



Hery Winarsi &lt;winarsi12@gmail.com&gt;

---

**Manuscript ID: FR-2020-500**

---

**Food Research** <foodresearch.my@outlook.com>  
To: Hery Winarsi <winarsi12@gmail.com>

Thu, Sep 10, 2020 at 5:28 PM

Dear Dr. Hery Winarsi,

This message is to acknowledge receipt of the above manuscript that you submitted via email to Food Research. Your manuscript has been successfully checked-in. Please refer to the assigned manuscript ID number in any correspondence with the Food Research Editorial Office or with the editor.

Your paper will be reviewed by three or more reviewers assigned by the Food Research editorial board and final decision made by the editor will be informed by email in due course. Reviewers' suggestions and editor's comments will be then made available via email attached file. You can monitor the review process for your paper by emailing us on the "Status of my manuscript".

If your manuscript is accepted for publication, Food Research editorial office will contact you for the production of your manuscript.

Thank you very much for submitting your manuscript to Food Research.

Sincerely,

Professor Dr. Son Radu  
Chief Editor  
Email: [foodresearch.my@outlook.com](mailto:foodresearch.my@outlook.com)



---

**From:** Hery Winarsi <winarsi12@gmail.com>  
**Sent:** Thursday, 10 September, 2020 3:40 PM  
**To:** Food Research <[foodresearch.my@outlook.com](mailto:foodresearch.my@outlook.com)>  
**Subject:** manuscript ID

Dear FR editor,

On August 22, 2020, I submitted a manuscript entitled "Polyphenol rich mung bean (*Vigna radiata*) yogurt for obesity prevention", but until now I have not been given a manuscript ID. Please clarify.

Regards,

Dr. Hery Winarsi, MS  
Department of Nutrition Science, Faculty of Health Sciences,  
University of Jenderal Soedirman. Purwokerto, 53123.  
Central Java. Indonesia.

Phone/fax : +62281621122  
Mobile phone : +628161488133  
website: [www.gizi.unsoed.ac.id](http://www.gizi.unsoed.ac.id)

---

 **Letter to Author FR-2020-500.pdf**  
27K

10<sup>th</sup> September 2020

Authors: Winarsi, H.1, Agustia, F.C., Ramadhan, G.R., Zaki, I., Putri, W.A.K., Sulistyaning, A.R., Farida, F., Sari, H.P.

Manuscript title: Polyphenol rich mung bean (*Vigna radiata*) yogurt for obesity prevention

Manuscript ID: FR-2020-500

Dear Dr. Hery Winarsi,

This message is to acknowledge receipt of the above manuscript that you submitted via email to Food Research. Your manuscript has been successfully checked-in. Please refer to the assigned manuscript ID number in any correspondence with the Food Research Editorial Office or with the editor.

Your paper will be reviewed by three or more reviewers assigned by the Food Research editorial board and final decision made by the editor will be informed by email in due course. Reviewers' suggestions and editor's comments will be then made available via email attached file. You can monitor the review process for your paper by emailing us on the "Status of my manuscript".

If your manuscript is accepted for publication, Food Research editorial office will contact you for the production of your manuscript.

Thank you very much for submitting your manuscript to Food Research.

Sincerely,



Professor Dr. Son Radu  
Chief Editor  
Email: foodresearch.my@outlook.com



Hery Winarsi <winarsi12@gmail.com>

---

**Manuscript ID: FR-2020-500**

---

**Hery Winarsi** <winarsi12@gmail.com>  
To: Food Research <foodresearch.my@outlook.com>

Tue, Nov 24, 2020 at 4:58 PM

Dear Prof Son Radu,

Could it be that there is progress and good news for the ID FR-2020-500 article?  
We are looking forward to it

Regards,  
[Quoted text hidden]



Hery Winarsi &lt;winarsi12@gmail.com&gt;

---

**Manuscript ID: FR-2020-500**

---

**Food Research** <foodresearch.my@outlook.com>  
To: Hery Winarsi <winarsi12@gmail.com>

Wed, Nov 25, 2020 at 12:08 AM

Dear Dr. Hery,

Your manuscript is currently under technical review by our editorial board. We are currently experiencing high loads of publications, please expect some delay from our side. Thank you for your patience.

