

Physicochemical Properties and Optimization of Pigeon Peas (*Cajanus cajan*) Yogurt: Influence of Starter Culture and Incubation Techniques

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Abstract

Pigeon peas (Cajanus cajan (L.)) are a type of legume with potential for development as a sustainable alternative source of plant-based protein. These beans have a relatively high protein content, approximately 27–28%. This study aims to analyze the effects of variations in lactic acid bacteria cultures and incubation methods on the production of yogurt made from local Pigeon peas beans. The study employed a Completely Randomized Design (CRD) to test variations in Lactic Acid Bacteria (LAB) cultures (comprising Greek yogurt culture and powdered culture) combined with three different incubation methods: incubation at room temperature, in a thermos, and in a modified incubator. Data were analyzed using a two-way Anova. The results showed that variations in LAB cultures had a significant effect on protein content, moisture content, fat content, viscosity, and pH. Meanwhile, the incubation method had a significant effect on protein content, moisture content, ash content, total lactic acid, fat content, viscosity, and pH. The best Pigeon peas bean yogurt was obtained from the powdered culture treatment using the incubation method with a modified incubator, yielding a protein content of 3.47%; ash content of 0.69%; total lactic acid of 0.66%; fat content of 1%; viscosity of 0.47 cP; pH of 3.82; and moisture content of 83.19%. The results of the organoleptic test showed that the criteria for color, aroma, and taste were met, while on average, the panelists strongly liked the texture of the yogurt.

Keyword: Yogurt, Pigeon pea, Lactic Acid Bacteria, Incubation method, Fermentation

INTRODUCTION

Global demand for plant-based fermented beverages has surged significantly, driven by the rising prevalence of lactose intolerance and milk protein allergies, as well as a growing shift toward vegan and sustainable diets and the natural ingredients industry—spanning food, pharmaceuticals, and other applications. This has led to an intensified exploration of various plant species and food sources across the globe, including the investigation of plant potential in Indonesia. Indonesia is currently known to possess 100 to 150 plant families with potential for use as industrial crops, alternative foods, fruits, spices, and medicinal plants for health (Rasdianah et al., 2024). Indonesia is also one of the leading producers of various cultivated legumes, such as peanuts, soybeans, mung beans, and pigeon peas. Legumes hold significant potential for development into functional foods, such as plant-based milk products, and their application in fermented dairy beverages, specifically yogurt. Yogurt is one of the oldest and most popular fermented dairy products worldwide. With their superior nutritional profiles, various fermented dairy products and yogurt-based foods are emerging as increasingly popular alternative foods.

Although soy milk and almond milk generally dominate the market, there is significant potential to explore legumes that have not yet been fully utilized, such as pigeon peas (*Cajanus cajan*), which are promising candidates due to their high protein content (18–25%), rich dietary fiber, and an abundance of essential amino acids, particularly lysine, which is often lacking in cereal-based diets. Despite its great potential, the use of pigeon peas in the functional food industry, particularly as a substrate for yogurt drinks, remains under-explored compared to soybeans or chickpeas.

Transforming Pigeon peas bean milk into yogurt through fermentation will not only improve digestibility by reducing anti-nutritional factors such as phytic acid and oligosaccharides (which cause bloating) but also enhance the bioavailability of bioactive compounds. However, producing plant-based yogurt with desired physicochemical properties presents a significant technical challenge. Unlike cow's milk, plant-based extracts lack a casein micelle network, which often results in poor texture integrity, excessive syneresis, and a lack of the characteristic creamy mouthfeel.

Recent research findings indicate that the quality of legume-based yogurt is significantly influenced by the synergy between the composition of the starter culture and the incubation technique. The selection of lactic acid bacteria (LAB) strains—such as the traditional consortium of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* versus probiotic strains like *Lactobacillus acidophilus*—critically influences the rate of acidification and the production of

exopolysaccharides (EPS), which act as natural thickeners. Additionally, incubation parameters—particularly temperature and duration—determine the metabolic activity of the starter culture, which directly influences the profile of volatile flavor compounds and the rheological behavior of the final product.

To transform a substrate like Pigeon peas bean milk into a specific desired product, such as yogurt, a well-controlled fermentation process using microbial assistance is required. Yogurt fermentation can be carried out and proceeds with the assistance of lactic acid bacteria. During the fermentation process, lactose is converted into lactic acid, which lowers the pH and produces various volatile and non-volatile compounds as well as polysaccharides that can affect the texture and quality of the yogurt. These bacteria are also capable of surviving in acidic conditions (Astuty et al., 2021). The lactic acid bacteria used in yogurt production can be single-strain or a mixture (Suhartatik et al., 2020).

Several studies have addressed the basic fermentation process of pigeon pea milk; however, there remains a significant gap in systematic optimization that integrates the interactions between specific starter cultures and modern incubation strategies to achieve standardized physicochemical profiles. Most of the existing literature focuses on raw nutritional content, leaving a gap in understanding how processing variables can be manipulated to minimize the unpleasant nutty flavor and improve the colloidal stability of *Cajanus cajan* yogurt.

