

CHARACTERIZATION AND PHYTOCHEMICAL COMPOUNDS IDENTIFICATION OF YOGHURT WITH THE ADDITION OF CARRAGEENAN AND *Spirulina* sp.

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Abstract

Yoghurt is a fermented milk product containing lactic acid bacteria, such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Carrageenan is added as a stabilizer to improve the texture and stability of yoghurt, while *Spirulina* sp. is rich in bioactive compounds, such as proteins, amino acids, and antioxidants, that can enhance the nutritional and functional properties of the product. This study aimed to determine the formulation of carrageenan and *Spirulina* sp. in yoghurt based on physicochemical characteristics. The study used a completely randomized design (CRD) with four treatments (carrageenan + *Spirulina* sp.): P0 (0.00% + 0.00%), P1 (0.01% + 0.03%), P2 (0.03% + 0.05%), and P3 (0.05% + 0.07%) with three replicates. The analysis results showed that the best treatment was yoghurt with 0.05% carrageenan and 0.07% *Spirulina* sp. with pH 4.0, TAT 0.9%, LAB 9.3 log CFU/mL, and high water content (89.59%). The yoghurt contained lower ash (0.78%), fat (0.94%), and energy (43.22 Kcal/100 g) than commercial yoghurt. The protein content of this yoghurt was 3.65%, while that of commercial yoghurt was 3.63%. Yoghurt with the addition of carrageenan and *Spirulina* sp. met the quality standards based on SNI 2981:2009. Identification of phytochemical compounds showed the presence of terpenoid compounds, which have antioxidant activity and play a role in warding off free radicals from the body. This yoghurt has the potential to be a functional food product that is low in fat and energy and supports a healthy lifestyle.

Keywords: fermentation, lactid acid bacteria, phytochemicals, proximate, *S. thermophilus*

Karakterisasi dan Identifikasi Senyawa Fitokimia Yoghurt dengan Penambahan Karagenan dan *Spirulina* sp.

Abstrak

Yoghurt merupakan produk fermentasi susu dengan bakteri asam laktat misalnya *Lactobacillus bulgaricus* dan *Streptococcus thermophilus*. Karagenan ditambahkan sebagai *stabilizer* yang dapat memperbaiki tekstur dan kestabilan yoghurt, sedangkan *Spirulina* sp. kaya akan senyawa bioaktif, yaitu protein, asam amino, dan antioksidan yang berpotensi meningkatkan nilai gizi dan aktivitas fungsional yoghurt. Penelitian ini bertujuan untuk menentukan formulasi karagenan dan *Spirulina* sp. pada yoghurt berdasarkan karakteristik fisikokimia. Penelitian menggunakan rancangan acak lengkap (RAL) dengan empat perlakuan (karagenan + *Spirulina* sp.) yaitu P0 (0,00% + 0,00%); P1 (0,01% + 0,03%); P2 (0,03% + 0,05%); dan P3 (0,05% + 0,07%) dilakukan tiga kali ulangan. Hasil analisis menunjukkan perlakuan terbaik, yaitu yoghurt 0,05% karagenan + 0,07% *Spirulina* sp. dengan pH 4,0; TAT 0,9%; BAL 9,3 log CFU/mL;

serta kandungan air tinggi (89,59%). Yoghurt mengandung abu (0,78%), lemak (0,94%), dan energi (43,22 Kcal/100 g) lebih rendah dibanding yoghurt komersial. Kandungan protein yoghurt ini 3,65%, sedangkan yoghurt komersial 3,63%. Yoghurt dengan penambahan karagenan dan *Spirulina* sp. memenuhi standar mutu yoghurt berdasarkan SNI 2981:2009. Identifikasi senyawa fitokimia menunjukkan adanya senyawa terpenoid, yang memiliki aktivitas antioksidan dan berperan dalam menangkal radikal bebas. Yoghurt ini berpotensi sebagai produk pangan fungsional rendah lemak dan energi untuk mendukung gaya hidup sehat.

Kata kunci: bakteri asam laktat, fermentasi, fitokima, proksimat, *S. thermophilus*

INTRODUCTION

Consumer awareness of the importance of health has increased. Consumers are beginning to believe that the food they eat contributes to their health (Trajkovska *et al.,* 2024). This is indicated by changes in eating patterns, where the tendency to consume foods that are high in fat, salt, carbohydrates, cholesterol, and food additives (BTP) and low in fiber has become a trend in the country. Consumers choose natural and healthy foods that prevent or treat diseases.

Milk is a food that is easy to consume and contains high nutrition because it contains complete food substances such as protein, lipids, carbohydrates, glucose, minerals, and vitamins needed by humans (Shari, 2023). However, milk is a highly perishable food ingredient; therefore, further handling and processing are required to preserve its nutritional value. One such method is yogurt fermentation (Nugroho *et al.,* 2023). Yoghurt is a widely consumed product resulting from the fermentation of one or more lactic acid bacteria (LAB) (Fauziah *et al*., 2023). Yoghurt is known for its nutritional benefits and health effects (Setiadi *et al*., 2024). Yoghurt has various health benefits, such as helping to maintain the balance of intestinal microflora, reducing the risk of diarrhea, suppressing the growth of cancer cells, increasing body growth, and regulating cholesterol levels in the blood (Manihuruk *et al.,* 2025).

One of the innovations in the development of functional yoghurt is the addition of natural ingredients, such as carrageenan and *Spirulina*, which have specific but complementary benefits without compromising the processing or quality of the final product (Wang *et al.,* 2023; Rahman *et al.,* 2024). Carrageenan is one of the most widely used ingredients in the food industry for stabilizing product texture, including yoghurt, and it directly contributes to improving the quality of the final product (Nugraha *et al.,* 2022). Carrageenan is commonly used as a stabilizer, emulsifier, and thickening agent in food products. It contains hydrocolloid compounds that help maintain the quality of yoghurt. Carrageenan is produced by extracting red seaweed. It possesses excellent functional properties, including water-holding capacity and the ability to stabilize food systems (Anggraini *et al.,* 2016), and can reduce syneresis in yoghurt. Several food products developed using carrageenan include soyghurt (Ningsih *et al.,* 2024), yoghurt (Nurbaeti *et al.,* 2024), jelly drinks (Ismayana *et al.,* 2024), and puddings (Dewi *et al.,* 2025).

