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Functional Food Innovation of Yogurt From Breadnut Seeds (*Artocarpus camasi*): Basic Formulation and Quality Analysis

Putu Rima Sintyadewi*, Wahyu Krisna Yoga

Bachelor of Food Technology Program, Faculty of Technology, Institute of Technology and Health Bali, Denpasar, Bali, Indonesia
*E-mail: rima.itekesbali@gmail.com

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Abstract: Yogurt is one of the dairy products fermented by lactic acid bacteria (LAB) that has long been recognised as a functional food. Breadnut seeds (*Artocarpus camansi*) have the potential as prebiotics to stimulate LAB growth during yogurt fermentation so that the functional effects of this plant-based yogurt can be optimised. In addition, breadnut seeds are also reported to contain essential amino acids and have high antioxidant activity. The purpose of this study was to determine the best formulation of breadnut seed dairy and LAB type (*L. bulgaricus* and *S. termophilus*) adjusted to the quality standards of SNI (2981-2009) yogurt, including pH test, total titratable acid, proximate test (water, fat, protein, and ash content), and microbiological test (total LAB). The design used in this study was a completely randomized design (CRD) with two factorials. The first factor was the formulation of breadnut seed dairy with UHT milk with four treatments, namely P1 (70%:30%), P2 (50%:50%), P3 (30%:70%), and P4 (100% breadnut seed dairy). The second factor is the type of LAB stater with three treatments, namely S1 (*L. bulgaricus*), S2 (*S. thermophilus*), and S3 (*combination of both*). The recommended formulation for breadnut seed yogurt is 100% breadnut seed dairy with a combination of *L. bulgaricus and S. thermophilus* staters (P4S3). The P4S3 formulation has met the quality standards of yogurt by SNI 2981:2009, including ash content, fat content, protein content, total acid and pH, and total LAB. The P4S3 formulation has an advantage over other formulations by having a lower fat content of 2.37% and a higher protein content of 3.71% compared to animal milk. Breadnut seed yogurt with P4S3 formulation contains a total LAB 3.02 x 10^7, which has potential as an alternative functional drink.

Keywords: Breadnut Seeds; Functional Food; Yogurt.

Introduction

Much of the world's population, especially those in developed and developing countries, has increased awareness and interest in functional foods. Functional foods positively affect biological functions and human health and are expected to reduce the risk of several diseases [1]. Functional foods are becoming a global trend as awareness of the health benefits and prevention of disease risk through food consumption increases [2]. This interest, which spans several decades, has significantly influenced the development of the food industry and has also contributed to the transformation of research in the food industry[3].

Yogurt is one of the widespread lactic acid bacteria (LAB) fermented dairy products and has long been recognized as a functional food [4]. Yogurt consumption is claimed to be able to provide healthy and therapeutic effects for the body, such as reducing the risk of cardiovascular disease [5] and diabetes [6], modulating the immune system [7] and increasing colonization of gut microbiota to reduce the risk of colon cancer [8]. These therapeutic effects are inseparable from the role of LAB during the yogurt fermentation process. LAB produces lactic acid and antimicrobial compounds that are reported to inhibit the growth of *Escherichia coli*, *Salmonella thypi*, and *Staphlococcus aureus* in vitro, thereby reducing the risk of intestinal infections [9]. Lactic acid production by LAB during yogurt fermentation is reported to positively affect calcium absorption in the body. Calcium plays a vital role in blood glucose control and regulates blood lipid profile by increasing fecal fat; thus, yogurt consumption is claimed to reduce blood cholesterol levels [10]. In addition, LAB is also reported to be able to increase the antioxidant capacity of the final product by producing γ-Aminobutyric acid (GABA) [5].

The trend of plant-based non-dairy yogurt products continues to grow, especially as an alternative for consumers with specific dietary preferences, such as vegans and individuals with lactose intolerance [3]. Plant-based yogurts are reported to have high fiber content with low sugar and sodium levels and have antioxidant activity that can fight free radicals, reduce inflammation, and support cardiovascular health. Plant-based yogurts are also reported to contain magnesium, selenium, and zinc, which help the body fight infections and strengthen the immune system [2].

