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Microbiological and Physicochemical Properties of Legume-Based Yogurt Drink Formulated from Jack Bean (*Canavalia ensiformis* L.) Extract and Kidney Bean (*Phaseolus vulgaris* L.) Extract

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ABSTRACT

Yogurt is a fermented dairy product whose quality is largely governed by the metabolic activity of lactic acid bacteria (LAB). In recent years, the development of plant-based yogurt has gained attention as a strategy to diversify fermented products, particularly legumes. Jack bean and kidney bean are legumes rich in complex carbohydrates and proteins that may support LAB growth during fermentation. This study aimed to evaluate the microbiological and physicochemical properties of legume-based yogurt drinks from jack bean and kidney bean extracts. Yogurt drinks were fermented by Streptococcus thermophilus and Lactobacillus bulgaricus as starter cultures. The study employed a Completely Randomized Design with formulas which was divided of different ratio of jack bean extract and kidney bean extract (P1: 0%; P2: 80%:0%; P3: 0:80%; F1: 40%:40%; F2: 60%:20%; F3: 20%:60%). All formulations were evaluated for LAB viability, and the highest LAB formula count was analyzed for pH, lactic acid content, viscosity, solids-non-fat (SNF), and reducing sugar levels. The results showed that Formula 2 containing 60% jack bean extract and 20% kidney bean extract met all evaluated parameters of SNI 2981:2009 which exhibited the highest LAB viability with 10.70±0.09 log CFU/mL ($p<0.05$), exceeding the minimum requirement ($\geq$7 log CFU/mL). This formulation showed a pH of 4.23$\pm$0.00, lactic acid content of 1.954$\pm$0.03%, viscosity of 5.139 $\pm$ 0.05 cP, SNF content of 9.410 $\pm$ 0.09%, and relatively low reducing sugar content (2.017 $\pm$ 0.06%). These results suggest the combination of jack bean-kidney bean shows strong potential as raw materials for legume-based yogurt drinks with favorable microbiological viability and physicochemical properties.

Key Messages:

- Formula 2 (60% jack bean extract: 20% kidney bean extract) has the highest LAB viability
- Formula 2 has microbiological and physicochemical properties that meet requirements of SNI 2981:2009

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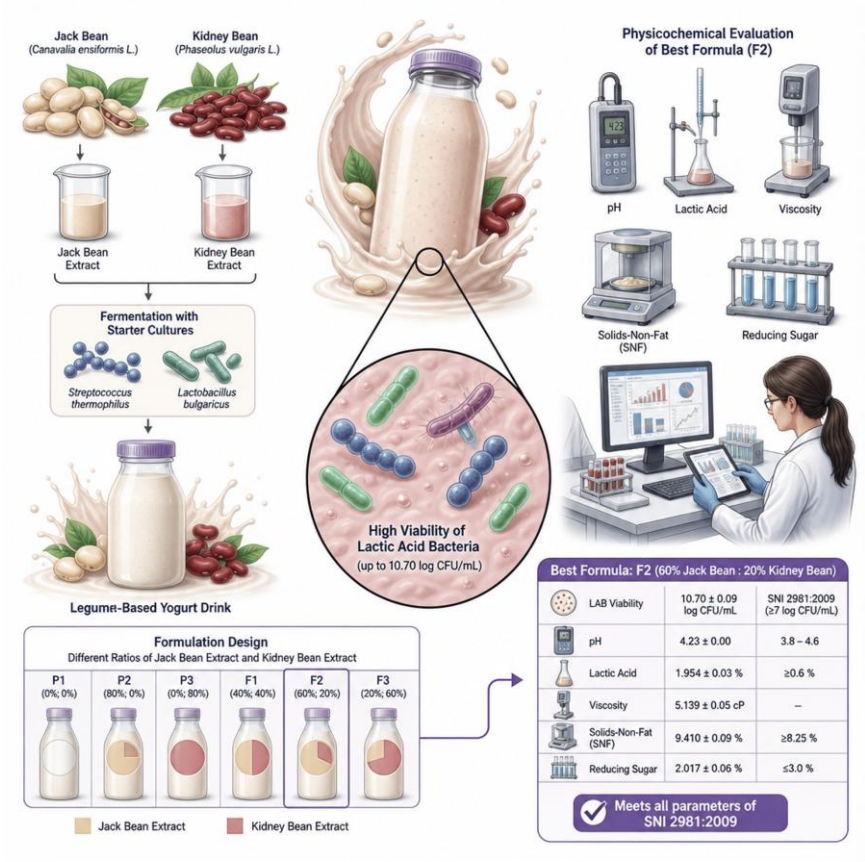
GRAPHICAL ABSTRACT