Meanwhile, *Spirulina* is a high-protein source (55-70% of dry weight) and contains amino acids and essential fatty acids, such as γ-linolenic acid and oleic acid (Fernandes *et al.,* 2023). *Spirulina* is also rich in antioxidants and iron and can support the growth of Lactic Acid Bacteria (LAB), which are important in the yoghurt fermentation process and help maintain product shelf life (Mesbah *et al.,* 2022). Food products developed from *Spirulina* sp. include cheese (Bosnea *et al.,* 2021), yoghurt (Mesbah *et al.,* 2022), and ayran (Çelekli *et al.,* 2020). The combination of these two ingredients not only improves the physical and nutritional quality of yoghurt but also represents a novel approach to functional yoghurt formulation by utilizing natural stabilizers and nutrient-rich microalgae. This innovation has been shown to enhance yoghurt texture, extend shelf life, and increase bioactive properties, such as antioxidant activity, aligning with consumer demand for functional and natural foods (Wang *et al.,* 2023; Fernandes *et al.,* 2023).

This study aimed to determine the optimal concentration of carrageenan and Spirulina sp. for producing selected yoghurt formulas, yoghurt characterization with the addition of carrageenan and *Spirulina* sp., and identification of phytochemical compounds. The hypothesis of this study is that the addition of carrageenan and *Spirulina* sp. at certain concentrations can produce yoghurt with physicochemical characteristics and nutritional content that comply with SNI 2981:2009, contain bioactive compounds, and have better quality than commercial yoghurt.

MATERIALS AND METHODS

Starter Culture

The lactic acid bacterial culture was obtained from the Food and Nutrition Culture Collection (FNCC), Food and Nutrition Study Center, Gadjah Mada University, Yogyakarta. The bacterial strains used were *L. bulgaricus* FNCC 0041 and *S. thermophilus* FNCC 0040. *L. bulgaricus* FNCC 0041 and *S. thermophilus* FNCC 0040 were verified before preparing the starter culture. The verification process included gram staining, motility, and catalase testing. Starter culture preparation began by inoculating the stock cultures of *L. bulgaricus* and *S. thermophilus* preserved in glycerol into de Man, Rogosa, and Sharpe Agar (MRS-A) slants (Oxoid). The slants were then incubated in a Thermocline type 4200 incubator at 37 °C for 48 h. After incubation, the bacterial colonies on MRS-A were transferred into 10 mL of de Man, Rogosa, and Sharpe Broth (MRS-B) (Oxoid) and incubated at 37 °C for 24 h. Subsequently, 10% of the resulting culture (5 mL) was inoculated into 45 mL of fresh MRS-B and incubated again at 37 °C for 24 h. The number of bacterial cells was determined using the pour-plate method (total plate count).

A total of 90% of the commercial milk was heated and then allowed to cool before 10% of the inoculum was added. The mixture was then incubated at 37 °C for 24 h. The parameters observed were pH, Total Titrated Acid (TTA), and total Lactic Acid Bacteria (LAB).

Yoghurt Formulation

The yoghurt formula used in this study involved variations in the concentrations of carrageenan and *Spirulina* sp. according to the three treatments presented in Table 1. Yoghurt was prepared based on the method described by Ramadhany (2019), with modifications.

The manufacturing process started with heating Greenfields low-fat commercial milk at 43°C, followed by cooling it to 37°C. Starter cultures of *L. bulgaricus* FNCC 0041 and *S. thermophilus* FNCC 0040 (1:1 ratio) were added to the commercial milk. The starter was added at a volume of 15% (v/v) with a bacterial population of 1×10^7 CFU/mL, in accordance with the Indonesian National Standard (SNI 2981:2009) for yoghurt. Carrageenan and *Spirulina* sp. were added according to the treatment formulation. The carrageenan used in this study was refined carrageenan obtained from Kappa Carrageenan Nusantara (Bekasi, Indonesia). The *Spirulina* sp. used was a fine green powder with a bland taste and good heat stability, obtained from PT Algaepark Indonesia Mandiri, Indonesia. It contains high protein levels and natural pigments, such as beta-carotene, carotenoids, and phycocyanobilin. Both materials were stored in airtight containers at room temperature until they were used. Carrageenan was added to the following formulas: 0 (0.00%), 1 (0.01%), 2 (0.03%), and 3 (0.05%). *Spirulina* sp. was then added based on the following formulations (w/v): formula 0 (0.00%), formula 1 (0.03%), formula 2 (0.05%), and formula 3 (0.07%). The

Table 1 Composition of yoghurt formulas

Ingredients	Control	Formula 1	Formula 2	Formula 3
Commercial milk (% v/v)	84.92	84.92	84.92	84.92
S. thermophilus FNCC 0040 (% v/v)	7.5	7.5	7.5	7.5
L. bulgaricus FNCC 0041 (% v/v)	7.5	7.5	7.5	7.5
Carragenan (% w/v)	0	0.01	0.03	0.05
Spirulina sp. (% w/v)	0	0.03	0.05	0.07

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mixture was fermented at 37°C for 16 h until coagulation occurred (Rachman *et al.,* 2018). After fermentation, the yoghurt was packaged in sterile glass containers with tight-fitting lids and stored at 4°C until further analysis. The parameters measured included the pH, TTA, and total LAB.

pH Analysis

pH testing was conducted using a pH meter, following the standard method SNI 06-6989-11-2004 (National Standardization Agency [BSN], 2004). The pH was determined using a pH meter calibrated with standard buffer solutions at pH 4 and 7, prior to measurement.