Therefore, this study aims to optimize the production of pigeon pea yogurt by evaluating the effects of various starter cultures and incubation techniques on its physicochemical properties. By applying a systematic optimization approach, this study seeks to establish a high-quality plant-based yogurt formulation that meets nutritional requirements and consumer sensory expectations, thereby serving as a viable alternative to dairy-based products in the functional food market

METHOD

Research Site

This study was conducted at the Agroindustry Quality Testing Laboratory and the Nutrition and Chemistry Laboratory of the Department of Aquaculture, Pangkajene Islands State Agricultural Polytechnic, from January to May 2025.

Tools and Materials

The equipment used in yogurt production included a digital scale, scissors, a mixing bowl, a pot, containers, a wooden spatula, a blender, a stove, a strainer, jars, a tray, cardboard, a



thermometer, a tablespoon, a thick cloth, a refrigerator, a thermos, duct tape, Petri dishes, an oven, Erlenmeyer flasks, a measuring flask, a dropper pipette, a volumetric pipette, Ostwald tubes, metal spatulas, pH meters, funnels, stainless steel trays, spatulas, manual and digital penetration testers, Kjeldahl steam distillation apparatus, pycnometers, gloves, combustion ovens, drying ovens, test tubes, test tube racks, filter paper, lab coats, acid digestion cabinets, stopwatches, and modified incubators. The modified incubator used is a simple, manually operated incubator made from a closed cardboard box equipped with an incandescent light bulb and a thermometer to control the temperature. The materials used were bintotoeng beans, water, Greek yogurt culture, powdered culture, B+ nutritional lactose, SGM soy milk, selenium, concentrated H_2SO_4 solution, distilled water, tissue, 1% H_3BO_3 solution, mixed indicator, 0.1% HCl solution, 0.1 M H_2SO_4 solution, phenolphthalein solution (pp) 1%, 0.1 N NaOH solution, and chloroform solution

METHODS

The study used a completely randomized design (CRD) to test variations in Lactic Acid Bacteria (LAB) cultures, consisting of Greek yogurt culture (A1) and powdered culture (A2), combined with three different incubation methods: incubation at room temperature (B1), in a thermos container (B2), and a modified incubator (B3). The data were analyzed using a two-way ANOVA. Data on the aroma, color, taste, and texture of the yogurt were obtained through panelist evaluations in an organoleptic test

Preparing Milk and Yogurt

The preparation of Pigeon peas bean milk begins by gathering 800 grams of Pigeon peas beans, followed by washing them. Next, the washed Pigeon peas beans are soaked for 3 hours. After soaking, the Pigeon peas beans are drained and rinsed under running water; the Pigeon peas beans are then boiled for 30 minutes and left to rest for 10 minutes. The Pigeon peas beans are separated from their skins. The Pigeon peas beans are rinsed again under running water until clean. The Pigeon peas beans are blended using a blender with a 1:2 water-to-bean ratio. The resulting mixture is then strained using a cheesecloth to obtain Pigeon peas bean milk. Next, the yogurt-making process is carried out by mixing the Pigeon peas bean milk with soy milk. The milk is then heated to 80°C , cooled to 40°C , and Lactose Nutrisi B+ is added at a concentration of 45% to the milk. The milk is then divided into two containers, designated as Sample A1 and Sample A2. Sample A1 is inoculated with 40% Greek yogurt culture. Sample A2 is inoculated with 40% powdered culture. Each sample is placed in a jar and labeled accordingly. Samples A1B1 and



A2B1 are fermented at room temperature by being wrapped in a thick cloth at 25°C. Samples A1B2 and A2B2 were fermented in thermoses containing warm water at 40°C, while samples A1B3 and A2B3 were fermented in a modified incubator at 50°C. Each fermentation process lasted 10 hours. All samples were cooled in a refrigerator to halt the fermentation process.

Protein Content

Protein content was determined using the Kjeldahl method (Bakhtra et al., 2017), in which 0.2–0.5 grams of sample were placed in a 100-ml Kjeldahl flask. Approximately 1 gram of selenium mixture and 6 ml of concentrated H₂SO₄ are added and then homogenized. The mixture is digested in a fume hood until clear (approximately 2 hours), after which the sample is allowed to cool. A 250-ml Erlenmeyer flask is prepared, and 1% H₃BO₃ solution and 4 drops of Michlin indicator solution are added. Distill for approximately 7 minutes or until the volume of the collected liquid reaches 75 ml. Titrate with 0.1 M HCl solution. The protein content can be calculated using equation (1)

$$\text{Protein Content (\%)} = \frac{\text{ml titration} \times 0.14 \times 6.25}{\text{Sample Weight}} \times 100 \% \dots\dots\dots(1)$$