Best regards,  
Son Radu, PhD  
Chief Editor

---

**From:** Hery Winarsi <winarsi12@gmail.com>**Sent:** Tuesday, 24 November, 2020 5:58 PM

[Quoted text hidden]

[Quoted text hidden]

30<sup>th</sup> November 2020

Dear Dr Winarsi

**ACCEPTANCE LETTER**

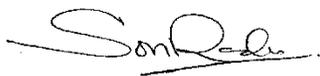
Food Research, is pleased to inform you that the following manuscript has been accepted for publication in Food Research journal.

Manuscript Title : Polyphenol rich mung bean (*Vigna radiata*) yogurt for obesity prevention

Authors : Winarsi, H., Agustia, F.C., Ramadhan, G.R., Zaki, I., Putri, W.A.K., Sulistyning, A.R., Farida, F. and Sari, H.P.

We thank you for your fine contribution to the Food Research journal and encourage you to submit other articles to the Journal.

Yours sincerely,



**Professor Dr. Son Radu**  
Chief Editor  
Food Research

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

\*Winarsi, H., Agustia, F.C., Ramadhan, G.R., Zaki, I., Putri, W.A.K., Sulistyning, A.R., Farida, F. and Sari, H.P.

Department of Nutrition Science, Faculty of Health Sciences, Universitas Jenderal Soedirman, Purwokerto, 53123, Central Java, Indonesia

### Article history:

Received: 10 September 2020

Received in revised form: 14 October 2020

Accepted: 30 November 2020

Available Online: 7 March 2021

### Keywords:

Mung beans yogurt,  
Phenolic,  
Soluble protein,  
Sensory

### DOI:

[https://doi.org/10.26656/fr.2017.5\(2\).500](https://doi.org/10.26656/fr.2017.5(2).500)

### 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  $525.958\pm 48.9$  mg GAE/L and  $43.179\pm 1.87\%$ , 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.

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 essential 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, tannin, 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.

Mung beans (*Vigna radiata*) contains 20-24%

\*Corresponding author.

Email: [winarsi12@gmail.com](mailto:winarsi12@gmail.com)

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, as well 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 four 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 (preferable) 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 Na<sub>2</sub>CO<sub>3</sub> 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 Na<sub>2</sub>CO<sub>3</sub> 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

Formula	Color	Taste	Flavor	Viscosity
P0B1	2.1 <sup>b</sup>	2.1 <sup>dc</sup>	2.3 <sup>c</sup>	1.8 <sup>c</sup>
P0B2	2.3 <sup>b</sup>	2.1 <sup>c</sup>	2.4 <sup>c</sup>	1.9 <sup>bc</sup>
P1B1	2.6 <sup>b</sup>	2.4 <sup>cdc</sup>	2.5 <sup>c</sup>	2.2 <sup>bc</sup>
P1B2	2.7 <sup>b</sup>	2.6 <sup>bcd</sup>	2.6 <sup>bc</sup>	2.5 <sup>b</sup>
P2B1	3.5 <sup>a</sup>	3.0 <sup>abc</sup>	3.0 <sup>ab</sup>	3.5 <sup>a</sup>
P2B2	3.6 <sup>a</sup>	3.1 <sup>ab</sup>	3.1 <sup>ab</sup>	3.6 <sup>a</sup>
P3B1	3.9 <sup>a</sup>	3.4 <sup>a</sup>	3.5 <sup>a</sup>	3.6 <sup>a</sup>
P3B2	3.9 <sup>a</sup>	3.5 <sup>a</sup>	3.6 <sup>a</sup>	3.6 <sup>a</sup>

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

Formula	Soluble Protein (%)	Total Phenolic (mg GAE/L)
P0	40.648±2.92 <sup>b</sup>	392.415±16.53 <sup>b</sup>
P1	42.742±8.68 <sup>b</sup>	396.374±15.45 <sup>b</sup>
P2	45.304±7.88 <sup>ab</sup>	352.001±10.00 <sup>b</sup>
P3	49.502±6.47 <sup>a</sup>	556.895±81.2 <sup>a</sup>
B1	40.955±8.2 <sup>b</sup>	335.749±11.8 <sup>b</sup>
B2	48.143±2.13 <sup>a</sup>	513.094±2.9 <sup>a</sup>
P0B1	34.617±1.20 <sup>d</sup>	237.830±17.80 <sup>b</sup>
P0B2	50.867±1.70 <sup>a</sup>	547.000±13.90 <sup>a</sup>
P1B1	46.158±8.33 <sup>ab</sup>	313.458±10.59 <sup>b</sup>
P1B2	46.845±5.28 <sup>ab</sup>	479.290±61.6 <sup>a</sup>
P2B1	36.866±10.17 <sup>cd</sup>	265.750±54.7 <sup>b</sup>
P2B2	44.429±1.83 <sup>ab</sup>	438.253±22.6 <sup>a</sup>
P3B1	43.179±1.87 <sup>bc</sup>	525.958±48.9 <sup>a</sup>
P3B2	47.429±2.09 <sup>ab</sup>	587.833±10.23 <sup>a</sup>

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 (Laforgue 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. Santamaría *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, acetaldehyde, 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  $587.83 \pm 10.23$  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 adenylyl 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  $\beta$ -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 yogurt 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.