INTRODUCTION

Yogurt is a fermented dairy product produced through the metabolic activity of lactic acid bacteria (LAB), primarily *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, which convert lactose into lactic acid (1). This fermentation process induces pH reduction, protein gel formation, and the development of characteristic yogurt texture and flavor. According to BPOM classification, fermented milk products include fermented milk, stirred yogurt, and yogurt drinks, each exhibiting distinct quality attributes (2).

Yogurt quality is defined by measurable physicochemical and microbiological parameters. The Indonesian National Standard (1) (SNI 2981:2009) specifies key quality requirements, including lactic acid content of 0.5–2.0%, solids-non-fat content of at least 8.2%, and LAB viability not less than 7 log CFU/mL. These parameters serve as essential indicators of fermentation efficiency, product stability, and microbiological safety.

Recent yogurt innovations have increasingly explored the use of plant-based ingredients for dairy alternatives due to lactose intolerance or environmental concerns, particularly legumes. Previous studies have demonstrated that the substitution or combination of milk with legume-based materials can significantly influence LAB viability as well as the physicochemical and sensory properties of yogurt due to their bioactive compounds such as olygosaccharide, protein, and phytochemical components (3–7).

Jack bean is a locally available legume with high protein and carbohydrate contents, making it a promising raw material for yogurt production. However, the presence of cyanide and phytic acid as toxic agent and antinutritional compounds in raw jack bean necessitates appropriate processing, as these substances may cause toxicity and interfere with mineral bioavailability. Fermentation has been reported to effectively reduce these compounds while improving its functional properties (8–10). Although jack bean has considerable potential as a plant-based substrate for yogurt production, previous studies indicate that formulations based primarily on jack bean do not consistently achieve the required LAB viability standards. While improvements in viscosity and LAB growth have been observed, the fermentation

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performance remains suboptimal, suggesting that complementary substrates may be required to provide sufficient fermentable nutrients for LAB proliferation (11).

Combining jack bean with kidney bean represents a potential strategy to improve yogurt quality throught a synergistic formulation. Jack bean contributes high protein and carbohydrates that may support the formation of a stable protein matrix and viscosity (8), while kidney bean provides fermentable oligosaccharides, dietary fiber, and naturally sweet flavor that may enhance LAB growth and product acceptability (12–14). In addition, kidney bean contains phytochemicals such ad flavonoids and polyphenols, whose levels have been reported to increase during fermentation (15).

Despite extensive research on legume-based yogurt diversification, studies specifically addressing the combined use of jack bean and kidney bean and their interactive effects on yogurt physicochemical and microbiological characteristics remain limited. Previous investigation on jack bean-based yogurt have reported LAB viability levels below the minimum requirement specified by the Indonesia National Standard for yogurt (SNI 2981:2009; >7 log CFU/mL). Incorporating kidney bean as a complementary substrate may provide additional fermentable nutrients that support LAB growth and improve fermentation performance. Therefore, This study aims to determine the optimal ratio of jack bean and kidney bean extracts to produce a yogurt drink that meets SNI 2981:2009 quality standards, with particular emphasis on maximizing LAB viability and maintaining favorable physicochemical characteristics.

METHODS

This study employed an experimental research design using a Completely Randomized Design (CRD), with three replications for each formulation and two analytical measurements for all evaluated parameters. Jack beans were obtained from Rumah Canavalia (Bogor, Indonesia), while kidney beans (Angkasa®) were obtained from local supermarkets in Bogor. Product development and pH analysis were conducted at the Pharmaceutical Laboratory, while LAB enumeration was performed at the Microbiology Laboratory, and analyses of lactic acid content, viscosity, SNF, and reducing sugar levels were conducted at the ISO 17025:2017 and KAN certified Service Laboratory, Faculty of Mathematics and Natural Sciences, Pakuan University.