Notes:

Titration volume = Weight of the original sample after titration, expressed in mL

6.25 = Protein factor for yogurt

Sample weight = Weight of the original sample in grams

Moisture Content

Moisture content was measured using the oven method (Soeparyo et al., 2018). The process began by drying an empty porcelain dish in an oven at 70°C for 15 minutes. An 18-gram sample is placed in a porcelain dish and then placed in an oven/dryer for 24 hours at 80°C. The dish is allowed to cool for 3–5 minutes. Once cooled, the sample is weighed again. The moisture content is calculated using Equation (2)

$$\text{Moisture Content (\%)} = \frac{(A+B)-C}{B} \times 100 \% \dots\dots\dots(2)$$

Notes:

A = Weight of empty dish

B = Weight of sample

C = Weight of dish and sample after drying

The ash content

The ash content percentage is measured using the oven method, in which an empty porcelain ash dish is placed in an incineration furnace. The temperature is gradually increased until it reaches 550°C. Next, the combustion temperature is lowered to 40°C. The porcelain ash dish is removed and cooled for 30 minutes, then the weight of the empty porcelain ash dish is measured (A grams). Approximately 1–2 grams of the sample is placed into the homogenized porcelain ash dish, then placed in an oven at 100°C for 24 hours. The porcelain ash dish is transferred to the combustion furnace, and the temperature is gradually increased to approximately 550°C (±5°C). Maintain this temperature for 8 hours/overnight until white ash is obtained. Upon completion, the temperature of the combustion furnace is lowered to 40°C. The porcelain ash dish is removed using tongs and allowed to cool. Once cooled, the weight of the porcelain ash dish is measured (B grams). The ash content can be calculated using equation (3).

$$\text{Ash Content (\%)} = \frac{B-A}{\text{Sample (g)}} \times 100 \% \dots\dots\dots (3)$$

Notes:

A = Weight of the porcelain dish, in grams

B = Weight of the dish with ash, in grams

Total Lactic Acid Content

The percentage of total lactic acid was calculated using the method described by Nugroho (2023), which involves preparing a 5-gram sample of the yogurt to be titrated. The 5-gram sample was placed in a 100-ml volumetric flask, then distilled water was added up to the mark and the mixture was homogenized. Add 2 drops of 1% phenolphthalein (PP) to the sample before titration. Titrate with 0.1 N NaOH solution until the color turns to a constant pink. The total lactic acid content can be calculated using equation (4)

$$\text{Lactic Acid Content (\%)} = \frac{\text{Vol NaOH} \times \text{N NaOH} \times \text{FP} \times \text{BM}}{\text{Sample Weight (mg)}} \times 100 \% \dots\dots\dots (4)$$

Notes:

MW = Molecular Weight

DF = Dilution Factor

N = Normality

Vol = Volume

Fat Content

The percentage of Fat content can be calculated using the method described by Soeparyo et al. (2018). One gram of the sample was prepared and placed in a 10-ml graduated test tube, and chloroform was added to near the mark. The tube is then tightly sealed, homogenized, and left for approximately 3 hours. The fat is then brought up to the 10 ml mark with the same fat-soluble solvent using a dropper pipette, homogenized again, and filtered through filter paper into a test tube. The sample is pipetted into a pre-weighed dish (A grams) and then oven-dried at 100°C for approximately 45 minutes, after which it is cooled. Once cooled, the fat dish is weighed (B grams). The fat content can be calculated using The Equation (5)

$$\text{Lipid Content (\%)} = \frac{B-A}{\text{Sample (g)}} \times 100 \% \dots\dots\dots (5)$$

Notes:

A = Weight of the dish, in grams

B = Weight of the dish with Fat, in grams

Viscosity

The viscosity percentage was calculated using the method described by Bayu et al. (2017). An empty pycnometer was weighed to determine its mass. A sample was drawn using a 10-ml volumetric pipette, then placed into the pycnometer and weighed. A 15-ml sample was pipetted using a 10-ml volumetric pipette and placed into an Ostwald tube. The sample was drawn into the Ostwald tube up to the upper calibration mark, and the time it took for the sample to fall to the lower calibration mark was measured using a stopwatch. The viscosity was then calculated using the formula. The viscosity level can be determined using equation (6) and (7)

$$\rho \text{ sampel} = \frac{m'-m}{v} \dots\dots\dots (6)$$

$$\text{Viscosity (cp)} = \frac{\rho \text{ sampel} \times t \text{ sampel} \times \eta \text{ air}}{\rho \text{ air} \times t \text{ air}} \dots\dots\dots (7)$$

Notes:

m = Mass of empty pycnometer (g)

m' = Mass of pycnometer + sample (g)

v = Volume of pycnometer (ml)

η = Viscosity of water (0.1 cP)

ρ = Density (g/ml) (water = 1 g/ml)

t = Time (s)

pH Test

The pH test was performed on samples weighing 25 grams each. The samples were placed in Erlenmeyer flasks, and the pH of each sample was measured using a pH meter.