Observing this trend, some researchers began to innovate to utilise plant-based dairy raw materials from grains and nuts to improve the nutritional value of yogurt. Some examples are the utilization of jackfruit seeds [11], corn and mung bean seeds [12], soybeans [13], etc. Seeds are known to be one of the sources of prebiotics. Prebiotics are compounds in food that cannot be digested in the body, which can selectively stimulate the growth or activity of

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beneficial microorganisms for the body, one of which is LAB [4]. Yogurt beverage development can be done by adding prebiotics to increase its functional value. Breadnut (*Artocarpus camansi*) seeds, known in Bali as "timbul," are food ingredients the community has not widely utilized.

Breadnut seeds are reported to contain 75% carbohydrates, 6.16% protein, and 9.07% vegetable fat [14]. These results indicate that breadnut seeds can act as a source of prebiotics. Carbohydrates (polysaccharides and oligosaccharides) in breadnut seeds, which are pretty high, can stimulate LAB growth during yogurt fermentation. Apart from being a source of prebiotics, breadnut seeds are also reported to contain essential amino acids such as leucine, phenylalanine, lysine, and isoleucine, which can regulate blood sugar levels and boost the immune system [15]. Breadnut seeds are also reported to have a high DPPH free radical scavenging activity of 97.33% [14]. Based on the results of these studies, there is a synergistic potential between breadnut seeds and LAB to produce functional food products, in this case, plant-based yogurt.

Developing plant-based yogurt products using breadnut seeds can contribute to the government's efforts to diversify food sources, particularly local food. As a local plant, the raw material of breadnut seeds will be more affordable compared to other plant-based yogurt raw materials, especially in tropical countries, thus supporting the production of more sustainable food products.

By observing the potential, research on processing breadnut seeds into yogurt raw materials is a critical need. Most plant-based yogurt research so far has only focused on more popular ingredients such as soy [13], almond, or oat [2]. The use of breadnut seeds as yogurt raw material has not been widely explored, such as formulating plant-based yogurt to meet the quality standards of yogurt as required by SNI (2981-2009). Food products, in this case yogurt, must meet the Indonesian National Standard (SNI) [16] to ensure product quality, safety, and competitiveness in national and international markets.

Based on the context, the author is interested in examining the best composition of breadnut seed dairy formulation and variations in LAB stater types. Several studies reported that the quality of yogurt is not only influenced by the raw materials but also to the kind of LAB used. Soy dairy-based yogurt fermentation showed the best quality with a combination of LAB staters (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) [17], while in cow's milk yogurt, *Streptococcus thermophilus* staters showed the best yogurt quality [18]. Based on this, it is necessary to do a formulation to determine the correct type of LAB in making breadnut seed yogurt, which is adjusted to SNI standards (2981-2009), including a pH test, a total titratable acid, a proximate test (water, fat, protein, and ash content), and a microbiological test (total lactic acid bacteria).

This research aims to determine the basic formulation of breadnut seed yogurt by considering the composition of raw materials and the type of LAB stater. The results of this research are expected to provide added value to breadnut seeds, which are often considered secondary food ingredients, through transformation into yogurt products with higher economic value and can be used as an alternative functional food product based on local ingredients.

Research Methods

This experimental research was conducted in the Integrated Laboratory of Food Technology Study Program, Faculty of Technology, ITEKES Bali. The design used was a completely randomized design (CRD) with two factorials. The first factor is the formulation of breadnut seeds with UHT milk with four treatments, namely: (1) P1 70% UHT milk: 30% breadnut seed dairy; (2) P2 50% UHT milk: 50% breadnut seed dairy; (3) P3 30% UHT dairy; 70% breadnut seed dairy; (4) P4 100% breadnut seed dairy; and P0 as control (UHT milk without addition of breadnut seed dairy).

