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RESEARCH

Development and Characterization of Gelatin-Free Set-Yoghurt Incorporated with Seaweed Phycocolloids

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ABSTRACT

The potential use of seaweed phycocolloids as stabilizing agents in gelatin-free set-yoghurt was evaluated. Three seaweed phycocolloids, namely carrageenan, alginate, and agar-agar, were extracted from *Kappaphycus alvarazii*, *Sargassum wightii,* and *Gracilaria salicornia*, respectively. Preliminary trials were conducted to determine the optimal incorporation levels of phycocolloids in gelatin-free set-yoghurts. Based on syneresis percentage, 0.12% (w/v) carrageenan, 0.2% (w/v) alginate, and 0.3% (w/v) agar-agar levels were selected as the best incorporation levels for set-yoghurt production. The hardness, syneresis, color, proximate composition, and sensory properties of the set-yoghurt containing selected levels of phycocolloids were assessed in comparison with the control, a set-yoghurt containing gelatin (0.8% w/v). All the phycocolloids-incorporated set-yoghurt showed lower ($p<0.05$) hardness and yellowness and higher syneresis compared to gelatin incorporated set-yoghurt (0.8% w/v), and higher lactic acid bacteria count on day 7 of storage at 4 °C. Protein content was high ($p<0.05$) in gelatin added set-yoghurt and lowest in agar-agar set-yoghurt. Alginate-added set-yoghurt had a similar protein content as the gelatin-incorporated yoghurt. Consumer preferences for all sensory attributes of alginate yoghurt were comparable to or higher than those of gelatin-added yoghurt, whereas carrageenan and agar-agar-incorporated set-yoghurt received lower preference for certain sensory attributes such as flavor, texture, and mouth feeling. In conclusion, carrageenan, alginate, and agar-agar derived from seaweed can be effectively utilized as stabilizers in set-yoghurt. Out of the tested phycocolloids, alginate stands out as it maintains the sensory attributes and protein content, making it a particularly suitable choice to use as a stabilizing agent in set-yoghurt.

INTRODUCTION

Yoghurt is one of the most widely consumed fermented dairy products. It is valued for its high protein content, vitamin B, and essential minerals, and well known for its numerous nutritional and health benefits (Altemimi, 2018). Gelatin is the most commonly used and preferred stabilizer in yoghurt production (Arab et al., 2022). It is a multifunctional ingredient that enhances gel strength, viscosity, and water-binding capacity (Temesgen, 2015). In addition, the melt-in-mouth property of gelatin imparts a fat-like sensory perception to low-fat yoghurt (Pang *et al*., 2017). Gelatin is typically produced from animal by-products, mainly from bovine and porcine sources (Alipal *et al*., 2019). This makes gelatin-containing yoghurt less acceptable to certain consumer groups, particularly those seeking Halal-certified or natural plant-based stabilizers. Growing consumer concern regarding the use of animal-derived gelatin has also led many informed lacto-vegetarians to avoid this otherwise nutritious dairy product (Altemimi, 2018). Therefore, developing alternative stabilizing agents for yoghurt offers a valuable opportunity to enhance consumer acceptance.

Seaweeds are marine, macroscopic, photosynthetic eukaryotic organisms capable of performing photosynthesis (Coppejans *et al*., 2009). Their cell walls contain a mixture of high–molecular weight polysaccharides known as phycocolloids (Anbuchezhian *et al*., 2015). Phycocolloids are water-soluble and can be extracted using hot water or alkaline solutions (Abdul Khalil et al., 2018). The major seaweed-derived phycocolloids include agar-agar, carrageenan, and alginate (Narayan *et al*., 2008). These compounds possess high viscosity and demonstrate excellent gelling, stabilizing, and emulsifying properties across various food and industrial applications (Anbuchezhian *et al*., 2015).

