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The Effect of Kelulut Honey (*Trigona spp.*) Concentration as a Natural Sugar on the Quality of Mung Bean Soyghurt (*Vigna radiata L.*)

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Abstract: Mung bean soyghurt is a fermented functional food product developed from plant-based milk derived from mung beans (*Vigna radiata* L.). Lactic acid bacteria (LAB) are employed as the fermentative agents in the fermentation process, with the addition of kelulut honey (*Trigona spp.*) serving as a natural nutrient source. This product contains both probiotic microorganisms and prebiotic components, offering potential health-promoting properties. This research aimed to was conducted to assess the impact of varying concentrations of kelulut honey, used as a natural sugar, on the quality of mung bean soyghurt. Fermentation was carried out at 37 °C℃ for 8 hours with kelulut honey incorporated at concentrations of 0%, 5%, 10%, and 15% (v/v), along with 0,5 gram of probiotic starter. The resulting products were analyzed for LAB growth, total titratable acidity (TTA), and pH. LAB populations were determined using the Total Plate Count (TPC) method, titratable acidity was determined through acid-base titration, and pH was assessed using a calibrated pH meter. The results showed that increasing concentrations of kelulut honey significantly enhanced LAB growth and TTA while reducing pH values. The honey also influenced organoleptic properties, including color, aroma, and taste. The best treatment was observed in the formulation containing 10% kelulut honey, which yielded a LAB count of $4,20 \times 10^8$ CFU/mL, a TTA of 1,638%, a pH of 4,00, and organoleptic characteristics that received favourable evaluations from the panellists across all assessed parameters.

Keywords: Kelulut Honey; LAB; Mung bean soyghurt; pH; TTA.

Introduction

Mung bean (*Vigna radiata L.*) is a legume recognized for its rich nutritional content, including proteins, carbohydrates, and bioactive compounds such as polyphenols, peptides, and polysaccharides [1][2]. Mung bean protein is recognized as an excellent source of amino acids, particularly essential amino acids such as phenylalanine/tyrosine, leucine, lysine, isoleucine, valine, and histidine, which are present in high amounts and are crucial for the development and regeneration of body tissues [3][4][5]. Due to their rich nutritional profile, mung beans have been recognized as a functional food with various health benefits. The high fiber content in mung beans supports a healthy gut microbiome, leading to improved nutrient absorption and overall digestive health. Research by [6] indicates that the low glycemic index of mung beans can slow glucose absorption and improve insulin sensitivity, making them beneficial for blood glucose control. In addition, their high antioxidant content, including phenolic compounds and flavonoids, helps reduce oxidative stress and inflammation both of which are major contributors to cardiovascular disease [7].

Despite their high nutritional value, mung beans also contain antinutritional factors such as tannins, trypsin inhibitors, hemagglutinins, and phytic acid, which can reduce the bioavailability of nutrients [8]. Several studies have demonstrated that appropriate processing methods can effectively reduce these antinutrients. One such method is fermentation, whereby mung beans are processed into soyghurt, enhancing their nutritional quality and functional properties [9].

Soyghurt is a fermented product derived from plant based milk using lactic acid bacteria (LAB), such as Streptococcus thermophilus and Lactobacillus bulgaricus [10]. This fermentation process not only extends the shelf life of the product through natural preservation but also enhances its nutritional value, improves sensory characteristics, and offers additional health benefits [11]. Soyghurt has several advantages over animal-based yoghurt, including lower fat content, higher antioxidant levels, and suitability as an alternative for individuals with lactose intolerance [12].

The most important factor that makes mung bean suitable as a raw material for soyghurt production is its high α-galactooligosaccharide (α-GOS) content [13]. High α-GOS levels function as a prebiotic by promoting probiotic growth and supporting the equilibrium of colonic microflora [14][15]. During fermentation, oligosaccharides present in mung beans are hydrolyzed by the enzyme α-galactosidase into free galactose and glucose, which are subsequently utilized by lactic acid bacteria (LAB) for cell growth. This utilization reduces the availability of sugars for lactic acid synthesis, potentially leading to suboptimal acid production. To enhance lactic acid formation, additional sugar sources such as glucose or fructose are required. Kelulut honey, a natural product rich in simple sugars, may serve as an effective alternative nutrient source during fermentation [16].

