Polyphenol rich mung bean (Vigna radiata) yogurt for obesity prevention


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Abstract

This study was aimed to obtain yogurt formula rich in antioxidant phenolic and soluble protein of mung beans. Mung beans were washed, soaked, and then drained. With a ratio of 1:8, mung-beans and water, blended, and then filtered to produce mung beans juice. Mung beans yogurt (Mungbe-Yo) were made with the proportion of mung bean juice and skim milk were 100:0; 95:5; 90:10, and 85:15, before added with sugar as much as 10%, pasteurized in a 70°C temperature for 20 mins. After that, each Mungbe-Yo formula was cooled to 45°C, then added lactic acid bacteria (LAB) as a starter, as much as 2% and 4%, followed by incubation at room temperature for 24 hrs. Sensory tests included color, taste, flavor, and viscosity were performed by fifty-five panelists on a 5-point score basis. Antioxidant phenolic content was measured using the Folin-Ciocalteu method, while soluble protein was using the Lowry method. The highest score and most likable color, flavor, viscosity, and taste by panelists were formula P3B2, it was not different significantly with P3B1, P2B1, P2B2, and P1B2 (p>0.05). The proportion of mung bean juice-skim milk and LAB significantly affected the total phenolic and soluble protein (P<0.05), meanwhile its interaction was also statistically significant (P<0.05). Using effectivity index, the best Mungbe-Yo formula according to its total phenol and soluble protein content, as well as sensory was P3B1 formula, which was 85% mung bean juice, 15% skim milk and 2% LAB, containing phenolics and soluble protein were 525.958±48.9 mg GAE/L and 43.179±1.87%, respectively. Mungbe-Yo is suitable for individuals with obesity.

1. Introduction

Obesity affects individual health, thus accelerating the progression of degenerative diseases such as coronary heart disease, atherosclerosis, diabetes mellitus, and arthritis (Owen et al., 2015). Numerous factors might trigger obesity condition, one of them was low antioxidant status. Winarsi et al. (2012) reported that antioxidant status in obese women indicated SOD activity was lower than normal weight, which also worsened by a high level of free radicals in the forms of MDA plasma. It was also reported that oxidative stress was significantly correlated with high Body Mass Index (BMI) (Marseglia et al., 2015). According to Winarsi et al. (2016), high antioxidant food products could help improve antioxidant status in patients with oxidative stress. One of the rich antioxidant content in the nuts family is mung beans.

Mung beans (Vigna radiata) contains 20-24% protein, dominated with globulin and albumin as much as 60% and 25% from total protein, respectively (Kudre et al., 2013). Mung beans protein is rich in essentials amino acids such as leucine isoleucine, and valine, however, they were lack threonine, lysine, and tryptophan. Meanwhile, its carbohydrate contents were higher (50-60%) than soybeans. However, mung beans contain several anti-nutrition compounds which usually interfere with nutrition absorption processes such as phytic acid, tannin, lectin, and protease inhibitor (Cabrera-Orozco et al., 2013). Soaking could help reduce its phytic acid, protease inhibitor, lectin, and oxalate calcium content. Efriwati et al. (2013) reported that soaking combines with lactic acid fermentation as well as the cooking process would improve the benefits of a food product. Some fermented food products are yogurt, cheese, wine, beer, and many more.

Yogurt is a fermented food product rich in protein.
Protein benefitted the human body by giving prolonged satiety, even its thermal effect are higher than carbohydrate and fat (Jørgensen et al., 2019), hence affect the availability of hormone cholecystokinin, GLP-1 and ghrelin (Yamada and Takahashi, 2012). Bacterial that is normally used for yogurt fermentation was lactic acid bacteria (LAB).

Some scientists have reported that yogurt production was influenced by the amount of LAB used in the process, lactose content, and fermentation time. Winarsi et al. (2019) reported that 2% of LAB content with 24 hr fermentation provides a more likable yogurt sensory test and the highest phenolic content. On the other hand, Wardhani et al. (2015) stated that yogurt fermentation for 15 hrs with 3% of LAB starter could improve its fat and protein nutrient contents. The optimum condition to achieve the highest protein content was yogurt fermentation for 10 h using LAB of 3% (Yilmaz et al., 2015).