Total Titrated Acid Analysis

The measurement of total titrated acid (TTA) was conducted according to the Indonesian National Standard for yoghurt (SNI 2981:2009) using 0.1 N NaOH titration with phenolphthalein as an indicator. The total titrated acid was calculated using the following equation:

$$TTA = \frac{V\ NaOH \times N\ NaOH \times 90}{W} \times 100\%$$

Information:
V NaOH= volume of NaOH used
N NaOH = normality of NaOH measured
W = sample weight
90 = molecular weight of lactic acid

Total LAB Analysis

Total LAB was enumerated using a modified method of SNI (2009) through serial dilution and pour plating on de Man Rogosa and Sharpe Agar (MRSA) supplemented with 0.5% $CaCO_3$. The plates were incubated at 37°C for 48 h, and colonies (25-250) were counted to calculate the LAB population, expressed as CFU/mL. The following formula was used for the calculation:

$$N = \frac{\Sigma C}{[(1 \times n1)+(0,1+n2)\times(d)]}$$

Information:
N = number of product colonies per mL or colonies per gram;
ΣC = number of colonies on all plates counted;
n1 = number of plates in the first dilution counted;
n2 = number of plates in the second dilution counted;
d = first dilution counted

Nutritional Composition Analysis

The nutritional composition of the yoghurt samples was analyzed using standard methods. The ash content was determined according to SNI 01-2891-1992 (point 6.1), while the moisture content was measured following SNI 01-2891-1992 (point 5.1). The total fat content was analyzed using the gravimetric method (18-8-5/MU), and the protein content was determined using titrimetric analysis (18-8-31/MU). Carbohydrate content was calculated by difference based on the 18-8-9/MU method, whereas energy from fat and total energy (kcal/100 g) were calculated using the 18-8-9/MU/SMM-SIG procedures.

Fractionation of Active Compound in Yoghurt with the Addition of Carrageenan and *Spirulina* sp. using TLC

Fractionation or separation of active components from yoghurt extract with the addition of carrageenan and *Spirulina* sp. was carried out using thin-layer chromatography (TLC). The main objective of this technique was to determine the most effective eluent ratio for separating active compounds. The separation process was performed using silica gel 60 F_{254} plates and observed under UV light at wavelengths of 254 and 366 nm.

Three variations of eluent ratios were used in this process, namely: A) chloroform: ethyl acetate: ethanol (7:1:3), B) chloroform: ethyl acetate: ethanol (7:1:2), and C) chloroform: ethyl acetate: ethanol (8:1:1). The selection of this eluent system was based on the difference in polarity of each solvent, where chloroform was non-polar, ethyl acetate was semi-polar, and ethanol was polar. This combination provides a wide polarity range, allowing the separation of compounds based on their affinity for the stationary phase (silica gel) and mobile phase (eluent) (Lade *et al.,* 2014; Notonegoro *et al.,* 2022).

The procedure started with the saturation of the chamber with the eluent for

30 min. The extracts were dissolved in the original solvent and spotted on a silica plate using a capillary tube. The plate was then inserted into the chamber until the mobile phase rose to approximately 1 cm from the upper limit of the plate. After the elution process was completed, the plates were dried at room temperature and observed under UV light at two wavelengths to detect compound spots.

The visualization of terpenoid compound patches was performed using two approaches. First, the samples were observed under UV light at 254 and 366 nm to identify fluorescent or dark spots that appeared above the fluorescent background. The plates were then sprayed with anisaldehyde-sulfuric acid reagent and heated at 105°C for 5-10 minutes. This reaction produced characteristic colored spots, such as green, purple, pink, or blue, which indicated the presence of terpenoid compounds (Harborne, 1984).

The assessment was carried out by calculating the Retardation Factor (Rf), which was then compared with the threshold range of 0.2-0.8. If the Rf value was too high, the polarity of the eluent was reduced and vice versa (Aritonang, 2022). To strengthen the identification, the comparison compounds artemisinin and terpineol were used, which are known to have Rf values of 0.32 and 0.375, respectively (Nuria *et al.,* 2014; Arundina *et al.,* 2015). The Rf values were calculated using the following formula:

$$Rf = \frac{\text{Distance traveled by the components}}{\text{Total distance traveled by the eluent}}$$

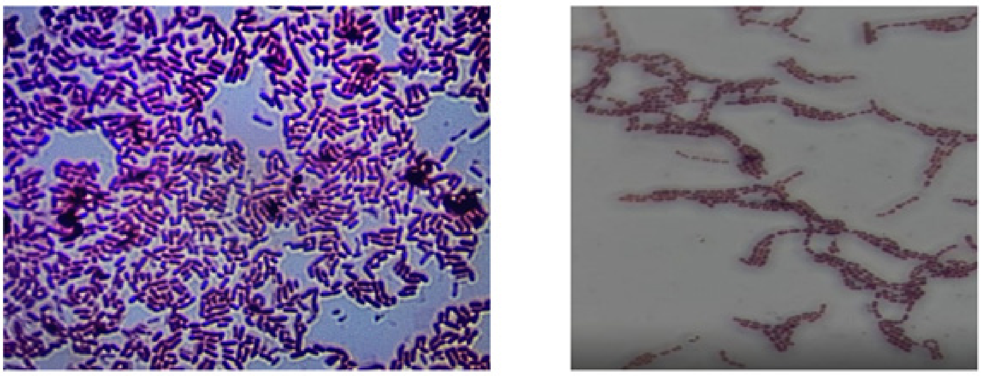
Experimental Design and Data Analysis

The experimental design used in this study was a completely randomized design (CRD). Quantitative data on pH, TTA, and total LAB were analyzed using analysis of variance (ANOVA) with the Statistical Package for the Social Sciences (SPSS) version 22.0. If significant differences were found, the analysis was followed by the least significant difference (LSD) test. The verification data for LAB and proximate analysis were analyzed descriptively and presented in tabular form. Phytochemical identification was performed using thin-layer chromatography (TLC) with various eluents. The Rf values were calculated and analyzed descriptively based on color changes and separation patterns on the chromatogram. The results showed that the spots did not tail, and the distance between the spots was clear. The Rf data are presented in the form of images.