RESULTS AND DISCUSSION

Protein Content

Protein content was determined using the Kjeldahl method, as this method is commonly used for protein analysis in food and beverages. The Kjeldahl method works by converting organic compounds into inorganic compounds (Rosaini et al., 2017). The protein content results for Pigeon peas peanut yogurt are shown in Figure 1.

The histogram data in Figure 1 show that the protein content of Pigeon peas peanut yogurt treated with powdered culture is higher. This is consistent with the finding (Purwanti et al., 2022) that the protein content of powdered culture is higher because its production process involves the use of specific bacterial starters, such as *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus thermophilus*, *Streptococcus thermophilus*, *Bifidobacterium lactis*, and *Bifidobacterium animalis*, which are known to increase protein levels in yogurt.

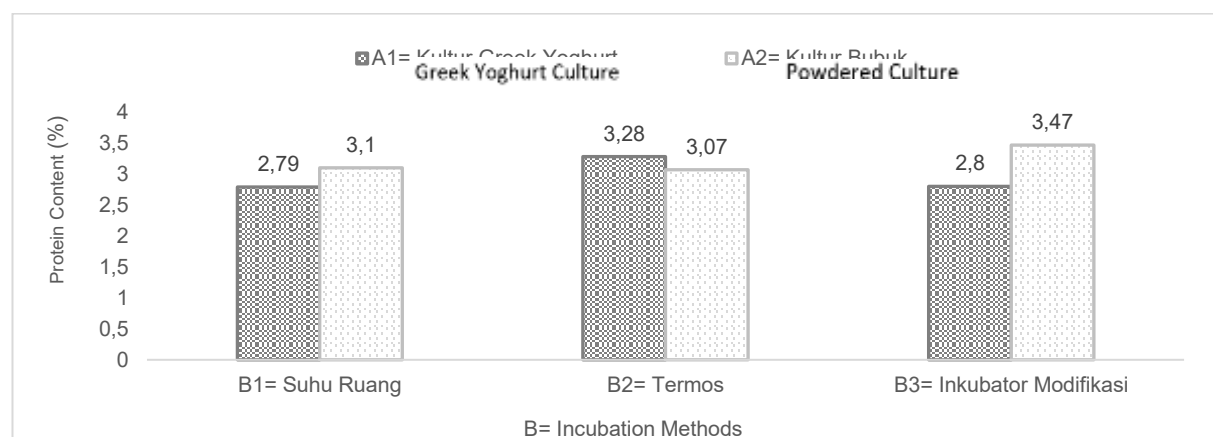


Figure 1. Protein Content (%) of Pigeon peas peanut yogurt treated with Greek Yogurt Culture (A1) and Powdered Culture (A2) across three incubation methods: Room temperature (B1), Thermos container (B2), and Modified incubator (B3).

Moisture Content

Moisture content is an important parameter of a food product because it is closely related to the product's shelf life. Moisture content can affect the appearance, texture, and flavor of food ingredients (Fikriyah and Nasution, 2021). The moisture content results for Pigeon peas yogurt are shown in Figure 2.

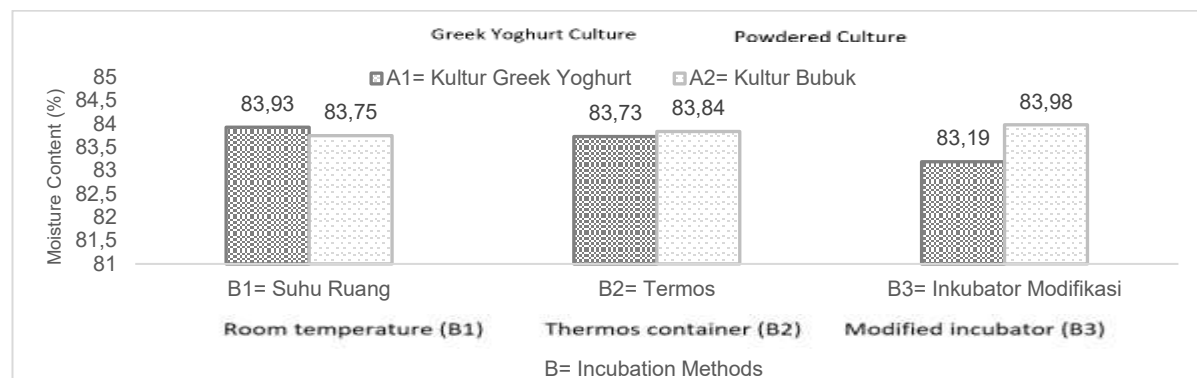


Figure 2. Moisture Content of Yogurt Under Different Lactic Acid Bacteria Cultures and Incubation Methods

The histogram in Figure 2 shows that Greek yogurt culture has a low moisture content, resulting in a relatively long shelf life. This is consistent with the finding (Putri et al., 2021) that Greek yogurt culture has a lower moisture content than powdered culture due to the production process, which involves whey filtration. This whey filtration produces yogurt with a firmer texture and reduces the moisture content.

