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Functional Food Innovation of Moringa Oleifera L. Seed-Based Plant Yogurt: Fermentation Optimization with Three Lactic Acid Bacteria.

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Abstract: Moringa (*Moringa oleifera* L.) is a nutrient-dense plant with significant potential as a functional food source. Beyond its widely utilized leaves, moringa seeds possess substantial potential due to their high protein and bioactive compound content; however, their current utilization remains limited. This study aimed to evaluate the effect of varying inoculum proportions of three Lactic Acid Bacteria (LAB) species on the characteristics of moringa seed-based plant-based yogurt. The research followed a Completely Randomized Design (CRD), employing various inoculum proportions of *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus plantarum*. Data were statistically analyzed using ANOVA followed by Duncan's Multiple Range Test (DMRT) at a 95% confidence level. The results indicated that varying inoculum proportions significantly affected the total LAB count, total soluble solids, total acidity, viscosity, pH, proximate composition, and antioxidant activity. The formulation with a 1%:2%:2% ratio (P7) produced moringa seed yogurt with the optimal functional characteristics and consumer acceptance. This study concludes that the synergy among the three LAB species effectively optimizes the quality of moringa seed-based plant-based yogurt. This product has potential as a functional food alternative for individuals with lactose intolerance, while contributing to food security by downstreaming nutrient-rich local commodities.

Keywords: Fermentation; Moringa; Moringa Seeds; Probiotics; Yogurt.

Introduction

Moringa (Moringa oleifera L.) is known for its extraordinary health benefits and is often referred to as a "superfood" due to its rich nutritional content. This plant contains high levels of protein, vitamins, minerals, and antioxidants, making it a potential food ingredient for further development [1]. Until now, the use of Moringa has focused more on its leaves, while the seeds are still rarely utilized optimally, even though the seeds contain high protein and bioactive compounds that play a role in improving body health. One innovation that can be done to increase the use of Moringa seeds is through a fermentation process, which not only increases nutritional value but also enriches the probiotic content in the resulting product [2].

Fermentation has long been used in the food industry to improve a product's nutritional, functional, and organoleptic characteristics. One of the most well-known fermented products is yogurt, which is derived from the fermentation of milk using lactic acid bacteria (LAB). Yogurt has health benefits, particularly in maintaining the balance of gut microbiota, boosting the immune system, and possessing anti-inflammatory and antioxidant effects [3].

According to data from the Indonesian Pediatrician Association (IDAI), the prevalence of cow's milk protein allergy in children under five years of age in Indonesia ranges from 2% to 7.5%, or approximately more than 1.6 million children at risk of developing a cow's milk allergy [4]. Furthermore, lactose intolerance is also a common condition that affects an individual's ability to digest animal milk products [5]. In Indonesia, the prevalence of lactose intolerance is quite high in several regions, such as Jakarta (23.1%) and Manado (63.2%), according to a study of children with diarrhea [6]. Plant-based yogurt is an important alternative, especially for individuals with animal protein allergies, lactose intolerance, or those who choose a plant-based diet [7]. This product can help meet protein and other nutritional needs previously only available from animal products. However, plant-based yogurt made from moringa seeds is still rare, and research on optimizing fermentation to improve the nutritional and functional quality of this product is also very limited. In this context, moringa seeds could be a promising base ingredient for developing functional plant-based yogurt.

To produce high-quality yogurt, the combination of LAB used in fermentation is a key factor. In this study, three LAB types will be used: Lactobacillus bulgaricus, Streptococcus thermophilus, and Lactobacillus plantarum, which have been shown to improve the microbiological, physical, nutritional, and sensory characteristics of fermented products. Lactobacillus bulgaricus plays a role in producing lactic acid, which lowers pH, accelerates fermentation, and increases the viscosity and texture of yogurt [8]. Streptococcus thermophilus functions as the main starter that plays a role in the initial fermentation by producing exopolysaccharide compounds that increase the viscosity of yogurt and provide a distinctive taste [9]. Meanwhile, Lactobacillus plantarum has good adaptive abilities to plant substrates, can ferment various types of plant sugars, can act as a probiotic, with high antioxidant activity that can increase the shelf life and health benefits of plant-based yogurt [10].

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In addition to microbiological, physical, and nutritional aspects, this study will also evaluate the sensory acceptability of moringa seed-based yogurt by consumers. Taste, texture, aroma, and color are crucial factors in determining a product's acceptance in the market. Therefore, this study focuses not only on the scientific aspects but also considers the product's appeal to the wider community. With increasing public awareness of healthy and functional foods, innovation in developing fermented products made with local ingredients, such as moringa seeds, has great potential for broader adoption.

Research Methods

This research is an experimental study. The research design used was a Completely Randomized Design (CRD) with varying proportions of lactic acid bacteria inoculum (Lactobacillus bulgaricus, Streptococcus thermophilus, and Lactobacillus plantarum). The ratio of milk and moringa seed infusion for all treatments was 90% milk and 10% moringa seed infusion. This experiment was repeated three times, resulting in 27 experimental units.