RESULTS AND DISCUSSION

Characteristics of Lactic Acid Bacteria (LAB) Starter Culture

The starters used in this study were *L. bulgaricus* FNCC 0041 and *S. thermophilus* FNCC 0040. Based on data from the Food and Nutrition Culture Collection ([FNCC], 2022), *L. bulgaricus* FNCC 0041 is a gram-positive, rod-shaped, non-motile, non-spore-forming, and catalase-negative bacterium. *S. thermophillus* FNCC 0040 is also a gram-positive, coccus-shaped, non-motile, non-endospore-producing, and catalase-negative bacterium. A starter culture is a collection of live microorganisms that are specially selected



(A) (B)

Figure 1 Gram staining results of starter culture isolates and used to initiate the fermentation process in food products.

The morphology and physiological characteristics of both isolates were verified through Gram staining, motility, and catalase tests. Gram staining results (observed under 1000× magnification) showed that *L. bulgaricus* FNCC 0041 had a long rod morphology, while *S. thermophilus* FNCC 0040 was coccus-shaped, with both showing a purple color typical of Gram-positive bacteria (Figures 1A and 1bB). The motility test showed that both isolates were non-motile, characterized by a limited growth pattern only along the inoculation line on semi-solid media. In the catalase test, no bubbles were formed after the addition of 3% H_2O_2, indicating that neither of the isolates produced the enzyme catalase.

The Gram staining test showed that the isolates *L. bulgaricus* FNCC 0041 and *S. thermophilus* FNCC 0040 were Gram-positive bacteria. *L. bulgaricus* and *S. thermophilus* are the two primary lactic acid bacteria used as starter cultures in yoghurt production. *L. bulgaricus* is a Gram-positive, rod-shaped, anaerobic bacterium that does not form endospores. It is homofermentative, producing lactic acid as the main fermentation product. Additionally, it is microaerophilic, does not digest casein, does not produce indole or hydrogen sulfide (H_2S), does not produce the catalase enzyme, and is nonpathogenic. Its optimal growth temperature ranges between 40-44°C. In contrast, *S. thermophilus* is a gram-positive, coccus-shaped, non-motile, facultatively anaerobic bacterium that lacks spores. It is catalase-negative and exhibits optimal growth at temperatures between 35-53°C, classifying it as a thermotolerant bacterium. This species is aerotolerant and demonstrates high peptidase activity, although its proteolytic capability is lower than that of *L. bulgaricus* (Sfakianakis & Tzia, 2014; Hendarto *et al.*, 2019).

pH Value and Total Titrated Acid of Yoghurt

Total titrated acid (TTA) and pH were the main chemical indicators used to assess the success of the fermentation process, where TTA represented the total amount of acid present in the sample. TTA was measured using a titration method with a standard base solution to accurately determine the acid concentration. Research on the addition of carrageenan to yoghurt products, such as that by Nugraha *et al.* (2022), showed that the addition of carrageenan to yoghurt had a significant effect on pH, total LAB, and TTA. The changes in pH and TTA values during the different fermentation periods are presented in Figures 2.

The results of the analysis of variance (ANOVA) showed that the addition of carrageenan and *Spirulina* sp. to yoghurt significantly affected the pH value ($p<0.05$). Based on the results of the least significant difference (LSD) test, the pH of the control

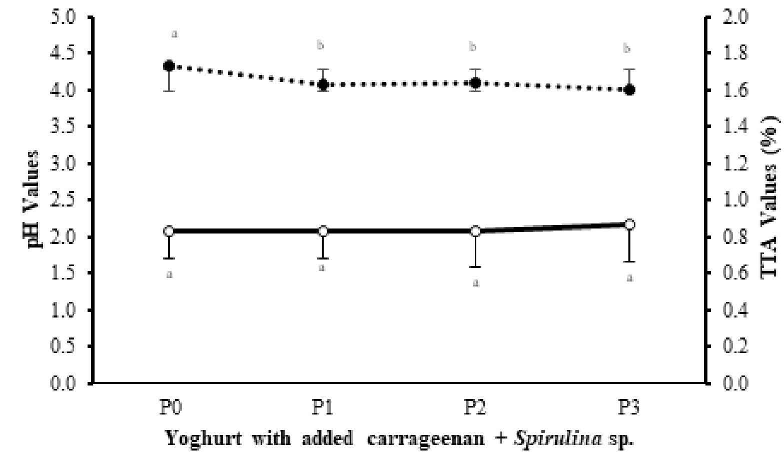


Figure 2 pH values (··•··) and TTA (—) of yoghurt with added carrageenan + *Spirulina* sp. Note: P0 (0.00% + 0.00%); P1 (0.01% + 0.03%); P2 (0.03% + 0.05%); and P3 (0.05% + 0.07%)

yoghurt (P0), which did not contain carrageenan or *Spirulina* sp., was significantly higher than that of all treatment groups (P1, P2, and P3). This indicates that the addition of carrageenan and *Spirulina* sp., combined with the fermentation activity of *L. bulgaricus* and *S. thermophilus*, was able to reduce the pH of the yoghurt. Treatment P3 had the lowest pH value (4.0), which was significantly different from P0 but not significantly different from P1 and P2, both of which had pH values of 4.1. No significant difference was observed between P1 and P2. The pH range of 4.0 to 4.3 across all samples confirmed that the yoghurt remained in an acidic condition. This decrease in pH was associated with the metabolic activity of lactic acid bacteria, which converted lactose into lactic acid. This process increased the concentration of hydrogen ions (H^+) in yoghurt, thereby lowering the pH value.

The results of this study showed that increasing carrageenan concentration decreased the pH of yoghurt. This finding differed from the results reported by Nugraha *et al.* (2022), who found that pH increased with higher concentrations of carrageenan, namely, pH 4.64 at 0.1% carrageenan, pH 4.80 at 0.3%, and pH 5.2 at 0.5%. This difference was presumably due to the combination of additional ingredients used in this study, namely carrageenan and *Spirulina*. Carrageenan is known to stabilize the system and slow down the diffusion of acid from the matrix, while *Spirulina* also contributed to maintaining pH due to its nutritional properties that supported the growth of lactic acid bacteria. Therefore, the combination of carrageenan and *Spirulina* sp. in this study was effective in maintaining the acidity of yoghurt in accordance with the quality standards for fermented milk products set by the FDA (2019), which require a pH value below 4.6.

