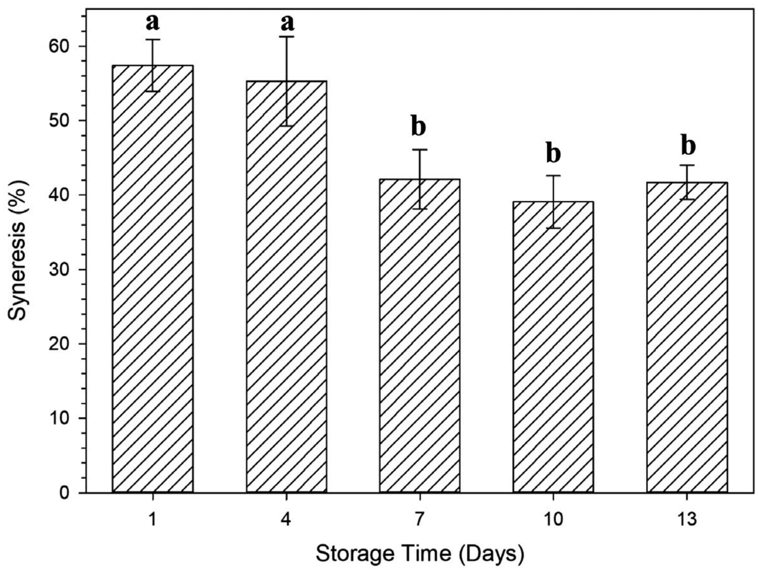

FIGURE 6 Syneresis values of fermented soy gel samples produced with T2: starter culture bacteria + Bifidobacteria, measured during the storage time

3.3.4 | Syneresis

Changes in the syneresis values during the storage period of fermented soy-gel is shown in Figure 6. The highest syneresis of the sample was observed in the first day of storage while the lowest was observed on the 10th day at storage. Syneresis of the sample continues to reduce until the 10th day of the storage. After the 10th day, a slight increase in the syneresis was observed. The results are in agreement with Ghorbani et al. (2012), who reported that the syneresis of the yoghurt decreases significantly during the storage. This might be because, refrigerated storage for sufficient time rearranges yoghurt gel structure and increases the water activity that leads to a decrease in the syneresis (Ghorbani et al., 2012). The upward trend of syneresis after a certain time of storage (i.e.,10th day) is in agreement with Shahbandari et al. (2016), who reported an increase in syneresis after 14 days of storage of stirred soy yoghurts at 4°C. The probable reason for increasing the syneresis after a certain time may be attributed due to hydrolysis and digestion of the proteins in the product due to the action of microorganisms, which then lead to losing the protein structural properties and their interactions with water.

3.4 | Total lactic acid bacteria and Bifidobacteria viable counts during storage of fermented soy-gels

Changes in viable cell counts (log CFU/ml) for LAB and BB in fermented soy-gel is shown in Table 2. The viable count of LAB and BB on Day 7 is significantly ($p < .05$) higher than the counts of 1st and the 14th day. The lowest values of the viable counts of both LAB and BB was at thefirst day of storage. The increase in the viable cell counts of the LAB during refrigerated storage might due to LAB's capability in metabolizing stachyose and raffinose in soy milk and survive, as previously reported by Wang et al. (2002, 2003). The reduction of *Lactobacillus* spp. viable cell counts during extended storage could be associated with the post-acidification which leads to a further reduction in pH (Shah, 2000). Moreover, the increase of organic acids and metabolites, such as hydrogen peroxide produced by yoghurt bacteria may negatively influence the survival of *Lactobacillus* spp. (Shah, 2000).

Viable counts of the BB of the soy-gel at theseventh day was higher ($p < .05$) than that of the other 2 days. The lower values of the viable counts of the sample were at thefirst day of storage. The viable cell counts reduced gradually to 8.60 log CFU/ml by Day 14 of storage. According to Kailasapathy (2006), the low pH of yoghurt and its further reduction and post acidification contributes to the low viability of probiotics. Thus, further pH reduction of fermented soy-gel may be the reason for the loss in viability of BB after theseventh day of storage. Nevertheless, the result is in contrast to a similar experiment conducted by Ghorbani et al. (2012), who reported that the highest viable *B. lactis* B-12 count was observed at the 14th day of storage.

3.5 | Viable counts between fermented soy-gels and cow milk yoghurt

Changes in viable cell counts of fermented soy-gel were compared with a cow milk yoghurt and results are presented in Figure 7. In the first day of storage, the viable counts of LAB were higher in cow milk yoghurt than in fermented soy-gel (Figure 7a). However, by Day 7, the viable counts of LAB were higher in fermented soy-gels than in cow milk yoghurt (Figure 7a). This likely to associate with oligosaccharides present in soybeans which have been proven to be prebiotics (Lan et al., 2007), that may contribute to higher growth of LAB in fermented soy-gel compared to the cow milk yoghurt during the storage. However, as reported by Canganella et al. (2000), significantly higher survival of *S. thermophilus* in soy milk fermented gels compared to cow milk yoghurt was observed only during storage at 12°C, but the survival was not significant during storage at 4°C.