The second factor is the type of Lactic Acid Bacteria (LAB) stater with three treatments, namely S1 (*Lactobacillus bulgaricus*), S2 (*Streptococcus thermophilus),* and S3 (combination of both). Each treatment combination was repeated three (three) times. The data obtained were analyzed by the statistical method of *analysis of variance* (ANOVA) and continued with Duncan's multiple tests if there were significant differences ($P \leq 0.05$) between treatments.

The research stages include the rejuvenation of stater bacteria and the making and formulation of yogurt from breadnut seeds. The analysis included chemical analysis and total LAB analysis. Furthermore, the data were compared with SNI standards (2981-2009) related to yogurt quality requirements to obtain the treatment with the best formulation.

Bacteria Stater Refreshment

Refreshment of bacterial cultures (*L. bulgaricus and S. thermophilus*) from the Microbiology Laboratory of ITEKES Bali was carried out by inoculating bacterial cultures using a ratio of 1:9 into UHT milk that had been sterilized by autoclave at 115ºC for 3 minutes. Furthermore, milk inoculated by LAB culture was incubated at 37ºC for 18 hours until it formed coagulation. This refreshment is done to cultivate and adapt the culture to the media before being used as a starter in the actual fermentation [19].

Preparation and Formulation of Breadnut Seed Yogurt *(Artocarpus camansi)*

Breadnut seeds were separated from the pulp and washed. The seeds were soaked for 12 hours. It is boiled in 500 mL of water, then removed and drained. After cooling, the seeds are separated from the skin and cut into small pieces. Breadnut seed pieces They were blended for 3 minutes until smooth. The breadnut seed dairy is filtered and then boiled at 85ºC while stirring for 2 minutes. The next stage is the formulation of breadnut seed dairy with UHT milk according to the treatment. The dairy formulation was cooled to a temperature (40-44ºC), then 5% (v/v) yogurt was added according to the treatment. Yogurt was then fermented in an incubator at 37ºC for 18 hours, and the samples obtained were analyzed [11]

pH Test

The pH value was measured using a pH meter. The pH meter is calibrated at pH 4 and 7. The electrode is inserted in the sample and left until the numbers listed on the pH meter do not show a shift in numbers [20].

Total Acid Titration Test

A total of 1 mL of sample was put into an Erlemeyer, diluted with 10 mL of distilled water, then titrated with a 0,1 N NaOH solution. A phenolphthalin indicator was used to determine the endpoint of the titration, and the amount of NaOH used was recorded [20].

Water Content Test

An empty cup was dried in an oven for 15 minutes, then cooled in a desiccator and weighed. Then, the cup was added with 5 gr of sample and dried in the oven at 105ºC for 6 hours. The cup with its contents was then cooled in a desiccator and weighed. Drying is carried out until a constant weight is obtained. Moisture content is calculated as the difference between the sample's initial weight before and after drying [20].

Kjeldahl Method Protein Content Test

The sample was weighed at 0,2 gr in a 30 mL Kjeldahl flask and added 1,9 + 0,1 gr of concentrated K2SO4 and 2,0 + 0,1 mL of concentrated H_2SO_4. The sample is deconstructed for 1–1,5 hours until the liquid becomes clear. The liquid was cooled, and 8–10 mL of NaOH was added to the distillation device. At the bottom of the condenser, place an Erlenmeyer containing 5 mL of H_3BO_3 solution and a few drops of methyl red indicator. The end of the condenser hose must be submerged in the solution to accommodate the distillate of about 15 mL. The distillate was titrated with 0,02 N HCl until a gray color occurred. The same procedure was also carried out on the blank (without sample). The amount of sample titration (a) and blank titration (b) is expressed in mL of 0,02 N HCl [20].

$$\text{Protein Content (\%)} = \text{N Content (\%)} \times 6{,}25$$

Fat Content Test

The fat flask was dried in an oven, cooled in a desiccator and weighed. A 5-gr sample was wrapped in filter paper and put into the Soxhlet. Hexene solvent was put into the fat flask to taste. Then reflux for at least 5 hours until the solvent that drops back into the fat flask is apparent. The solvent in the fat flask is distilled and collected again. The extracted fat flask is heated in an oven at 105ºC until it reaches a constant weight, then cooled in a desiccator. Next, the flask and fat are weighed [20].