Seaweeds belonging to the orders *Gracilariales* and *Gelidiales* are the economically important sources of agar-agar (Anbuchezhian *et al*., 2015). Agar-agar is widely used in the food and pharmaceutical industries due to its gelation properties, biocompatibility, biodegradability, and non-toxicity. Moreover, agar-agar is composed of two major fractions: agarose, the neutral fraction responsible for gelling ability; and agaropectin, the charged polymer fraction arising from the existence of several substituent groups such as sulphates, methyl ethers, and pyruvates (Ferreira *et al*., 2019). Alginate can commercially be produced from seaweeds, including *Sargassum wightii*, *Ascophyllum nodosum*, *Laminaria* spp., *Lessonia nigrescens*, *Ecklonia maxima, Macrocystis pyrifera,* and *Durvillea antartica* (Abdul Khalil et al., 2018). Alginates are often employed as additives to modify and stabilize the texture of foods, owing to their ability to form gels and stabilize aqueous mixtures and emulsions (Torres *et al*., 2007). Carrageenan is mostly produced from the seaweeds *Kappaphycus alvarazii* and *Euchema denticulatum* (Narayan *et al*., 2008). Carrageenan serves as a gelling, thickening, and stabilizing agent in a variety of food products (Anbuchezhian *et al*., 2015). Hence, seaweed phycocolloids possess the potential to be used as stabilizers in dairy products. There have been some previous attempts to replace gelatin in yoghurt with agar from the seaweed *Gracilaria verrucosa* (Paththuwe Arachchi *et al*., 2018), but no studies have been conducted to compare the effects of different types of phycocolloids in yoghurt production. Therefore, the present study was conducted to assess the feasibility of using three phycocolloids (carrageenan, agar-agar, and alginate) as stabilizers in gelatin-free set-yoghurt.

METHODOLOGY

Extraction of seaweed phycocolloids

Three seaweed phycocolloids, namely carrageenan, alginate, and agar-agar were extracted from seaweed species, including *Kappaphycus alvarazii*, *Sargassum wightii,* and *Gracilaria salicornia*, respectively. *K. alvarazii* was collected from the Thuraiyoor Farm, Jaffna, Sri Lanka (9°37'43.22"N, 79°53'1.09"E), and *S. wightii* and *G. salicornia* were collected from Mandaithivu seaside in Jaffna, Sri Lanka (9°35'51.58"N, 79°58'53.51"E). The collected seaweeds were thoroughly washed three to four times with tap water to eliminate saltiness and remove any unwanted material such as salt, epiphytes, and sand. After drying in air, *K. alvarazii* and *S. wightii* were dried at 50

°C while *G. salicornia* was dried at 60 °C in a drying oven (CHF097XX2.5, GALLENKAMP, United Kingdom). Dried seaweeds were ground into powder and used for the extraction of phycocolloids. Carrageenan was extracted with hot water according to the method described by Rhein-knudsen *et al*. (2017), and precipitated with 95% of ethanol (1:3 v/v) to obtain a fibrous mass. Briefly, *K. alvarazii* powder was dissolved in distilled water and heated at 85 °C for 1.5 h in a hot water bath. The mixture was filtered using muscling cloth, and ethanol was added to precipitate carrageenan. The precipitated carrageenan was dried at 50 °C until a constant weight was reached. Agar-agar was extracted with hot water at 90 °C according to the method by De Alwis & Wijesekara (2022). In brief, *G. salicornia* powder was dissolved in distilled water, and heated at 90 °C for 1 h in a hot water bath. The mixture was filtered using muslin cloth and oven dried at 60 °C. Alginate was extracted according to the method described by Chee *et al*. (2011). Briefly, *S. wightii* powder was soaked in 300 mL of 1% $CaCl_2$ while heating at 50 °C for 3 h. After washing three times with distilled water, a 5% HCl solution was added to the mixture and left for 1 h. The mixture was washed again three times with 300 mL of distilled water. A 300 mL of 3% Na_2CO_3 solution was then added to the mixture and left for 1 h. Afterward, 250 mL of water was added to it before leaving it to stand overnight. The viscous mixture was centrifuged at 14,000 ×g for 10 min. The extracted sodium alginate was precipitated with 95% of ethanol (1:3, v/v) and filtered using a muslin cloth. The precipitate was oven-dried at 50 °C (Torres *et al*., 2007). The dried extracts were cooled to room temperature (20-25°C) and ground (< 2 mm) to prepare carrageenan, agar-agar, and alginate powder.

