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Hery Winarsi <winarsi12@gmail.com>

FR-2021-176

Hery Winarsi <winarsi12@gmail.com> Wed, Apr 14, 2021 at 9:59 PM
To: Food Research <foodresearch.my@outlook.com>

Dear Prof. Son Radu, Ph.D.

A month ago we sent the manuscript ID FR-2021-176.
Please tell me how the progress is.

Regards,

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

Gmail

Hery Winarsi <winarsi12@gmail.com>

FR-2021-176

Food Research <foodresearch.my@outlook.com> Thu, Apr 15, 2021 at 10:34 PM
To: Hery Winarsi <winarsi12@gmail.com>

Dear Dr. Hery Winarsi,

Apologies for the delayed response. Your manuscript is still under review.

Best regards,
Son Radu, PhD
Chief Editor

From: Hery Winarsi <winarsi12@gmail.com>
Sent: Wednesday, 14 April, 2021 10:59 PM
To: Food Research <foodresearch.my@outlook.com>
Subject: FR-2021-176

[Quoted text hidden]

Gmail

Hery Winarsi <winarsi12@gmail.com>

Re: FR-2021-176 - Article Production

Food Research <foodresearch.my@outlook.com> Sun, Feb 20, 2022 at 1:49 PM
To: Hery Winarsi <winarsi12@gmail.com>

Dear Dr Winarsi,

Manuscript ID: FR-2021-176
Manuscript Title: Mung bean sprouts yoghurt rich in antioxidants as a functional drink during pandemic

Before we can proceed with the article production, I would like to clarify a few points that I have commented in the manuscript. Please refer to the attachment. Please address the issues raised in the comments.

Please use the attached copy to make your revisions as it has been corrected to the Journal's format. Once you have done, kindly revert the copy to me as soon as possible. Please note the faster you respond, the quicker we will process your manuscript.

Thanks & Regards,
Vivian New
Editor
Food Research

From: Food Research <foodresearch.my@outlook.com>
Sent: Friday, 14 January, 2022 2:40 PM
To: Hery Winarsi <winarsi12@gmail.com>
Subject: Re: FR-2021-176 - Decision on your manuscript

Dear Dr Winarsi,

Apologies for the delay. We had a large volume of manuscript for production. We're getting to your manuscript soon.

Thanks & Regards,
Vivian New
Editor
Food Research

From: Hery Winarsi <winarsi12@gmail.com>
Sent: Thursday, 13 January, 2022 10:44 PM
To: Food Research <foodresearch.my@outlook.com>
Subject: Re: FR-2021-176 - Decision on your manuscript

Dear Vivian,

Once again I ask when is the article with ID FR-2021-176 published? Last August, you said this article would probably be published in 3 months' time.

Regards,

On Wed, Aug 4, 2021 at 7:23 PM Food Research <foodresearch.my@outlook.com> wrote:

> Dear Dr Winarsi,
>
> Probably in 3 months' time.

Thanks & Regards,
Vivian New
Editor
Food Research

From: Hery Winarsi <winarsi12@gmail.com>
Sent: Wednesday, 4 August, 2021 5:08 PM
To: Food Research <foodresearch.my@outlook.com>
Subject: Re: FR-2021-176 - Decision on your manuscript

Dear Vivian New

When is the accepted article (FR-2021-176) published?

Regards,

On Sat, Jul 10, 2021 at 2:44 PM Food Research <foodresearch.my@outlook.com> wrote:

> Dear Dr Winarsi,
>
> It is a pleasure to accept your manuscript for publication in Food Research journal. Please refer to the attachment for your acceptance letter. I will contact you again once the galley proof is ready for viewing and approval.
>
> Thank you for your fine contribution. We look forward to your continued contributions to the Journal.
>
> Sincerely,
> Dr Vivian New
> Editor
> Food Research

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

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

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Dear Dr Winarsi,

Your manuscript is currently available online and in press on our website https://www.myfoodresearch.com
Please note that the version in press is the 'Corrected Proof' version. The manuscript information e.g. volur
number and DOI, will be provided soon once payment is made. We will then inform you of the update of yo
details.

Thank you for your fine contribution. We hope that you continue to submit other articles to the Journal.

Thanks & Regards,
Vivian New
Editor
Food Research

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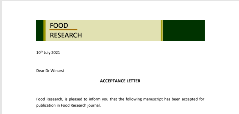
FOOD RESEARCH

ACCEPTANCE LETTER

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Hery Winarsi Thu, Jan 13, 2

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Re: FR-2021-176 - Article Production

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Food Research <foodresearch.my@outlook.com> Sun, Feb 20, 2022, 1:49 PM
to me

Dear Dr Winarsi,

Manuscript ID: FR-2021-176
Manuscript Title: Mung bean sprouts yoghurt rich in antioxidants as a functional drink during pandemic

Before we can proceed with the article production, I would like to clarify a few points that I have commente
manuscript. Please refer to the attachment. Please address the issues raised in the comments.