Table 1. Lactic Acid Bacteria Combination

Treatments	*L.bulgaricus: S. thermophilus: L. plantarum*
P1	1%:1%:1%
P2	2%:1%:1%
P3	1%:2%:1%
P4	1%:1%:2%
P5	2%:2%:1%
P6	2%:1%:2%
P7	1%:2%:2%
P8	2%:2%:2%
P9	Control (without fermentation)

Place and Time of Research

This research was conducted at the Integrated Laboratory of the Food Technology Study Program, ITEKES Bali and at the Integrated Service Laboratory, Faculty of Agricultural Technology, Udayana University from June to August 2025.

Research Procedures

Starter Bacteria Rejuvenation

The bacterial culture (Lactobacillus bulgaricus, Streptococcus thermophilus, and Lactobacillus plantarum) isolated from the ITEKES Bali Microbiology Laboratory was refreshed by inoculating it at a 1:9 ratio into moringa seed milk. The milk, inoculated with the LAB starter, was then incubated at 37°C for 18 hours until coagulation occurred [11].

Moringa Seed Yogurt Making

The process of making moringa seed yogurt begins with brewing the moringa seeds and pasteurizing the milk. Next, lactic acid bacteria were inoculated by adding a combination of Lactobacillus bulgaricus, Streptococcus thermophilus, and Lactobacillus plantarum according to the prescribed treatment. Fermentation was carried out at 37°C for 18 hours. After fermentation is complete, the yogurt is cooled to 4°C before further analysis [12].

Total LAB Test

The total number of Lactic Acid Bacteria (LAB) is determined using the Total Plate Count (TPC) method specified in ISO 15214:1998. Initially, the yogurt sample is homogenized and undergoes a series of decimal dilutions using sterile physiological saline solution. Each dilution is then inoculated onto de Man, Rogosa, and Sharpe (MRS) agar, a medium specifically formulated to support the growth of LAB. The plates are incubated at 37°C for 48 hours to allow optimal colony development. After the incubation period, colonies that emerge are counted, and the results are expressed as Colony Forming Units per milliliter (CFU/mL). This enumeration is critical to ensure that the product meets the minimum probiotic requirements for functional yogurt [13].

Total Soluble Solids Test

Total soluble solids are determined using the gravimetric method, which quantifies the non-volatile components of the yogurt. The procedure begins by weighing a specific amount of the sample into a pre-weighed porcelain dish. This sample is then placed in a drying oven set at 105°C to facilitate the complete evaporation of its water content. The drying process continues until a constant weight is achieved, ensuring that only the solid residue remains. The final weight of the residue is compared to the initial sample weight to calculate the percentage of total solids. This parameter provides insights into the concentration of proteins, fats, and sugars present after the fermentation process [14].

Total Acidity Test

The total acidity of the yogurt is analyzed by an acid-base titration method using a standardized 0.1 N NaOH solution. A measured volume of the sample is diluted with distilled water and homogenized thoroughly before testing. Phenolphthalein is added to the mixture as a pH indicator, which signals the endpoint of the titration by changing to a faint pink color. The volume of titrant used is then recorded to calculate the total acidity, expressed as the percentage of lactic acid (% w/v). Monitoring total acidity is essential because it reflects the metabolic activity of the starter cultures in converting sugars into organic acids [15].

Viscosity Test

Viscosity analysis is performed using a Brookfield Viscometer to assess the flow properties and internal friction of the yogurt. Before measurement, the sample is maintained at a specific cold temperature to mimic standard storage conditions. A suitable spindle and rotation speed are selected based on the expected thickness of the moringa seed yogurt. The viscometer measures the fluid's resistance to rotation of the spindle, providing data in centipoise (cP). This analysis is an important parameter in determining the product's texture and its overall consistency as a drinkable or set yogurt [15].

pH Test

The pH of the samples is measured with a digital pH meter to monitor the acidity achieved during fermentation. The instrument is first calibrated using standard buffer solutions at pH 4.0 and 7.0 to ensure accurate readings. The electrode is then carefully immersed in the yogurt sample at room temperature until a stable value appears on the display. This measurement is crucial for identifying the completion of the fermentation process and the formation of the gel structure. A consistent pH value also serves as a quality control indicator to prevent over-acidification of the final product [16].

Water Content Test

Water content is measured using the oven-drying method, which involves heating the sample at 105°C until all moisture is removed. A small amount of the yogurt is placed in a crucible and weighed accurately before being subjected to the heating process. The sample remains in the oven until it reaches a constant weight, indicating that no further water loss is occurring. After drying, the sample is cooled in a desiccator to prevent it from re-absorbing moisture from the surrounding air. The percentage of water content is then calculated based on the difference between the initial and final weights of the sample [17].

Protein Content Test

The total protein content is analyzed using the Kjeldahl method, which consists of three distinct stages: digestion, distillation, and titration. During digestion, the sample is heated with concentrated sulfuric acid to break down organic nitrogen into ammonium sulfate. The resulting solution is then distilled to release ammonia, which is subsequently captured in a boric acid solution. The captured ammonia is titrated with a standard hydrochloric acid solution to determine the total nitrogen concentration. Finally, the nitrogen content is multiplied by a conversion factor of 6.25 to calculate the total crude protein percentage [17].