Set-yoghurt preparation

The set-yoghurt production was conducted at the Dairy Processing Laboratory, Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Sri Lanka. Preliminary trials were conducted to determine the optimal incorporation levels of three phycocolloids in gelatin-free set-yoghurt based on visual observations and syneresis percentage. Different incorporation levels (0.12%, 0.15%, 0.2%, 0.3%, o.5%, 0.8%, 1.0%, 1.2% (w/v)) of carrageenan, alginate, and agar-agar were tested in set-yoghurt preparation. According to the visual observations and syneresis values, the selected optimal levels for carrageenan, alginate, and agar-agar were 0.12%, 0.2% and 0.3%, respectively.

Four set-yoghurt treatments were prepared, i.e., Gelatin yoghurt: Gelatin incorporated set-yoghurt (0.8%) (w/v); Alginate yoghurt: Alginate incorporated gelatin-free set-yoghurt (0.2%) (w/v); Agar-agar yoghurt: Agar-agar incorporated gelatin-free set-yoghurt (0.3%) (w/v), and Carrageenan yoghurt: Carrageenan incorporated gelatin-free set-yoghurt (0.12%) (w/v). Yoghurts were prepared according to the procedure shown in Figure 1. Bovine milk was heated to 55-60 °C and homogenized using a homogenizer (HR3741/00, Philips Hand Mixture, China). While milk was being heated, sugar (10%) (w/v) was added at 70 °C, and phycocolloids were added at their optimum solubility temperatures. The optimum solubility temperatures maintained for carrageenan, alginate, and agar-agar set-yoghurts were 70 °C, 85 °C, and 85 °C, respectively. The mixture was then cooled in an ice bath, and the starter culture (0.04%; Yoflex-0.028%, ABT-5- 0.012%) was added at 42°C. The milk mixture was incubated in an incubator (DT-120-699422, ALP Co., Ltd. Tokyo, Japan) for 4 h, and the set-yoghurts were then stored in refrigerator at 4 °C until further analyses.

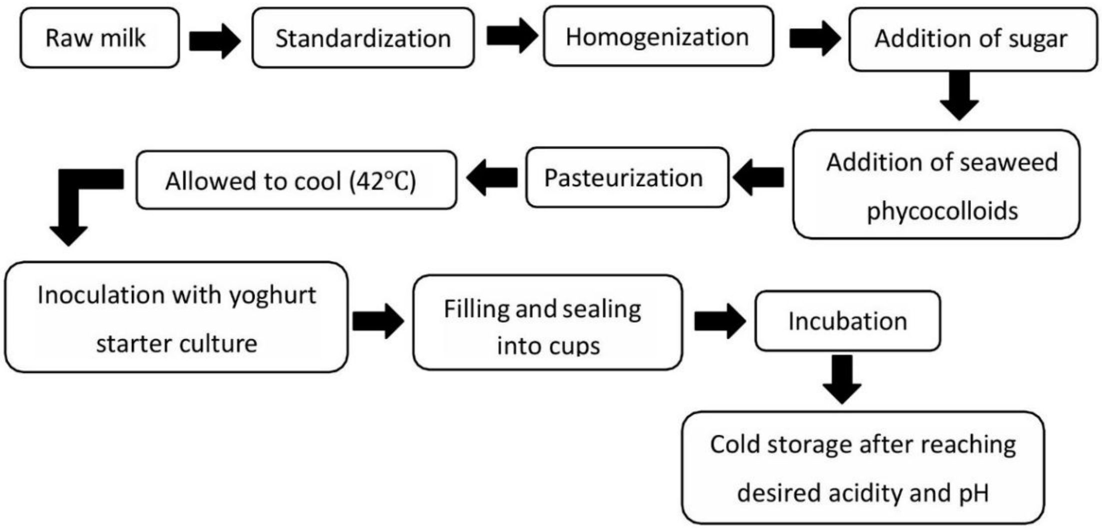

Figure 1: Yoghurt production procedure.