Please use the attached copy to make your revisions as it has been corrected to the Journal's format. Onc
done, kindly revert the copy to me as soon as possible. Please note the faster you respond, the quicker we

FOOD
RESEARCH

10th July 2021

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	:	Mung bean sprouts yoghurt rich in antioxidants as a functional drink during pandemic
Authors	:	Winarsi, H., Erminawati, E., Andreas, A. and Nuraeni, I.

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

Food Research 6 (1) : 287 - 295 (February 2022)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Mung bean sprouts yoghurt rich in antioxidants as a functional drink during pandemic

[1,*]Winarsi, H., [2]Erminawati, E., [3]Andreas, A. and [1]Nuraeni, I.

[1]*Department of Nutrition Science, Faculty of Health Sciences, Universitas Jenderal Soedirman, Purwokerto, 53123, Central Java, Indonesia*
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Phenolic,
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Antioxidants

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https://doi.org/10.26656/fr.2017.6(1).176

Abstract

This study was aimed to determine the effect of Lactic Acid Bacteria (LAB) and the proportion of mung bean sprouts milk - skim milk on phenolic content, protein, and fibre of mung bean sprouts yoghurt, and the best formula. Green beans are germinated for 10 hrs, blended until smooth, and then fermented into yoghurt. LAB levels of 0.25% and 0.5%, as well as the proportion of mung bean sprouts milk - skim milk 100: 0, 90:10, 80:20, and 70:30, with three replications. Phenolic levels were determined by the Folin-Ciocalteu method; dissolved protein by the Lowry method; and fibre by the AOAC method. LAB levels of 0.5% increased the content of phenolic (P = 0.02), protein (P = 0.0003), and fibre (P = 0.046), as well as the proportion of 70% mung bean sprouts milk and 30% skim milk also increased the content of phenolic (P = 0.0014), protein (P = 0.014), and fibre (P = 0.036) of mung bean sprouts yoghurt, but the treatment interaction had no effect (P<0.05). The best mung bean sprouts yoghurt formula was mung bean sprouts yoghurt made with the proportion of mung bean sprouts milk: skim milk, 70:30 with LAB 0.5%, with the content of phenolic 1273.11 mg GAE/L, 41.40% protein, and 12.17% fibre. Mung bean sprouted yoghurt was rich in phenolic antioxidants, protein, and fibre and suitable as an antioxidant-rich drink during the COVID-19 pandemic.

1. Introduction

In times of the COVID-19 pandemic like today, maintaining health is very important, especially the body's immune system. In addition to macronutrient intake, it is no less important to consume food products rich in antioxidants, because some researchers report that foods rich in antioxidants can overcome oxidative stress and have the potential to act as immunomodulators (Middleton *et al*., 2000; Winarsi *et al*., 2012; Winarsi *et al*., 2013). Excellent immune status can ward off exposure to bacteria and coronaviruses.

One type of nut that are rich in antioxidants is mung beans (*Vigna radiata*). Besides, mung beans are also high in protein, amino acids, oligosaccharides, and polyphenols. Polyphenol compounds are known to be the main contributors as antioxidants, anti-inflammatory, and anti-tumour. However, high oligosaccharides in nuts can cause flatulence, it is important to do processing with soaking and germination. According to Winarsi *et al.* (2019), the phenolic content of mung bean-based yoghurt that has been soaked for 12 hrs reaches 525.958±48.9 mg GAE/L, but there are still many flavours. Germination can eliminate the many flavours in the red bean sprouts yoghurt, and even increase its phenolic content. Thus, germination provides several benefits, such as reducing the anti-nutritional content, increasing digestibility (Sokrab *et al*., 2012), and the total phenolic content (Guo *et al*., 2012; Winarsi *et al*., 2019). This occurs because the activity of protease, lipase and carbohydrase increases during the germination process, the content of amino acids, fatty acids, and glucose is higher than ungerminated nuts. Glucose, amino acids, vitamins, and minerals are simple molecules that are easy to digest. More than that, these molecules can be essential nutrients for the growth of lactic acid bacteria (LAB).