The study was conducted in two stages which were product development and analytical evaluation. All formulations were initially analyzed for lactic acid bacteria (LAB) viability. The formulation exhibiting the highest LAB count among the legume-based yogurts (tested formulas) was subsequently selected for further analyses, including pH, lactic acid content, viscosity, solids-non-fat (SNF) content, and reducing sugar levels. All chemical analyses were conducted in accordance with the Indonesian National Standar for yogurt (SNI 2981:2009) to ensure that the developed yogurt met the nationally established quality requirements.

Enumeration of lactic acid bacteria (LAB)

Total LAB was determined using the Total Plate Count (TPC) method on de Man, Rogosa, and Sharpe agar (MRS agar). The medium was prepared by dissolving 65.13 g of MRS agar in 1000 mL of distilled water and sterilized at 121°C for 15 min. Yogurt samples were serially diluted in sterile distilled water at a ratio of 1:9 to obtain dilutions ranging from 10^{-1} to 10^{-9}. Aliquots (1 mL) from appropriate dilutions (10^{-8}–10^{-9}) were plated in duplicate into petri dishes containing approximately 10 mL of semi-solid MRS agar and gently mixed to ensure uniform distribution of the sample. The plates were incubated at 37°C for 48 h under aerobic condition. Colonies were counted from dishes containing 25–250 colonies, and the results were expressed as colony-forming units per milliliter (CFU/mL). The total LAB count was calculated using the following equation:

$$\text{CFU/mL} = n \; x \; F$$

where **n** represents the average colony count from duplicate plates at a given dilution, and **F** is the corresponding dilution factor. LAB counts were evaluated against the minimum requirement of the Indonesian National Standard for yogurt (SNI 2981:2009), which specifies ≥7 log CFU/mL.

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Physicochemical analysis

pH measurement

The pH of yogurt samples was measured using a calibrated digital pH meter. The instrument was calibrated with standard buffer solutions at pH 4.0 and 7.0 prior to measurement. The electrode was immersed directly into 10 mL of sample until a stable reading was obtained. All measurements were performed in duplicate.

Lactic acid determination

Lactic acid content was determined using a titration method. Approximately 20 g of yogurt sample was diluted with CO_2-free distilled water to twice its volume. Phenolphthalein indicator (1%, 2–3 drops) was added, and the solution was titrated with 0.1 N NaOH until a faint pink endpoint was obtained. Lactic acid content was calculated using the following equation:

$$\text{Lactic acid content (\%)} = \frac{V \times N \times 90}{W} \times 100\%$$

where V is the volume of NaOH used (mL), N is the normality of NaOH, W is the sample weight, and 90 represents the equivalent weight of lactic acid.

Viscosity measurement

Viscosity was measured using a Brookfield rotational viscometer with spindle No. 4 at 20 rpm. Approximately 100 mL of yogurt sample was placed in a beaker and equilibrated at 25°C before measurement. The spindle was immersed to the designated mark, and viscosity values were recorded in centipoise (cP) once a stable reading was obtained. Measurements were performed in duplicate.

Solids-non-fat (SNF) determination

Total solids were determined by the oven-drying method**.** Approximately 3 g of sample was weighed in a pre-dried weighing dish and dried in an oven at 100 ± 1°C for 4 h. The dish was then cooled in a desiccator for 30 min and reweighed. Total solids (%) were calculated based on weight loss during drying. Solids-not-fat (SNF) content was subsequently calculated as:

$$\text{SNF (\%)} = \frac{(W1-W2)-(B1-B2)}{W1-W2} \times 100\%$$

Reducing sugar determination

Reducing sugar content was determined using the Luff–Schoorl method. Approximately 5–10 g of sample was weighed and diluted in a 250 mL volumetric flask. The solution was clarified using lead acetate and excess lead was removed by the addition of sodium carbonate. An aliquot of 10 mL of the clarified sample solution was transferred into an Erlenmeyer flask and mixed with 25 mL of Luff–Schoorl reagent and boiling chips. The mixture was heated to boiling for 10 min and subsequently cooled. A blank solution was prepared by mixing 25 mL of Luff–Schoorl reagent with 25 mL of distilled water and treated under the same conditions. After cooling, 15 mL of 20% KI and 25 mL of H_2SO_4 were added to both sample and blank solutions. The solutions were then titrated with 0.1 N $Na_2S_2O_3$ using starch as an indicator until the blue color disappeared. Reducing sugar content was calculated based on the Luff–Schoorl table and expressed as percentage using the following equation:

$$\text{Reducing sugar (\%)} = \frac{\text{AT X Fp}}{\text{Berat sampel x 1000}} \times 100\%$$

where AT represents the Luff–Schoorl table value and Fp represents the dilution factor