Sensory evaluation of set-yoghurts

Sensory evaluation of set-yoghurts was conducted on the first day after preparation, with thirty untrained panelists using a 5-point hedonic scale. The evaluated sensory attributes were aroma, color, flavor, texture, mouth feel, and overall acceptability.

Compositional analysis of set-yoghurts

The proximate composition of the set-yoghurts was evaluated according to the AOAC procedure (AOAC, 1980). The dry matter content was measured by drying set-yoghurt samples in a drying oven (CHF097XX2.5, GALLENKAMP, United Kingdom) at 105 °C until a constant weight was reached, and the ash content was measured by combusting the samples at 600 °C for 4 h in a muffle furnace. The Kjeldhal method was used for the determination of crude protein content in set-yoghurt. Crude fat content was measured by extracting fat with acetone using a Soxhlet extraction unit (code 6002316, JP Selecta, Spain).

Microbial assessment of set-yoghurts

Lactic acid bacteria, yeast, and mold counts in the set-yoghurt were determined on day 1, 7, and 14. The lactic acid bacteria were enumerated on DE Man-Rogosa-Sharpe agar (MRS agar) and incubated at 37 °C for 3 days under aerobic conditions. Yeast and mold were enumerated on yeast and mold agar and incubated at 37 °C for 5 days under aerobic conditions.

Statistical analysis

The data were statistically analyzed using SPSS 29.0 software. The proximate composition, physicochemical properties, and microbiological counts were analyzed using one-way ANOVA, followed by Tukey's multiple comparison test for mean comparison. The sensory evaluation data were analyzed using an independent sample median test.

RESULTS AND DISCUSSION

Sensory properties of set-yoghurt

Sensory panel results are shown in Figure 2. Consumer preferences for all sensory attributes, i.e., color, aroma, flavor, texture, mouth feeling, and overall acceptability, of alginate yoghurt were comparable to or higher than those for gelatin yoghurt. The preferences for the flavor, texture, mouth feeling, and overall acceptability of agar-agar and carrageenan yoghurts were lower ($p<0.05$) than gelatin yoghurts, yet agar-agar yoghurt had similar aroma and color as gelatin yoghurts. Yoghurt incorporated with carrageenan scored the lowest ($p < 0.05$) in all sensory attributes, which may be attributed to the gel-like texture characteristic of carrageenan yoghurts, resembling that of traditionally fermented buffalo milk curd.

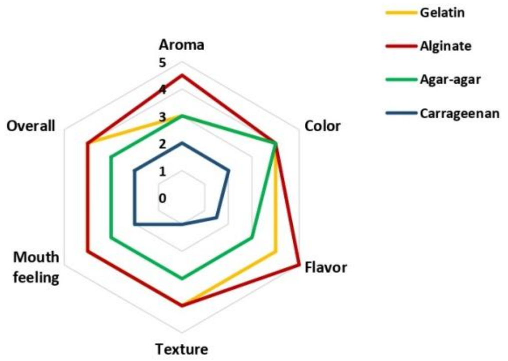

Figure 2: Preferences for sensory attributes of phycocolloids-incorporated yoghurt and gelatin yoghurt[1].

Proximate composition of yoghurt

Figure 3 shows the proximate composition of phycocolloids-incorporated yoghurts and gelatin yoghurts. Yoghurt incorporated with gelatin had a higher ($p<0.05$) protein content than yoghurt with phycocolloids. This is because gelatin, derived from animal sources, contains more protein (Alipal *et al.*, 2019), whereas phycocolloids are polysaccharides (Pangestuti & Kim, 2015). The fat content was higher ($p<0.05$) in agar-agar and carrageenan yoghurt, whereas it was lower ($p<0.05$) in alginate yoghurt compared to gelatin yoghurt.