More of it, LAB main substrate was lactose (milk sugar), thus, in the making of mung beans yogurt in this research was also using skim milk. That lactic acid production could improve taste, reduced milk acidity levels, as well as improve its acceptability (Teshome, 2015). Meanwhile, LAB could help produced phenolic compounds through secondary metabolites. But, until now there was no mung beans yogurt formula found, which was preferable, high soluble protein, and as phenolic antioxidant content that might be beneficial to obese individuals. This study aims to obtain mung beans yogurt formula that was likable, high soluble protein, and antioxidant phenolic contents.

2. Materials and methods

This research used a randomized group design with 2 factors, which were P (proportion of mung beans juice and skim milk) with 4 stages, and B (LAB level) with 2 stages. Mung beans that were washed thoroughly, were soaked in a hot water for 12 hrs before being drained. One part of mung beans (skin included) was added with 8 parts of water, blended to produce a smooth result, and followed by a filtration process using filtrate paper, were called mung beans juice. This study protocol was reviewed and approved by the ethics committee of the Medical Faculty of Universitas Jenderal Soedirman, Purwokerto, Indonesia (083/KEPK/III/2019).

2.1 Production of mung beans yogurt

Mung beans yogurt (Mungbe-Yo) were made by mixing mung beans juice and skim milk with proportion variances of P0 = 100:0; P1 = 95:5; P2 = 90:10, and P3 = 85:15, with additional LAB of B1 = 2% and B2 = 4%, performed in three repetitions, to produce 24 units of trial products. Sucrose (10%) was added to improve the final taste of yogurt. The solution was then pasteurized at 70°C temperature for 10 mins before it was being cooled down to a 45°C temperature. Then, the solutions were added with LAB starter for 2% and 4% from total volume and followed by fermentation process a room temperature for 24-hrs (Winarsi et al., 2019, with slight modifications).

2.2 Sensory test of Mungbe-Yo

Sensory test was measured using hedonic test (preference) performed by fifty-five panelists, including color, taste, flavor, and viscosity with a 5-point score ranged from 1–5, which are 1 = extremely dislike; 2 = dislike; 3 = slightly like; 4 = like; 5 = extremely like (Rocha et al., 2015).

2.3 Total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu method following Orak (2007). Gallic acid solutions (in methanol) were made in 300, 400, 500, 600, and 700 mg/L concentrations. As much as 0.2 mL of solutions were pipetted and then added with 15.8 mL aquadest and 1 mL of Folin-Ciocalteu reagent, then mixed to create homogenous solutions with a clear yellowish colour. The solutions were left for 8 mins before added with 3 mL of Na2CO3 20% solutions and then mixed until homogeneous. The solutions were again left for 30 mins in a room temperature until the colour changed to blue. The solutions absorbance was measured using UV-Vis spectrophotometric in a 765 nm wavelength, then the calibration curve was made to illustrate the correlation between gallic acid concentrations (mg/L) with absorbance.

A 0.1 mL sample was pipetted to be inserted in a reaction tube before added with 5 mL aquadest, then added with 0.2 mL Folin Ciocalteu that was diluted with aquadest in a ratio of 1:1, then mixed. The solutions were left for 5 mins before added with 1 mL of Na2CO3 5% solutions than being vortexed. The solutions were then left for 1 hr in a dark room. The solutions absorbance were measured in a maximum wavelength that has been obtained and replicated twice previously. This measurement was conducted in five replications.

2.4 Determination of soluble protein content using the Lowry method

It was started with making soluble protein solutions, Bovine Serum Albumin (BSA) in various concentration levels ranged from 30-300 µg/mL, then one mL of
sample was taken from each concentration before inserted into a reaction tube. Approximately 8 mL of Lowry B reagent were added and then left at room temperature for 10 mins. After that, 1 mL of Lowry A reagent was added, mixed, and then left for 20 mins. The solution absorbance was read at a 600 nm wavelength (Lee et al., 2015).

2.5 Statistical analysis

Total phenolic and protein contents were analyzed using the F test. If there was a significant effect, that was continued with Duncan’s Multiple Range Test (DMRT) at a 5% significance level. The sensory variables were analyzed using the Friedman test. If there was a significant result, the multiple comparison tests were performed at a 5% significance level. The effectiveness index was used to find out the best of Mungbe-Yo formula based on the sensory, phenolic, and soluble protein content.