Yoghurt, one of the probiotic products produced through LAB fermentation, contains *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Yoghurt is reported to increase nutritional and antioxidant content

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(Guo *et al*., 2012), vitamins and minerals, and dietary fibre (Winarsi *et al*., 2019) it is beneficial for health, and to improve antioxidant status and immunity (Winarsi *et al*., 2016).

The success of making yoghurt depends on LAB levels, the amount of milk as a source of lactose, temperature, and duration of fermentation. LAB concentration with a level of 1% and 24 hrs of fermentation time produced yoghurt with high acidity (Siregar *et al*., 2014). Likewise, according to Polyorach *et al*. (2016), the optimum LAB activity occurs in 24-hour fermentation. Prasetyo (2010) adds that the use of a yoghurt starter with a level of 6-8% does not affect the characteristics of the resulting yoghurt, he suggests using a LAB starter at a lower level. Dendi (2013) reported the use of LAB of 2-4% can produce a good viscosity of yoghurt.

Meanwhile, green bean yoghurt was reported by Winarsi *et al*. (2021) using LAB 2- 4%, followed by incubation at room temperature for 24 hrs. As much as 85% mung bean juice, 15% skim milk, and 2% LAB, containing the highest phenolics and soluble protein. However, pre-research found a LAB of 0.25-0.5% gave sensory mung bean sprouts yoghurt the favoured one.

The problem is how many labs and the proportion of milk that can be used to make mung bean sprouts yoghurt to obtain products rich in phenolics, protein, and fibre, which are useful as antioxidant-rich drinks during the COVID-19 pandemic. The purpose of this study was to determine the effect of LAB levels and the proportion of mung bean sprouts milk – skim milk on phenolic content, protein, and fibre, and to obtain the best mung bean sprouts yoghurt formula.

2. Materials and methods

This experimental study used a randomized block design with 2 factors. The first factor, LAB levels (0.25 and 0.5%), and the second factor is the proportion of mung bean sprouts milk and skim milk (100: 0; 90:10; 80:20; 70:30). Each treatment was repeated 3 times, there were 24 experimental units.

2.1 Mung Bean Sprouted Yoghurt (MungbeS-Yo) production

Mung bean sprouted yoghurt (MungbeS-Yo) was produced according to Winarsi *et al.* (2021) with slight modifications. Mung beans that have been sprouted for 10 hrs. As much as one part of the sprouts plus six parts of water, then blend it until you get a smooth liquid called mung bean sprouts milk (MungbeS-Milk). For each proportion of mung bean sprouts and skim milk, 10% sucrose was added, then heated at 70°C for 10 mins. Cooled to a temperature of 40-45ºC, then inoculated with a LAB starter 0.25% and 0.5% of the total volume, and incubated at room temperature for 24 hrs, to obtain mung bean sprouted yoghurt (MungbeS-Yo).

2.2 Determination of phenolic levels

The total phenolic test by calorimetry with visible spectrophotometry using the Folin-Ciocalteu reagent via the Folin-Ciocalteu method. The principle of this method is the formation of complex compounds that produce blue colour (Alfian and Hari, 2012). Visible spectrophotometry is a simple method for determining levels by measuring the absorption that occurs from chemical interactions between electromagnetic radiation and molecules or atoms of a chemical substance in the visible region. The wavelength of visible light ranges from 400-800 nm. The principle of spectrophotometry is that if there is monochromatic light passing through a solution, some of the incoming light will be reflected, some will be absorbed and partially transmitted. The absorbed light is converted into a spectrum which is a function of wavelength and absorbance value.

2.3 Determination of fibre content

Fiber content was determined according to the AOAC method (1999). The filter paper was prepared, and a sample of 5 mL was measured and put into the Erlenmeyer. H_2SO_4 is added to 150 mL, then the Erlenmeyer is closed using aluminium foil and boiled using an electric heater until the internal temperature reaches 95-100ºC and cooled to room temperature. Filtered using filter paper, then the residue obtained is put into the Erlenmeyer and closed again. The residue that has been put into the Erlenmeyer is then added with NaOH 0.255 N to 150 mL and covered with aluminium foil, boiled with an electric heater until its internal temperature is 95-100ºC, then cooled to room temperature. Filtered using filter paper that has been weighed beforehand. The filter paper was drenched with 20 mL of 95% alcohol, then poured again using 20 mL of hot water. The filter paper is put in a petri dish and heated in the oven for 12 hrs. After that, the weight of the filter paper is weighed, and the residual weight is calculated. By reducing the weight of the final filter paper, the weight of the initial filter paper is the amount of yoghurt fibre, green bean sprouts milk. The number of fibres is calculated using the formula:

Fibre content (%) = [(residual weight, g)/(sample weight, g)] × 100%

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2.4 Determination of dissolved protein content

The dissolved protein content was determined using the Lowry method. Dissolved protein solutions of Bovine Serum Albumin (BSA) in various concentrations ranging from 30-300 µg/mL were prepared, then 1 mL of the sample from each concentration was taken before inserting it into the test tube. Lowry B reagent (8 mL) were added and then left at room temperature for 10 mins. After that, 1 mL of Lowry A reagent was added, stirred, left for 20 mins. The absorbance of the solution was read at a wavelength of 600 nm (Lee *et al*., 2015).