Fat Content Test

The fat content is measured using the Soxhlet method, which utilizes a continuous extraction process with an organic solvent. The dried yogurt sample is placed in an extraction thimble and treated with n-hexane or petroleum ether for several hours. This solvent effectively dissolves and removes the lipid components from the sample's solid matrix. Once the extraction is complete, the solvent is evaporated, leaving only the fat residue in the collection flask. The remaining fat is then weighed to determine the total fat percentage, which contributes significantly to the yogurt's flavor and mouthfeel [17].

Ash Content Test

Ash content is determined by placing the sample in a muffle furnace at 550°C to oxidize all organic matter. The process begins with pre-ashing the sample over a flame to prevent splattering and excessive smoke within the furnace. The sample is heated to high temperatures until the remaining residue turns into a greyish-white ash. This residue represents the total inorganic or mineral content present in the moringa seed-based yogurt. The final weight is recorded after cooling to calculate the mineral percentage, which is a vital part of the product's nutritional profile [17].

Carbohydrate Test

Carbohydrate content is determined using the by-difference method, an indirect calculation based on the remaining nutritional components. This method assumes that carbohydrates make up the portion of the sample that is not water, protein, fat, or ash. The calculation is performed by subtracting the combined percentages of these four components from 100%. This resulting value includes both digestible sugars and any dietary fibers that may be present in the plant-based ingredients. It is a standard approach in food analysis to complete the proximate composition profile of a new food product [17].

Calorie Testing

The calorie content of the yogurt is measured using a bomb calorimeter to determine its total energy value. A dried and compressed sample is placed inside a combustion chamber that is pressurized with pure oxygen. Upon ignition, the sample undergoes complete combustion, and the heat released is absorbed by a surrounding water jacket. The increase in water temperature is used to calculate the gross energy content produced by the sample. This energy value is then converted into kilocalories (kcal) to provide information on the caloric density of the product [18].

Antioxidant Activity Test IC50

The antioxidant activity is evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. Yogurt samples are extracted using a suitable solvent, and the resulting extract is mixed with a DPPH solution. The mixture is then incubated in the dark for a specific period to allow the reaction to reach equilibrium. After incubation, the solution's absorbance is measured at 517 nm using a UV-Vis spectrophotometer. The IC50 value is calculated from the inhibition curve to determine the concentration required to neutralize 50% of the free radicals [19].

Sensory Testing

The hedonic sensory test is conducted to assess the level of preference among potential consumers for the developed yogurt. A panel of 30 untrained individuals, primarily university students, is asked to evaluate attributes such as color, aroma, texture, and taste. Participants record their scores on a hedonic scale of 1 to 9, where 1 represents "dislike extremely" and 9 represents "like extremely." The test is carried out in a controlled environment to minimize external distractions and ensure the validity of the results. These sensory findings are essential for determining the marketability and consumer acceptance of the moringa seed yogurt [20].

Texture Testing

Texture Profile Analysis (TPA) is conducted using a texture analyzer to objectively measure the mechanical properties of the yogurt. The sample is subjected to a double-compression cycle using a cylindrical probe to simulate the physical action of chewing. During this test, the instrument records the force-time data to calculate various parameters such as hardness, springiness, and adhesiveness. It also measures cohesiveness, which indicates how well the yogurt structure holds together during consumption. This objective data complements the sensory testing by providing a precise scientific description of the product's physical texture [21].

Data Analysis

The data obtained from all experimental procedures are analyzed using the Analysis of Variance (ANOVA) statistical method. This analysis is used to determine if the different inoculum proportions have a statistically significant effect on the yogurt's characteristics. If the ANOVA indicates significant differences, a post hoc test is performed to identify specific differences between treatments. Duncan's Multiple Range Test (DMRT) is applied at a significance level of $P \leq 0.05$ for this purpose. This rigorous statistical approach ensures that the conclusions drawn from the research are scientifically sound and reliable.

Results and Discussion

Total LAB Test

Total Lactic Acid Bacteria (LAB) is a count of the number of viable lactic acid bacteria colonies in a product. This test is typically performed by plate count on selective media. The purpose of the lactic acid bacteria test is to determine the viability of probiotics in yogurt products, as LAB plays a crucial role in the fermentation of lactose into lactic acid and provides health benefits to the digestive tract [22].

Table 2. Results of the Total LAB Test

No.	Treatments	Composition	Average of Total LAB (CFU/g)
1	P1	1%:1%:1%	1.23×10^{7b}
2	P2	2%:1%:1%	1.52×10^{7cd}
3	P3	1%:2%:1%	1.44×10^{7c}
4	P4	1%:1%:2%	1.42×10^{7c}
5	P5	2%:2%:1%	1.59×10^{7cd}
6	P6	2%:1%:2%	1.64×10^{7d}
7	P7	1%:2%:2%	1.67×10^{7d}
8	P8	2%:2%:2%	1.71×10^{7d}
9	P9	Control	0^{a}

Based on the test results shown in Table 3, the total lactic acid bacteria in moringa seed yogurt ranged from 0–1.71×107 CFU/g. ANOVA results showed that variations in the inoculum proportion in moringa seed yogurt significantly affected the total lactic acid bacteria parameters in moringa seed yogurt. The lowest total lactic acid bacteria count was observed in treatment P9 (control), which lacked fermentation, with 0 bacteria. The highest total lactic acid bacteria count was found in treatment P8 (2%:2%:2%) with a total of 1.71×107 CFU/g. The higher the total LAB, the better the quality of the probiotics in the yogurt. According to SNI 2981:2009 concerning yogurt quality requirements, the minimum number of starter bacteria contained in a product is 107 colonies/g. Based on this, it can be concluded that the moringa seed yogurt in treatments P1 to P8 met SNI standards.