Yogurt Drink Preparation

The yogurt drink formulations consisted of three control formulas and three tested formulas with different ratio of jack bean extract and kidney bean extract. The control formulas included a non-legume (P1, 90% skim milk), and two single-legume formulas (P2, 80% jack bean extract; P3, 80% kidney bean extract), while the tested formulas were F1 (40% jack bean extract : 40% kidney bean extract), F2 (60% jack bean extract : 20% kidney bean extract), and F3 (20% jack bean extract : 60% kidney bean extract). In

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addition to the main substrate composition, P1 contained 5% starter culture and 5% sugar, whereas P2, P3, F1, F2, and F3 were formulated with 10% skim milk, 5% starter culture, and 5% sugar.

Yogurt drink production consisted of four stages: starter culture preparation, preparation of jack bean extract, preparation of kidney bean extract, and yogurt fermentation. The starter culture was prepared using a commercial powdered culture (Lactina by YOE®, 1 g) containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Full-cream milk (Greenfields®, 1000 mL) was pasteurized at 80–85°C, cooled to 30–45°C, inoculated with the starter culture, homogenized, and incubated at 42°C for 18 h until a thick starter culture was obtained. Jack bean extract was prepared from 500 g of sorted and washed beans. The beans were soaked in 1 L of water containing 1% $NaHCO_3$ for 12 h to soften the texture and reduce hydrogen cyanide and phytic acid content. After soaking, the beans were rinsed, dehulled, boiled at 100°C for 15 min, and rinsed three times with hot water to reduce beany-off-flavor. The cooked beans were blended with hot water (80–100°C) at a ratio of 1:8 (w/v) (based on preliminary trials), filtered through muslin cloth, and the filtrate was heated to 80°C without boiling. Kidney bean extract was prepared similarly. Kidney beans (500 g) were sorted, washed, soaked in 1 L of water for approximately 12 h, dehulled, and boiled at 100°C for 15 min to reduce the characteristic beany odor. The beans were then blended with hot water at a ratio of 1:3 (w/v) (based on preliminary trials) and filtered through muslin cloth to obtain the extract. For yogurt drink production, skim milk (Greenfields®) and the legume extracts were mixed according to the formulation design (P1–F3). Skim milk (90% for P1; 10% for P2-F3) and sugar (5%) were added and homogenized. The mixture was pasteurized at 80–85°C for 15 min, cooled to 40°C, inoculated with 5% starter culture, and incubated at 40°C for 12 h to obtain yogurt drink.