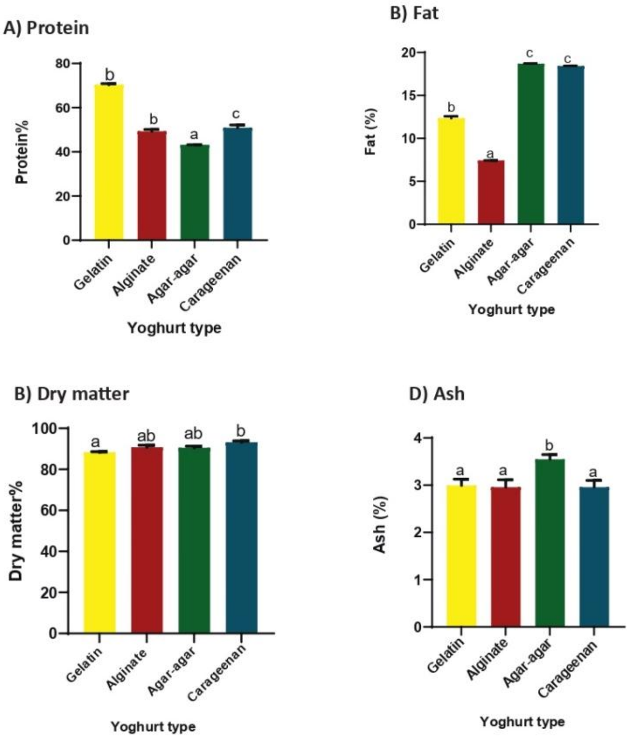

Figure 3: Protein (A), fat (B), dry matter (C) and ash (D) contents of phycocolloids-incorporated yoghurts and gelatin yoghurt[2].

These differences may be attributed to variations in the composition of the gelling agents, their interactions with other ingredients, processing conditions, emulsifying properties, and the resulting yoghurt microstructure, all of which can influence fat content (Winarni *et al.*, 2021).

[1] Gelatin: 0.8% gelatin incorporated yoghurt, Alginate: 0.2% alginate incorporated yoghurt, Agar-agar: 0.3% agar-agar incorporated yoghurt, Carrageenan: 0.12% carrageenan incorporated yoghurt.

[2] Bars with different letters in each graph are significantly different (P<0.05). Gelatin: 0.8% gelatin incorporated yoghurt, Alginate: 0.2% alginate incorporated yoghurt, Agar-agar: 0.3% agar-agar incorporated yoghurt, Carrageenan: 0.12% carrageenan incorporated yoghurt.

Physicochemical parameters of yoghurt

Color is one of the most significant characteristics of foods and beverages and is often the first attribute noticed by consumers. The color of food plays a crucial role in its appeal to our sense of taste (Gawai *et al*., 2017). The color values of phycocolloids-incorporated yoghurts and gelatin yoghurts are shown in Table 1. There were no differences ($p>0.05$) in brightness (L^* values) and greenness (a^* values) between phycocolloids-incorporated yoghurts and gelatin yoghurt. Nevertheless, the yellowness (b^* values) ($p<0.05$) was lower in phycocolloids- incorporated yoghurt compared to gelatin, which could be due to the differing chemical compositions and properties of phycocolloids and gelatin. These different additives and major ingredients may interact differently with the yoghurt matrix, affecting its color (Alipal *et al*., 2019).

Table 1: The color and gel strength of phycocolloids-incorporated set-yoghurt and gelatin yoghurt

	Gelatin	Alginate	Agar-agar	Carrageenan	p value
Color					
L*	82.9 ± 3.2	77.4 ± 16.9	86.40 ± 2.4	84.01 ± 4.0	0.38
a*	-3.01± 0.5	-2.74 ± 1.2	-48.24± 111.0	-3.43 ± 1.3	0.42
b*	15.75± 1.3^{c}	9.50 ± 0.8^{a}	12.75 ± 2.5^{b}	10.59 ± 0.8^{ab}	<0.001
Gel strength					
Hardness (N)	2.31 ± 0.1^{d}	1.35± 0.0^{c}	0.13 ± 0.0^{a}	0.84 ± 0.2^{b}	<0.001
Compact energy (J)	0.02 ± 0.0^{c}	0.01± 0.0^{ab}	0.00 ± 0.0^{a}	0.01 ± 0.0^{b}	0.003

Values are expressed as mean ± standard deviation. Values in the same row with different superscript letters are significantly different ($P<0.05$). Gelatin: 0.8% gelatin incorporated yoghurt, Alginate: 0.2% alginate incorporated yoghurt, Agar-agar: 0.3% agar-agar incorporated yoghurt, Carrageenan: 0.12% carrageenan incorporated yoghurt.

