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Corn Milk Yoghurt: Nutritional Composition Analysis Based On Fermentation Starter Variatons

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Abstract

This study explores the nutritional profile of corn milk yoghurt developed using different fermentation starters. Corn (Zea mays L.), rich in carbohydrates, protein, and essential micronutrients, offers a sustainable and affordable plant-based alternative to dairy in yoghurt production. With increasing consumer demand for functional, health-promoting foods and the challenges of animal milk availability, corn milk presents a promising base for fermented products. This experimental research applied a Completely Randomized Design (CRD) with four treatments: Lactobacillus bulgaricus, Lactobacillus casei, a combination of both, and a control (no starter). The objective was to evaluate the impact of starter variation on lactic acid content, protein concentration, and fat levels in the resulting yoghurt. Findings revealed that the combined culture produced the highest lactic acid level (0.7%), while yoghurt fermented solely with L. bulgaricus yielded the highest protein content (3.6%) and the lowest fat content (1.05%). Although the lactic acid content fell below the Indonesian National Standard (SNI) threshold, all yoghurt variants met the SNI minimum for protein and were classified as low-fat yoghurts. These outcomes indicate that fermentation starter selection significantly influences the nutritional characteristics of corn milk yoghurt. Moreover, the study demonstrates the potential of corn milk as a functional food base that aligns with both nutritional standards and sustainable food innovation. L. bulgaricus emerged as the most effective starter, optimizing both protein enrichment and fat reduction. These insights may guide future development of non-dairy fermented products for health-conscious and lactose-intolerant populations.

Keywords: Corn Milk Yogurt; Fermentation Starter; *Lactobacillus Bulgaricus; Lactobacillus Casei*; Nutritional Composition

Introduction

Maize (*Zea mays* L.) is one of the world's primary cereal commodities and plays a significant role in food security. It serves not only as a major source of carbohydrates but also provides essential proteins, fibers, vitamins, and minerals (Erenstein et al., 2022). In Indonesia, maize

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is the second most important staple food after rice and is a vital component in both food and feed industries (Naisali et al., 2023). With the growing public awareness of health and nutrition, the development of functional food products based on local ingredients has become increasingly relevant.

Yogurt is a fermented milk product widely appreciated for its probiotic benefits, particularly in promoting digestive and immune health (Sugito & Sutriswanto, 2018) . Traditionally, yogurt is produced from animal milk; however, limitations in milk supply, high production costs, and consumer preference for plant-based alternatives have encouraged the exploration of non-dairy ingredients. Plant-based milks such as soy, almond, and oat have been extensively utilized in developing yogurt-like fermented products (Arise et al., 2022). In this context, corn milk offers significant potential as a fermentation substrate due to its abundance, unique nutritional profile, and lower production cost compared to animal milk (Tshering et al., 2024).

The production of corn milk involves an extraction process that yields a nutrient-rich liquid, though its sensory and functional characteristics still require optimization. Fermentation is key to transforming corn milk into yogurt, with the fermentation starter playing a central role. Various types of lactic acid bacteria (LAB) starters, particularly *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, are known to ferment sugars into lactic acid. This not only preserves the product but also contributes to its texture, aroma, and distinctive taste (YASNI & MAULIDYA, 2014). However, variations in starter strains and combinations can significantly affect the acidity profile, volatile compound production, and physicochemical characteristics of the final product, all of which directly influence its nutritional composition (Dan et al., 2023).

This study aims to comprehensively analyze the nutritional composition of corn milk yogurt fermented with different starter cultures. The analysis focuses on macronutrients (carbohydrates, proteins, fats) and examines potential changes in micronutrients and bioactive compounds resulting from microbial activity during fermentation. The findings are expected to provide valuable scientific insights into the feasibility of corn milk as a functional yogurt base and offer guidance on selecting suitable starter cultures to optimize nutritional quality and product performance.

Literature Review

1. The Potential of Corn Milk as a Fermentation Substrate

Corn (*Zea mays L.*) is one of the world's leading cereal commodities, valued not only as a carbohydrate source but also for its content of protein, fiber, vitamins, and essential minerals (Erenstein et al., 2022). In Indonesia, corn ranks as the second most important staple after rice (Naisali et al., 2023). Corn milk, extracted from sweet corn kernels, presents a promising plant-based alternative for fermentation due to its high availability, unique nutritional profile, and relatively low production cost compared to animal milk (Tshering et al., 2024).