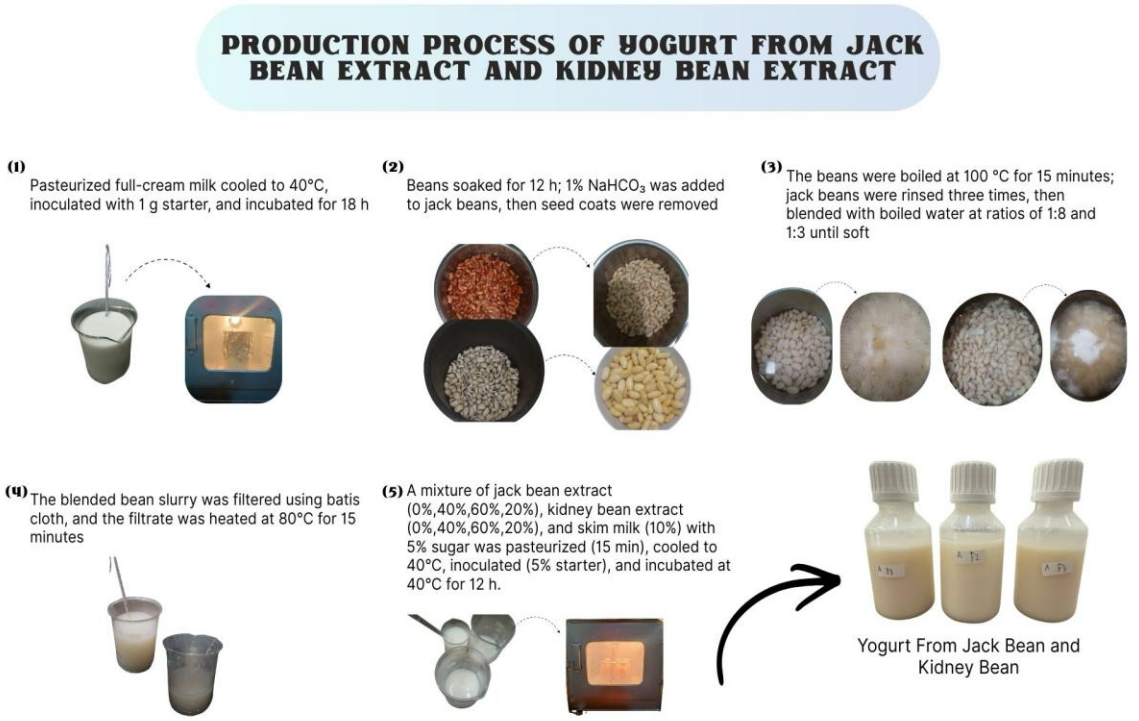

Figure 1. Production process of yogurt from jack bean extract and kidney bean extract

The LAB data were subjected to statistical analysis using IBM SPSS Statistics version 31.0.0.0 (IBM Corp., Armonk, NY, USA). Differences among formulations were evaluated by one-way Analysis of Variance (ANOVA). When statistically significant differences were observed ($p<0.05$), Duncan's Multiple Range Test (DMRT) was employed as a post-hoc analysis to compare the mean values among formulas, while physicochemical parameters including pH, lactic acid content, viscosity, solids-non-fat (SNF) content, and reducing sugar levels were evaluated descriptively.

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RESULTS

Quality Characteristics of Yogurt Made from Jack Bean and Kidney bean Extract

Differences in yogurt characteristics between yogurt legume-based formulas and the non-legume control were reflected in observed lactic acid bacteria (LAB) populations. In the present study, yogurt formulation containing jack bean and kidney bean extracts exhibited higher LAB counts compared with the control formulation without legume addition.

Table 1. LAB viability of yogurt formulas

Formula	LAB Viability (log CFU/mL)	Standard of SNI 2981:2009 (1)
P1	10.23 ± 0.01[a]	Minimum requirement: 7 log CFU/mL
P2	10.38 ± 0.11[b]	
P3	10.25 ± 0.07[a]	
F1	10.45 ± 0.03[b]	
F2	10.70 ± 0.09[c]	
F3	10.44 ± 0.06[b]	

Data are presented as mean ± SD (n=3). Different superscript letters indicate significant differences according to Duncan's Multiple Range Test ($p<0.05$)