The results of the analysis of variance (ANOVA) showed that the addition of carrageenan and *Spirulina* sp. to yoghurt did not have a significant effect on total titratable acidity, with a significance value of p = 0.994. This value, which was much higher than the 0.05 threshold, indicated that there were no significant differences among the treatments in terms of the acid content in the yoghurt. The average total titratable acidity ranged from 0.8%-0.9%, which remained within the standard quality range for yoghurt, as specified by SNI 2981:2009 (0.5%-2%) (BSN, 2009). The absence of significant differences in titratable acid concentrations among treatments suggests that the addition of carrageenan and *Spirulina* sp. in the yoghurt formulation did not significantly affect the activity of lactic acid bacteria (*L. bulgaricus* and *S. thermophilus*) in producing organic acids during fermentation. Nugraha *et al.* (2022) showed that the best physicochemical properties were achieved by the addition of 0.5% carrageenan, which resulted in a total acid value of 0.56%.

The addition of carrageenan and *Spirulina* sp. did not directly affect the metabolism of lactose to lactic acid. The total titrated acid (TTA) in yoghurt is primarily a result of the fermentative activity of lactic acid bacteria, which convert lactose into lactic acid. During the fermentation process, *S. thermophilus* plays an active role in the early stages by breaking down amino acids and other nutrients and utilizing oxygen, thus creating anaerobic conditions that favor the growth of *L. bulgaricus* (Sieuwerts, 2016). The production of organic acids, such as lactic and acetic acids, by these two bacteria contributed to a decrease in pH and an increase in TTA. The acidity of yoghurt is mainly influenced by the metabolic products of organic acids generated during fermentation (Chen *et al.*, 2017). The TTA value represents the total acid content in both the dissociated and undissociated forms; therefore, the greater the accumulation of acid during fermentation, the lower the resulting pH value, and vice versa (Meilanie *et al.,* 2018).

Total Lactic Acid Bacteria *L. bulgaricus* and *S. thermophillus*

Lactic acid bacteria (LAB) are a group of gram-positive, non-spore-forming bacteria that appear as cocci or bacilli and are generally catalase-negative. The main characteristic of LAB is their ability to produce lactic acid as the primary metabolic product during fermentation (Nudyanto & Zubaidah, 2015). LAB are commonly found in various fermented food products and are recognized for their health benefits. Owing to their role in preventing and treating various diseases, LAB are classified as probiotic bacteria (Rambitan *et al.,* 2018). The presence of LAB is essential not only for human health but also in the food industry. LAB contribute to digestive health, enhance the immune system, increase nutritional value, and improve the taste and texture of food products. Additionally, LAB play a role in inhibiting the growth of pathogenic bacteria, thus serving as natural preservatives.

The analysis of variance (ANOVA) results showed that carrageenan and Spirulina sp. addition significantly affected the total number of lactic acid bacteria ($p < 0.05$). Based on further testing using the least significant difference (LSD) method, treatment P1 (10.6 log CFU/mL) was significantly different from P0 (8.5 log CFU/mL) and P3 (9.3 log CFU/mL). This indicates that treatment P1 significantly increased the number of lactic acid bacteria.

The number of LAB in P1 was higher than that in the other treatments, indicating that the combination of carrageenan and *Spirulina* sp. in the formulation supported the growth of lactic acid, such as *L. bulgaricus* and *S. thermophilus*. The addition of *Spirulina* contributed not only as a nutritional supplement due to its high protein, vitamin, and mineral content, but also provided growth-promoting factors such as iron and essential amino acids that enhanced bacterial metabolism (Mesbah *et al.,* 2022). Furthermore, *Spirulina* contains bioactive compounds that may have prebiotic-like effects, stimulating LAB proliferation. Carrageenan, a natural polysaccharide, helped form a stable gel matrix that protected the bacteria and maintained a favorable environment for their activity during fermentation. This structural stability likely reduces physical stress on the cells and improves oxygen diffusion control, creating conditions more suitable for anaerobic LAB growth.

The higher total LAB in P1 than in P2 and P3 may be due to the lower concentration of carrageenan and *Spirulina* sp., which creates a more conducive environment for the growth of lactic acid bacteria. In small amounts, *Spirulina* can act as a source of nutrients and antioxidants, but at high concentrations, it can inhibit microbial activity due to the content of bioactive compounds such as phenols and saponins, which are antimicrobial (Gabr *et al.,* 2020; Winahyu *et al.,* 2020). This is in line with the research of Ramayani *et al.* (2018), who reported that the decrease in the number of lactic acid bacteria in synbiotic herbal yoghurt with the addition of cinnamon is caused by the antimicrobials contained in it. Cinnamon contains cinnamaldehyde components. *Cinnamaldehyde* at 0.2% can reduce the 3-log number of lactic acid bacteria. Cinnamon extract can inhibit the development of bacteria

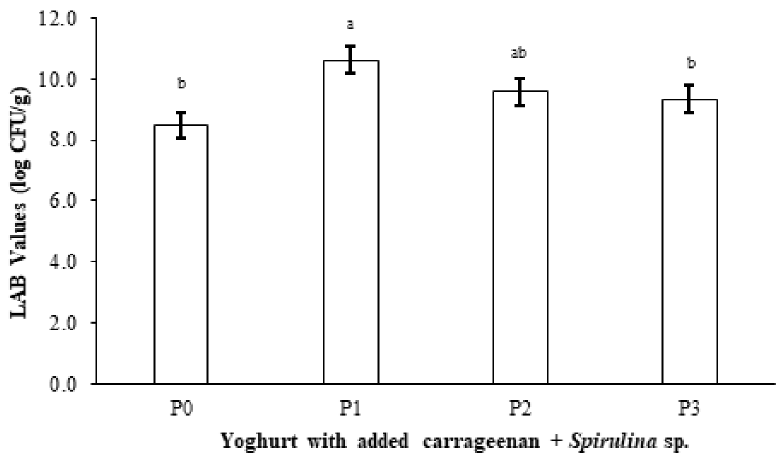


Figure 3 Total LAB of yoghurt with added carrageenan + *Spirulina* sp. Note: P0 (0.00% + 0.00%); P1 (0.01% + 0.03%); P2 (0.03% + 0.05%); and P3 (0.05% + 0.07%)

during yoghurt fermentation because of its antibacterial content that inhibits bacterial growth (Choi *et al.,* 2016).