### Conflict of interest

The authors declare no conflict of interest.

### Acknowledgments

The author offered the deepest gratitude towards Universitas Jenderal Soedirman (Unsoed) Rector through Lembaga Penelitian dan Pengabdian Masyarakat (LPPM) Chairman for funding this research in the skim of Riset Unggulan Unsoed 2019, as well as the facilities.

### References

- Andarwulan, N., Kurniasih, D., Apriady, R.A., Rahmat, H., Roto, A.V. and Bolling, B.W. (2012). Polyphenols, carotenoids, and ascorbic acid in underutilized medicinal vegetables. *Journal of Functional Foods*, 4(1), 339-347. <https://doi.org/10.1016/j.jff.2012.01.003>
- Cabrera-Orozco, A., Jiménez-Martínez, C. and Dávila-Ortiz, G. (2013). Soybean: Non-Nutritional Factors and Their Biological Functionality. In El-Shemy, H. (Ed.) Soybean - Bio-Active Compounds. InTech Open E-Book.
- Castillo Martinez, F.A., Balciunas, E.M., Salgado, J.M., Domínguez González, J.M., Converti, A. and Oliveira, R.P.D.S. (2013). Lactic acid properties, applications and production: A review. *Trends in Food Science and Technology*, 30(1), 70-83. <https://doi.org/10.1016/j.tifs.2012.11.007>
- Chairunnisa, H., Baila, R.L., Pratama, A. and Hadiat, D.R. (2017). Chemical characteristics of yoghurt set with flour milk as raw material with the addition of beet juice (*Beta Vulgaris* L.). *Jurnal Ilmu Ternak*, 17 (1), 35-39.
- Chen, C., Zhao, S., Hao, G., Yu, H., Tian, H. and Zhao, G. (2017). Role of lactic acid bacteria on the yogurt flavour: A review. *International Journal of Food Properties*, 20(Suppl. 1), S316-S330. <https://doi.org/10.1080/10942912.2017.1295988>
- Costa, G.M., Paula, M.M., Barão, C.E., Klososki, S.J., Bonafé, E.G., Visentainer, J.V. and Pimentel, T.C. (2019). Yoghurt added with *Lactobacillus casei* and sweetened with natural sweeteners and/or prebiotics: Implications on quality parameters and probiotic survival. *International Dairy Journal*, 97, 139-148. <https://doi.org/10.1016/j.idairyj.2019.05.007>
- DeGarmo, E.P., Sullivan, W.G. and Canada, J.R. (1984). *Engineering Economy*. 7th ed. New York, USA: Mac Milan .
- De Vuyst, L. and Leroy, F. (2020). Functional role of yeasts, lactic acid bacteria, and acetic acid bacteria in cocoa fermentation processes. *FEMS Microbiology Reviews*, 44(4), 432-453. <https://doi.org/10.1093/femsre/fuaa014>