Total Soluble Solids Test

Total soluble solids is a measurement of all dissolved components (such as sugar, protein, and minerals) in yogurt. Total soluble solids testing assesses the consistency, texture quality, and density of the product. The correct solids value will support a stable yogurt texture and comply with food quality standards [23]. Based on the test results shown in Table 4, the total soluble solids in moringa seed yogurt ranged from 8.45-9.38% Brix. Based on the results of the ANOVA test, variations in the proportion of inoculum in moringa seed yogurt significantly affected the total soluble solids parameter of moringa seed yogurt. The lowest total soluble solids were obtained in treatment P1 (1%:1%:1%), with a total soluble solids of 8.45%, and the highest total soluble solids were obtained in treatment P9 (control), which is without fermentation, with a total soluble solids reaching 9.38% Brix.

Table 3. Results of Total Solids Test

No.	Treatmens	Composition	Average of Total Soluble Solids (%Brix)
1	P1	1%:1%:1%	8.45^{a}
2	P2	2%:1%:1%	8.95^{c}
3	P3	1%:2%:1%	8.46^{a}
4	P4	1%:1%:2%	8.61^{ab}
5	P5	2%:2%:1%	8.90^{c}
6	P6	2%:1%:2%	8.83^{bc}
7	P7	1%:2%:2%	8.75^{bc}
8	P8	2%:2%:2%	8.76^{bc}
9	P9	Control	9.38^{d}

Total Acid Test

The total acid test was conducted to assess the product's acidity. Total acid will determine the yogurt's distinctive sour taste and its shelf life. A balanced acid level is also an indicator of successful fermentation [24]. Based on the test results shown in Table 5, the total acid in moringa seed yogurt ranged from 0.71 to 8.36%. Based on the ANOVA test results, variations in the inoculum proportion in moringa seed yogurt significantly affected the total acid parameter of moringa seed yogurt. The lowest total acid was found in treatment P9 (control), which lacked fermentation, at 0.71%. While the highest total acid was found in treatment P3 (1%:2%:1%) with a total acid of 8.36%.

Table 4. Results of Total Acid Test

No.	Treatments	Composition	Average of Total Acid (%)
1	P1	1%:1%:1%	8.13[bc]
2	P2	2%:1%:1%	7.96[b]
3	P3	1%:2%:1%	8.36[c]
4	P4	1%:1%:2%	8.31[c]
5	P5	2%:2%:1%	8.09[bc]
6	P6	2%:1%:2%	8.14[bc]
7	P7	1%:2%:2%	7.97[b]
8	P8	2%:2%:2%	8.23[bc]
9	P9	Control	0.71[a]

Viscosity Test

Viscosity is a measure of the thickness of a liquid or semi-solid. Viscosity testing on yogurt products plays a crucial role in measuring viscosity because it is related to consumer acceptance, texture, and product stability during storage. Viscosity is influenced by the number of LAB, protein content, and degree of fermentation [22]. Based on the test results shown in Table 6, the viscosity of moringa seed yogurt ranged from 1,260.18 to 1,657.61 mPa*s. Based on the ANOVA test results, variations in the proportion of inoculum in moringa seed yogurt significantly affected the viscosity of moringa seed yogurt. The lowest viscosity was obtained in treatment P9 (control), namely without fermentation, with a viscosity of 1,260.18 mPa*s, while the highest viscosity was obtained in treatment P7 (1%:2%:2%) with a viscosity reaching 1,657.61 mPa*s.

Table 5. Results of Viscosity Test

No.	Treatments	Composition	Average of Viscosity (mPa*s)
1	P1	1%:1%:1%	1.609,32[cd]
2	P2	2%:1%:1%	1.636,26[cd]
3	P3	1%:2%:1%	1.582,08[bc]
4	P4	1%:1%:2%	1.527,22[b]
5	P5	2%:2%:1%	1.623,95[cd]
6	P6	2%:1%:2%	1.571,71[bc]
7	P7	1%:2%:2%	1.657,61[d]
8	P8	2%:2%:2%	1.593,82[cd]
10	P9	Control	1.260,18[a]

pH Test

pH measurement is used to determine the acidity of yogurt. A low pH value indicates the formation of lactic acid during fermentation. This test is important for controlling product quality, preventing the growth of pathogenic microbes, and ensuring sensory characteristics remain up to standard [25]. Based on the test results shown in Table 7, the pH of moringa seed yogurt ranged from 3.65–6.76. Based on the ANOVA test results, variations in the proportion of inoculum in moringa seed yogurt significantly affected the pH of moringa seed yogurt. The lowest pH value was obtained in treatment P3 (1%:2%:1%) with a value of 3.65, while the highest pH was obtained in treatment P9 (control), which is without fermentation with a value of 6.76. The lower the pH value obtained, the more acidic the moringa seed yogurt.