Ash Content

Ash content is the residue remaining from a food sample that has been completely incinerated during the ashing process to break down the remaining organic and mineral compounds (Smith et al., 2023). The ash content results for Pigeon peas peanut yogurt are shown in Figure 3.

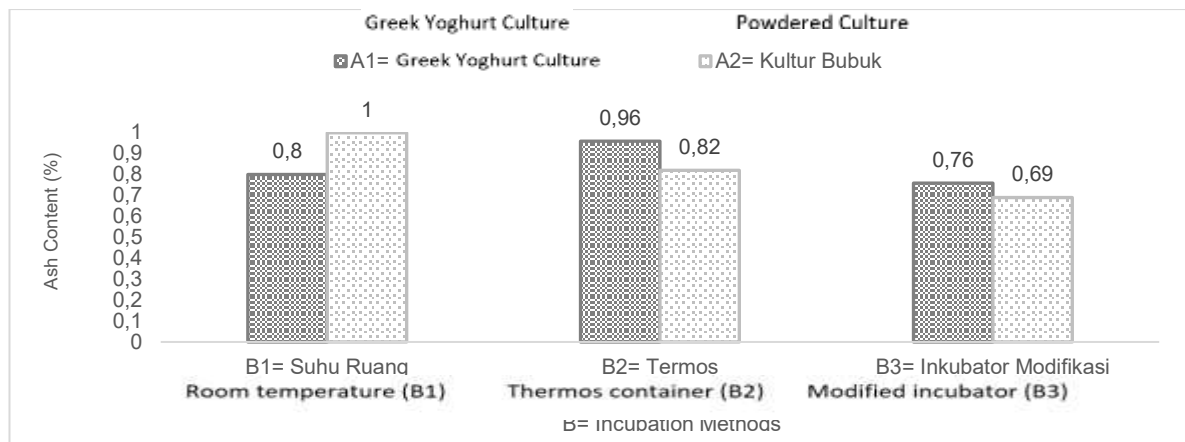


Figure 3. Ash Content of Yogurt Under Different Lactic Acid Bacteria Cultures and Incubation Methods

Figure 3 shows the ash content data for Pigeon peas peanut yogurt, where the lowest ash content was observed in the modified incubator treatment. This aligns with the findings of (Junita et al., 2023), who noted that the fermentation process of milk into yogurt produces not only lactic acid but also minerals such as magnesium as byproducts. An increase in ash content may indicate contamination from (dust, soil, or other foreign materials), the use of raw materials that do not meet standards, and a suboptimal yogurt production process.

Total Lactic Acid

Lactic acid is a chemical compound produced during the body's metabolic processes; it can also be produced by lactic acid bacteria during fermentation (Adrianto et al., 2020). The total lactic acid content of Pigeon peas peanut yogurt is shown in Figure 4.

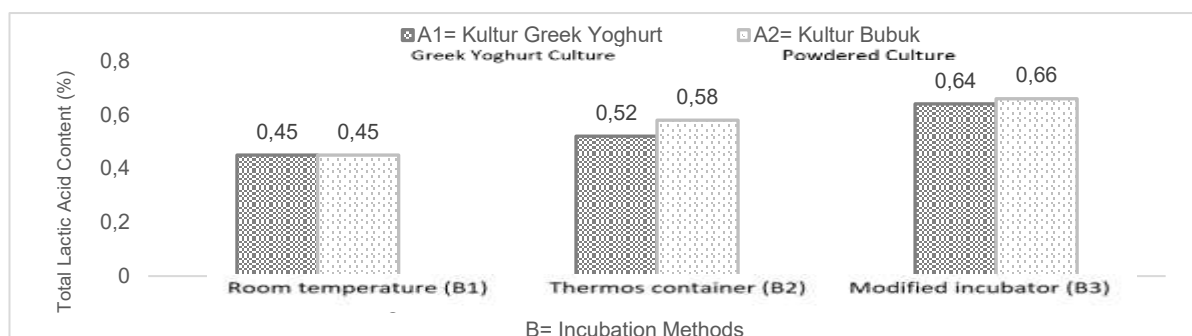


Figure 4. Total Lactic Acid Content of Yogurt Under Different Lactic Acid Bacteria Cultures and Incubation Methods

Figure 4 show that the powdered culture treatments using the room-temperature incubation, thermos, and modified incubator methods resulted in an increase in total lactic acid from 0.45% to 0.66% in Pigeon peas peanut milk yogurt. This is consistent with the statement by Nugroho (2023), who noted that total acid content increases due to the activity of *Lactobacillus acidophilus* (BAL), which breaks down lactose and simple sugars into lactic acid. During their growth phase, the bacteria utilize lactose as an energy source.

