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Formulation and evaluation of a probiotic drink fortified chlorella for enhanced health benefits

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Abstract. Probiotics are microorganisms that have benefits when consumed, they can be found in yogurt. Yogurt commercially lack protein; the addition of chlorella is a solution due to chlorella contains high protein. The research aimed to determine the effect of chlorella fortification on the quality characteristics of yogurt and the best concentration of chlorella addition in the manufacturing of yogurt. The experimental method using a non-factorial Completely Randomized Design (CRD) consisting of 4 treatment levels: without the addition of chlorella (PC_0), 0.75% of chlorella (PC_1), 1% of chlorella (PC_2), and 1.25% of chlorella (PC_3). The parameters tested in this research were hedonic testing and quality, chemical composition, physical properties, and microbiological assay. The results showed that yogurt fortified with 1% chlorella was most favorable by consumer acceptance, with organoleptic characteristics: green and thick color, slightly characteristic aroma of chlorella, and slightly sour taste typical of chlorella. Meanwhile, the proximate composition of this yogurt contains 81.26% moisture, 3.77% protein, 2.30% fat, 0.89% ash, 1.47% fiber; physical properties of pH 4.65, Aw 0.75, viscosity 205.85 cP; and microbiological value of acid total 1.95%, and lactic acid bacteria 2.1×10^7 cfu/g. Therefore, the yogurt chlorella is suitable for consumption and can enhance health benefits.

1 Introduction

Probiotics are live microorganisms intended to have health benefits when consumed or introduced to the body. They can be found in yogurt and other fermented foods, dietary supplements, and beauty products. Yogurt is a fermented beverage drink, produced by lactic acid bacteria such as lactobacillus. Probiotics are living microorganisms beneficial to their hosts when given in sufficient quantities [1]. The health benefits of functional drinks can be further enhanced by adding certain lactic acid bacteria (LAB) to the drink. LAB probiotic cultures are the most commonly used in dairy products and beverages [2-3].

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Yogurt has several health advantages, such as the prevention of osteoporosis, diabetes, and cardiovascular diseases, as well as the promotion of gut health and immune system modulation [4]. Apart from its advantages, yogurt has disadvantages because the content of iron, vitamins, protein tends to be low and saturated fat is high, its nutritional content is not as good as fresh milk and is slightly reduced due to heating at high temperatures. Therefore, it is necessary to fortify microalgae chlorella to increase its nutritional value.

Microalgae are micro-sized algae that can grow and develop rapidly [5]. Chlorella is a type of microalgae that has a green color because it contains chlorophyll pigment and provides health benefits. Chlorella is called single cell protein because of its high protein content of 50-60% DW, and contains all the amino acids needed for body growth and health [6], moreover, it contains other nutrients needed by the body such as 11% fat, 20.1% carbohydrates, vitamins, 4.6% minerals, 0.2% fiber, chlorophyll, β-carotene and antioxidants [7-8], so that chlorella can be applied to food products as health food [9].

Health benefits of chlorella such as reducing cholesterol, antitumor, antidiabetic, anticancer, antimutagenic-anticarcinogenic, and suppressing the increase in blood pressure in experimental mice and humans [10]. Chlorella fortification in food products has been widely carried out, including in cookies [9] and cosmetic facial mask products [11]. However, in preparing drinks there is still limited, but there has been previous research on making yogurt from microalgae *Spriulina platensis* [12]. Therefore, chlorella fortification can be carried out in making yogurt as a probiotic drink.

Based on the description above, an important problem can be seen, namely the large number of people, especially in developing countries who experience nutritional deficiencies. Lack of nutrition can cause various health problems, ranging from stunting in children to degenerative diseases in adults. Nutrition is one of the essential components of human life. A balanced nutritional intake is very important for maintaining body health, increasing endurance and supporting optimal growth. Efforts that can be made to overcome the problem of malnutrition are through food fortification. In increasing the nutritional value of the community towards the consumption value of yogurt, one of which is making yogurt by utilizing natural ingredients, namely chlorella which contains various nutrients, namely protein, vitamins, minerals and antioxidants in high amounts [8]. Seeing the potential of chlorella in the development of yogurt is very relevant. Chlorella fortification in making yogurt has the potential and role in regulating the digestive tract, anti-diarrhea, increasing growth, helping people with lactose intolerance and regulating cholesterol levels in the blood [10].

With a distinctive texture and taste and having a nutritional content that has been fortified with chlorella, it can contribute to the processing and development of products as a functional drink.