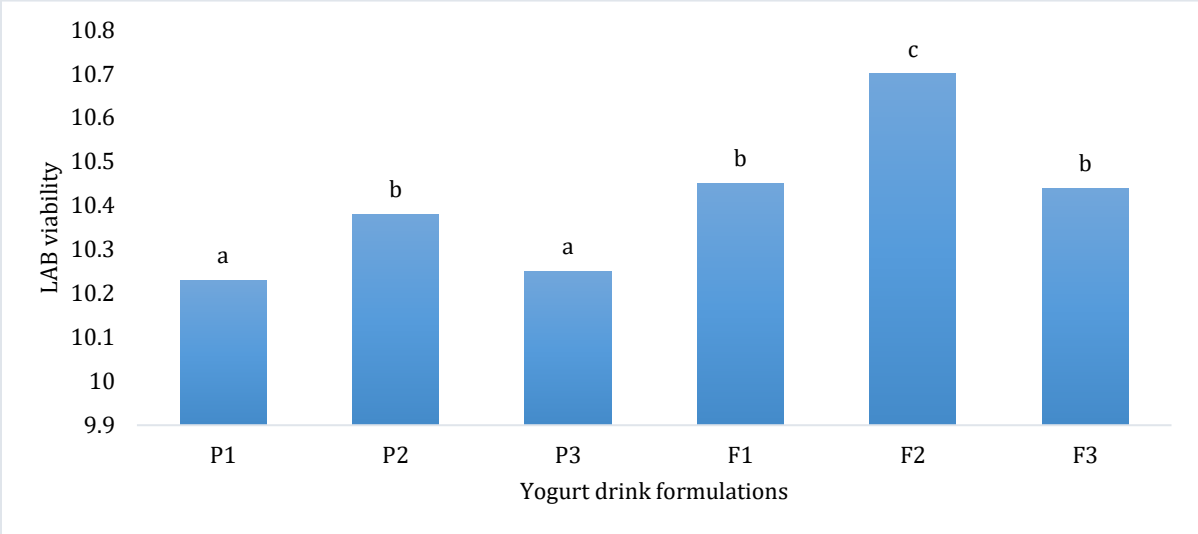

Figure 2. LAB viability of yogurt drink formulations (P1-F3). Values represent mean ± SD (n=3). Different letters above the bars indicate significant differences among formulations ($p<0.05$) according to Duncan's Multiple Range Test

The analysis of variance demonstrated that yogurt formulation had a significant effect ($p<0.05$) on the total LAB count. LAB counts varied among formulations, ranging from 10.23 ± 0.01 to 10.70 ± 0.09 log CFU/mL. Formula 2 containing 60% jack bean extract and 20% kidney bean extract exhibited the highest lactic acid bacteria (LAB) population (10.70 ± 0.09 log CFU/mL), which was significantly higher ($p<0.05$) than those obeserved in the control formulas P1 (10.23 ± 0.01 log CFU/mL), P2 (10.38 ± 0.11 log CFU/mL), and P3 (10.25 ± 0.07 log CFU/mL) as well as in Formula 1 (10.45 ± 0.03 log CFU/mL) and Formula 3 (10.44 ± 0.06 log CFU/mL). All yogurt formulations exceeded the minimum LAB requirement of 7 log CFU/mL specified by the Indonesian National Standard for yogurt (SNI 2981:2009). Considering its significantly highest LAB viability, Formula 2 was selected for further physicochemical characterization to assess its fermentation performance and product quality. The evaluated parameters included lactic acid content, pH, viscosity, solids-non-fat content, and reducing sugar levels, as summarized in Table 2.

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Table 2. Lactic acid content, ph, viscosity, solid-non-fat, and reducing sugar level of Formula 2 (60% jack bean extract and 20% kidney bean extract)

Quality Characteristics of Formula 2	Value	Standard of SNI 2981:2009
pH	4.23 ± 0.00	Not specified
Lactic acid content (%)	1.954 ± 0.03	0.5-2.0%
Viscosity (cP)	5.139 ± 0.05	Not specified
Solid-non-fat (%)	9.410 ± 0.09	Min 8.2%
Reducing sugar level (%)	2.017 ± 0.06	Not specified

Formula 2 exhibited a pH of 4.23 ± 0.00 and **a** lactic acid content of 1.954 ± 0.03%**,** which is within the acceptable range of 0.5–2.0% specified by SNI 2981:2009. The product showed a viscosity of 5.139 ± 0.05 cP and a solids-non-fat (SNF) content of 9.410 ± 0.09%**,** exceeding the minimum requirement **of** 8.2% established by SNI 2981:2009**.** In addition, the reducing sugar level was 2.017 ± 0.06%**.** Overall, the physicochemical parameters of Formula 2 complied with the quality standards for yogurt specified by the Indonesian National Standard.

DISCUSSION

Lactic acid bacteria (LAB) comprise a group of Gram-positive microorganisms with facultative anaerobic characteristics that primarily convert carbohydrates into lactic acid through fermentation (16). Among these, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* are the most widely applied starter cultures in yogurt production, where they exhibit a well-documented synergistic interaction during fermentation (16–18). In the present work, yogurt formulated with jack bean and kidney bean extracts demonstrated superior LAB viability compared to yogurt produced solely from a milk base.