Fat Content

Fat content refers to the percentage of fat in a food product, which is used to ensure food safety and quality. Testing the fat content of a food product can be done by extracting the fat using the oven-drying method (Sirait and Nasution, 2024). The fat content results for Pigeon peas peanut yogurt are shown in Figure 5.

Figure 5 shows the effect of fat content on the culture of Pigeon peas peanut yogurt using a gradually reduced incubation method. This aligns with the findings of Purba et al. (2023), who noted that low-fat yogurt is considered superior due to its lower calorie and fat content. Low-fat yogurt is rich in essential nutrients such as protein, which is beneficial for overall health and the immune system.

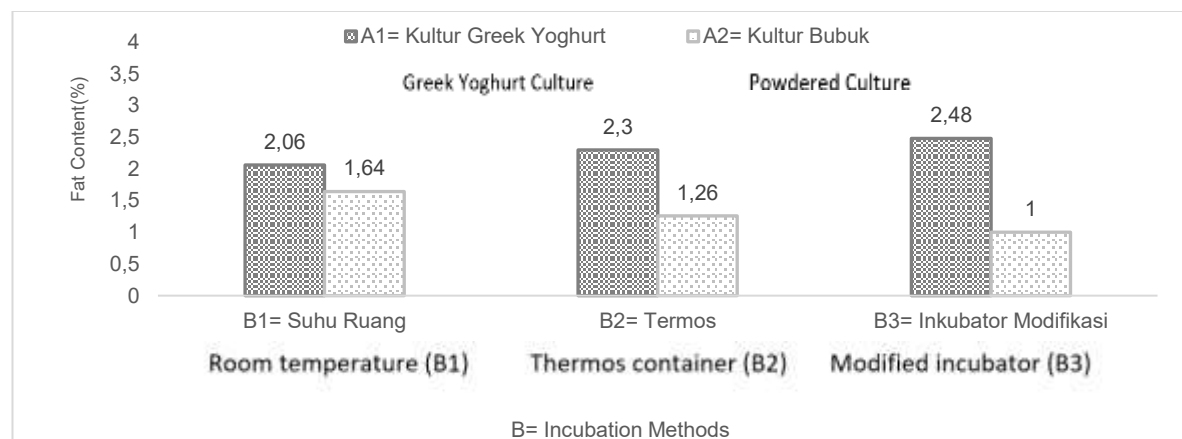


Figure 5. Fat Content of Yogurt Under Different Lactic Acid Bacteria Cultures and Incubation Methods

Viscosity

Viscosity is a property of liquids used to determine changes in the thickness of the resulting yogurt. The increase in yogurt viscosity is caused by bacteria, higher total acidity, and the gel formed during the fermentation process (Evadewi, 2021). The results of the viscosity measurements for Pigeon peas peanut yogurt are shown in Figure 6.

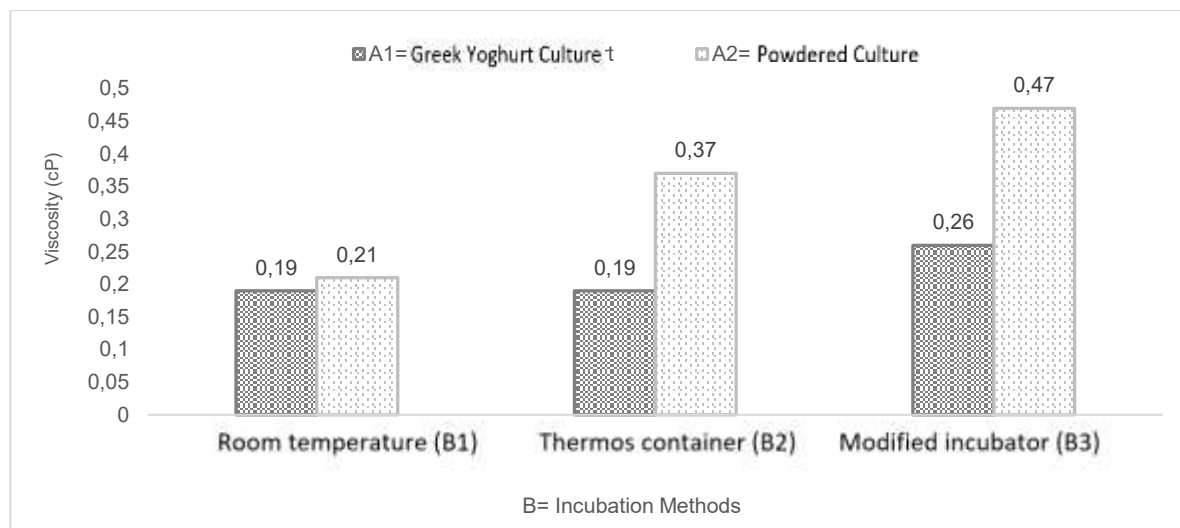


Figure 6. Fat Content of Yogurt Under Different Lactic Acid Bacteria Cultures and Incubation Methods