3. Results and discussion

3.1 Mungbe-Yo sensory

Table 1 illustrates Mungbe-Yo sensory test results made according to the proportions of mung beans juice and skim milk as well as LAB content. In this study, the highest taste score was found in Mungbe-Yo formula P3B2, which was not different significantly from P3B1, P2B2, and P2B1, plausibly due to the higher proportion of skim milk in yogurt. During the fermentation process, lactose was changed into lactic acid by LAB. More glucose content (from a higher proportion of skim milk) was utilized to produce lactic acid also indicating higher LAB activities. According to Chen et al. (2017), lactic acid produced during the fermentation process could improve taste and acidity by reducing pH. The formation of lactic acid and LAB metabolite results in the fermentation process affected yogurt taste. The characteristic taste of yogurt was one of the main factors that affect consumer's preferability towards yogurt product.

The highest score of the color attribute was found in formula P3B2, which was not different significantly with P3B1, P2B2, and P2B1, possibly due to skim milk protein content. Milk’s protein was consist of casein, lactalbumin, lactoglobulin with casein level reached 80% of total protein. In milk, the protein was dispersed as particles in various sizes. Milk casein color has whitish yellow-colored with granule structures, odorless, and bland taste. Hence, a higher proportion of skim milk resulted in a more interesting colour of Mungbe-Yo affecting its high score measurement.

Similar findings were also found in the flavour attribute. The highest score occurred in formula P3B2, which was not different significantly with P3B1, P2B2, and P2B1, likely related to LAB activity and the amount of skim milk in Mungbe-Yo. L. bulgaricus and S. thermophilus bacteria produce the final fermentation product that is lactic acid. L. bulgaricus and S. thermophilus bacteria decompose lactose (milk sugar) into lactic acid and various flavour and taste components. In this case, L. bulgaricus played more roles in flavour formation, while S. thermophilus played more roles in taste formation (Teshome, 2015; Chen et al., 2017). Lactic acid resulted from the decomposition of various organic compounds. Lactose fermentation from LAB produced lactic acid. Lactose that was available in milk was a suitable substrate for Streptococcus lactis and Lactobacillus. Streptococcus lactis produced 1% lactic acid before reaching the pH which suppresses it, then Lactobacillus produces lactic acid reaching 4%.

Table 1. The effect of mung beans juice – skim milk proportions and LAB levels towards Mungbe-Yo sensorial properties

<table>
<thead>
<tr>
<th>Formula</th>
<th>Color</th>
<th>Taste</th>
<th>Flavor</th>
<th>Viscosity</th>
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<tbody>
<tr>
<td>P0B1</td>
<td>2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>P0B2</td>
<td>2.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.9&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>P1B1</td>
<td>2.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.4&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>2.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>P1B2</td>
<td>2.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>2.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>P2B1</td>
<td>3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>P2B2</td>
<td>3.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.1&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>P3B1</td>
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<td>3.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>P3B2</td>
<td>3.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Numbers followed with superscript letter from the same column were not statistically significant with alpha 5%. P0B1, mung beans juice: skim milk proportions = 100:0 with LAB 2%; P0B2, mung beans juice: skim milk proportions = 100:0 with LAB 4%; P1B1, mung beans juice: skim milk proportions = 95:5 with LAB 2%; P1B2, mung beans juice: skim milk proportions = 95:5 with LAB 4%; P2B1, mung beans juice: skim milk proportions = 90:10 with LAB 2%; P2B2, mung beans juice: skim milk proportions = 90:10 with LAB 4%; P3B1, mung beans juice: skim milk proportions = 85:15 with LAB 2%; P3B2, mung beans juice: skim milk proportions = 85:15 with LAB 4%.

The highest viscosity score was found in Mungbe-Yo formula P3B2, which was not different significantly with P3B1, P2B2, and P2B1. Viscosity is related to the amount of agglomerate protein by the presence of acids (Pedras et al., 2013). The more amount of skim milk proportion used in the production of Mungbe-Yo, the more protein occurred in its content. More skim milk content resulted in more lactose content, thus elevating LAB growth to produce more lactic acid, affecting more agglomerate protein hence increased the viscosity and preferable of a product. In this study, yogurt has a
slightly viscose texture with homogeneous consistency, possibly due to agglomerate protein resulted from organic acid produced by the starter culture.  