2.5 Data analysis

Data were analysed using ANOVA, followed by Duncan's Multiple Range Test (DMRT) at the 5% level if there is significance. Determination of the best formula using the effectiveness index (deGarmo *et al*., 1984).

3. Results and discussion

3.1 Effect of LAB levels and proportion of mung bean sprout milk - skim milk on phenolics content of MungbeS-Yo

LAB levels affect the phenolic levels of MungbeS-Yo; likewise, the proportion of mung bean sprout milk - skim milk also affected the antioxidant levels, however, the interaction of LAB and the proportion of mung bean sprout milk - skim milk did not affect the phenolic content of yoghurt (Table 1). The use of LAB 0.5% increased phenolic levels (P = 0.022), and the highest level was 1245.27 mg GAE/L. During germination (spontaneous fermentation) the elements of green beans are degraded by various enzymes and endogenous bacteria, causing the dissolution of some of the components and active molecules in them (Hur *et al*., 2014). Moreover, during the fermentation of LAB in the production of yoghurt, the overall bio-accessibility of nutrients and produce compounds with antioxidant and anti-inflammatory activity, such as phenolics (Greco *et al*., 2011; Winarsi *et al*., 2016).

According to Celep *et al*. (2014), phenolic compounds can be produced by LAB through secondary metabolites. Primurdia and Kusnadi (2014) argued that LAB performs metabolism to produce secondary metabolites (phenol compounds) by degrading ferulic acid and cinnamic acid into 4-vinyl phenol and 4-vinyl guaiacol. Cinnamic acid is a type of phenolic acid, therefore the higher the LAB levels used in the manufacture of yoghurt, the more phenolic content in MungbeS-Yo increases.

The fermentation process increases the biological activity in green bean sprouts because the microbial enzymes in them make the phenolic content more active (Worku and Sahu, 2017). Limon *et al*. (2015) argued that during the fermentation process, LAB produces the enzyme β-glucosidase, an enzyme that hydrolyses phenolic glucosides in green bean sprouts and produces aglycone compounds called phenolic compounds that the concentration is increased. Besides, LAB is capable of producing molecules as a result of certain bacterial synthesis pathways (Turpin *et al*., 2016), some of which have antioxidant activity (Kaizu *et al*., 1993).

Apart from the use of LAB levels, the proportion of green bean sprouts milk-skim milk also affected phenolic levels (P = 0.0014) (Table 2). The highest level of 1073.68 mg GAE/L was found in the proportion of 70% green bean sprouts milk and 30% skim milk. The higher

Table 1. Effect of LAB levels on phenolic, protein, and fibre content of MungbeS-Yo

	A	B	*P-value*
Phenolics (mg GAE/L)	1041.04±216.04^{a}	1245.27±171.05^{b}	0.022
Protein (%)	27.29±2.84^{a}	34.61±4.84^{b}	0.0003
Fibre (%)	8.06±1.49^{a}	9.77±2.23^{b}	0.046
TSS (°Brix)	9.88±0.2^{a}	9.75±0.4^{a}	0.44

Values are presented as mean±SD, n = 12. Values with different superscripts within the same row are statistically significant with α= 5%. A: 0.25% LAB level, B: 0.5% LAB Level

Table 2. Effect of the proportion of mung bean sprouts milk and skim milk on phenolic, protein, and the fibre content of MungbeS-Yo

	P1	P2	P3	P4
Phenolics (mg GAE/L)	1418.49±80.09^{a}	1068.03±147^{b}	1012.41±118^{b}	1073.68±219^{c}
Protein (%)	26.46±3.6^{a}	29.67±4.2^{a}	31.73±2.5^{a}	35.94±5.7^{b}
Fibre (%)	8.94±1.3^{a}	9.45±2.2^{a}	6.99±0.7^{a}	10.29±2.1^{b}
TSS (°Brix)	9.5±0.35^{a}	9.75±0.25^{a}	10±0^{b}	10±0^{b}