2 Materials and methods of research

2.1 Materials

The ingredients used in this study were chlorella which was cultivated in the Laboratory of Microbiology and Biotechnology of Fishery Product, Faculty of Fisheries and Marine Science Universitas Riau, skim milk, UHT milk, sugar and starter culture of *Lactobacillus acidophilus* bacteria. The chemicals for analysis: NaOH, H_2SO_4, H_3BO_3, HCl, K_2SO_4, Hexane, alcohol, KI, HgO, NaS_2O_3, distilled water, pp indicator, MRS broth media,

The equipment used were centrifuge, hot plate, jar, tray, analytical balance, digital scale, dropper pipette, Soxhlet, Kjeldhal flask, Erlenmeyer flask, filter paper, porcelain cup, measuring flask, electric furnace, oven, desiccator, distillation, measuring cup, spectrophotometer, beaker glass, micropipette, pH meter, filter paper, thermometer, incubator, test tube rack, petri dish, autoclave, test tube, 100 mesh sieve, mortar, label paper, aluminum foil, tissue, tissue, gloves, and masks.

2.2 Research method

The research method used was an experimental method using a non-factorial Completely Randomized Design (CRD) consisting of 4 treatment levels: without the addition of chlorella powder (PC_0), 0.75 % of chlorella powder (PC_1), 1.0 % of chlorella powder (PC_2), and 1.25 % of chlorella powder (PC_3).

The parameters tested in this research were: hedonic testing and quality (appearance, taste, aroma) with 25 panelists [13]; chemical composition [14] (moisture, fat, protein, ash, fiber content); physical properties [15] pH, Aw [14], a viscosity [16]; microbiological assay Acid lactic total [13] and Lactic acid bacteria [17].

2.3 Preparation of chlorella powder

Chlorella which has been cultivated for 12 days is harvested using a centrifuge at 4500 rpm for 3 minutes, the paste that has been obtained is dried naturally using sunlight for 5 hours at a temperature of ± 30ºC. The dried chlorella powder was pulverized using a mortar and then sieved using a 100 mesh sieve until the desired chlorella powder was obtained.

2.4 Preparation of rejuvenated *Lactobacillus acidhopilus* bacteria

Probiotic bacteria were taken from the rejuvenated stock culture by taking 1 one loopful, then inoculated into fresh MRSA media, then incubated for 24 hours at 37 ºC, transferred to oblique agar media and incubated for 24 hours, then the incubated bacteria were taken 1 one loopful after which they are inoculated with MRS broth solubility and incubated for 24 hours [18].

2.5 Preparation of yogurt starter

Skim milk media 10% (b/v) was made by weighing 10 grams of skim milk powder, then dissolved in 100 mL of distilled water and 15 grams of glucose then heated and stirred until dissolved until it reached 85°C for 10 minutes. Then, skim milk was left at room temperature until it reached 32ºC and then added active culture 1 mL MRS broth, was stirred well and incubated for 24 hours in an incubator at 37ºC [17].

2.6 Formulation of yogurt fortified chlorella

The yogurt formulation for each treatment was modified and following the formula of the previous study [18] is shown in Table 1, for the amount of chlorella powder used was obtained from the percentage of milk used.

Table 1. The formulation of yogurt fortified chlorella

Materials	Composition			
	PC_0	**PC_1**	**PC_2**	**PC_3**
Chlorella (g)	0	1.5	2.0	2.5
UHT Milk (mL)	200	200	200	200
Sugar (g)	30	30	30	30
Starter culture of *Lactobacillus acidophilus* bacteria (mL)	10	10	10	10

2.7 Production of yogurt [19]

200 mL of UHT milk for each treatment is poured into Erlenmeyer each for pasteurization, then heated for 10 minutes at a temperature 85°C, and added 30 grams of sugar, cooling to a temperature of 45°C, then put the milk into the jar. After that, 10 ml of starter culture is inoculated. Incubate in a closed room at room temperature for 24 hours (make sure the storage room is clean and protected from light), and add chlorella powder in the last 2 hours of the fermentation process.

2.8 Data analysis

The experiment was carried out in 3 repetitions and the results were expressed as the mean ± standard deviation. The experimental results were analyzed using ANOVA, and differences between treatments ($p<0.05$) were carried out using Duncan test software SPSS version 26.

3 Results

3.1 Hedonic value and quality

The hedonic value of yogurt fortified chlorella is shown in Table 2. The PC_2 with 1% chlorella is the best treatment for all parameters (appearance, taste, aroma), with the criteria green and thick color, slightly characteristic aroma of chlorella, and slightly sour taste typical of chlorella. The green color is caused by chlorophyll pigments contained in chlorella [8]. In fermentation process of yogurt is carried out by utilizing lactic acid bacteria (*Lactobacillus acidhopilus*) to produce a sour taste that can break down carbohydrates that produce lactic acid [20].