Table 2: The pH, titratable acidity, and syneresis of phycocolloids-incorporated yoghurt and gelatin yoghurt during storage at 4 ^{0}C for 14 days

	Gelatin	Alginate	Agar-agar	Carrageenan	p value
pH					
Day 1	4.41 ± 0.02	4.39 ± 0.03	4.25 ± 0.07	4.33 ± 0.02	0.06
Day 7	4.37 ± 0.04	4.36 ± 0.05	4.24 ± 0.06	4.29± 0.01	0.11
Day 14	4.35 ± 0.04^{b}	4.35 ± 0.05^{b}	4.14± 0.01^{a}	4.27 ± 0.02^{ab}	0.01
Titratable acidity (%)					
Day 1	0.78 ± 0.01^{b}	0.65 ± 0.02^{a}	0.82 ± 0.02^{b}	0.78 ± 0.01^{b}	0.003
Day 7	0.78 ± 0.01^{b}	0.67 ± 0.00^{a}	0.82 ± 0.00^{c}	0.74 ± 0.01^{b}	0.001
Day 14	0.76 ± 0.01^{b}	0.68 ± 0.01^{a}	0.82 ± 0.01^{c}	0.74 ± 0.01^{b}	0.002
Syneresis (%)					
Day 1	0.0 ±0.0^{a}	37.5 ± 2.1^{c}	25.5 ± 0.7^{b}	43.0 ±0.0^{d}	<0.001
Day 7	0.0 ± 0.0^{a}	46.0 ± 1.4^{c}	36.0 ± 2.8^{b}	43.5 ± 0.7^{c}	<0.001
Day14	0.0 ± 0.0^{a}	60.5 ± 0.7^{c}	47.5± 0.7^{b}	45.0 ± 1.4^{b}	<0.001

Values are expressed as mean ± standard deviation. Values in the same row with different superscript letters are significantly different ($P<0.05$). Gelatin: 0.8% gelatin incorporated yoghurt, Alginate: 0.2% alginate incorporated yoghurt, Agar-agar: 0.3% agar-agar incorporated yoghurt, Carrageenan: 0.12% carrageenan incorporated yoghurt.

The hardness and compact energy of yoghurt containing phycocolloids were lower ($p<0.05$) compared to the gelatin yoghurt (Table 1). The stabilizing ability of protein is higher than polysaccharides, and thereby phycocolloids-incorporated yoghurt can have less hardness compared to gelatin yoghurt (Skryplonek *et al*., 2019). Furthermore, phycocolloids from seaweeds have been shown to contain, high sulphate content, which can be associated with weak gel formation (Yarnpakdee *et al*., 2015).

The acidity level plays a critical role in yoghurt production, affecting the fermentation process, microbial safety, texture, consistency, and overall flavor of the product (De Brabandere & De Baerdemaeker, 1999). The pH values of phycocolloids-incorporated yoghurts did not differ ($p<0.05$) from gelatin yoghurt until 7 days of the storage period. However, agar-agar yoghurt showed lower ($p<0.05$) pH on day 14 (Table 2). The composition of phycocolloids, fermentation efficiency, and microbial growth can affect the slight differences in the pH of yoghurts (Bhattarai *et al*., 2016).

The titratable acidity serves as a quality control measure during yoghurt production and storage, as well as it also reflects the extent of fermentation that has been occurred during yoghurt production (Matela *et al*., 2019). The titratable acidity of carrageenan yoghurt did not differ from gelatin yoghurt throughout the storage period. Alginate yoghurt showed lower ($p<0.05$) titratable acidity than gelatin yoghurt on day 1, 7, and 14, whereas agar-agar yoghurt showed higher ($p<0.05$) titratable acidity on day 7 and 14 (Table 2). However, a previous study has reported that the addition of polysaccharides had no effect on the activity of yoghurt starter culture bacteria (Xu *et al.*, 2019).