Values are presented as mean±SD, n = 6. Values with different superscripts within the same row are statistically significant with α= 5%. P1: 100 sprouts milk: 0 skim milk, P2: 90 sprouts milk: 10 skim milk, P3: 80 sprouts milk: 20 skim milk, P4: 70 sprouts milk: 30 skim milk, TSS: Total solid soluble.

the addition of skim milk, the higher the total dissolved solids value. In this study the higher the proportion of skim milk, the higher the total amount of dissolved solids ($p = 0.044$). During the fermentation process, most of the milk lactose is broken down into lactic acid by the starter culture. The remaining lactose and organic acids formed are counted as total dissolved solids. Organic acids (including lactic acid) are a type of total dissolved solids apart from sugars, pigments, and vitamins (Sintasari *et al*., 2014). Total dissolved solids also come from the breakdown of proteins into simple and water-soluble molecules, such as amino acids and peptones, the breakdown of carbohydrates, and the breakdown of fats into free fatty acids and glycerol.

Dissolved solid components other than pigments, organic acids and proteins, also include sucrose. The longer the fermentation the more dissolved components and causes the softening of the cell wall tissue and makes it easier for water to penetrate the material, more solid molecules are extracted, thereby increasing the total dissolved solids. In this study, the skin of mung bean sprouts was not removed. The high phenolic content of MungbeS-Yo is thought to be related to the presence of green bean skins carried in the product, according to the results of research by Christman *et al*. (2018) and Winarsi *et al*. (2019).

Towaha *et al*. (2012) revealed that the longer the fermentation process, the lower the pH because organic acids such as lactic and acetic acids were formed. Dissolved organic acids release protons (H^+) thereby lowering the pH. MungbeS-Yo fermented for 24 hrs has a pH of 3.67-3.76. According to Indah and Susanto (2005), lactic acid, acetic acid, citric acid, succinic acid, malic acid, acetaldehyde, diacetyl, and acetoin are compounds that can increase antioxidants and stabilize them during fermentation, by synergizing in regenerating antioxidant compounds.

The highest total phenolic content of mung bean sprouts milk yoghurt was 1073.68±219 mg GAE/L, whereas according to Winarsi *et al*. (2021) the level in green bean milk yoghurt (without being germinated) is only 556.89±81.2 mg GAE/L. Thus, the MungbeS-Yo antioxidant level is 1.93 times the level in green bean milk yoghurt, therefore it can be believed that germination also contributes to increasing total phenolic levels.

Germination is a spontaneous fermentation. In spontaneous fermentation, microorganisms are not added in the process, on the other hand, a starter is not added in spontaneous fermentation. According to Yes'se (2014), the spontaneous or non-spontaneous fermentation process can increase the phenolic content of cocoa beans. This statement indicates that unfermented beans have a lower total phenol content than fermented beans. In this study MungbeS-Yo was made through non-spontaneous fermentation, then followed by LAB fermentation, so, logically, the phenolic levels increased significantly.

Moreover, microbial enzymes during fermentation activate phenolic MungbeS-Yo (Alberto *et al*., 2006). Limon *et al*. (2015) also argued that during the fermentation process LAB was able to produce β-glucosidase enzymes, an enzyme that hydrolyses phenolic glucosides in green bean sprouts and produces phenolic aglycone compounds, that the concentration increases. The same finding occurred in the research of Towaha *et al*. (2012) which has fermented cocoa beans, the content of antioxidant compounds has also increased.

In this study, the skin of mung bean sprouts was not removed. Christman *et al*. (2018) reported that many phenolic compounds are present in the skin. Nurjanati *et al*. (2018) confirmed that the total phenolic content of red bean milk added for 0 hrs 10 hrs (without skin) was 337 mg GAE/L and 418 mg GAE/L, lower than red bean sprouts milk, 452 mg GAE/L and 475 mg GAE/L. LAB fermentation can also increase the phenolic levels of green bean sprouts milk yoghurt because these bacteria produce secondary metabolites (phenolic compounds) (Primurdia and Kusnadi, 2014). Thus, the increase in total phenol content is not only due to the role of LAB but also because of the green bean germination process.

3.2 Effect of LAB levels and proportion of mung bean sprout milk - skim milk on the dissolved protein content of MungbeS-Yo

LAB levels increased MungbeS-Yo protein levels, and the highest level (34.61%) was found in the use of 0.5% LAB which was different from other levels ($P = 0.0027$). Likewise, the proportion of 70% MungbeS-Milk and 30% skim milk also gave the highest protein content of 35.94% ($P = 0.014$), but the interaction of LAB levels and the proportion of MungbeS-Milk-skim did not change the MungbeS-Yo protein content ($P = 0.89$).