All treatments in this study produced LAB counts that exceeded the minimum requirement set by the Indonesian National Standard (SNI 2981-2009), which is $\geq 10^7$ CFU/mL (equivalent to ≥7 log CFU/mL). The observed values ranged from 8.5-10.6 log CFU/mL, indicating that fermentation occurred optimally in all treatments and resulted in microbiologically viable yoghurt. Nugraha *et al.* (2022) also reported that the addition of 0.5% carrageenan resulted in a total LAB count of 8.94 log CFU/mL. Pangestu (2017) reported that the best result was obtained with the addition of 3% carrageenan, which showed LAB viability in the range of 5.78–6.29 log CFU/mL. Ningsih *et al.* (2024) stated that carrageenan contains polysaccharides that serve as energy-producing substrates for the growth of lactic acid bacteria. The addition of 1% carrageenan resulted in a total LAB count of 9.98 logCFU/mL.

The addition of *Spirulina* to the yoghurt-making process has been shown to enhance the growth of lactic acid bacteria (LAB), thereby accelerating the fermentation process (Barkallah *et al.,* 2017). Research has indicated that the average total LAB count in several commercial yoghurt products reached 9.17 log CFU/mL, while yoghurt fermented using a mixed starter culture and supplemented with 1% *Spirulina* showed a higher LAB count of 9.23 log CFU/mL (Agustini *et al.,* 2017). The increase in the LAB population suggests that *Spirulina* can serve as an additional nutrient source that supports the growth of probiotic microorganisms in yoghurt. Therefore, the inclusion of *Spirulina* not only accelerated the fermentation process but also improved the microbiological quality and functional value of the final yoghurt product.

Nutritional Content of Yoghurt with Added Carrageenan and *Spirulina* sp. Formula Selected

Yoghurt with 0.05% carrageenan + 0.07% Spirulina sp. (P3) was selected as the preferred yoghurt because all treatments met the quality requirements based on SNI 2981-2009 regarding yoghurt. This formula contained the highest concentrations of carrageenan and *Spirulina* sp. and was therefore considered to represent the treatment with the highest level of functional ingredient addition.

This study aimed to compare the nutritional content of selected yoghurt samples with 0.05% carrageenan + 0.07% Spirulina sp. (P3) with that of commercially available yoghurt. The nutritional parameters observed included ash content, energy from fat, total fat content, moisture content, total energy, carbohydrate content *(by difference),* and protein content. The results of the nutritional content analysis are shown in Table 2.

The nutritional composition of the selected yoghurt formula (P3) was compared with that of commercial plain yoghurt (Table 2). Commercial yoghurt was chosen as a reference because it represents a product commonly consumed by the public, although detailed information regarding the use of additives such as carrageenan was not

Table 2 Nutrient content of yoghurt with added carrageenan and *Spirulina* sp. selected formula formula (P3) and commercial yoghurt

Nutrient content	Selected formula	Commercial
Ash (%)	0.78±0.01	0.86±0.02
Energy From Fat	8.46±2.26	22.32±0.51
Total Fat (%)	0.94±0.25	2.48±0.06
Water (%)	89.59±0.13	74.47±0.11
Total Energy (Kcal/100 g)	43.22±1.69	111.10±0.82
Carbohydrate (%)	5.04±0.26	18.58±0.06
Protein (%)	3.65±0.28	3.62±0.02

provided on the product label. In contrast, formula P3 explicitly contained carrageenan as a stabilizer to improve texture and water retention, and *Spirulina* sp. as a functional ingredient with the potential to enhance nutritional value through protein, amino acids, and bioactive compounds. However, the contribution of *Spirulina* to the increase in protein and mineral content was relatively limited because of the small amount added. In addition, the addition of *Spirulina* could also affect sensory attributes such as taste, aroma, and color, which need to be considered in product development. The following section presents a comparative analysis of the proximate composition, including ash, energy, fat, water, carbohydrates, and protein, between the selected formula (P3) and commercial yoghurt.

Ash content

The average ash content in the selected yoghurt formula (P3) was 0.78%, whereas that of commercial yoghurt was 0.84%. This indicates that there was no substantial difference in the overall mineral content between the two types of yoghurt. Although *Spirulina* is known to be rich in minerals such as calcium (Ca), iron (Fe), magnesium (Mg), phosphorus (P), and zinc (Zn) (Józsa *et al.,* 2020), the amount incorporated into the yoghurt was likely too low to significantly increase the ash content. Both the P3 formula and commercial yoghurt met the standard requirements set by the Indonesian National Standard (SNI 2981-2009), which allows a maximum ash content of 1.0% (w/w) (BSN, 2009). The ash content of commercial yoghurt typically ranges from 0.28% to 0.95% (Matela *et al.,* 2019), and the values observed in this study fell within that range.

Energy

The total energy of the selected formula yoghurt (P3) was 43.22 kcal/100 g, which was considerably lower than that of the commercial yoghurt (111.10 kcal/100 g). The energy derived from fat in the P3 yoghurt was 8.46 kcal/100 g, whereas in the commercial yoghurt, it reached 22.68 kcal/100 g. These results indicate that commercial yoghurt contains a higher fat content, thereby contributing more energy from fat. The lower fat content in the P3 formulation was likely due to the use of low-fat ingredients and the inclusion of *Spirulina,* which contains limited amounts of unsaturated fatty acids, such as gamma-linolenic acid (Józsa *et al.,* 2020). Therefore, the P3 formula may serve as a potential low-calorie dietary alternative.