Syneresis refers to the release of whey from the gel structure, resulting in the formation of a liquid layer on the surface of the yoghurt. It is an important measure of the consistency of yoghurt (Paththuwe Arachchi *et al*., 2018). In the context of set-yoghurt, syneresis measures the amount of whey separation or water loss that occurs over time (Matela *et al*., 2019). All three phycocolloids-incorporated set-yoghurts had higher ($p<0.05$) syneresis than gelatin set-yoghurt on day 1, 7, and 14 (Table 2). The concentration of phycocolloids plays a significant role in stabilizing the protein network in yoghurt. Higher levels of phycocolloids, due to swelling or excessive negative charge, might disrupt protein clusters and enhance whey drainage (Khubber *et al*., 2021). Thus, the observed results can be due to the differences in incorporation levels of three phycocolloids in set-yoghurt.

Microbial assessment of set-yoghurt

Lactic acid bacteria and yeast and mold counts of phycocolloids-incorporated yoghurt and gelatin yoghurt during storage at 4 °C for 14 days are shown in the Table 3. Lactic acid bacteria, as well as yeast and mold counts, are crucial for ensuring food safety and quality control. On day 1, there was no difference ($p>0.05$) in lactic acid bacteria count among four set-yoghurt types. Nevertheless, lactic acid bacteria count was higher ($p<0.05$) in phycocolloids-incorporated yoghurts on day 7. On day 1, phycocolloids-incorporated set-yoghurt had higher ($p<0.05$) yeast and mold count than gelatin yoghurt. The yeast and mold counts in phycocolloid-incorporated set-yoghurt did not differ from that in gelatin yoghurt on days 7 and 14, possibly due to identical storage and production conditions.

Table 3: Lactic acid bacteria and yeast and mold counts (log CFU/g) of phycocolloids-incorporated yoghurt and gelatin yoghurt during storage at 4 ^{0}C for 14 days

	Gelatin	Alginate	Agar-agar	Carrageenan	p value
Lactic acid bacteria					
Day 1	4.15 ± 0.21	4.25 ± 0.07	4.40 ± 0.00	4.15 ± 0.21	0.42
Day 7	3.00 ± 0.00^a	4.40 ± 0.14^c	4.15 ± 0.07^c	3.55 ± 0.07^b	<0.001
Day 14	3.20 ± 0.07^a	3.40 ± 0.14^{ab}	4.10 ± 0.14^b	3.50 ± 0.14^{ab}	0.03
Yeast and mold					
Day 1	0.00 ± 0.00^a	2.65 ±0.50^b	3.90 ± 0.14^b	2.65 ± 0.50^b	0.002
Day 7	1.00 ± 0.14^{ab}	0.00 ± 0.00^a	3.15 ± 0.21^b	3.10 ± 0.14^b	0.03
Day 14	3.50 ± 0.07	3.05 ± 0.35	3.50 ± 0.00	3.75 ± 0.21	0.07

Values are expressed as mean ± standard deviation. Values in the same row with different superscript letters are significantly different (P<0.05). Gelatin: 0.8% gelatin incorporated yoghurt, Alginate: 0.2% alginate incorporated yoghurt, Agar-agar: 0.3% agar-agar incorporated yoghurt, Carrageenan: 0.12% carrageenan incorporated yoghurt.

CONCLUSION

In conclusion, carrageenan, alginate and agar-agar from seaweeds can be used as stabilizing agents in set-yoghurt at the incorporation levels of 0.12% (w/v), 0.2% (w/v) and 0.3% (w/v), respectively. The incorporation of alginate did not compromise sensory attributes of set-yoghurt, while carrageenan and agar-agar incorporated set-yoghurt showed lower preference for attributes such as flavor, texture and mouth feeling. Hence, alginate stands out as it maintains the sensory attributes, making it a particularly suitable choice as a stabilizing agent in set-yoghurt. The present results would be beneficial in meeting the demand for dairy products among the lacto-vegetarian community and religious groups that prefer gelatin-free foods.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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