Dissolved protein is part of the total protein contained in food products, which is in the form of oligopeptides or amino acids that are easily absorbed by the digestive system (Purwoko *et al*., 2007). In this study, yoghurt protein was derived from the total protein raw materials used, such as green bean sprouts, skim milk, and LAB (Yusmarini *et al*., 2004). LAB uses skim milk protein as a nutrient for growth (Ardi *et al*., 2017). The higher the LAB levels, the more protein is degraded by lactic acid bacteria which are proteolytic, resulting in many oligopeptides and amino acids (Widodo, 2003).

According to Amani *et al*. (2016), high concentrations of LAB can increase the number of protease enzymes that can hydrolyze complex proteins into simple amino acids. Thus, the increase in dissolved protein content of MungbeS-Yo is related to the proteolytic activity of lactic acid bacteria, which converts them into amino acids and peptides (Hassan *et al*., 2010).

Bacteria are known to have 4 growth phases including the lag phase and the log phase. During the lag phase, the bacteria adapt to the new growth medium. In the lag phase, there has not been an increase in the number of bacteria, but metabolic activity has begun to occur, although not significant. During the log (exponential) phase, very rapid bacterial growth occurred (Nishino *et al*., 2018). In this phase, there is a protein breakdown process by LAB (Purkan *et al*., 2017). Chairunnisa *et al*. (2017) added that bacteria have proteolytic activity to break down complex proteins into simple proteins in the form of oligopeptides and amino acids with the help of protease enzymes.

Mung bean sprouts are the raw material for making this yoghurt. According to Farghaly *et al*. (2019), legume germination products increase protein digestibility by suppressing their phytic acid content. Thus, products obtained with LAB fermentation make food products therapeutic benefits, including antioxidant activity (Winarsi *et al*., 2020; Winarsi *et al*., 2021), anticarcinogenic (Hirayama, 1999), and immunostimulants (Winarsi *et al*., 2016).

Related to its benefits as an immunostimulant, MungbeS-Yo which is made with the role of LAB was also reported by Riaz Rajoka *et al*. (2017) has other therapeutic properties, such as prophylactic (preventing infection) against several types of intestinal infections, improving tolerance to foods containing lactose, and preventing cancer initiation. Fermented foods are also useful in preventing diarrhoea that causes infant death (Kok and Hutkins, 2018). Therefore, developing and utilizing fermentation technology is one of the efforts to obtain healthy food products.

3.3 Effect of LAB levels and proportion of mung bean sprout milk - skim milk on fibre content of MungbeS-Yo

In fact, the higher the LAB content used for the manufacture of MungbeS-Yo products, increased the fibre content (P = 0.018), the highest level was 9.77±2.23% when using 0.5% LAB. The highest fibre content of 10.29±2.1% was found in MungbeS-Yo with a proportion of 70 MungbeS-Milk and 30 skims. However, the interaction between LAB and MungbeS-Milk-skim proportion did not affect MungbeS-Yo fibre content (P = 0.533).

Guo and Beta (2013) state that more than 1% of phenolic antioxidants are covalently bound to insoluble fibre, therefore, bioavailability is low. Phenolic antioxidants are difficult to extract because of the many covalent bonds in insoluble fibre. However, the covalent bonds in insoluble fibre can be hydrolysed by microbial enzymes, including the enzymes secreted by LAB. The bioavailability of phenolic antioxidants bound to insoluble fibre can be increased by utilizing microbes that have enzymes to metabolize fibre, including lactic acid bacteria (LAB). Lactic acid bacteria have hydrolytic enzymes to metabolize insoluble fibre (cellulose, hemicellulose). Apart from covalent bonds in insoluble fibre, the bioavailability of phenolic antioxidants increases (Laddomada *et al*., 2015).

As reported by Zubaidah *et al*. (2012), the growth of LAB increases metabolic processes, as well as the biomass that is formed. The increase in the amount of biomass increases the fibre content in yoghurt. Majesty *et al*. (2015) added that the increase in fibre weight was related to LAB activity in forming more cellulose and bonding to one another in the form of fibres. This is presumably because LAB can break down sugar into cellulose during fermentation. Krabi *et al*. (2015) argued that the longer the fermentation time, the greater the ability of LAB to produce cellulose fibres which are part of these fibres. In the MungbeS-Milk-skim fermentation medium, components that have the potential as antioxidants are phenolics, fibre, vitamins A, E, amino acids, and proteins that have sulfhydryl groups. Organic acids produced during fermentation lower the pH (pH 3.67-3.76). This condition can maintain the antioxidant activity in MungbeS-Yo.