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2. Plant-Based Yogurt and the Role of Fermentation

Traditional yogurt, made from dairy milk, is widely consumed for its probiotic benefits that support gut health and immunity (Sugito & Sutriswanto, 2018). However, limitations in dairy supply and growing consumer preference for plant-based diets have driven innovation in non-dairy yogurt alternatives using soy, almond, oat, and more recently, corn milk (Arise et al., 2022). The fermentation process, particularly by lactic acid bacteria (LAB) such as *Lactobacillus bulgaricus* and *Lactobacillus casei*, is key to converting plant substrates into yogurt-like products with desirable texture, aroma, and nutritional enhancement (Dan et al., 2023).

3. Fermentation Starters and Nutritional Quality

The type and combination of fermentation starters significantly affect the physicochemical and nutritional quality of the final yogurt product. Different strains of LAB exhibit distinct metabolic pathways that influence the levels of lactic acid, protein, and fat (Xu et al., 2015; McFarland, 2015). *L. bulgaricus* is known for its ability to increase protein content through microbial growth and biosynthesis (Mardiana et al., 2020), while *L. casei* contributes to acid production and has high tolerance to fermentation conditions (Zhaoping et al., 2012). Combining both strains often leads to synergistic effects that improve enzymatic activity and overall nutrient density (Widodo et al., 2019).

4. National Standards and the Significance of Quality Assessment

According to the Indonesian National Standard (SNI, 2009), yogurt products are classified by fat and protein content into standard, low-fat, and non-fat categories. Evaluating plant-based yogurt against these benchmarks is crucial for ensuring consumer safety, nutritional adequacy, and industrial competitiveness. Moreover, adhering to quality standards can enhance the market viability of functional food products made from local ingredients such as corn milk (Behm et al., 2022).

Research Methodology

Type and Time of Research

This study was conducted in February at the Central Laboratory of Health (Balai Besar Laboratorium Kesehatan), Makassar. The research employed a quantitative experimental design to examine the effect of different fermentation starters on the quality of corn milk yogurt. The experimental setup followed a Completely Randomized Design (CRD) with three treatments and one replication for each treatment.

The treatments were as follows:

LB : Corn Milk + Lactobacillus bulgaricus

LC : Corn Milk + Lactobacillus casei

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LC + LB : Corn Milk + combination of both starters

Control : Corn milk without any starter

Equipment

The equipment used included: stirrers, analytical balances, thermometers, volumetric pipettes, burettes, Erlenmeyer flasks, beakers, boiling stones, tissue paper, droppers, Kjeldahl flasks, laminar air flow cabinet, electric heaters, fume hoods, Markham stills, porcelain dishes, and ovens.

Materials

The chemical reagents used included distilled water (aquadest), 0.1 N sodium hydroxide (NaOH), sulfuric acid (H_2SO_4), 0.1 N hydrochloric acid (HCl), phenolphthalein indicator, selenium mixture, boiling stones, boric acid (H_3BO_3), and chloroform.

Preparation of Corn Milk

Sweet corn was peeled, and the silk was removed, followed by thorough washing. The kernels were separated to obtain 700 grams of sweet corn. These kernels were then blended with water in a 1:6 ratio (corn:water). The blended mixture was filtered to extract pure corn milk.

Experimental Procedures

a. Preparation of Fermentation Medium

1) A total of 2000 ml of corn milk was heated and supplemented with 5% (b/v) granulated sugar and 15% (b/v) skim milk powder.

2) The mixture was pasteurized at 90°C for 15 minutes, then cooled to 40–43°C

b. Fermentation Stage

1) Starter cultures of *Lactobacillus bulgaricus* and *Lactobacillus casei* were prepared. Once the medium reached 45°C, 10 ml of each starter culture was added to the fermentation samples with the following treatments:

   a. Treatment I: 500 ml corn milk + 10 ml *L. bulgaricus*

   b. Treatment II: 500 ml corn milk + 10 ml *L. casei*

   c. Treatment III: 500 ml corn milk + 5 ml *L. bulgaricus* + 5 ml *L. Casei*

2) The mixtures were stirred thoroughly and transferred to sterile containers, sealed with aluminum foil, and incubated at 45°C for 8 hours. After incubation, the samples were immediately refrigerated to halt further fermentation.