Table 6. Results of pH Test

No.	Perlakuan	Komposisi	Average of pH
1	P1	1%:1%:1%	3.75[abc]
2	P2	2%:1%:1%	3.91[c]
3	P3	1%:2%:1%	3.65[a]
4	P4	1%:1%:2%	3.73[ab]
5	P5	2%:2%:1%	3.85[bc]
6	P6	2%:1%:2%	3.78[abc]
7	P7	1%:2%:2%	3.76[abc]
8	P8	2%:2%:2%	3.72[ab]
9	P9	Control	6.76[d]

Water Content Test

The water content test was conducted to determine the water content in yogurt under various treatments. This test is important for assessing the shelf life and consistency of yogurt, as high water content can affect texture stability and accelerate spoilage. Yogurt products generally have a high water content, so it is important to control it to maintain quality [26]. Based on the test results shown in Table 8, the water content of moringa seed yogurt ranged from 82.50 to 89.86%. Based on the ANOVA test results, variations in the inoculum proportion in moringa seed yogurt significantly affected the water content of the yogurt. The lowest water content was observed in treatment P3 (1%:2%:1%) at 82.50%, while the highest was observed in treatment P2 (2%:1%:1%) at 89.86%.

Table 7. Results of Water Content Test

No.	Treatments	Composition	Average of Water Content (%bb)
1	P1	1%:1%:1%	86.21[b]
2	P2	2%:1%:1%	89.86[d]
3	P3	1%:2%:1%	82.50[a]
4	P4	1%:1%:2%	86.62[b]
5	P5	2%:2%:1%	86.84[bc]
6	P6	2%:1%:2%	89.27[d]
7	P7	1%:2%:2%	83.16[a]
8	P8	2%:2%:2%	84.55[ab]
9	P9	Control	89.15[cd]

Protein Content Test

Protein is an important component in milk that plays a role in forming the gel structure of yogurt. Protein content tests assess the nutrient content and nutritional quality of the product. Protein in yogurt also affects viscosity and texture [25]. Based on the test results shown in Table 9, the protein content in moringa seed yogurt ranged from 3.13-3.42%. Based on the results of the ANOVA test, variations in the proportion of inoculum in moringa seed yogurt significantly affected the protein content of moringa seed yogurt. The lowest protein content was found in treatment P3 (1%:2%:1%) with a value of 3.13%, while the highest protein content was found in treatment P2 (2%:1%:1%) with a value of 3.42%. According to SNI 2981:2009 concerning yogurt quality requirements, the minimum protein content in the product is 2.7%. Based on this, it can be concluded that the protein content of moringa seed yogurt in all treatments, from P1 to P9, meets SNI standards.

Table 8. Results of Protein Content Test

No.	Treatments	Composition	Average of Protein Content (%bb)
1	P1	1%:1%:1%	3.24[bc]
2	P2	2%:1%:1%	3.42[d]
3	P3	1%:2%:1%	3.13[a]
4	P4	1%:1%:2%	3.32[c]
5	P5	2%:2%:1%	3.31[c]
6	P6	2%:1%:2%	3.33[cd]
7	P7	1%:2%:2%	3.28[bc]
8	P8	2%:2%:2%	3.18[ab]
9	P9	Control	3.27[bc]

Fat Content Test

Fat content testing is performed to assess the amount of fat in yogurt products. Fat plays a role in providing flavor, a soft texture, and increasing energy value. Furthermore, controlled fat content is important for consumers on a low-fat diet [26]. Based on the test results shown in Table 10, the fat content in moringa seed yogurt ranged from 2.18 to 2.42%. Based on the ANOVA test results, variations in the inoculum proportion in moringa seed yogurt significantly affected the fat content of the moringa seed yogurt. The lowest fat content was found in treatment P3 (1%:2%:1%) with a value of 2.18%, while the highest fat content was found in treatment P2 (2%:1%:1%) with a value of 2.42%. According to SNI 2981:2009 concerning yogurt quality requirements, the minimum fat content in yogurt products is 3.0%, while the quality requirements for low-fat yogurt are 0.6-2.9%. Based on this, it can be concluded that the fat content of moringa seed yogurt in treatments P1 to P9 does not meet the SNI standard for conventional yogurt, but meets the SNI standard as low-fat yogurt.

Table 9. Results of Fat Content Test

No.	Treatments	Composition	Average of Fat Content (%bb)
1	P1	1%:1%:1%	2,31[cd]
2	P2	2%:1%:1%	2,42[e]
3	P3	1%:2%:1%	2,18[a]
4	P4	1%:1%:2%	2,27[bc]
5	P5	2%:2%:1%	2,38[de]
6	P6	2%:1%:2%	2,38[de]
7	P7	1%:2%:2%	2,20[ab]
8	P8	2%:2%:2%	2,33[cde]
9	P9	Control	2,29[bcd]

Ash Content Test

Ash content is the mineral residue remaining after the sample is burned. This test is important for determining the levels of minerals such as calcium and phosphorus, which are beneficial for bone health. Appropriate ash content also indicates the quality of raw materials such as milk and moringa seed powder [26]. Based on the test results shown in Table 11, the ash content of moringa seed yogurt ranged from 0.73–0.79%. Based on the ANOVA test results, variations in the inoculum proportion in moringa seed yogurt significantly affected the ash content of the moringa seed yogurt. The lowest ash content was found in treatment P3 (1%:2%:1%) with a value of 0.73%, while the highest ash content was found in treatment P2 (2%:1%:1%) with a value of 0.79%. According to SNI 2981:2009 concerning yogurt quality requirements, the maximum ash content in a product is 1%. Based on this, it can be concluded that the ash content of moringa seed yogurt in all treatments, from P1 to P9, meets SNI standards.