Moreover, the skin of green bean sprouts is not removed, allowing it to increase the fibre content of yoghurt (Christman *et al*., 2018). Winarsi *et al.* (2019) added that the fibre content of red bean yoghurt (which was not germinated) was 0.24%, while the red kidney bean sprouts milk yoghurt fibre was 26.21 times higher. Therefore, germination also contributes to the high fibre content of green bean sprouted milk yoghurt.

3.4 The best MungbeS-Yo formula

The selection of the best formula is carried out using the effective index method according to deGarmo *et al*. (1984). By giving the weight of the phenolic value 1, protein 0.9, and fibre 0.8 followed by calculating the effective value and product value, the best formula is MungbeS-Yo which is made from 70% MungbeS-Milk and 30% skim, and 0.5. % BAL. The best formula contains Phenolic 1273.11 mg GAE/L, 41.40% protein, and 12.17% fibre.

In general, antioxidant-rich products have the potential as antioxidants and immunostimulants. As an antioxidant, probiotics work to reduce reactive compounds (Amaretti *et al.,* 2013), chelate transition metal ions, inhibit several enzymes associated with ascorbate autoxidation (Talwalkar and Kailasapathy, 2003), and scavenge oxidant compounds in the intestine (Azcárate-Peril *et al*., 2011), induces transcription of genes involved in the biosynthesis of glutathione in the intestinal mucosa (Lutgendorff *et al*., 2009), increases glutathione synthesis in pancreatic cells, and stimulates the action of intestinal microflora enzymes (Lutgendorff *et al.,* 2008).

The bacteria in probiotic yoghurt can increase the growth of beneficial gut microbiota and affect intestinal and tissue function through immune system regulation (Maynard *et al*., 2012). This effect can prevent weight gain (Poutahidis *et al*., 2013).

MungbeS-Yo, with its phenolic, protein, and fibre content, is thought to be useful as a product to support the immune system during the COVID-19 pandemic. Phenolic acid is a simple phenolic compound that is associated with a lower risk of chronic disease and various types of cancer (Reboredo-Rodríguez *et al*., 2018), as well as various viruses (Kaila *et al*., 1994).

MungbeS-Yo is rich in fibre content (10.29%). The health effects of dietary fibre sources are closely related to the physiological properties of the fibre. Fibre provides high viscosity in the digestive tract, and adequate consumption can reduce the absorption of glucose and cholesterol. High dietary fibre consumption can prevent diabetes and hypercholesterolemia. The fibre in the fermented colon produces short-chain fatty acids (acetate, propionate, and butyrate) which can prevent the increase in cholesterol (propionate) or prevent colon cancer (butyrate). The large water-binding capacity of the fibres results in bulky digestion and high-water content, thus preventing both constipation and diverticulosis. The type of probiotic *Lactobacillus* in MungbeS-Yo provides the highest antioxidant effect (Mishra *et al*., 2015) that this yoghurt product can improve antioxidant status and reduce oxidative stress, ultimately maintaining excellent health status in the current COVID-19 pandemic.

4. Conclusion

The higher the LAB level increases the phenolic antioxidant content, protein, and fibre of green bean sprouts yoghurt. The highest proportion of MungbeS-Milk (no skim) yields MungbeS-Yo with the highest phenolic content, but it is not preferred. The lower the proportion of green bean sprouts milk, or the higher the skim milk increased the levels of phenolic antioxidants, protein, and yoghurt fibre of green bean sprouts, but the interaction of LAB levels and the proportion of mung bean sprouts and skim milk did not change the phenolic, protein, and the fibre content of yoghurt-based products green bean sprouts. The best formula is found in MungbeS-Yo which is made from 70% MungbeS-Milk, 30% skim, and 0.5% LAB. The best MungbeS-Yo contains phenolic at 1245.57 mg GAE/L, 35.94% protein, and 10.29% fibre. This antioxidant-rich yoghurt is useful as a product to support antioxidant status and immune status in the current COVID-19 pandemic.

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References

Alberto, M.R., Rinsdahl Canavosio, M.A. and Manca de Nadra, M.C. (2006). Antimicrobial effect of polyphenols from apple skins on human bacterial pathogens. *Electronic Journal of Biotechnology,* 9 (3), 205-209. https://doi.org/10.2225/vol9-issue3-fulltext-1

Alfian, R. and Hari S. (2012). Penetapan kadar fenolik total ekstrak metanol kelopak bunga rosella merah (*Hibiscus Sabdariffa* Linn) dengan variasi tempat tumbuh secara spektrofotometri. *Jurnal Ilmiah Kefarmasian*, 2(1), 73-80. https://doi.org/10.12928/pharmaciana.v2i1.655 [In Bahasa Indonesia].