Ash Content Test

The porcelain cup was heated in the oven for 15 minutes, then cooled in a desiccator and weighed. A total of 5 gr of the sample was put in a porcelain cup, weighed, then burned until it was no more smoke and fumigated in a furnace (temperature 550ºC) until white (all samples became ash). After that, it is cooled in a desiccator until it gets a constant weight [20].

Total LAB Test

Samples were diluted to 10^{-10} in physiological solution, and then 1 mL of sample was planted on MRSA media using the spread plate method and incubated anaerobically at 37ºC for 24 hours. Growing colonies were counted using a colony counter. The total colonies counted must meet the International Commission Microbiology Food (ICMF) standard, which is between 30-300 colonies [19]

Results and Discussion

Chemical Quality of Breadnut Seed (*Artocarpus camansi*) yogurt

The results of the chemical quality analysis of breadnut seed yogurt are in Table 1. The statistical analysis results with ANOVA showed that all treatments had a significant effect at $P < 0,05$ on ash content, water content, fat content, protein content, and total acid content of breadnut seed yogurt (*Artocarpus camansi*).

Table 1 shows that the ash content was higher in the 100% breadnut seed dairy formulation compared to the 0%, 25%, and 50% breadnut seed dairy formulations. This is because adding breadnut seed dairy can increase the content of calcium, magnesium, phosphorus, potassium, and other minerals in yogurt, which positively correlates with the increase in ash content in the sample. However, based on the Duncan test, it is known that the use of a combination of staters (*L. bulgaricus* and *S. thermophilus*) is similar to the ash content, which can be seen from the notation in each treatment (Table 1). This result is in accordance with research reported by Rahayu et al. [21] that the highest ash content in jackfruit seed yogurt increased with increasing concentration of jackfruit seed flour addition in the tested sample but was not significantly different in the formulation with the combination of staters. Based on the results obtained, the ash content of breadnut seed yogurt in all treatments has met the SNI 2981:2009 standard for yogurt quality requirements, which is a maximum of 0,1% (b/b).

The water content analysis results showed a decrease in yogurt added with breadnut seed dairy, which had the lowest water content in the P4S3 treatment, 77,25% (Table 1). A higher concentration of plant-based dairy leads to an increase in the proportion of solid materials (such as protein, fat, and carbohydrates), thus reducing the relative water content in yogurt. This will result in yogurt with a thicker texture and lower water content per volume [22].

Table 1 shows the range of fat content in breadnut seed yogurt is 2,37–2,81. These results show that the fat content in all treatment formulations has met the SNI 2981:2009 standard for yogurt quality requirements, which is a maximum of 2,9% (b/b). According to Ciron et al. [23], fat gives yogurt a thick and creamy consistency. The concentration of fat content will affect the texture and viscosity of yogurt. The results of this study are in line with the data in Table 1, which shows that yogurt with high-fat content has a low water content, so the yogurt has a thicker texture.

Table 1. The proximate and lactic acid content of breadnut seed (*Artocarpus camansi*) yogurt