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Kelulut honey (*Trigona spp.*) is a type of honey produced by stingless bees of the genus Trigona. It contains enzymes, vitamins, minerals, and other bioactive compounds such as hydrogen peroxide, polyphenols, and peptides. These components can support the immune system, accelerate wound healing, and help prevent various diseases [17]. Kelulut honey contains various types of natural sugars, primarily simple sugars such as glucose and fructose, which account for approximately 32–38% of the total sugar content. These sugars can be readily absorbed by bacterial cells as an energy source during the fermentation process. In addition to glucose and fructose, kelulut honey also contains disaccharides and oligosaccharides [18][19].

The addition of kelulut honey is expected to support the growth and activity of lactic acid bacteria (LAB) during the fermentation process. In this study, kelulut honey was selected due to its content of simple sugars, which can be readily absorbed by LAB cells and serve as an energy source during fermentation. However, an excessively high concentration of honey may inhibit bacterial growth because of the hygroscopic nature of sugars, which can draw water and reduce its availability for microbial activity [20]. Therefore, it is important to investigate the effect of varying concentrations of kelulut honey on the quality of mung bean soyghurt.

Research Methods

Type of Research

This research was conducted using a true experimental method in a laboratory setting. A Completely Randomized Design (CRD) was implemented, featuring a single factor of honey concentration (%) with four treatment groups (0%, 5%, 10%, and 15%), each repeated three times. Observational data comprising pH, total titratable acidity (TTA), and total lactic acid bacteria (LAB) were subjected to one-way ANOVA, followed by a post hoc LSD test using SPSS version 27. These analyses were conducted to assess the impact of varying honey concentrations on soyghurt quality.

Preparation of Mung Bean Juice

Mung beans were initially sorted to select those in good and intact condition, then cleaned and soaked in clean water at a ratio of 1:3 for 10 hours. After soaking, the seed coats were removed and discarded, followed by rinsing with clean water. Mung beans were mixed with water in a 1:5 proportion, followed by filtration to obtain the mung bean juice, which was subsequently pasteurized at 70 °C for 15 minutes [21].

Preparation of Mung Bean Soyghurt

Pasteurized mung bean juice was mixed with kelulut honey at concentrations of 0%, 5%, 10%, and 15% (v/v) to a final volume of 100 mL. The mixture was then cooled to a temperature of 40–45℃. Subsequently, 0,5 grams of probiotic starter culture were added to each treatment, and the mixtures were incubated at 37℃ for 8 hours [22].

pH Test (Degree of Acidity)

To determine the pH of mung bean soyghurt, a calibrated pH meter (using pH 4.0 and 7.0 buffer solutions) was employed. The electrode was immersed in 10 mL of the sample, and the pH was recorded after stabilization [23].

TTA Test (Total Titratable Acid)

Total titratable acidity (TTA), expressed as a percentage of lactic acid, was determined using an acid-base titration method. A 10 mL sample was diluted to 100 mL, and 20 mL of this dilution was titrated with 0,1 N NaOH after adding phenolphthalein indicator. The titration was continued until a stable pink color appeared. All titrations were conducted in triplicate to ensure analytical precision [23]. The lactic acid content (%) was determined using the following equation:

$$\text{TTA} = \frac{\text{V NaOH} \times \text{N NaOH} \times \text{fp} \times \text{BM}}{\text{weight of sample} \times 1000} \times 100\%$$

Where *fp* is the dilution factor and *BM* is the molecular weight of lactic acid.

Total LAB (Lactic Acid Bacteria) Test

Total lactic acid bacteria (LAB) counts were determined through the application of the Total Plate Count (TPC) method. Prior to testing, all equipment was sterilized using an autoclave at 121 °C℃ for 15 minutes. A 1 mL sample of mung bean soyghurt was transferred into a test tube containing 9 mL of 0,85% NaCl solution to obtain a 10^{-1} dilution. Then, 1 mL of this first dilution was transferred into another test tube with 9 mL of the same diluent to achieve a 10^{-2} dilution, and the serial dilution process was continued up to 10^{-8}. From the 10^{-6} to 10^{-8} dilutions, 1 mL of each sample was taken using a micropipette and placed into sterile petri dishes. Sterile MRS-A media supplemented with 1% $CaCO_3$ was then poured into the petri dishes. To ensure uniform distribution of the inoculum, the plates were gently swirled in a figure-eight pattern and then allowed to solidify. The plates were incubated at 37 °C℃ for 48 hours [24]. Following incubation, the number of colony-forming units (CFUs) was counted and multiplied by the dilution factor to estimate the total viable bacteria.