3.2 The effect of mung beans juice–skim milk proportions and LAB level to the soluble protein content of Mungbe-Yo

In this study, higher proportions of mung bean juice resulted in higher soluble protein content (P<0.05); higher LAB content also resulted in higher soluble protein content (P<0.05), as well as its interactions (P<0.05) (Table 2). Soluble protein is an oligopeptide or amino acids that were easily digested by digestive tracts, whereas total protein is nitrogen (N) content measurement in a sample (Joye, 2019). Andarwulan et al. (2012) also stated that soluble protein was part of the total protein that is available in a food product. Those proteins have chains with total amino acids that are less than 10, hence they are easier to be digested in the human digestive tract. In other words, the higher content the better its utilization in the human body.

Table 2. The effect of mung beans juice – skim milk proportions and LAB level to soluble protein and total phenolic content of Mungbe-Yo

<table>
<thead>
<tr>
<th>Formula</th>
<th>Soluble Protein (%)</th>
<th>Total Phenolic (mg GAE/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>40.64±2.92b</td>
<td>392.415±16.53b</td>
</tr>
<tr>
<td>P1</td>
<td>42.74±8.68b</td>
<td>396.374±15.45b</td>
</tr>
<tr>
<td>P2</td>
<td>45.30±7.88ab</td>
<td>352.001±10.00b</td>
</tr>
<tr>
<td>P3</td>
<td>49.50±6.47a</td>
<td>556.895±81.2a</td>
</tr>
<tr>
<td>B1</td>
<td>40.95±8.2b</td>
<td>335.749±11.8b</td>
</tr>
<tr>
<td>B2</td>
<td>48.14±2.13a</td>
<td>513.094±2.9a</td>
</tr>
<tr>
<td>P0B1</td>
<td>34.61±1.20d</td>
<td>237.830±17.80b</td>
</tr>
<tr>
<td>P0B2</td>
<td>50.86±1.70d</td>
<td>547.000±13.90a</td>
</tr>
<tr>
<td>P1B1</td>
<td>46.15±8.33ab</td>
<td>313.458±10.59b</td>
</tr>
<tr>
<td>P1B2</td>
<td>46.84±5.28ab</td>
<td>479.290±61.6a</td>
</tr>
<tr>
<td>P2B1</td>
<td>36.86±10.17cd</td>
<td>265.750±54.7b</td>
</tr>
<tr>
<td>P2B2</td>
<td>44.42±1.83ab</td>
<td>438.253±22.6a</td>
</tr>
<tr>
<td>P3B1</td>
<td>43.17±1.87bc</td>
<td>525.958±48.99a</td>
</tr>
<tr>
<td>P3B2</td>
<td>47.42±2.09ab</td>
<td>587.833±10.23a</td>
</tr>
</tbody>
</table>

Numbers followed with superscript letter from the same column were not statistically significant with alpha 5%. P0B1, mung beans juice: skim milk proportions = 100:0 with LAB 2%; P0B2, mung beans juice: skim milk proportions = 100:0 with LAB 4%; P1B1, mung beans juice: skim milk proportions = 95:5 with LAB 2%; P1B2, mung beans juice: skim milk proportions = 95:5 with LAB 4%; P2B1, mung beans juice: skim milk proportions = 90:10 with LAB 2%; P2B2, mung beans juice: skim milk proportions = 90:10 with LAB 4%; P3B1, mung beans juice: skim milk proportions = 85:15 with LAB 2%; P3B2, mung beans juice: skim milk proportions = 85:15 with LAB 4%.

Higher content of soluble protein might be correlated with a higher proportion of skim milk and the amount of LAB. According to Hall et al. (2014), bacteria has 4 phase of developments, among them, are lag phase and log phase. In the lag phase, bacteria adapted itself in a new growth media. During this phase, the growing number of bacteria had not escalated yet, however, metabolic activities were seen to be active although still insignificant. Later in the log phase (exponential), the rapid growth of bacteria was seen to happen. In this phase, the protein breakdown process by LAB is happening (Purkan et al., 2017). Castillo Martinez et al. (2013) also added that S. thermophilus and L. bulgaricus bacteria worked mutualistic. At the beginning of fermentation, S. thermophilus started to grow earlier and produced lactic acid and formic acid that were beneficial for the growth of L. Bulgaricus. After exposed to a sufficient amount of nutrients, L. bulgaricus started to grow rapidly resulted in an elevation of L. bulgaricus numbers (Sieuwerts, 2016). Moreover, L. bulgaricus bacteria have proteolytic activity (Chairunnisa et al., 2017), which able to break down complex proteins to simple proteins such as oligopeptides and amino acids with the help of protease enzyme. Thus, a higher concentration of LAB used in Mungbe-Yo production process has higher soluble protein content. In this study, the protein levels of formula P3B2, P2B2, P1B2, and P1B1 were not different significantly with P0B2 (P>0.05), which means the amount of skim milk did not affect the protein content that was reflected in control formulas.