Table 2. The hedonic value of yogurt fortified chlorella

Parameters	Treatments			
	PC_0	**PC_1**	**PC_2**	**PC_3**
Appearance	$6.65±0.33^b$	$6.76±0.21^{bc}$	$7.29±0.17^c$	$5.53±0.30^a$
Taste	$6.65±0.33^b$	$6.68±0.21^b$	$7.37±0.23^c$	$5.21±0.16^a$
Aroma	$6.65±0.33^b$	$6.65±0.12^b$	$7.24±0.08^c$	$5.64±0.29^a$

The addition of chlorella in a high concentration (1.25%) makes the product less acceptable to consumers, due to a bit bitter taste. Based on ANOVA was shown that for appearance PC_2 was not significantly different from PC_1, while for taste and aroma, PC_2 was significantly different among treatments.

Table 3. The hedonic quality of yogurt fortified chlorella

Parameters	Treatments			
	PC_0	PC_1	PC_2	PC_3
Appearance	$6.52{\pm}0.24^{b}$	$6.81{\pm}0.05^{bc}$	$7.21{\pm}0.09^{c}$	$5.32{\pm}0.21^{a}$
Taste	$6.68{\pm}0.24^{b}$	$6.89{\pm}0.12^{bc}$	$7.24{\pm}0.08^{c}$	$6.01{\pm}0.09^{a}$
Aroma	$6.55{\pm}0.16^{b}$	$6.73{\pm}0.12^{bc}$	$7.21{\pm}0.12^{c}$	$5.69{\pm}0.16^{a}$

Table 3 shows that PC_2 obtained the highest value among treatments. It means that PC_2 had better hedonic quality than other treatments. Based on ANOVA showed that PC_2 was not significantly different from PC_1 treatments for all parameters.

The more chlorella powder fortification added to yogurt will give a bitter taste because chlorella contains substances that can cause a bitter taste, namely phycocyanin substances [21].

3.2 Proximate value

The proximate values of yogurt fortified chlorella are presented in Table 4. The moisture content of yogurt fortified chlorella 81.16-83.83% in accordance with SNI where the max yogurt water content is 84% [13]. According to [22] this can happen because there is a polar chain bond in the protein, namely hydroxyl, carboxyl, sulfhydryl and carbonyl which will bind to hydrogen in water.

Table 4. The proximate values of yogurt fortified chlorella (%)

Parameters	Treatments			
	PC_0	PC_1	PC_2	PC_3
Moisture	$83.83{\pm}0.04^{c}$	$81.63{\pm}0.21^{b}$	$81.26{\pm}0.01^{a}$	$81.16{\pm}0.02^{a}$
Fat	$1.96{\pm}0.03^{a}$	$2.11{\pm}0.01^{ab}$	$2.30{\pm}0.02^{b}$	$2.67{\pm}0.17^{a}$
Protein	$2.77{\pm}0.19^{a}$	$2.92{\pm}0.09^{a}$	$3.77{\pm}0.04^{b}$	$4.59{\pm}0.24^{c}$
Ash	$0.66{\pm}0.01^{a}$	$0.80{\pm}0.02^{b}$	$0.89{\pm}0.03^{bc}$	$0.98{\pm}0.05^{c}$
Fiber	$0.98{\pm}0.01^{a}$	$1.16{\pm}0.04^{b}$	$1.47{\pm}0.05^{c}$	$1.97{\pm}0.06^{d}$

The best treatment in PC_2 shows that the fat content of yogurt fortified chlorella is 2.30%, it meets the Indonesian National Standard (SNI) below 3% [13]; protein content 3.77%. It meets SNI min 2.7%. This protein value is higher than the protein content of probiotic yogurt with added red fruit ranging from 2.60%-2.84% [23]. Testing protein and fat content is one of the parameters of product durability, this test is important to assess the nutrition and quality of food ingredients [24], ash content 0.89% meets SNI namely 1%. Fermentation in yogurt can increase mineral content, especially calcium, magnesium, and zinc [25]. This can occur because the phytase enzyme produced by *Lactobacillus* sp. can break mineral bonds in phytic acid that has interacted with minerals and protein residues [26].

Based on ANOVA shows that PC_2 treatment was significantly different among treatments for parameters of moisture, protein, and fiber content. Meanwhile, the fat content of PC_2 was not significantly different from PC_3, and for ash content, PC_2 was significantly different from PC_1 and PC_3. The fiber value of chlorella fortified yogurt is higher than the fiber content contained in commercial yogurt, which is 0.75% [27]. The increase in fiber content is also influenced by chlorella based on its dry weight chlorella contains 60% protein, 12% lipid, 11% carbohydrate, 4% crude fiber, 7% ash and 4% water, 1% carotenoid and 4% chlorophyll [28].