Organoleptic Test

Organoleptic quality of the mung bean soyghurt was assessed through a hedonic test, in which panellists evaluated their preferences for specific sensory attributes including color, taste, and aroma. The test was conducted with 15 untrained panellists, consisting of chemistry students. Sensory analysis was performed using a five-point hedonic scale, with ratings from 1 (strongly dislike) to 5 (strongly like), including intermediate scores of 2 (dislike), 3 (neutral), and 4 (like) [22]. This study involved human subjects, specifically panellists, to evaluate product acceptability. Ethical approval was granted by the Health Research Ethics Commission, Faculty of Medicine, Surabaya State University, under approval number 75-KEPK.

Result and Discussion

Total Lactic Acid Bacteria (LAB)

The objective of this study was to investigate the effect of varying concentrations of kelulut honey on the total population of lactic acid bacteria (LAB) in mung bean soyghurt. The LAB counts were quantified through the Total Plate Count (TPC) method by enumerating the colonies grown on a selective agar medium, allowing for precise assessment of microbial growth in response to the different honey concentrations. The fundamental principle of the TPC method involves cultivating viable microbial cells on a specific agar medium. In this study, the medium consisted of a mixture of de Man, Rogosa, and Sharpe agar (MRS-A) and $CaCO_3$ dissolved in 0,85% NaCl, which facilitates optimal microbial growth and colony formation for direct observation and enumeration [25]. Data on total LAB were analyzed using a one-way ANOVA to determine the effect of kelulut honey concentration on total LAB. The results of the One Way ANOVA showed that kelulut honey concentration had a significant effect on total LAB, with a p-value < 0,05. The results of the total LAB count are summarized in Table 1.

Table 1. The result of the total LAB test in mung bean soyghurt

Sample	Kelulut Honey Concentration (%)	Total LAB (CFU/mL)
A	0	1.68×10^{8a}
B	5	2.92×10^{8b}
C	10	4.20×10^{8d}
D	15	3.82×10^{8c}

Note: Values followed by the same letter are not significantly different ($\alpha > 0.05$).

Based on the results presented in Table 1, the concentration of kelulut honey significantly affects lactic acid bacteria. The addition of kelulut honey results in an increase in total LAB from $1,68 \times 10^8$ to $4,20 \times 10^8$ CFU/mL. The total LAB value in mung bean soyghurt increased significantly with the addition of kelulut honey up to a concentration of 10%, followed by a decrease at 15%. Maximum LAB growth occurred at 10% kelulut honey addition, with an increase from $1,68 \times 10^8$ to $4,20 \times 10^8$ CFU/mL. These total LAB values meet the requirements of SNI 2981:2009, which specifies a minimum total LAB of 1×10^7 CFU/mL.

The addition of kelulut honey can increase the total lactic acid bacteria (LAB) because kelulut honey serves as a natural sugar source that provides energy and nutrients essential for LAB growth during the soyghurt fermentation process [18]. The amino acids, minerals, vitamins, fiber, and enzymes present in kelulut honey also fulfil the nutritional requirements of LAB, enabling bacterial cells to efficiently utilize glucose and degrade various sugars, including monosaccharides, disaccharides, and polysaccharides, to support LAB proliferation. LAB growth in mung bean soygurt occurs due to the activity of the enzyme α-galactosidase, which breaks down α-GOS into free galactose and simple sugars such as glucose through hydrolysis reactions. The glucose produced from the hydrolysis process enters the glycolytic pathway, where it is metabolized into pyruvate. Additionally, glucose from kelulut honey serves as an essential substrate for the growth of lactic acid bacteria (LAB) during fermentation by providing energy and the necessary components for metabolic activity and cell proliferation. LAB utilize glucose as their primary substrate in the fermentation process to generate energy and metabolic products, primarily lactic acid [15]. The main mechanism involves glycolysis, where glucose is broken down into pyruvate. Pyruvate, the end product of glycolysis, is subsequently converted into lactic acid by the enzyme lactate dehydrogenase via the Embden-Meyerhof-Parnas (EMP) pathway. This reaction occurs under anaerobic conditions, in the absence of oxygen. During this process, NADH generated in glycolysis is oxidized back to NAD^+, ensuring the continuity of glycolysis [11][15].