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Yogurht Analisys

The parameters analyzed included proximate composition (protein, fat) and total acidity (expressed as % lactic acid). Total acidity was measured via titration using 0.1 N NaOH. Proximate analysis followed the procedures outlined by AOC (Aricetti & Tubino, 2012) .

a. Chemical Analysis

a). Lactic Acid Determination

1. Weigh 10 g of yogurt sample into a 100 ml Erlenmeyer flask..
2. Dilute with distilled water to twice the original volume.
3. Add 4 drops of phenolphthalein and titrate with 0.1 N NaOH until a color change indicates the endpoint.
4. Calculate total acidity as % lactic acid using.

$$\text{Lactic Acid (\%)} = \frac{\text{A x B x 0,009 x 90.08}}{\text{C}}$$

Where

A = ml NaOH 0,01 N

B = normality of NaOH (0,09142 mgrek/ml)

C = weight of sample (gr)

90.08 = molar mass of lactic acid (mg/mgerk)(Czauderna & Kowalczyk, 2008)

b). Protein Content (Kjeldahl Method)

1. Weigh 0.5 grams of sample and place it in a Kjeldahl flask. Then, add 15 ml of H2SO4 (sulfuric acid), 0.5 g of selenium mixture, and a boiling stone.
2. Heat the mixture in an electric heater until boiling, until the solution becomes clear and greenish in color. Do this in a fume hood.
3. Cool and dilute with 200 ml of distilled water.
4. Transfer to a distillation flask.
5. Add 5 drops of 0.1% PP (Phenolphthalein) indicator.
6. Add NaOH (Sodium Hydroxide) Pellets, stirring until the solution turns red.

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7. Collect the distillation results with 20 ml of H3BO3 (Boric Acid) until the distillate volume reaches 100 ml.
8. Add Bromcresol Green indicator.
9. Titrate with 0.1 N HCl (Hydrochloric Acid) until a purple color forms.

Determination of Blank Content

1. Weigh 0.5 g of the sample into a Kjeldahl flask.
2. Add 15 ml H_2SO_4, 0.5 g selenium mixture, and boiling stones.
3. Digest until the solution becomes clear green.
4. Dilute with 200 ml distilled water and distill the solution.
5. Add 5 drops of phenolphthalein and sodium hydroxide pellets.
6. Distillate is collected in 20 ml of 4% boric acid and titrated with 0.1 N HCl using Bromocresol Green.

$$\text{Protein Content} = \frac{(\text{A}-\text{B}) x\ 0{,}09234\ x\ 14{,}008\ x\ 6{,}25}{Sample\ weight} x\ 100\%$$

Where

A = ml titran sampel (ml)

B = ml titran blanko (ml)

0.09234 = Normalitas HCl 0.1 N (mgrek/ml)

14.008 = Berat molekul Nitrogen (mg/mgrek)

6.25 = Faktor protein untuk susu (Fujihara et al., 2009)

c). Fat Content (Chloroform Extraction Method)

1. Heat a porcelain dish for approximately 1.5 hours at 105°C.
2. Weigh the fixed weight of the porcelain dish (e.g., 1 gram).
3. Place 10 grams of sample in a separating funnel and add 15 ml of chloroform. Then, extract the sample and let it separate.
4. Collect the bottom layer in a porcelain dish of known weight.
5. Wash the top layer again with 10 ml of chloroform and extract until dissolved.
6. Collect the bottom layer in a porcelain dish.

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7. Place the porcelain dish containing the extract in an oven and evaporate it at approximately 105°C for 1.5 hours.
8. Weigh the evaporated porcelain dish.

   Can be calculated using the formula ;

$$\text{Fat} = \frac{b-a}{c} x\ 100\%$$

Description :

b = Weight of flask + fat (gr)

a = Weight of empty flask (gr)

c = Weight of sample(gr)(Fujihara et al., 2009)

Results and Discussion

Research Findings

The quality of corn milk yogurt was evaluated based on several parameters, including lactic acid content, protein content, and fat content.

Table 1.1 **Effect of Fermentation Starter on the Quality of Corn Milk Yogurt**

Sampel Code	Test Result		
	Laktit Acid (%)	Protein (%)	Fat (%)
LB	0.5	3.6	1.051
LC	0.4	3.25	1.33
LB + LC	0.7	3.31	1.05
Kontrol	0.2	3.03	1.33

Source: Primary data processed, 2014

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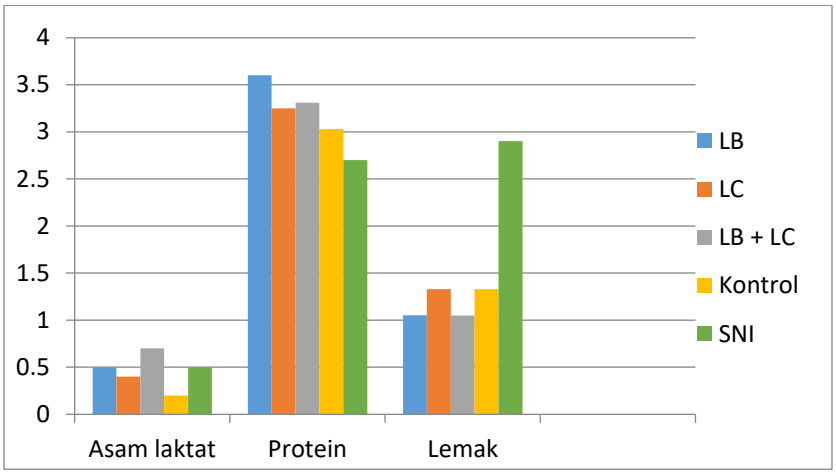