- Efriwati, Suwanto, A., Rahayu, G. and Nuraida, L. (2013). Population dynamics of yeasts and lactic acid bacteria (LAB) during tempeh production. *HAYATI Journal of Biosciences*, 20(2), 57-64. <https://doi.org/10.4308/hjb.20.2.57>
- El-Seedi, H.R., Taher, E.A., Sheikh, B.Y., Anjum, S., Saeed, A., AlAjmi, M.F. and Göransson, U. (2018). Hydroxycinnamic Acids: Natural Sources, Biosynthesis, Possible Biological Activities, and Roles in Islamic Medicine. *Studies in Natural Products Chemistry*, 55, 269-292.
- Fazilah, N.F., Ariff, A.B., Khayat, M.E., Rios-Solis, L. and Halim, M. (2018). Influence of probiotics, prebiotics, synbiotics and bioactive phytochemicals on the formulation of functional yogurt. *Journal of Functional Foods*, 48, 387-399. <https://doi.org/10.1016/j.jff.2018.07.039>
- Gaur, G., Oh, J.-H., Filannino, P., Gobetti, M., van Pijkeren, J.-P. and Gänzle, M.G. (2019). Genetic determinants of hydroxycinnamic acid metabolism in heterofermentative *Lactobacilli*. *Applied and Environmental Microbiology*, 86, e02461-1. <https://doi.org/10.1128/AEM.02461-19>
- Hall, B.G., Acar, H., Nandipati, A. and Barlow, M. (2014). Growth rates made easy. *Molecular Biology and Evolution*, 31(1), 232-238. <https://doi.org/10.1093/molbev/mst187>
- Jørgensen, C.E., Abrahamsen, R.K., Rukke, E.-O., Hoffmann, T.K., Johansen, A.-G. and Skeie, S.B. (2019). Processing of high-protein yoghurt – A review. *International Dairy Journal*, 88, 42-59. <https://doi.org/10.1016/j.idairyj.2018.08.002>
- Joye, I. (2019). Protein digestibility of cereal products. *Foods*, 8(6), 199. <https://doi.org/10.3390/foods8060199>
- Kudre, T.G., Benjakul, S. and Kishimura, H. (2013). Comparative study on chemical compositions and properties of protein isolates from mung bean, black bean, and Bambara groundnut. *Journal of the Science of Food and Agriculture*, 93(10), 2429-2436. <https://doi.org/10.1002/jsfa.6052>
- Laforgue, R. and Lonvaud-Funel, A. (2012). Hydroxycinnamic acid decarboxylase activity of *Brettanomyces bruxellensis* involved in volatile phenol production: relationship with cell viability. *Food Microbiology*, 32(2), 230-234. <https://doi.org/10.1016/j.fm.2012.06.004>
- Lee, N., Shin, S., Chung, H.J., Kim, D.K., Lim, J., Park, H. and Oh, H.J. (2015). Improved quantification of protein in vaccines containing aluminum hydroxide by simple modification of the Lowry method. *Vaccine*, 33(39), 5031-5034. <https://doi.org/10.1016/j.vaccine.2015.08.004>
- Li, D., Li, J., Zhao, F., Wang, G., Qin, Q. and Hao, Y. (2016). The influence of fermentation condition on production and molecular mass of EPS produced by *Streptococcus thermophilus* 05-34 in milk-based medium. *Food Chemistry*, 197(Part A), 367-372. <https://doi.org/10.1016/j.foodchem.2015.10.129>
- Marseglia, L., Manti, S., D'Angelo, G., Nicotera, A., Parisi, E., Di Rosa, G., Gitto, E. and Arrigo, T. (2015). Oxidative stress in obesity: a critical component in human diseases. *International Journal of Molecular Sciences*, 16(1), 378-400. <https://doi.org/10.3390/ijms16010378>
- Orak, H.H. (2007). Total antioxidant activities, phenolics, anthocyanins, polyphenol oxidase activities of selected red grape cultivars, and their correlations. *Scientia Horticulturae*, 111(3), 235-241. <https://doi.org/10.1016/j.scienta.2006.10.019>
- Owen, C.G., Kapetanakis, V.V. and Rudnicka, A.R. (2015). Body mass index in early and middle adult life: prospective associations with myocardial infarction, stroke, and diabetes over 30 years: the British Regional Heart Study. *British Medical Journal*, 5(9), e008105. <https://doi.org/10.1136/bmjopen-2015-008105>
- Pedras, M.M., Tribst, A.A.L. and Cristianini, M. (2013). Effects of high-pressure homogenisation on physicochemical characteristics of partially skimmed milk. *International Journal of Food Science and Technology*, 49(3), 861-866. <https://doi.org/10.1111/ijfs.12378>
- Pertiwi, S., Aminah, S. and Nurhidajah. (2013). Antioxidant activity, chemical characteristics, and organoleptic properties of black soybean sprouts milk based on variation in germination time. *Jurnal Pangan dan Gizi*, 4(2), 1-8.
- Pesta, D.H. and Samuel, V.T. (2014). A high-protein diet for reducing body fat: Mechanisms and possible caveats. *Nutrition and Metabolism*, 11(1), 1-8. <https://doi.org/10.1186/1743-7075-11-53>
- Primurdia, G.E. and Kusnadi, J. (2014). Antioxidant activity of probiotic drink from dates extract (*Phoenix dactylifera* L.) with isolates of *L. plantarum* and *L. casei*. *Jurnal Pangan dan Agroindustri*, 2(3), 98-109.
- Purkan, P., Laila, N.N. and Sumarsih, S. (2017). *Lactobacillus bulgaricus* as a probiotic to improve the quality of tofu dregs for earthworm feed. *Jurnal Kimia Riset*, 2(1), 1-9. <https://doi.org/10.20473/jkr.v2i1.3688>
- Qiu, L., LeBel, R.P., Storm, D.R. and Chen, X. (2016).