3.3 The effect of mung bean juice - skim milk proportions and LAB levels to total phenolic content of Mungbe-Yo

The proportions of mung beans juice – skim milk was significantly affect total phenolic contents (P<0.05), with the highest phenolic compounds of 556.89±81.2 mg GAE/L, which was found in the P3 (85% mung beans juice and 15% skim milk). The higher LAB content utilized in yogurt production, the higher its total phenolic content (P<0.05). Besides, the interaction of mung bean juice and skim milk proportions, as well as LAB content, were different significantly (P<0.05), with the highest content, was 587.83±10.23 mg GAE/L available in formula P3B2.

Phenolic compounds were originally generated from mung beans, not skim milk. Even though formula P3B2 seems to have a small proportion of mung bean juice, but the amount was 85% from all total materials, or 5.7 times higher than skim milk, thus still dominated the effect over skim milk. According to El-Seedi et al. (2018), the cereal cell wall as in red beans has cinnamic hydroxy acid content that was esterified in a polysaccharide cell wall. Cinnamic hydroxy acids experienced significant loss during fermentation (LaFargue and Lonvaud-Funel, 2012). The increase of phenolic compounds in Mungbe-
Yo during fermentation possibly due to decarboxylation of cinnamic acid by microorganisms, such as trans-4-hydroxy-3-methoxy cinnamic acid (ferulic acid) and trans-4-hydroxycinnamic acid (p-coumaric acid) producing phenolic compound 4-vinyl guaiacol and 4-vinyl phenol. Santamaria et al. (2018) reported that cinnamic acid decarboxylation into vinyl phenol by LAB was happened because of vinyl phenol reductase enzyme activity.

Gaur et al. (2019) also stated that Lactobacillus has ferulic acid reductase enzyme and vinyl phenol reductase enzyme to degrade ferulic acid and cinnamic acid, which were the component of the polysaccharides cell wall, into 4-vinyl phenol and 4-vinyl guaiacol. Hence, LAB roles in this Mungbe-Yo fermentation were really important in the production of phenolic antioxidant compounds.

The LAB fermentation could also improve phenolic content Mungbe-Yo since that bacteria produced secondary metabolites, such as phenolic compounds (Primurdia and Kusnadi, 2014). The longer fermentation time resulted in lower pH due to the formation of organic acids such as lactic acid and acetic acid. Soluble organic acids released proton (H⁺), thus lowering pH. Mungbe-Yo with the highest phenolic content had pH 4.68. During the fermentation process, the LAB metabolized itself and produced some organic acids (De Vuyst and Leroy, 2010). Lactic acid, acetic acid, citric acid, succinate acid, malate acid, acetaldelyde, diacetyl, and acetoin were compounds that can be improved and stabilized antioxidant during fermentation. The organic acids that were produced during fermentation played synergetic roles and could regenerate antioxidant compounds. Furthermore, an acidic condition due to the availability of those organic acids during fermentation could increase antioxidant activity because it was more stable in an acidic atmosphere.

Mungbe-Yo phenolic content was 587.83±10.23 mg GAE/L, lower than red kidney beans sprouts yogurt (993.08 mg GAE /L) (Winarsi et al., 2019), but higher than black soybeans sprout milk yogurt (112.39 mg GAE /L) (Pertiwi et al., 2013). The acidic condition of Mungbe-Yo was increased along with the addition of skim milk. The growth of L. acidophilus stopped below pH 4.0, whereas the growth of Bifidobacterium spp. was restricted below pH 5.0. The decrease of Mungbe-Yo pH was correlated with LAB activity is broken down from lactose to lactic acid. The production of lactic acid as a result of glucose metabolism affects pH yogurt decreased. The condition was correlated with the elevation number of lactic acid bacteria that utilized lactose. The more metabolizable glucose sources affect the increase of organic acids that automatically affect the reduction of pH. The more sugar sources that can be metabolized, the more organic acids are produced so that the pH is automatically lower.