This increase in total LAB is consistent with previous research by [16], which reported a significant increase in total LAB in orange sweet potato yoghurt with increasing honey concentration up to 10%. However, the increase in total LAB did not continue with the addition of higher concentrations of honey. The results showed a decrease in total LAB at the 15% concentration of kelulut honey. This decline occurred because, at this concentration, the high sugar content may increase osmotic pressure, which can inhibit enzymatic activity and nutrient absorption by LAB. Similarly, study [26] demonstrated that honey addition to yoghurt significantly affects total LAB levels. However, the increase in total LAB does not continue indefinitely with the addition of honey, as excessive amounts can inhibit the growth of lactic acid bacteria. High concentrations of honey may create suboptimal conditions in the medium for LAB growth, causing plasmolysis of bacterial cells due to reduced water availability. This is consistent with the findings of [16], which reported that the high sugar content in honey inhibits bacterial growth because the hygroscopic nature of sugar draws water away, thereby reducing or even eliminating the water available for microbial growth. The higher the sugar concentration added, the more the activity of the bacterial starter is suppressed [27].

Total Titratable Acid (TTA)

The total titratable acidity (TTA) test was conducted to evaluate the impact of kelulut honey concentration on the overall acid content in mung bean soyghurt, utilizing the acid-base titration method. The reaction equation is as follows:

$$C_3H_6O_3\ (aq) + NaOH\ (aq) \rightleftharpoons C_3H_6O_3Na\ (aq) + H_2O\ (l)$$

The fundamental principle of acid-base titration involves the neutralization reaction between acidic and basic components, where the titrant (NaOH solution) is added to the titrate (soyghurt sample) until the equivalence point is reached. This point is indicated by a color change in the phenolphthalein indicator to pink [28]. Data on TTA were analyzed using a one-way ANOVA to determine the effect of kelulut honey concentration on TTA. The results of the One Way ANOVA showed that kelulut honey concentration had a significant effect on total LAB, with a p-value < 0,05. The results of the total titratable acid test are summarized in Table 2.

Table 2. The result oftotal titratable acid test in mung bean soyghurt

Sample	Kelulut Honey Concentration (%)	TTA (%)
A	0	0.755[a]
B	5	1.155[b]
C	10	1.638[d]
D	15	1.462[c]

Note: Values followed by the same letter are not significantly different (α > 0.05).

As shown in Table 2, kelulut honey concentration significantly influenced the total titratable acidity of the samples. The addition of kelulut honey results in an increase in total titratable acid value from 0,755 to 1,638. The TTA value of mung bean soyghurt increased with the addition of kelulut honey up to a concentration of 10%, then decreased at 15%. The highest TTA value was observed in mung bean soyghurt with 10% kelulut honey, measuring 1,638, while the lowest TTA value was found at 0% honey, measuring 0,755. These titratable acid values meet the requirements of SNI 2981:2009, which specifies a TAT range of 0,5 to 2,0.

The increase in lactic acid bacteria (LAB) growth is accompanied by an increase in the total acid produced. As LAB growth increases, the total titratable acid (TTA) or total lactic acid also rises, and vice versa. This is because the total acid is influenced by LAB activity, with lactic acid being a metabolite produced by LAB [16]. The increase in total acid results from the greater amount of organic acids formed during the fermentation process. This rise in TTA occurs because, during fermentation, LAB degrade the glucose present in kelulut honey into lactic acid, which lowers the pH [29]. The lactic acid fermentation process begins with the glycolysis pathway, which produces pyruvic acid. Due to the lack of oxygen during fermentation, pyruvic acid is metabolized via anaerobic degradation catalyzed by lactic acid dehydrogenase. This enzyme facilitates the reduction of pyruvate by NADH, resulting in the production of energy and lactic acid[30].

The addition of kelulut honey can increase the fermentation rate of LAB, as it provides numerous substrates that can be converted into lactic acid, thereby accelerating the acidification process. In addition to serving as an energy source, the sugar content also supports the growth and proliferation of LAB. Furthermore, kelulut honey not only provides carbohydrates but also contains small amounts of vitamins and minerals that help balance nutrients for the bacteria [27]. This study is supported by research [16], which shows that increased LAB activity with the addition of honey during fermentation triggers the degradation of glucose in the sugar, resulting in lactic acid production. This observation aligns with the study by [27], indicating that higher honey concentrations result in decreased pH values due to enhanced glucose fermentation to lactic acid by LAB.

pH Value (Degree of Acidity)

pH, a parameter indicative of acidity, was measured using a pH meter calibrated with standard buffer solutions of known hydrogen ion concentrations. This measurement was conducted to assess the acidity level of mung bean soyghurt [31]. Data on pH were analyzed using a one-way *ANOVA* to determine the effect of kelulut honey concentration on total LAB. The results of the *One Way ANOVA* showed that kelulut honey concentration had a significant effect on pH, with a p-value < 0,05. The results of the pH test are summarized in Table 3.