Formulations	Water Content (%)	Ash Content SNI (BSN, 2009) maks 1.0% (b/b)	Fat Content SNI (BSN, 2009) maks 3.5% (b/b)	Protein Content SNI (BSN, 2009) min 2.7% (b/b)	Total Acid SNI (BSN, 2009) 0.2 sd 0.9 % (b/b)
P0S1	89.33 ± 0.26^{m}	0.54 ± 0.11^{b}	3.03 ± 0.37^{h}	3.37 ± 0.02^{a}	0.54 ± 0.02^{a}
P0S2	89.23 ±0.35^{l}	0.55 ± 0.10^{b}	3.02 ± 0.20^{h}	3.40 ± 0.01^{b}	0.55 ± 0.10^{ab}
P0S3	89.30 ± 0.25^{m}	0.47 ± 0.20^{a}	3.17 ± 0.20^{i}	3.43 ± 0.02^{b}	0.57 ± 0.10^{ab}
P1S1	86.18 ± 0.25^{j}	0.57 ± 0.15^{b}	2.73 ± 0.01^{f}	3.46± 0.01^{c}	0.57 ± 0.01bc
P1S2	86.31 ± 0.17^{k}	0.60 ± 0.15^{c}	2.81 ± 0.17^{g}	3.49 ± 0.02^{cd}	0.54 ± 0.10^{ab}
P1S3	86.21 ± 0.15^{j}	0.63 ± 0.23^{c}	2.71 ± 0.40^{f}	3.51 ± 0.15^{de}	0.66 ± 0.01^{e}
P2S1	82.52 ± 0.15^{h}	0.71 ± 0.10^{d}	2.67 ± 0.02^{e}	3.61 ±0.02^{g}	0.60 ± 0.15^{cd}
P2S2	83.08 ± 0.10^{i}	0.74 ± 0.10^{e}	2.64 ± 0.15^{de}	3.57 ± 0.05^{f}	0.57 ± 0.02^{bc}
P2S3	81.64 ± 0.36^{g}	0.72 ± 0.20^{de}	2.62 ± 0.30^{d}	3.63 ± 0.17^{gh}	0.72 ±0.02^{fg}
P3S1	78.44 ± 0.19^{e}	0.81 ± 0.15^{f}	2.54 ±0.25^{c}	3.68 ±0.25^{j}	0.67 ±0.05^{e}
P3S2	79.23 ± 0.25^{f}	0.80 ± 0.20^{f}	2.51 ± 0.20^{c}	3.57 ± 0.02^{f}	0.61 ±0.02^{d}
P3S3	78.26 ± 0.20^{d}	0.82 ± 0.05^{f}	2.52 ± 0.10^{c}	3.60 ±0.05^{g}	0.74 ±0.05^{gh}
P4S1	76.66 ± 0.11^{a}	0.88 ± 0.05^{h}	2.46 ± 0.20^{b}	3.54 ± 0.23^{e}	0.71 ±0.20^{f}
P4S2	76.81 ± 0.17^{b}	0.87 ±0.06^{gh}	2.41 ± 0.23^{a}	3.64 ±0.17^{i}	0.68 ± 0.70^{e}
P4S3	77.25 ± 0.25^{c}	0.86 ± 0.13^{g}	2.37 ± 0.02^{a}	3.71 ±0.10^{j}	0.77 ± 0.15^{h}

Description :
Data are presented as mean ± standard deviation (SD) of 3 replicates
[ab]Data followed by the same letter in the same column is not significantly different in the Duncan test at the 0.05 level.

The protein content in yogurt has essential functions for the health of the body, such as supporting tissue growth and repair, increasing muscle strength, and supporting the immune system and digestive tract health. The data in Table 1 shows that the addition of breadnut seed dairy can increase the protein content in yogurt. The highest protein content was found in the formulation of adding 100% breadnut seed dairy with a combination of *L. bulgaricus* and *S. thermophilus* staters (P4S3), which amounted to 3.71% (b/b). These results have met the SNI 2981: 2009 standard for yogurt quality requirements, which is a minimum of 2.7% (w/b). This result is supported by [19], who reported that the protein content of sweet corn yogurt is higher than the protein content of dairy-based yogurt. According to [20], staters *L. bulgaricus* and *S. thermophilus* are able to degrade plant-based dairy proteins into peptides and amino acids during fermentation. A high protein concentration in yogurt will result in better nutritional value, as more protein will be converted into a more easily absorbed form.

Total acid in yogurt is the total amount of organic acid produced during fermentation. Total acid in yogurt shows the acidity produced by lactic acid bacteria that ferment sugar into lactic acid. Table 1 shows the total acid in breadnut seed yogurt ranged from 0.57-0.77% (b/b). These results show that all treatment formulations have met the SNI 2981:2009 standard for yogurt quality requirements, which is 0.2-0.9% (b/b). The increase in total acid occurred along with the addition of breadnut seed dairy concentration in yogurt. Research on jackfruit seed flour yogurt also reported similar results, namely, the total acid increased to 1.99% in the 5% jackfruit seed flour formulation compared to 4% jackfruit seed flour, which only reached 1.85% [21]. This is because, at higher concentrations of breadnut seed dairy, the amount of substrate available is more significant so that LAB can produce more lactic acid during fermentation.