The yogurt formulations developed in this study incorporated legume-derived ingredients, specifically jack bean and kidney bean, both of which are characterized by high carbohydrate content, particularly oligosaccharides. These carbohydrates have been extensively reported to act as prebiotic compounds that selectively enhance the growth of probiotic microorganisms, including LAB (11,12,19,20). Formula 2 consisting of 60% jack bean extract and 20% kidney bean extract exhibited the highest LAB viability at 10.70 ± 0.09 log CFU/mL ($p<0.05$)**,** which substantially exceeds the minimum level required by the Indonesian National Standard for yogurt (SNI 2981:2009; ≥7 log CFU/mL). This finding is consistent with previous reports indicating that jack bean is rich in carbohydrates (50.6%) and protein (28.6%) (8), thereby could be supplying both carbon and nitrogen sources necessary to sustain LAB growth during fermentation. In addition, kidney bean contains considerable amounts of oligosaccharides, including raffinose family oligosaccharides, which can serve as fermentable substrates for LAB and may act as prebiotic compounds that stimulate microbial metabolism. In this context, jack bean primarily contributes carbohydrate and protein components that support carbon and nitrogen availability, while kidney bean supplies additional fermentable carbohydrates. Carbon compounds function mainly as energy substrates in microbial metabolism, whereas nitrogen is essential for the synthesis of amino acids, proteins, and other cellular components required for bacterial proliferation. Previous studies have shown that the availability and combination of carbon and nitrogen sources strongly influence LAB growth and metabolic activity during fermentation (21). Moreover, variations in carbon–nitrogen composition can significantly affect viable cell populations and fermentation performance of LAB strains (22). Therefore, the higher LAB viability observed in Formula 2 may reflect a more favorable nutritional balance provided by the combined legume substrates, supporting enhanced microbial proliferation during fermentation. The exceptionally high LAB count observed in this study also suggests that the pre-processing steps applied to the legume were effective in reducing inhibitory anti-nutritional compounds. Jack bean is known to contain toxic and anti-nutritional substances such as hydrogen cyanide and phytic acid, which may inhibit microbial activity if present at high concentrations. The $NaHCO_3$ soaking followed by boiling likely reduced these compounds to levels that no longer exert inhibitory effects of microbial growth and metabolism.

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Beyond its role in supporting microbial growth during fermentation, the nutritional composition of the legume substrates may also contribute to the functional properties of the resulting plant-based yogurt. In particular, the inclusion of kidney bean may provide additional nutritional value, as it contains bioactive compounds such as flavonoids and polyphenols with potential antioxidant properties. Although these compounds were not quantified in the present study, fermentation has been reported to enhance the bioavailability of plant-derived phytochemicals (15). Therefore, the combination of high LAB viability and phytochemical rich legume substrates suggests that this formulation may hold promise as a plant-based functional fermented product.

Fermentation mediated by LAB plays a pivotal role in defining the physicochemical attributes of yogurt. The metabolic processes occurring during fermentation lead to the formation of lactic acid and other metabolites, which collectively drive changes in pH, lactic acid concentration, viscosity, and total soluble milk solids. A reduction in pH represents an early indicator of LAB fermentation. Through lactose fermentation, LAB generate lactic acid, resulting in a decrease in yogurt pH to values ≤4.6. This acidification not only contributes to microbial preservation by suppressing spoilage miroorganisms but also promotes milk protein coagulation, particularly casein, which forms the basis of yogurt's thick consistency (16). In this study, the yogurt exhibited a pH of 4.23 ± 0.00 which is comparable to the pH reported for mung bean yogurt (4.56) (23). The decrease in pH was accompanied by a concomitant rise in lactic acid content. Formula 2 contained 1.954 ± 0.03% lactic acid, corresponding to the recommended quality range of 0.5–2.0% and notably higher than those reported for soy–pea soygurt (0.54–0.58%) (24) and mung bean yogurt (0.52–0.77%) (23). Given that lactic acid is the principal organic acid formed during LAB metabolism, higher concentrations may indicate greater metabolic activity during fermentation (16). The increased lactic acid content observed in the present study is therefore likely attributable to the greater LAB population supported by the jack bean-kidney bean matrix.