Table 3. The result of the pH value in mung bean soyghurt

Sample	Kelulut Honey Concentration (%)	pH
A	0	4.77[a]
B	5	4.35[b]
C	10	4.00[d]
D	15	4.12[c]

Note: Values followed by the same letter are not significantly different (α > 0.05).

As shown in Table 3, the pH value is significantly influenced by the concentration of kelulut honey. The addition of kelulut honey results in an increase in total titratable acid value from 4,77 to 4,00. The pH of mung bean soyghurt decreased with the addition of kelulut honey up to a concentration of 10%, followed by an increase at 15%. The highest pH value was observed in mung bean soyghurt with 0% kelulut honey (4,77), while the lowest pH value was found at 10% honey concentration (4,00). These pH values comply with the SNI 2981:2009 standard, which specifies an acceptable pH range of 3,80–4,50.

An increase in lactic acid bacteria (LAB) growth corresponded with a decrease in pH value. The observed decrease in pH, concomitant with an increase in total acidity, is attributed to the accumulation of organic acids produced during the fermentation process [32]. This pH reduction can occur because, during fermentation, LAB degrade glucose found in kelulut honey into lactic acid, which decreases the pH. Lactic acid bacteria (LAB) commonly ferment carbohydrate substrates to produce significant quantities of lactic acid. The accumulation of lactic acid during fermentation decreases the pH of the environment, contributing to the characteristic sour taste. As the amount of lactic acid increases, the number of hydrogen ions (H^+) also increases, leading to a further decrease in pH [33][34].

The metabolic activity of microorganisms during fermentation leads to a decrease in pH and the production of antimicrobial compounds, including alcohol and bacteriocins, which inhibit the proliferation of spoilage microbes. In this process, each fermented sugar molecule yields two molecules of ATP and pyruvate. The pyruvate is subsequently converted into lactate through the oxidation of NADH to NAD^+. The accumulation of lactate as a metabolic end product leads to a decrease in pH, resulting in acidification of the substrate [35]. These research findings are supported by [16], which stated that increasing LAB activity along with honey addition during the fermentation process triggers glucose degradation, resulting in lactic acid production. As lactic acid increases during fermentation, it decreases the pH of the growth environment and contributes to a sour taste. These results are consistent with those reported by [27], which indicate that higher concentrations of honey lead to a decrease in pH due to the conversion of glucose into lactic acid by lactic acid bacteria (LAB).

Research [36] shows that reducing the pH of fermentation products to below 4.5 is known to inhibit the growth of pathogenic bacteria. An acidic environment can disrupt the function of enzymes essential for bacterial growth and alter the structure of proteins necessary for bacterial survival. In addition, low pH can cause protonation of biological molecules, which may interfere with the structure and function of bacterial cells. The fermentation process involving various types of lactic acid bacteria can further inhibit the growth of pathogenic bacteria, as these bacteria are classified as probiotics that provide health benefits and suppress the proliferation of harmful microbes [37]. This is consistent with research [38], which states that fermented foods and beverages with low pH levels inhibit the growth of harmful bacteria and contribute to improved digestive health.

Organoleptic Quality

Organoleptic quality was evaluated using a hedonic test questionnaire to assess panellists' preferences for specific attributes such as color, taste, and aroma of the mung bean soyghurt. The test was conducted with 15 untrained panellists, consisting of chemistry students. Sensory evaluation was conducted using a five-point hedonic scale, where scores ranged from 1 (strongly dislike) to 5 (strongly like), including intermediate scores of 2 (dislike), 3 (neutral), and 4 (like). The effect of kelulut honey addition on the organoleptic characteristics of mung bean soyghurt is presented in Figure 1.