Total Lactic Acid Bacteria (LAB) and pH of Breadnut Seed (*Artocarpus camansi*) Yogurt

Based on statistical tests using ANOVA, it was found that the addition of breadnut seed dairy and the combination of *L. bulgaricus* and *S. thermophilus* staters had a very significant effect ($P < 0.05$) on the total LAB and pH value of breadnut seed yogurt compared to the control (treatment that did not add breadnut seed dairy). The results of the analysis of the total LAB and pH value of breadnut seed dairy yogurt are listed in Table 2. Based on Table 2, it is known that the addition of breadnut seed dairy to yogurt can increase total LAB and decrease the pH value of yogurt.

The pH value is one of the safety parameters in fermented dairy beverage products. Table 2 shows that the pH value in the control is higher than the treatment with adding breadnut seed dairy with a combination of *L. bulgaricus* and *S. thermophilus* staters. The control had a pH ranging from 4.43–4.51, while the pH value in the treatment of adding breadnut seed dairy with a combination of *L. bulgaricus* and *S. thermophilus* (P1S3, P2S3, P3S3, and P4S3) ranged from 4.26-4.05. The decrease in pH in the treatment of adding breadnut seed dairy with a combination of *L. bulgaricus* and *S. thermophilus* staters is in line with the results of total LAB analysis. The higher the total LAB in yogurt, the lower the pH of yogurt (Table 2). This is because the nutrients contained in breadnut seed dairy can support the growth of LAB to produce organic compounds during the fermentation process, which causes a decrease in the pH value of yogurt.

According to Vinderola et al. [26], in the early stages of fermentation, *S. thermophilus* is active in fermenting lactose into lactic acid, causing a decrease in pH. This decrease in pH stimulates the metabolic activity of *L. bulgaricus* to produce more other lactic acids, such as peptides, that cause a reduction in pH in yogurt. This decrease in pH will support the growth of starter bacteria and inhibit the growth of unwanted pathogenic bacteria [9].

Based on the results obtained, it is known that the pH value in all treatments has met the SNI 2981:2009 standard for yogurt quality requirements, which is 4.0-4.5.

Table 2. Total Lactic Acid Bacteria and pH of Breadnut (*Artocarpus camansi*) seed yogurt

Formulations	Total Lactic Acid Bacteria (LAB) (CFU/mL)	pH
P0S1	$1.36 \times 10^4 \pm 4.16^a$	4.51 ± 0.01^h
P0S2	$1.34 \times 10^4 \pm 2.08^a$	4.55 ± 0.01^i
P0S3	$1.19 \times 10^7 \pm 4.22^b$	4.43 ± 0.30^f
P1S1	$2.34 \times 10^4 \pm 4.16^a$	4.46 ± 0.11^g
P1S2	$2.45 \times 10^4 \pm 4.72^a$	4.51 ± 0.11^h
P1S3	$1.35 \times 10^7 \pm 4.22^c$	4.26 ± 0.15^e
P2S1	$2.83 \times 10^4 \pm 4.22^a$	4.21 ± 0.15^d
P2S2	$2.64 \times 10^4 \pm 4.04^a$	4.47 ± 0.15^g
P2S3	$1.82 \times 10^7 \pm 4.22^d$	4.19 ± 0.11^{cd}
P3S1	$1.22 \times 10^5 \pm 2.51^a$	4.16 ± 0.15^c
P3S2	$1.68 \times 10^4 \pm 1.15^a$	4.21 ± 0.10^d
P3S3	$1.34 \times 10^7 \pm 4.22^e$	4.11 ± 0.11^b
P4S1	$1.36 \times 10^5 \pm 2.51^a$	4.21 ± 0.11^d
P4S2	$1.72 \times 10^5 \pm 2.53^a$	4.17 ± 0.17^c
P4S3	$3.02 \times 10^7 \pm 4.22^f$	4.05 ± 0.30^a

Description :
Data are presented as mean ± standard deviation (SD) of 3 replicates
[ab]Data followed by the same letter in the same column is not significantly different in the Duncan test at the 0.05 level.