Figure 1.1 Bar Chart Presentation for Viewing the Quality of Corn Milk Yogurt.

Description:

Lb : *Lactobacillus bulgaricus*

Lc : *Lactobacillus casei*

Lb + Lc : Combination of *L. bulgaricus* and *L.*

Control : Corn milk without starter

SNI : Indonesian National Standard

1. Lactic Acid Content

Titratable acidity (TAT) in yogurt is commonly expressed as a percentage of lactic acid. Lactic acid ($C_3H_6O_3$) is the primary acid produced during the fermentation of milk into yogurt and contributes significantly to its characteristic flavor (Afriani, 2010). In this study, diluted corn milk yogurt samples were titrated using phenolphthalein as an indicator. The titration endpoint was marked by a color change, indicating a shift in pH due to acid neutralization by sodium hydroxide (NaOH).

In the lactic acid test, the PP (Phenolphthalein) indicator is added to the corn yogurt diluted in the Erlenmeyer flask. This indicator plays a crucial role in distinguishing between acidic and basic solutions and marks the endpoint of the titration. Next, the NaOH (Sodium Hydroxide) solution in the burette is slowly dripped into the Erlenmeyer flask containing the corn yogurt. The observed color change, from clear to pink or reddish purple, indicates a significant pH change in the corn yogurt, which validates the PP indicator's function as an indicator.

Fermentation leads to increased acidity as lactose is broken down by bacteria into lactic acid. Prolonged fermentation—up to an optimal point—encourages bacterial growth and intensifies lactose conversion (Akira, 2012). Higher concentrations of skim milk also increase lactic acid levels due to greater lactose availability(Zhaoping et al., 2012).

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According to SNI 2009, lactic acid content in yogurt should range from 0.5% to 2%. Table 1.1 shows that lactic acid levels in the corn milk yogurt were: 0.5% (LB), 0.4% (LC), 0.7% (LB+LC), and 0.2% (control). Although the LB+LC combination achieved the highest value, all values were below the minimum required standard. The low levels suggest that corn milk yogurt in this study did not meet the SNI standard for acidity.

Corn milk served as a control to assess the effectiveness of different starter cultures. While starter treatments showed increased acidity compared to the control (0.2%), the levels were still relatively low. In general, high acidity or low pH in fermented products reflects a greater degree of lactose conversion(Garrote et al., 2015). The variation in lactic acid content can be attributed to the efficiency and strain specificity of the starter cultures (Widodo et al., 2019). as well as to differences in Each starter strain has unique metabolic capabilities, which explains the variation in acid production (Xu et al., 2015). Higher concentrations of starter culture typically result in more microorganisms, which increases lactose metabolism and leads to greater lactic acid accumulation (McFarland, 2015).

2. Protein Content

Protein is an essential macronutrient necessary for energy, tissue repair, and the regulation of bodily functions (Whitney et al., 2023). Microbial cell composition includes a significant proportion of proteins, with microbial proteins accounting for about 15% of total cell mass(Madigan et al., 2018). Therefore, protein content in yogurt is influenced by the number and activity of microorganisms present. Higher microbial growth generally correlates with higher protein levels due to microbial biomass (Mardiana et al., 2020).

Protein analysis was conducted using the Kjeldahl method, which measures nitrogen content in the sample through digestion, distillation, and titration(Vinklárková et al., 2015). The nitrogen is converted to ammonia, trapped in boric acid, and titrated with HCl, allowing estimation of total protein(Sáez-Plaza et al., 2013).

The protein content in yogurt is largely determined by the initial protein composition of the raw materials used, such as corn milk. The higher the protein concentration in the raw materials, the higher the protein content in the final yogurt product (Boeck et al., 2021). In the context of this study, the corn used has a relatively high protein content, around 10%.

Skim milk is known as the milk residue after some or all of the cream (milk fat) has been removed. Skim milk is rich in protein and is often referred to as "whey milk" due to its significant protein concentration after fat separation. (Tamime & Robinson K, 2007).