TABLE 2 Viable cell counts of total lactic acid bacteria and bifidobacteria

Storage time (day)	Total lactic acid bacteria (log CFU/ml)	Bifidobacteria (log CFU/ml)
1	8.6^{b}	8.5^{b}
7	8.9^{a}	8.9^{a}
14	8.7^{b}	8.6^{b}

Abbreviation: CFU, colony forming units.
Means in the same column followed by different superscript are significantly different ($p < .05$).

On the first day of storage, the viable count of BB was significantly ($p < .05$) higher in cow milk yoghurt than in soy yoghurt (Figure 7b). Hence, the comparison of viable counts of total LAB and BB of fermented soy-gel with a cow milk yoghurt revealed higher initial viable counts of LAB and BB in cow milk yoghurt than in fermented soy-gel, and this probably due to readily available lactose in cow milk than zero level of lactose in soy milk (Hajirostamloo, 2009). BB have specific growth rates when cultured on different sugars (Rada et al., 2002). Thus, they might have an increased ability to utilize lactose more vigorously than other sugars and it may be the reason for observation of higher BB count in cow milk yoghurt at the first day of storage. However, as similar for LAB, soy oligosaccharides which act as prebiotics may contribute for a higher BB count in fermented soy-gels by the seventh day of storage. It has been reported that soy milk itself can support the growth of BB, owing to different sugars available in soy milk than in cow milk (Farnworth et al., 2007). BB have α-galactosidase that allows the probiotics to utilize sugars such as raffinose and stachyose (Scalabrini et al., 1998). The sufficient proteolytic activity of BB also supports their growth in soy milk (Kamaly, 1997). This also revisits the study by Murti et al. (1993), who confirmed that soy milk is a favorable medium for growth of BB alone or in combination with yoghurt SCB. Higher growth and survival of BB in fermented soy gels during

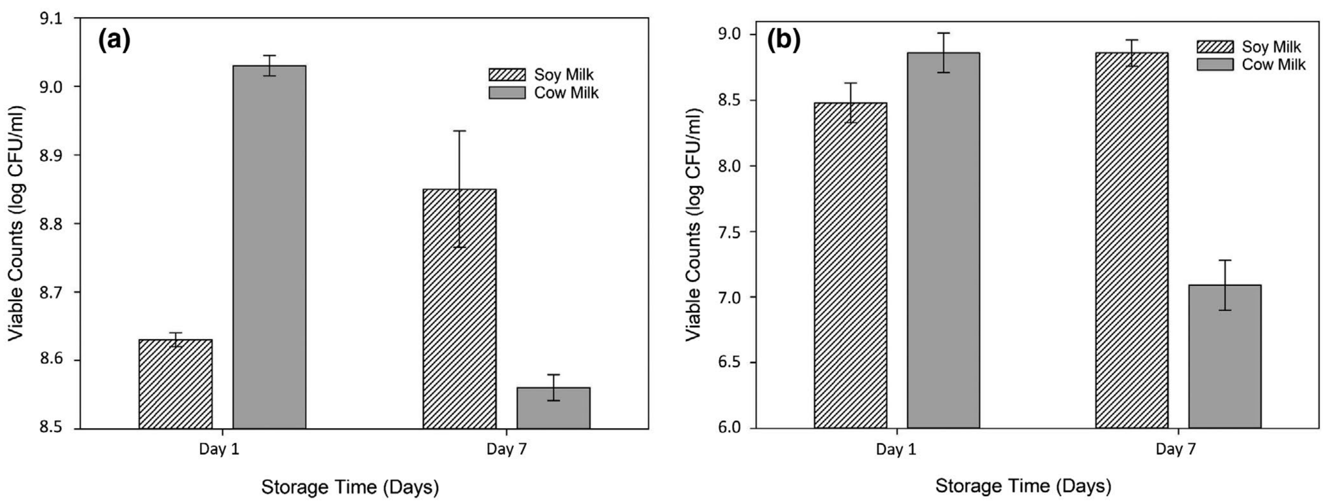

FIGURE 7 Viable cell counts of lactic acid bacteria (a) and Bifidobacteria (b) in cowmilk yoghurt and soy milk yoghurt upon storage (4°C) up to 7 days

storage than cow's milk yoghurt in the current study is in agreement with the findings of (Cui et al., 2021) who reported higher survival of BB(*B. animalis* subsp. *lactis* BB-12.) in probiotic soy yoghurt compared to probiotic cow's milk yoghurt during storage at 4°C. Thus, higher probiotic BB viability throughout the storage time of fermented soy-gel indicates its potential of being an excellent health-promoting alternative to cow milk yoghurt.

4 | CONCLUSIONS

Cow milk can be completely replaced with soy milk while gelatin can be perfectly replaced with agar-agar to produce technologically, functionally, and sensorial vice optimal fermented soy-gels. Incorporation of probiotic Bifidobacteria together with traditional yoghurt starter culture bacteria improve the overall flavor and texture of the soy-gels. Fermented soy-gels are effective to be used as probiotic carrier foods because recommended probiotic concentration level of 10^6 to over 10^7 or 10^8 can be achieved with Bifidobacteria. Higher viable counts of both lactic acid bacteria and Bifidobacteria can be achieved in fermented soy-gels compared to cow milk yoghurt during storage at 4°C for 7 days. Hence, fermented soy-gels would be a good alternative for dairy yoghurt as a probiotic carrier for vegans and people with lactose intolerance or milk protein allergy.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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