Table 10. Results of Ash Content Test

No.	Treatments	Composition	Average of Ash Content (%bb)
1	P1	1%:1%:1%	0.76[b]
2	P2	2%:1%:1%	0.79[c]
3	P3	1%:2%:1%	0.73[a]
4	P4	1%:1%:2%	0.75[ab]
5	P5	2%:2%:1%	0.76[b]
6	P6	2%:1%:2%	0.75[ab]
7	P7	1%:2%:2%	0.79[c]
8	P8	2%:2%:2%	0.74[ab]
9	P9	Control	0.75[b]

Carbohydrate Test

Carbohydrate content was calculated using the by-difference method. Carbohydrates (especially lactose) are the main substrate for LAB to produce lactic acid. This test is important to determine the residual sugar in the product and its relationship to sweetness and nutritional value [25]. Based on the test results shown in Table 12, the carbohydrate content in moringa seed yogurt ranged from 6.93–7.91%. Based on the ANOVA test results, variations in the inoculum proportion in moringa seed yogurt significantly affected the carbohydrate content of moringa seed yogurt. The lowest carbohydrate content was found in treatment P3 (1%:2%:1%) with a value of 6.93%, while the highest carbohydrate content was found in treatment P9 (control) with a value of 7.91%.

Table 11. Results of Carbohydrate Test

No.	Treatments	Composition	Average of Carbohydrate (%bb)
1	P1	1%:1%:1%	7.41[cd]
2	P2	2%:1%:1%	7.51[d]
3	P3	1%:2%:1%	6.93[a]
4	P4	1%:1%:2%	7.07[ab]
5	P5	2%:2%:1%	7.36[cd]
6	P6	2%:1%:2%	7.43[cd]
7	P7	1%:2%:2%	7.22[bc]
8	P8	2%:2%:2%	7.22[bc]
9	P9	Control	7.91[e]

Calorie Testing

The calorie test was conducted by calculating the total energy derived from protein, fat, and carbohydrates. The calorie test aims to determine the energy value consumers obtain from the product. Yogurt with the correct calorie calculation can be positioned as a functional food while meeting daily energy needs [26]. Based on the test results shown in Table 13, the calorie content of moringa seed yogurt ranges from 60.00–67.11 kcal/100g. Based on the ANOVA test results, variations in the inoculum proportion in moringa seed yogurt significantly affected the calorie content of moringa seed yogurt. The lowest calorie value was found in treatment P3 (1%:2%:1%) with a value of 60.00 kcal/100g, while the highest calorie content was found in treatment P9 (control) with a value of 67.11 kcal/100g.

Table 12. Results of Calorie Testing

No.	Treatments	Composition	Average of Calorie (Kkal/100g)
1	P1	1%:1%:1%	64.56[c]
2	P2	2%:1%:1%	66.84[d]
3	P3	1%:2%:1%	60.00[a]
4	P4	1%:1%:2%	61.94[b]
5	P5	2%:2%:1%	64.18[c]
6	P6	2%:1%:2%	62.96[bc]
7	P7	1%:2%:2%	61.19[ab]
8	P8	2%:2%:2%	63.08[bc]
9	P9	Control	67.11[d]

Antioxidant Activity Test IC50

Antioxidant activity testing assesses a compound's ability to neutralise free radicals, typically using the DPPH method and reporting IC_{50} values. The lower the IC_{50} value, the higher the antioxidant activity. In yogurt, this test is important because of the presence of additional ingredients such as moringa fruit, which can increase the content of bioactive compounds [22]. Based on the test results shown in Table 14, the antioxidant activity of moringa seed yogurt ranged from 269,549.73–335,905.82 ppm. Based on the ANOVA test results, variations in the proportion of inoculum in moringa seed yogurt significantly affected the antioxidant activity of moringa seed yogurt. The highest IC50 value was obtained in treatment P2 (2%:1%:1%) with a value of 335,905.82 ppm, while the lowest IC50 value was obtained in treatment P9 (control) with a value of 269,549.73 ppm.