Based on the 2009 Indonesian National Standard (SNI), the minimum protein content in yogurt is 2.7%. The results of the protein content analysis in corn yogurt from this study showed that all three samples—yogurt with *Lactobacillus bulgaricus* (3.6%), *Lactobacillus casei* (3.25%), and a combination of *Lactobacillus bulgaricus* and *Lactobacillus casei* (3.31%)—met the protein standards set by the 2009 SNI.

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In this study, corn milk also served as a control to compare the quality of the three yogurt types. Although the initial corn milk protein content was 3.09%, all corn yogurt samples produced in this study showed increased protein levels compared to the control.

3. Fat Content

Based on the 2009 Indonesian National Standard (SNI), the fat content in yogurt is categorized into three groups: standard yogurt with a minimum fat content of 3.0%, low-fat yogurt ranging from 0.6% to 2.9%, and non-fat yogurt with a maximum of 0.5%. In this study, the yogurt produced was classified as low-fat yogurt. The analysis of the fat content of corn yogurt showed that all three samples—*Lactobacillus bulgaricus* (1.051%), a combination of *Lactobacillus bulgaricus* and *Lactobacillus casei* (1.05%), and *Lactobacillus casei* (1.33%)—met the SNI 2009 standards established for low-fat yogurt..

Corn milk was used as a control in this study to compare the quality of the three yogurt types. The initial fat content of corn milk was 1.33%. Interestingly, two of the three yogurt variants showed a decrease in fat content compared to the control corn milk: yogurt with *Lactobacillus bulgaricus* (1.051%) and yogurt with a combination of *Lactobacillus bulgaricus* and *Lactobacillus casei* (1.05%). Meanwhile, yogurt with *Lactobacillus casei* had the same fat content as the control, at 1.33%.

The fat content of yogurt also depends heavily on the type of milk used as the raw material. Although corn and skim milk contribute fat, they do so in relatively low amounts. Yogurt made from whole milk will naturally have a higher fat content than yogurt made from skim milk (Tamime & Robinson K, 2007). Considering that the corn yogurt in this study used skim milk as one of its ingredients, this also contributed to its low fat content.

Based on the results of three tests conducted on corn yogurt, it can be concluded that the quality of the resulting yogurt meets Indonesian National Standard (SNI) standards. For lactic acid levels, all samples met the standard, with the highest levels found in the yogurt with a combination of *Lactobacillus bulgaricus* and *Lactobacillus casei* starters, followed by the yogurt with a *Lactobacillus bulgaricus* starter. In terms of protein content, all yogurt variants also met the specified SNI standards. The highest protein content was observed in the *Lactobacillus bulgaricus* yogurt, followed by the combination of *Lactobacillus bulgaricus* and *Lactobacillus casei* starters, and finally, the yogurt with a *Lactobacillus casei* starter. Finally, regarding fat content, all yogurts meet SNI standards as low-fat products, with the order of fat content from lowest to highest being *Lactobacillus bulgaricus* yogurt, then a combination of L*actobacillus bulgaricus* and *Lactobacillus casei yogurt*, and finally *Lactobacillus casei* yogurt. The implementation of the Indonesian National Standard (SNI) for yogurt in this study is crucial. The establishment of SNI aims to ensure the health, safety, and security of humans, animals, and plants. Furthermore, SNI also serves to preserve the environment, encourage healthy business competition, enhance product competitiveness, and improve industrial efficiency and performance. In the context of the global market, such as facing the ASEAN

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Economic Community (AEC), the existence of SNI is essential to strengthen the competitiveness of domestic products. The results of this study confirm that the levels of lactic acid, protein, and fat in the corn yogurt produced are within the range specified by SNI, which also aligns with the body's nutritional needs.

Conclusion

Based on the findings of this study, it can be concluded that the type of fermentation starter culture significantly affects the quality of corn milk yogurt. Specifically, the combination of *Lactobacillus bulgaricus* and *Lactobacillus casei* produced the highest lactic acid content, reaching 0.7%. Meanwhile, the yogurt fermented with *Lactobacillus bulgaricus* alone exhibited the highest protein concentration at 3.6%. Regarding fat content, the yogurt treated with *Lactobacillus bulgaricus* also achieved the lowest fat level of 1.05%. These results suggest that *Lactobacillus bulgaricus* is the most effective starter culture for producing high-quality, nutritionally enhanced corn milk yogurt. Its application, whether individually or in combination with *L. casei*, offers an optimal approach to improving the functional and nutritional characteristics of plant-based yogurt alternatives.

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