According to Li et al. (2016), Streptococcus bacteria were responsible for the decrease in the initial yogurt pH to 5.0. On the other hand, Lactobacilli were responsible for further decrease until the pH to 4.5. Probiotic bacteria, especially L. acidophilus when combined with S. thermophilus and L. bulgaricus bacteria could produce yogurt with a lower pH because they could ferment glucose into lactic acid quickly. According to Fazilah et al. (2018), yogurt bacteria L. acidophilus could improve acidity quickly with final pH below 4. That pH decreases along with the decrease of bacterial activity, marked by reduced survival of LABs. A similar condition occurred in the yogurt acidic level. The higher proportion of skim milk in yogurt production resulted in a higher acidic level in the final product. The increased of acidic level in Mungbe-Yo correlated with LAB activity towards lactose breakdown into lactic acid.

The more glucose content in the milk, the higher the acidic level in yogurt. Another possibility increased proportion of skim milk in this study could still be tolerated by LAB, proven that generated phenolic compounds content were even higher. The amount of sweeteners added in the making of Mungbe-Yo, which is sucrose, could help improved LAB growth and thus improved bacterial viabilities (Costa et al., 2019). Likewise, its activity in the production of its secondary metabolites (phenolic compounds).

The amount of LAB used in the making of Mungbe-Yo could also significantly affect the total phenolic content (P<0.05). The acid generated by Lactobacillus bulgaricus by converting glucose into lactic acid. The oxidation reaction that happened during the fermentation process could also induce phenolic to act as an antioxidant against that reaction. In microorganisms, glucose transport into cells could inhibit adenyl cyclase enzyme to produce cAMP (Qiu et al., 2016). The purpose of cAMP was stimulating various enzyme synthesis. The elevation of total phenolic content might also result from increased enzyme production by Lactobacillus bulgaricus that played a role in the formation of polyphenol through the decarboxylation process. Moreover, the reduction of polyphenol during fermentation was a consequence of protein deposition. The lower pH due to the higher proportion of skim milk resulted in protein deposition. The prolonged fermentation process increased the hydrogen bond between polyphenol and protein.
3.4 The selected of Mungbe-Yo formula

The selected or best Mungbe-Yo formula was determined using the effectivity index according to DeGarmo et al. (1984). Based on its phenolic content, soluble protein, flavour, taste, color, and viscosity, the selected formula was P3B1, which consists of 85% mung beans juice, 15% skim milk, and LAB 2%. That contained phenolic compound as much as 525.96±48.9 GAE/L and soluble proteins 43.18±1.87%. The Mungbe-Yo rich in phenolic and soluble proteins could be beneficial for individuals with obesity.

Some researchers report that phenolic as potential antioxidants. Phenolic mechanism towards bodyweight reduction could be explained through several mechanisms such as improved satiety, stimulate energy expenditure by induced thermogenesis in brown adipose tissues, modulating adipose tissues by inhibited adipocytes differentiation, and promoted adipocyte apoptosis, modulating lipolysis, and activating β-oxidation (Rupasinghe et al., 2016). Phenolic compounds are an important candidate as a beneficial diet for obesity. Continuous and prolonged intake of phenolic might resulted in lower blood pressure and adiposity effect, lipid profile improvement, as well as an anti-inflammation factor, which all combined played significant roles to inhibit obesity progression towards cardiovascular diseases (Tresserra-Rimbau et al., 2014).

Soluble protein content in the selected Mungbe-Yo was 24 times higher than the minimum threshold of soymilk protein (2%) in Standard Nasional Indonesia (1992). Pesta and Samuel (2014) reported that protein has a thermogenic effect and induced satiety higher than carbohydrates. Mungbe-Yo proteins could produce calories higher than carbohydrates. Therefore, the protein could suppress hunger and appetite which consequently helped reduced body weight. That high protein diet-induced satiety longer than low protein diet; hence suppressed subsequent calorie intake. The human body has no protein storage mechanisms; thus, protein intake was immediately metabolized into energy.

Thus it can be concluded that mung beans yoghurt made from 85% of mung beans juice and 15% skim milk added with 2% of LAB was the best formula, evidently containing phenolic antioxidants as much as 525.96±48.9 mg GAE/L and soluble protein 43.18±1.87%. Plausibly, this Mungbe-Yo product rich in phenolic and soluble proteins can be a potential functional drink to help control body weight in obese through several mechanisms.

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