The data in Figure 6 show that there was an increase in the viscosity of Pigeon peas peanut yogurt in the Greek yogurt culture and powdered culture treatments. The highest viscosity value was found in the powdered culture treatment using the modified incubator method, which employed a combination of ten probiotic strains: *Lactobacillus bulgaricus*, *Lactobacillus cidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus thermophilus*, *Streptococcus thermophilus*, *Bifidobacterium lactis*, and *Bifidobacterium animalis*, which influenced the viscosity of the yogurt. The addition of *Lactobacillus casei* to the powdered culture also affected the viscosity of the yogurt. This is consistent with the statement by Arzakayah et al. (2024) that *Lactobacillus casei* is an important probiotic bacterium in the fermentation process because it can produce lactic acid up to 90 cP and other organic acids. Higher levels of lactic acid cause casein to coagulate and form a gel. The more gel that forms, the higher the viscosity of the yogurt.

pH Value

The acidity or alkalinity of a solution is determined by its pH value. The pH value is determined in accordance with international standards (Hariyadi et al., 2020). The results of the pH analysis of Pigeon peas peanut yogurt are shown in Figure 7.

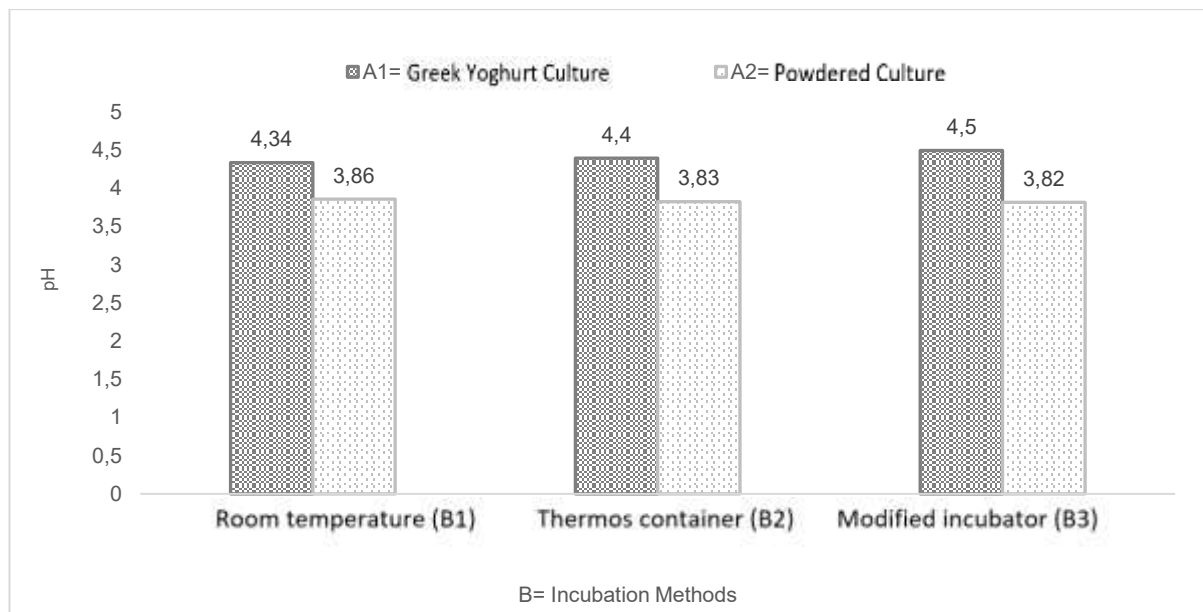


Figure 7. Fat Content of Yogurt Under Different Lactic Acid Bacteria Cultures and Incubation Methods

The pH values shown in Figure 7 indicate that the powdered culture treatment resulted in a decrease in pH in the Pigeon peas peanut yogurt. The pH value in the powdered culture treatment was lower than that in the Greek yogurt culture. This is consistent with the statement by Syafitri et al. (2022) that lactic acid bacteria growth in powdered cultures is more efficient, and the breakdown of the substrate into lactic acid is maximized, resulting in a lower pH. The powdered culture used contains several strains. These strains are known to produce lactic acid, which causes the product's pH to decrease.

Organoleptic

Organoleptic testing is a method that utilizes human senses to measure the level of acceptance of a product (Gusnandi et al., 2021). Organoleptic tests are conducted to determine panelists' preferences regarding specific characteristics such as color, aroma, taste, and consistency (Jonathan et al., 2022). The fat content results for Pigeon peas peanut yogurt are shown in Figure 8.