3.3 Physical properties

The physical properties of yogurt fortified chlorella are presented in Table 5. The pH values are in accordance with the quality requirements of yogurt based on SNI, which is 4.2-4.6. Based on the results of the study, the pH of the PC_2 product is 4.66 which indicates the condition of the yogurt is not too acid or neutral. The pH value can also indicate an indication of the presence or absence of microorganisms or enzyme activity in food by hydrolyzing or destroying macromolecules that form into smaller fractions that can affect the aroma and taste of food [29].

Table 5. The physical properties of yogurt fortified chlorella

Parameters	Treatments			
	PC_0	PC_1	PC_2	PC_3
pH	4.57 ± 0.02^{a}	4.64 ± 0.03^{a}	4.66 ± 0.03^{ab}	4.68 ± 0.01^{b}
Aw	0.79 ± 0.05^{a}	0.78 ± 0.01^{a}	0.81 ± 0.01^{ab}	0.83 ± 0.05^{b}
Viscosity (cP)	165.55 ± 9.65^{a}	170.55 ± 9.65^{a}	205.85 ± 4.75^{b}	397.45 ± 2.65^{c}

According to the quality standard of yogurt in SNI 01-2981-2009 [13], the maximum Aw in yogurt is 0.83, so it can be said that yogurt has met the SNI standard. Aw yogurt with chlorella powder fortification with different treatment concentrations according to the Aw range requirements with which it can be seen how long the product resistance to storage. Water is the most abundant food component and the most significant food safety parameter [30]. The addition of microalgae ingredients always reduces the growth energy of pathogenic bacteria compared to the control sample, because the addition of microalgae ingredients in the form of powdered material can absorb more water.

According to the quality standards of yogurt in SNI 01-2981-2009, the maximum viscosity in yogurt is 261.50 cP, so it can be said that yogurt has met the SNI standards. This viscosity value is higher than yogurt Spirulina which is 164.71% [31]. The increase in viscosity value occurs due to the increasing amount of chlorella powder fortification in each yogurt formula which increases the total solids in yogurt. In addition, several other factors that affect the viscosity of yogurt, namely protein and fat content.

3.4 Microbiological

The microbiological values yogurt fortified chlorella is presented in Table 6. The total acidity of yogurt is 1.42-2.00% in accordance with SNI where the maximum total acidity of yogurt is 0.5-2% [12], so it can be said that the yogurt has met the SNI standards.

Table 6. The microbiological values yogurt fortified chlorella

Parameters	Treatments			
	PC_0	PC_1	PC_2	PC_3
Acid total (%)	1.42 ± 0.08^{a}	1.89 ± 0.04^{ab}	1.95 ± 0.09^{b}	2.00 ± 0.04^{b}
Lactic Acid Bacteria (cfu/g)	$1.3x10^{7}\pm0.10^{a}$	$1.7x10^{7}\pm0.32^{ab}$	$2.1x10^{7}\pm0.15^{b}$	$2.9x10^{7}\pm0.32^{c}$

The results showed that the addition of spirulina at different concentrations into yogurt increased the titratable acidity from 81°D for the control to 90°D and 89°D for yogurt fortified with 1% dried and fresh spirulina respectively [11]. The titratable acidity of milk increased due to the buffering effect of added spirulina constituents such as protein, phosphate, citrate and lactate [32].

Based on the analysis of LAB growth in each yogurt formula, it still meets the SNI yogurt standard of at least 10^7 cfu/gram. The results showed that the addition of *Chlorella vulgaris* to yogurt significantly increased the growth of LAB starter culture compared to the control during fermentation, which was caused by a series of amino acids, proteins and vitamins in algal biomass.

4 Conclusions

Yogurt fortified with 1% chlorella powder was the best treatment with organoleptic characteristics: attractive yogurt, green and thick color, slightly characteristic aroma of chlorella and slightly sour taste typical of chlorella. Meanwhile, the proximate composition of yogurt fortified 1% chlorella powder contains moisture 81.26%, fat 2.30%, protein 3.77%, ash 0.89%, fiber 1.47%; physical properties of pH 4.66, Aw 0.81, viscosity 205.85; and microbiological value of acid total 1.95%, and lactic acid bacteria 2.1×10^7 cfu/g. Therefore, the yogurt produced is suitable for consumption, meets Indonesian national standards and can be used as a functional drink.

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