Table 13. Results of Antioxidant Activity Test IC50

No.	Treatments	Composition	Average of IC_{50} (ppm)
1	P1	1%:1%:1%	324.141,49[c]
2	P2	2%:1%:1%	335.905,82[d]
3	P3	1%:2%:1%	310.778,40[b]
4	P4	1%:1%:2%	318.291,51[bc]
5	P5	2%:2%:1%	315.730,32[bc]
6	P6	2%:1%:2%	323.630,56[c]
7	P7	1%:2%:2%	309.587,98[b]
8	P8	2%:2%:2%	316.583,42[bc]
9	P9	Control	269.549,73[a]

Sensory Test

Sensory testing was conducted to assess consumer acceptance. In this study, the parameters tested included color, aroma, taste, viscosity, and an overall product assessment. This testing is crucial in yogurt development because, despite the product's high nutritional value, organoleptic quality remains a key factor in successful market acceptance [23]. The assessment used a hedonic scale of 1–5, with 1 indicating "very dislike" and 5 indicating "very like." Twenty untrained panelists participated in the test to obtain a general overview of product acceptance.

Based on the sensory test results presented in Table 15, treatment P7 obtained the highest score for color, with a value of 4.6, indicating that the yogurt with treatment P7 was most preferred by consumers. The lowest score was obtained for treatment P9 (control), with a value of 3.15. Based on the aroma sensory test results, treatment P5 obtained the highest score, with a value of 4.0, while treatment P9 (control), with the lowest score, with a value of 2.35.

Based on the taste sensory test results, treatment P8 received the highest score (3.80), while treatment P9 (control) received the lowest (3.30). Based on the viscosity sensory test results, treatment P7 received the highest score (4.25), while treatment P9 (control) received the lowest (1.95). Based on the overall sensory test results, treatments P5 and P7 received the highest scores (3.95), while treatment P9 (control) received the lowest (3.1). Based on the overall average of the sensory parameters, it can be concluded that treatment P7 was the yogurt with the highest consumer acceptance, with a score of 3.98.

Table 14. Results of Sensoty Test

No.	Treatments	Combination	Color	Aroma	Taste	Viscosity	Overall	Average
1	P1	1%:1%:1%	4.20	3.10	3.75	4.05	3.90	3.80
2	P2	2%:1%:1%	4.25	3.15	3.55	4.15	3.80	3.78
3	P3	1%:2%:1%	4.05	3.05	3.40	2.80	3.40	3.34
4	P4	1%:1%:2%	4.35	3.45	3.45	3.00	3.85	3.62
5	P5	2%:2%:1%	4.15	4.00	3.55	3.95	3.95	3.92
6	P6	2%:1%:2%	4.20	3.60	3.65	3.10	3.80	3.67
7	P7	1%:2%:2%	4.60	3.50	3.60	4.25	3.95	3.98
8	P8	2%:2%:2%	4.50	3.40	3.80	3.45	3.90	3.81
9	P9	Control	3.15	2.35	3.30	1.95	3.10	2.77

Table 15. ANOVA Test

		Sum of Squares	df	Mean Square	F	Sig.
Moisture	Between Groups	171.459	8	21.432	11.808	.000
	Within Groups	32.672	18	1.815		
	Total	204.131	26			
Ash	Between Groups	.011	8	.001	7.415	.000
	Within Groups	.003	18	.000		
	Total	.014	26			

Protein	Between Groups	.185	8	.023	7.544	.000
	Within Groups	.055	18	.003		
	Total	.240	26			
Fat	Between Groups	.161	8	.020	8.143	.000
	Within Groups	.045	18	.002		
	Total	.206	26			
Carbohydrate	Between Groups	1.919	8	.240	17.094	.000
	Within Groups	.253	18	.014		
	Total	2.171	26			
Calorie	Between Groups	138.605	8	17.326	15.756	.000
	Within Groups	19.793	18	1.100		
	Total	158.398	26			
IC 50	Between Groups	8.127E9	8	1.016E9	26.039	.000
	Within Groups	7.022E8	18	39012208.179		
	Total	8.829E9	26			
Total acidity	Between Groups	148.172	8	18.522	782.109	.000
	Within Groups	.426	18	.024		
	Total	148.598	26			
pH	Between Groups	23.968	8	2.996	402.649	.000
	Within Groups	.134	18	.007		
	Total	24.102	26			
Total BAL	Between Groups	6.760E14	8	8.451E13	78.461	.000
	Within Groups	1.939E13	18	1.077E12		
	Total	6.954E14	26			
Viscosity	Between Groups	343629.543	8	42953.693	35.084	.000
	Within Groups	22037.366	18	1224.298		
	Total	365666.909	26			
Padatan	Between Groups	1.933	8	.242	14.381	.000
	Within Groups	.302	18	.017		
	Total	2.235	26			

Texture Testing

Hardness

Based on the test results shown in Table 16, the hardness value of moringa seed yogurt ranged from 11.36 to 24.98g. Based on the ANOVA results, variations in the proportion of inoculum in moringa seed yogurt significantly affected the hardness of the moringa seed yogurt. The lowest hardness value was found in the P9 (control) treatment, which was unfermented, at 11.36g. The highest hardness value was found in the P8 (2%:2%:2%) treatment, with a hardness of 24.98g.