Based on the results of Duncan's test, it is known that the treatment with P1S3 starter variation is significantly different at the 5% level with P2S3, P3S3, and P4S3 treatments (Table 2). These results show that using a combination of *L. bulgaricus* and S. *thermophilus* starters in plant-based yogurt fermentation dramatically affects the total LAB contained in the final yogurt product. Total LAB did not increase significantly when only one of the two starters was used. These results align with research by Ge et al. [27] that the combination of *L. delbrueckii subsp. bulgaricus* and *S. thermophilus* starter cultures are more effective in maintaining the stability and quality of the final yogurt product. *S. thermophilus* will ferment lactose and convert it into lactic acid, which causes a decrease in pH. This decrease in pH will increase the proteolytic activity of *L. bulgaricus* in forming peptides and amino acids that support LAB growth. Hence, the combination of these two states directly contributes to the increase in total LAB.

Formulation P4S3 (100% breadnut seed dairy with a combination of *L. bulgaricus* and *S. thermophilus* staters) showed the highest total LAB at 3.02×10^7 CFU/mL compared to the other formulations (P1S3, P2S3, and P3S3), which also met the SNI 2981:2009 standard for yogurt quality requirements (minimum number of 10^7 stater bacteria). Formulation P4S3 shows that breadnut seeds have the potential to support LAB growth in yogurt fermentation. Breadnut seeds contain nutrients such as carbohydrates, proteins, fats, vitamins, and minerals needed by LAB to accelerate fermentation. In this process, labs such as *L. bulgaricus* and *S. thermophilus* use carbon and nitrogen sources in their metabolism to produce lactic acid. The nutrients in breadnut seeds can support the biochemical mechanisms of LAB through the glycolysis pathway (EMP) [27], which converts carbohydrates into lactic acid. In addition, *kluwih* seeds also contain dietary fiber as a source of prebiotics that can support LAB growth and metabolism during fermentation [14].

Several studies have reported that plant-based dairy fortification can increase LAB growth during the yogurt fermentation process. This was reported by El-Shenaway et al. [3], who mentioned that LAB growth was relatively higher in plant-based yogurt formulations from tiger nut (Cyperus esculentus) than in animal milk. Similar research was reported by Ozturkoglu-budak et al. [2], who used various types of nuts, such as hazelnuts, almonds, and pistachio, in yogurt making. The results obtained showed that the three types of nuts were able to increase the total number of LAB staters (*S. thermophilus* and *L. bulgaricus*) compared to the control yogurt.

Total LAB in yogurt products (at least 10^7 CFU/mL) is one of the essential requirements that must be met to ensure that yogurt products have optimal benefits for the digestive tract. With a total of 10^7 CFU/mL, yogurt has enough live bacteria to reach the gut and provide benefits, such as helping to maintain the balance of the gut microbiota, helping to prevent digestive disorders caused by pathogenic bacteria, and boosting the immune system [28].

Conclusion

The recommended formulation for breadnut seed yogurt is 100% breadnut seed dairy with a combination of *L. bulgaricus and S. thermophilus* staters (P4S3). The P4S3 formulation has met the quality standards of yogurt in accordance with SNI 2981:2009, which includes parameters of ash content, fat content, protein content, total acid and pH, and total LAB. The P4S3 formulation has an advantage over other formulations by having a lower fat content of 2.37% and a higher protein content of 3.71% compared to animal milk. Breadnut seed yogurt with P4S3 formulation contains a total LAB 3.02×10^7, so it has the potential to be an alternative functional drink. Further research needs to be done is to conduct sensory tests to evaluate consumer acceptance of breadnut seed yogurt, assess the stability of yogurt during storage, and perform in vivo tests to see the functional effects of breadnut seed yogurt on experimental animals.

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