The results of the organoleptic test showed that the best average color was found in the modified incubator powder culture treatment, with an average score of 4 (category: like). Raw materials, storage duration, incubation temperature, and starter type influenced the resulting color. The color of the yogurt produced was nearly identical, as the raw materials used in making

this yogurt had similar compositions, so they did not significantly affect color formation. Sensory evaluation revealed that plain yogurt has a bone-white color. This aligns with the statement by Umaroh (2018) that the amount of added ingredients affects the final product. For aroma assessment, the best average score was found in the modified incubator powder culture treatment, with an average score of 4 (category: like). Pigeon peas red bean milk yogurt produced using the modified incubator method with powdered culture exhibits a sour aroma. This aligns with the statement by Pamela (2022) that bacteria can grow due to temperature and available nutrients; to meet these requirements, red bean milk is added because it contains protein (nitrogen), which microorganisms need to multiply

The average score from the organoleptic test for texture showed that the best texture rating was obtained from the powdered culture treatment using the modified incubator incubation method, with an average score of 5 (Category: very much like). Lactic acid bacteria influence the texture of yogurt. This is due to a decrease in pH, which causes the yogurt to become a thick liquid or semi-solid. Additionally, moisture content also affects yogurt texture. The lower the yogurt's moisture content, the thicker the resulting yogurt. Pigeon peas peanut milk yogurt in the powdered culture treatment using the modified incubator method has a thick texture. This aligns with the statement by Pamela (2022) that yogurt texture is influenced by the use of ingredients and fermentation temperature. The texture of red bean yogurt can also be influenced by the viscosity and pH of the yogurt.

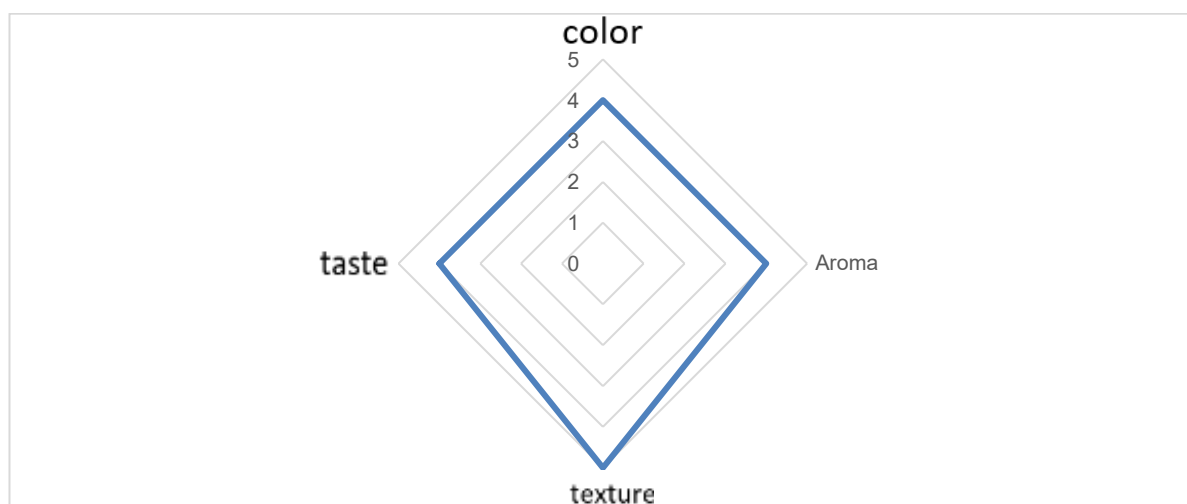


Figure 8. Organoleptic test results for Pigeon peas Peanut Yogurt regarding color, aroma, texture, and taste



In the taste evaluation, the best score was obtained from the modified incubator powdered culture treatment, with an average score of 4 (Category: like). The taste of the yogurt tends to be sour. The sour taste stems from the presence of organic acids, such as lactic acid, produced during the fermentation process. As the number of lactic acid bacteria in yogurt increases, so does the metabolic output—or the enzymatic and chemical reactions—that produce the flavor. This aligns with the findings of Rasbawati et al. (2019), who noted that the taste of yogurt is caused by the fermentation process. Lactic acid bacteria ferment carbohydrates to form lactic acid. The formation of lactic acid leads to an increase in acidity.

CONCLUSION

Based on the results of the tests and discussions that have been described, this study indicate that the BAL culture treatment had a significant effect on protein content, moisture content, fat content, viscosity, and pH. Meanwhile, the incubation method treatment had a significant effect on protein content, moisture content, ash content, total lactic acid, fat content, viscosity, and pH. The best quality of Pigeon peas peanut yogurt was obtained from the powdered culture treatment using the incubation method with a modified incubator, yielding a protein content of 3.47%; ash content of 0.69%; total lactic acid of 0.66%; fat content of 1%; viscosity of 0.47 cP; pH of 3.82; and moisture content of 83.19%. The results of the organoleptic test showed that the color, aroma, and taste scores met the “liked” criteria with an average score of 4, while the texture was classified as “very liked” with an average score of 5.

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