Total fat content

The fat content analysis showed that the selected yoghurt formula (P3) contained 0.94% fat, whereas the commercial yoghurt contained 2.48%. The P3 formula was classified as low-fat yoghurt, making it a healthier alternative for consumers concerned about fat intake. The lower fat content in P3 yoghurt was attributed to the use of low-fat milk as the main raw material. Additionally, *Spirulina*, which contains approximately 8% fat, did not significantly contribute to the total fat content because of its limited inclusion in the formulation (Jongkon *et al.,* 2008).

Water content

The water content in the selected yoghurt formula (P3) was higher at 89.59% compared to the commercial yoghurt, which contained only 74.47%. This value for commercial yoghurt was close to the typical water content of conventional yoghurt, which is approximately 85.0% (Rahman *et al.,* 1992). The elevated water content in the P3 formulation was likely due to the addition of carrageenan, a hydrophilic compound that can form gels and bind water molecules. However, water bonds within the gel matrix can undergo hydrolysis, resulting in an apparent increase in the free water content of the product (Imeson, 2011). Additionally, carrageenan, a hydrocolloid, is widely used in the food industry to improve texture and maintain the stability of liquid-based products. Water content is a critical factor influencing the quality and final characteristics of yoghurt. Ideally, the water content of yoghurt should be less than 84%, as excessive water content may negatively affect the texture and mouthfeel of the product (Matela *et al.,* 2019). Commercial yoghurt typically has a water content ranging from 76.08% to 80.07%. Therefore, the relatively high-water content in the selected formula (P3) should be considered during future reformulations to achieve a better balance between the texture and product stability.

The relatively high moisture content in the selected formula (P3) was suspected to be due to the use of low-fat milk, which had a lower total solids content than full-cream milk, thereby reducing the system's ability to retain water. This is in line with the findings of Djali *et al.* (2018), who stated that the reduction in fat content in yoghurt made from low-fat milk decreased the total solids, resulting in a poorer texture and increased whey separation in the yoghurt. In addition, the use of kappa-carrageenan as a stabilizer did not result in the formation of a dense and stable gel structure. In the study by Damian *et al.* (2012), iota-carrageenan (ι-carrageenan) and kappa-carrageenan (κ-carrageenan) were added at the same concentration, but the former resulted in a greater increase in yoghurt viscosity than the latter.

Carbohydrate

Carbohydrate content analysis was conducted using the by-difference method. The results showed a difference in carbohydrate levels between the selected yoghurt formula (P3), which contained 5.04% carbohydrate, and commercial yoghurt, which contained 18.58% carbohydrate. This difference was largely influenced by the composition of the ingredients used in each formulation. In commercial yoghurt, the carbohydrate content tends to be higher due to the addition of sweeteners or high-starch components aimed at enhancing flavor. In contrast, the selected formula (P3) did not contain added sugars, resulting in a relatively low carbohydrate content. The primary sources of carbohydrates in the selected formula were derived from the milk used (Greenfields brand) and *Spirulina* added during the formulation. *Spirulina* itself is known to contain approximately 16% carbohydrates (Jongkon *et al.,* 2008), which contributed to the overall carbohydrate content even in the absence of added sugars. According to the National Dairy Council (2013), yoghurt should ideally contain carbohydrates within the range of 13.7%-17.7%. Therefore, while the carbohydrate content of the commercial yoghurt was within the recommended range, the selected formula (P3) had a considerably lower carbohydrate level, which may appeal to consumers seeking low-carbohydrate or diabetic-friendly options.

Protein content

The protein content of the selected yoghurt formula (P3) was 3.65%, which was slightly higher than that of commercial yoghurt (3.62%). *Spirulina* is a rich source of protein, containing approximately 60-70% protein by weight (Józsa *et al.,* 2020). Nevertheless, the small amount of *Spirulina* used in the formulation may not have been sufficient to cause a meaningful increase in the protein content. In addition, an increase in the number of lactic acid bacteria in yoghurt is often followed by a slight increase in protein levels, as bacterial cells are largely composed of protein structures (Herawati & Wibawa, 2011). According to the Indonesian National Standard (SNI 2981-2009), the minimum protein content required in yoghurt is 2.7%. Based on these findings, both the selected formula and commercial yoghurt met the standard requirements for protein content.

Phytochemical Compounds in Yoghurt with Added Carrageenan and *Spirulina* sp. Selected Formula

The results of the separation of the compound components using the TLC method are presented in figure 4.

The separation of the compound components in the extract was carried out using a variety of eluent mixture ratios, namely: A) chloroform:ethyl acetate:ethanol (7:1:3), B) chloroform:ethyl acetate:ethanol (7:1:2), and C) chloroform:ethyl acetate:ethanol (8:1:1). The results showed that the eluent ratio had a significant effect on the effectiveness of separation, which was reflected in the number of spots and the Retardation factor (Rf) value on the chromatogram. The 8:1:1 ratio produced a single spot with an Rf value of 0.66, indicating a less than optimal separation. Meanwhile, the 7:1:2 ratio produced two spots