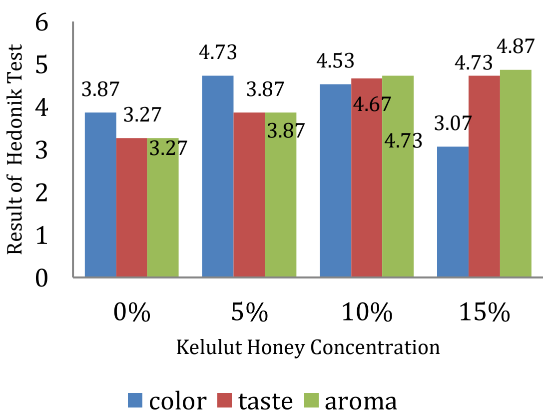

Figure 1. Results of the hedonic organoleptic evaluation of color, taste, and aroma.

Color

Color preference evaluation is a key factor influencing the visual appeal of a product, in this case, the color of mung bean soyghurt. The color of a product should be visually appealing, as it often reflects the expected flavor quality. As shown in Figure 1 that the average hedonic scores for the color attribute of mung bean soyghurt showed the highest value at a 5% kelulut honey concentration (mean = 4.73) and the lowest at a 15% concentration (mean = 3.07). These results indicate that panelists preferred the color of mung bean soyghurt with 5% kelulut honey and were less favorable toward the color at 15%. The level of color preference varied, influenced by the visual appearance of the fermented mung bean soyghurt. Mung bean juice has a white color, whereas kelulut honey is dark brown, resulting in a noticeable difference between the color of plain mung bean juice and that of the mung bean soyghurt. Consequently, increasing the concentration of kelulut honey results in a darker soyghurt appearance. The dark color of kelulut honey is attributed to its high polyphenol content and strong antioxidant activity. Furthermore, honey with elevated levels of reducing sugars can undergo Maillard reactions with amino acids, leading to the formation of dark brown pigments [39].

Taste

The hedonic evaluation of taste serves as an important indicator for assessing consumer acceptance of a product. As shown in Figure 1, the average panellist ratings for the taste attribute of mung bean soyghurt showed the highest score at a 15% kelulut honey concentration (mean = 4.73) and the lowest score at 0% concentration (mean = 3.27). This result may be due to the lower total acidity observed at 15% compared to 10%. Furthermore, increasing the concentration of kelulut honey tends to enhance the sour taste of mung bean soyghurt [40]. Kelulut honey serves as a nutrient source that promotes the growth of lactic acid bacteria (LAB) during the fermentation process in mung bean soyghurt production. Glucose present in the honey is utilized by LAB, which in turn produces lactic acid as a metabolic byproduct. As lactic acid levels increase throughout the fermentation process, the pH of the medium decreases, resulting in a more pronounced sour taste in the soyghurt. This sourness is a product of sugar fermentation into organic acids by LAB; thus, greater LAB proliferation leads to a more intense sour flavor in the final soyghurt product [30].

Aroma

As shown in Figure 1 that the average hedonic score for the taste attribute of mung bean soyghurt was highest at the 15% kelulut honey concentration, with a mean value of 4.87, and lowest at the 0% concentration, with a mean value of 3.27. The characteristic sour aroma of soyghurt is mainly due to the presence of volatile compounds such as acetaldehyde, acetic acid, and other organic acids, which are generated in small quantities during fermentation.Furthermore, during fermentation, lactic acid bacteria (LAB) metabolize kelulut honey as a nutrient source, promoting their growth and leading to the production of lactic acid, which contributes to the characteristic sour aroma of mung bean soyghurt. These findings align with those of [16], who reported that higher concentrations of honey enhance the sour taste in yoghurt.

Conclusion

The results of this study indicate that the addition of kelulut honey exerts a significant effect on the total lactic acid bacteria (LAB), total titratable acidity (TTA), and pH value of mung bean soyghurt.The best treatment was observed in mung bean soyghurt with 10% kelulut honey addition, which showed a total LAB value of 4,20 × 10^8 CFU/mL, total titratable acidity of 1,638%, and a pH value of 4,00. The incorporation of kelulut honey also affected the organoleptic properties of the mung bean soyghurt, with panellists showing acceptable levels of preference for color, aroma, and taste.

Author's Contribution
Eka Yuniar Retno Lamdari: contributed to conceiving and planning the experiments, gathering and processing the data used in the study, carrying out the experiments, analyzing the data, and writing, revising, and editing the manuscript; Rudiana Agustini: contributed to overseeing the research project and providing critical feedback.

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