Table 16. Hardness Test Results

No.	Treatments	Combinations	Average Hardness (g)
1	P1	1%:1%:1%	$17,90^{b}$
2	P2	2%:1%:1%	$20,35^{c}$
3	P3	1%:2%:1%	$18,42^{b}$
4	P4	1%:1%:2%	$20,73^{cd}$
5	P5	2%:2%:1%	$22,41^{de}$
6	P6	2%:1%:2%	$24,35^{ef}$
7	P7	1%:2%:2%	$22,92^{e}$
8	P8	2%:2%:2%	$24,98^{f}$
9	P9	control	$11,36^{a}$

Elasticity

Based on the test results shown in Table 17, the springiness value of moringa seed yogurt ranged from 0.78 to 0.86. Based on the ANOVA results, variations in the proportion of inoculum in moringa seed yogurt significantly affected the elasticity of the moringa seed yogurt. The lowest elasticity value was found in the P8 (2%:2%:2%) treatment, with a value of 0.78. The highest elasticity value was found in the P9 (control) treatment, which was unfermented, with a value of 0.86g.

Table 17. Elasticity Test Results

No.	Treatments	Combinations	Rerata Elastisitas (ratio)
1	P1	1%:1%:1%	0.84^{cd}
2	P2	2%:1%:1%	0.82^{bc}
3	P3	1%:2%:1%	0.82^{bc}
4	P4	1%:1%:2%	0.80^{ab}
5	P5	2%:2%:1%	0.79^{ab}
6	P6	2%:1%:2%	0.79^{ab}
7	P7	1%:2%:2%	0.81^{bc}
8	P8	2%:2%:2%	0.78^{a}
9	P9	control	0.86^{d}

Adhesiveness

Based on the test results shown in Table 18, the adhesiveness of moringa seed yogurt ranged from -54.58 to -18.45 g·s. Based on the ANOVA results, variations in the inoculum proportion in moringa seed yogurt significantly affected the adhesiveness. The lowest adhesiveness was observed in treatment P8 (2%:2%:2%), with a value of -54.38 g·s, while the highest was observed in treatment P9 (the control, with no fermentation), with a value of -18.45 g·s.

Table 18. Adhesion Test Results

No.	Treatments	Combinations	Rerata Daya Lekat (g·s)
1	P1	1%:1%:1%	-28.24^{f}
2	P2	2%:1%:1%	-37.56^{de}
3	P3	1%:2%:1%	-33.93^{e}
4	P4	1%:1%:2%	-46.90^{bc}
5	P5	2%:2%:1%	-38.32^{de}
6	P6	2%:1%:2%	-50.82^{ab}
7	P7	1%:2%:2%	-43.05^{cd}
8	P8	2%:2%:2%	-54.38^{a}
9	P9	control	-18.45^{g}

Cohesiveness

Based on the test results shown in Table 19, the cohesiveness of moringa seed yogurt ranged from 0.41 to 0.63. Based on the ANOVA results, variations in the inoculum proportion in moringa seed yogurt significantly affected the cohesiveness of moringa seed yogurt. The lowest cohesiveness value was obtained in the P9 (control) treatment, namely without fermentation, with a value of 0.41, while the highest cohesiveness value was obtained in the P8 treatment (2%:2%:2%) with a cohesiveness value of 0.63.

Table 19. Cohesiveness Test Results

No.	Treatments	Combinations	Rerata Kosehivitas (ratio)
1	P1	1%:1%:1%	0.52^{bc}
2	P2	2%:1%:1%	0.49^{b}
3	P3	1%:2%:1%	0.52^{bc}
4	P4	1%:1%:2%	0.57^{cde}
5	P5	2%:2%:1%	0.60^{de}
6	P6	2%:1%:2%	0.61^{de}
7	P7	1%:2%:2%	0.55^{cd}
8	P8	2%:2%:2%	0.63^{e}
9	P9	control	0.41^{a}

Table 20. ANOVA for Texture Testing

		Sum of Squares	df	Mean Square	F	Sig.
Hardness	Between Groups	416.918	8	52.115	42.448	.000
	Within Groups	22.099	18	1.228		
	Total	439.017	26			
Springiness	Between Groups	.016	8	.002	6.804	.000
	Within Groups	.005	18	.000		
	Total	.022	26			
Adhesiveness	Between Groups	3064.388	8	383.049	37.449	.000
	Within Groups	184.113	18	10.229		
	Total	3248.502	26			
Cohesiveness	Between Groups	.112	8	.014	13.146	.000
	Within Groups	.019	18	.001		
	Total	.132	26			

Conclusion

Variations in the inoculum proportions of *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus plantarum* significantly affected the microbiological, physical, chemical, and organoleptic characteristics of moringa seed yogurt. Based on a comprehensive analysis, formulation P7 (1%:2%:2%) was identified as the optimal treatment, yielding a product with the highest consumer acceptance and optimal viscosity and total lactic acid bacteria profiles. In terms of implications, this study demonstrates that moringa seeds, which have historically been underutilized, possess significant potential as a raw material for high-value functional foods. This plant-based yogurt innovation not only contributes to food security by utilizing local resources but also provides a strategic health solution as a probiotic alternative for individuals suffering from lactose intolerance and cow's milk protein allergy. Furthermore, the development of this product is expected to create new entrepreneurial opportunities in food technology rooted in local wisdom.

Author's Contribution

A.A.N.D.A.W Putra: served as the chief researcher (research designer and implementer). P.R. Sintyadewi: served as a research member (data collector and data analyst).

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