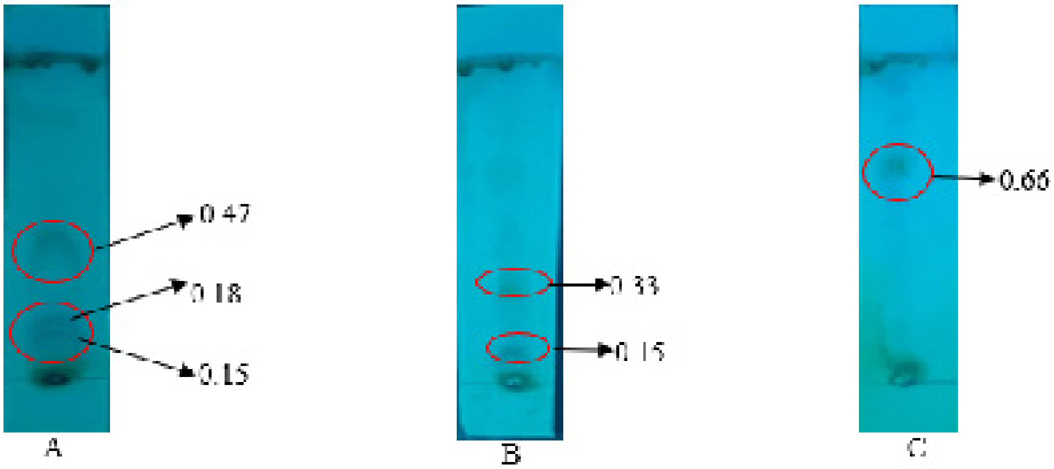


Figure 4 TLC results of yoghurt extract with the addition of carrageenan and *Spirulina* sp. visualized using anisaldehyde-sulfuric acid reagent: (A) chloroform: ethyl acetate: ethanol (7:1:3), (B) chloroform: ethyl acetate: ethanol (7:1:2), and (C) chloroform: ethyl acetate: ethanol (8:1:1)

with Rf values of 0.15 and 0.33, indicating the separation of compounds with intermediate polarities. The 7:1:3 ratio showed the best performance, producing three spots with Rf values of 0.15, 0.18, and 0.47, indicating the separation of more complex active components. To strengthen the results of compound identification using thin-layer chromatography (TLC), Table 3 presents the Rf values, observed spot colors, and comparisons with standard terpenoid compounds.

Some of the Rf values obtained were close to those of the terpenoid group comparison compounds. Arundina *et al.* (2015) reported that artemisinin has an Rf of 0.375, while Nuria *et al.* (2014) mentioned that terpineol has Rf 0.32. The closeness of this value to Rf in the results of this study indicates the possible presence of terpenoid compounds in the extract. In addition, the spots that appeared showed a greenish color after being sprayed with anisaldehyde-sulfuric acid reagent and were observed under UV light at wavelengths of 254 and 366 nm. This greenish color is a typical indicator of the presence of secondary metabolites such as terpenoids, steroids, triterpenoids, and saponins, as described by Harborne (1987) and Wagner and Bladt (1996).

The color reaction occurs because the anisaldehyde-sulfuric acid reagent is highly sensitive to the structure of terpenoid and steroid compounds, including monoterpenes, sesquiterpenes, diterpenes, and triterpenes.

Table 3 Rf values, spot colors, and comparison with standard terpenoid compounds

Eluent ratio (chloroform : ethyl acetate : ethanol)	Rf value	Spot color	Closest standard compound
7:1:3	0.15	Greenish	-
	0.18		-
	0.47		-
7:1:2	0.15		-
	0.33		Terpineol (Rf = 0,32) Artemisinin (Rf = 0,375)
8:1:1	0.66		-

Saponins are also known to produce a green to greenish-blue color with this reagent. This is consistent with the chemical nature of terpenoid compounds, which are generally nonpolar but contain hydroxyl groups (-OH); therefore, they can be extracted using semi-polar to polar solvents (Stachowiak *et al.,* 2020). Based on these results, it can be concluded that yoghurt extract with the addition of carrageenan and *Spirulina* sp. contains terpenoid bioactive compounds.

Terpenoid compounds are thought to have originated from *Spirulina* sp., which is known to contain various secondary metabolites, such as terpenoids, phenols, and active pigments. Terpenoids play a role in anti-inflammatory activity by inhibiting the production of proinflammatory cytokines, such as TNF-α (tumor necrosis factor alpha) (Wardani, 2020). Although carrageenan is not a direct source of terpenoid compounds, it contributes to the yoghurt system. Carrageenan is a sulfated polysaccharide derived from red seaweed that functions as a stabilizer and thickener (La Ega *et al.,* 2016). In addition, carrageenan could act as a protective matrix that helps maintain the stability of bioactive compounds from *Spirulina* sp., including terpenoids, during the fermentation process.

*Spirulin*a is known to contain active compounds such as phenol hydroquinone, steroids, flavonoids, and saponins (Gabr *et al.,* 2020). Steroid and saponin compounds found in *Spirulina* have anticancer activity (Akbarizare *et al.* 2019). The presence of various active compounds can improve the functional quality of food products. The active components in *Spirulina* sp. extracts have also been shown to have various pharmacological activities, such as antioxidant (Firdiyani *et al.,* 2015) and antifungal activities (Abdel-Moneim *et al.,* 2022). *Spirulina* extract shows potential as a breast anticancer agent by inhibiting the proliferation of MCF-7 breast cancer cells (Sirait *et al.,* 2019). Based on this potential, in this study, yoghurt was formulated with the addition of carrageenan and *Spirulina* sp. to increase the content of bioactive compounds and enrich the functional value of the final product.

The terpenoid compounds identified in this study are known to possess strong antioxidant activity by neutralizing free radicals, thereby reducing oxidative stress (Widyastuti *et al.,* 2021). This mechanism plays an important role in the prevention of chronic diseases, such as cardiovascular disease, cancer, and diabetes. Terpenoids also exhibit antibacterial, antimicrobial, antioxidant, and anti-inflammatory activities, further strengthening their role as bioactive compounds in functional foods (Prakash, 2017; Attamimi *et al.,* 2022). The presence of these compounds in yoghurt indicates its potential as a functional food that is beneficial for long-term health.

CONCLUSION

Yoghurt with carrageenan and *Spirulina* sp. demonstrated pH, Total Titrated Acid (TTA), and total LAB levels that complied with the Indonesian National Standard (SNI 2981:2009). Yoghurt with 0.05% carrageenan + 0.07% *Spirulina* sp. (P3) was the best treatment with lower calorie and fat contents than commercial yoghurt, indicating its potential as a low-fat and low-calorie food product. Phytochemical compounds identified as terpenoids suggest functional potential. Therefore, the selected formula yoghurt may be developed as a functional food product that supports a low-fat and low-energy diet, making it suitable for health-conscious consumers.

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