- Type 3 adenylyl cyclase: a key enzyme mediating the cAMP signaling in neuronal cilia. *International Journal of Physiology, Pathophysiology and Pharmacology*, 8(3), 95-108.
- Rocha, I.F.D.O. and Bolini, H.M.A. (2015). Passion fruit juice with different sweeteners: sensory profile by descriptive analysis and acceptance. *Food Science and Nutrition*, 3(2), 129-139. <https://doi.org/10.1002/fsn3.195>
- Rupasinghe, H.P.V., Sekhon-Loodu, S., Mantso, T. and Panayiotidis, M.I. (2016). Phytochemicals in regulating fatty acids  $\beta$ -oxidation: Potential underlying mechanisms and their involvement in obesity and weight loss. *Pharmacology and Therapeutics*, 165, 153-163. <https://doi.org/10.1016/j.pharmthera.2016.06.005>
- Santamaría, L., Reverón, I., López de Felipe, F., de Las Rivas, B. and Muñoz, R. (2018). Unraveling the reduction pathway as alternative metabolic route to hydroxycinnamate decarboxylation in *Lactobacillus plantarum*. *Applied and Environmental Microbiology*, 84, e01123-18. <https://doi.org/10.1128/AEM.01123-18>
- Sieuwerts, S. (2016). Microbial interactions in the yoghurt consortium: current status and product implications. *SOJ Microbiology and Infectious Diseases*, 4(2), 1-5. <https://doi.org/10.15226/sojmid/4/2/00150>
- Teshome, G. (2015). Review on lactic acid bacteria function in milk fermentation and preservation. *African Journal of Food Science*, 9(4), 170-175. <https://doi.org/10.5897/AJFS2015.1276>
- Tresserra-Rimbau, A., Rimm, E.B., Medina-Remón, A., Martínez-González, M.A., de la Torre, R., Corella, D. and Lamuela-Raventós, R.M. (2014). Inverse association between habitual polyphenol intake and incidence of cardiovascular events in the Predimed study. *Nutrition, Metabolism and Cardiovascular Diseases*, 24(6), 639-647. <https://doi.org/10.1016/j.numecd.2013.12.014>
- Wardhani, D.H, Diana, C.M. and Eko, A.P. (2015). Study of the effect of how to make corn milk, ratio, and fermentation time on the characteristics of sweet corn yogurt. *Momentum*, 11(1), 7-12.
- Winarsi, H., Sasongko, N.D. and Purwanto, A. (2016). Germinated-soy milk in suppressing inflammation and oxidative stress in blood plasma and breast milk of lactating mothers. *International Food Research Journal*, 23(2), 646-652.
- Winarsi, H., Septiana, A.T., Kartini and Hanifah, I.N. (2019). Lactic acid bacteria fermentation increases the phenolic content and yogurt fiber of red bean sprouts milk (*Phaseolus vulgaris* L.), a functional beverage for obesity. *Jurnal Gizi dan Pangan Soedirman*, 3(1), 64-75. <https://doi.org/10.20884/1.jgpps.2019.3.1.1526>
- Winarsi, H., Wijayanti, S.P.M. and Purwanto, A. (2012). Activity of Superoxide Dismutase, Catalase, and Glutathione Peroxidase enzymes in women with metabolic syndrome. *Majalah Kedokteran Bandung*, 44(1), 7-12. <https://doi.org/10.15395/mkb.v44n1.75>
- Yamada, H. and Takahashi, K. (2012). Ghrelin: Production, Action Mechanisms and Physiological Effects, p. 57-75. New York, USA: NOVA Science Publishers, Inc.
- Yilmaz, M.T., Dertli, E., Toker, O.S., Tatlisu, N.B., Sagdic, O. and Arici, M. (2015). Effect of in situ exopolysaccharide production on physicochemical, rheological, sensory, and microstructural properties of the yogurt drink ayran: An optimization study based on fermentation kinetics. *Journal of Dairy Science*, 98(3), 1604-1624. <https://doi.org/10.3168/jds.2014-8936>