Viscosity serves as a critical indicator of yogurt structure development during LAB fermentation. Acidification resulting from LAB activity promotes an increase in viscosity as the pH approaches the isoelectric point of milk proteins, leading to protein coagulation and thickening of the product (24). At this stage, denatured milk proteins aggregate into a three-dimensional gel network capable of retaining water, with stronger and more uniform networks producing higher viscosity values (18). The viscosity of jack bean and kidney bean yogurt reached 5.139 ± 0.05 cP, surpassing values reported for soy–pea soygurt (2.13–2.66 cP) (24) and mung bean-date yogurt (2.17–4.81 cP) (25). The higher viscosity observed in the present study is considered advantageous, as it reflects a more compact gel structure, improved water-holding capacity, and a reduced susceptibility of syneresis, thereby contributing to better texture and storage stability. The selected formulation ratio may also influence product characteristics, as jack bean is known to exhibit a beany or slightly bitter flavor when consumed alone. The incorporation of kidney bean at 20% in Formula 2 may help moderate these characteristic while maintaining the functional contribution of jack bean as the primary substrate.

LAB-driven fermentation also influences the solids-non-fat (SNF) fraction of yogurt. SNF encompasses proteins, lactose, minerals, and non-fat nitrogenous components that contribute to yogurt texture and stability. Quantitatively, LAB fermentation does not increase SNF content, rather it alters the functional state of these components by converting lactose into lactic acid and promoting protein denaturation and aggregation into a gel matrix (16,18). In the present study, Formula 2 yogurt exhibited an SNF value of 9.410 ± 0.09**%,** which is lower than those reported for yogurt formulated with tigernut, soybean, and cow's milk (10.59–12.27%) (26) and soybean–mango yogurt (12.37–14.56%) (27). Nevertheless, SNF value obtained in this study meets the the SNF requirement stipulated by SNI 2981:2009, which specifies a minimum SNF content of 8.2% for yogurt. Adequate SNF levels serve as an important quality indicator, as it ensures sufficient protein availability for robust gel formation and enhanced water-holding capacity, which collectively contribute to yogurt texture and shelf-life stability (16,18).

Reducing sugars refer to carbohydrates such as glucose, fructose, galactose, and lactose, that possess the ability to act as reducing agents. In yogurt, these sugars originate from residual unfermented lactose as well as from the enzymatic or microbial hydrolysis of complex carbohydrates and

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oligosaccharides during fermentation. The reducing sugar content of Formula 2 was 2.017 ± 0.06**%,** which is lower than values reported for rice bran yogurt (2.587%) (28), kimpul tuber–mango yogurt (3.0%) (29), and kefir supplemented with gembili tuber purée (6.08–6.34%) (30). The relatively low residual sugar level in this study may reflect more efficient substrate utilization during fermentation. This interpretation is supported by the concurrently high LAB viability (10.70 log CFU/mL) and lactic acid content (1.954 ± 0.03%), suggesting that fermentable carbohydrates were actively metabolized and converted into organic acids. As previously noted, jack bean and kidney bean are rich in carbohydrates particularly oligosaccharides, which function as prebiotics or readily available energy sources for LAB. The combination of high LAB counts, elevated lactic acid production, and lower residual reducing sugars therefore indicating that LAB population in Formula 2 actively metabolized available substrates into fermentation metabolites.

CONCLUSION

The yogurt drink developed from jack bean and kidney bean particularly Formula 2 (60% jack bean extract and 20% kidney bean extract), demonstrated a high viable count of LAB, accompanied by conformity to other key quality attributes. These findings highlight the potential of these legumes a substrates for plant-based yogurt and as alternative plant-based protein sources in fermented functional foods. Future research should focus on shelf-life, bioactive compound profiling, and probiotic validation through preclinical and clinical studies.

DECLARATION OF THE USE OF AI

The authors declare that no artificial intelligence (AI), AI-assisted technologies, or large language models (LLMs) were used in the conception of the study, data analysis, or the drafting, writing, and editing of this manuscript. The only exception is the graphical abstract, which was created using the design platform Illustrae (https://illustrae.co/). The authors take full responsibility for the content and accuracy of the graphical abstract and the entire manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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