Amaretti, A., di Nunzio, M., Pompei, A., Raimondi, S., Rossi, M. and Bordoni, A. (2012). Antioxidant properties of potentially probiotic bacteria: in vitro and in vivo activities. *Applied Microbiology and Biotechnology,* 97(2), 809-817. https://doi.org/10.1007/s00253-012-4241-7

AOAC. (1999). AOAC Official Methods of Analysis. 15th ed. Vol. 1. Washington D.C., USA: AOAC.

Azcárate-Peril, M.A., Sikes, M. and Bruno-Bárcena, J.M. (2011). The intestinal microbiota, gastrointestinal environment and colorectal cancer: a putative role for probiotics in prevention of colorectal cancer? *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 301(3), G401–G424. https://doi.org/10.1152/ajpgi.00110.2011

Celep, G.S., Rastmanesh, R. and Marotta F. (2014).

Chapter 43—Microbial metabolism of polyphenols and health. In Watson R.R., Preedy V.R. and Zibadi, S. (Eds.), Polyphenols in Human Health and Disease, p. 577-589. San Diego, USA: Academic Press. https://doi.org/10.1016/B978-0-12-398456-2.00043-8

Christman, L.M., Dean, L.L., Bueno Almeida, C. and Weissburg, J.R. (2018). Acceptability of peanut skins as a natural antioxidant in flavored coated peanuts. *Journal of Food Science*, 83(10), 2571-2577. https://doi.org/10.1111/1750-3841.14323

deGarmo, E.P., Sullivan, W.G. and Candra, C.R. (1984). Engineering Economy, 7th ed. New York, USA: Mc. Millan Publ., Co.

Dendi, P. (2013). Kajian Penambahan Skim dan Santan terhadap Karakteristik Yoghurt Whey. Bandung, Indonesia: Universitas Pasundan. [In Bahasa Indonesia].

Farghaly, M.M., Abdullah, M.A.M., Youssef, I.M.I., Abdel-Rahim, I.R. and Abouelezz, K. (2019). Effect of feeding hydroponic barley sprouts to sheep on feed intake, nutrient digestibility, nitrogen retention, rumen fermentation and ruminal enzymes activity. *Livestock Science,* 228, 31-37. https://doi.org/10.1016/j.livsci.2019.07.022

Greco, L., Gobbetti, M., Auricchio, R., Di Mase, R., Landolfo, F., Paparo, F., Di Cagno, R., De Angelis, M., Rizzello, C. G., Cassone, A., Terrone, G., Timpone, L., D'Aniello, M., Maglio, M., Troncone, R. and Auricchio, S. (2011). Safety for patients with celiac disease of baked goods made of wheat flour hydrolyzed during food processing. *Clinical Gastroenterology and Hepatology*, 9(1), 24-29. https://doi.org/10.1016/j.cgh.2010.09.025

Guo, W. and Beta, T. (2013). Phenolic acid composition and antioxidant potential of insoluble and soluble dietary fibre extracts derived from select whole-grain cereals. *Food Research International*, 51(2), 518-525. https://doi.org/10.1016/j.foodres.2013.01.008

Guo, X.-D., Wu, Ch.-S., Ma, Y.-J., Parry, J., Xu, Y.-Y., Liu, H. and Wang, M. (2012). Comparison of milling fractions of tartary buckwheat for their phenolics and antioxidant properties. *Food Research International*, 49(1), 53-59. https://doi.org/10.1016/j.foodres.2012.07.019

Hirayama, K.A.R.J. (1999). The role of lactic acid bacteria in colon cancer prevention: Mechanistic considerations. *Antonie Van Leeuwenhoek*, 76(1-4), 391-394. https://doi.org/10.1007/978-94-017-2027-4_25

Hur, S.J., Lee, S.Y., Kim, Y.-C., Choi, I. and Kim, G.-B. (2014). Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chemistry,* 160, 346-356. https://doi.org/10.1016/j.foodchem.2014.03.112

Kaila, M., Arvilommi, H., Soppi, E., Laine, S. and Isolauri, E. (1994). A prospective study of humoral immune responses to cow milk antigens in the first year of life. *Pediatric Allergy and Immunology, 5* (3), 164-169. https://doi.org/10.1111/j.1399-3038.1994.tb00233.x

Kaizu, H., Sasaki, M., Nakajima, H. and Suzuki, Y. (1993). Effect of antioxidative lactic acid bacteria on rats fed a diet deficient in vitamin E. *Journal of Dairy Science*, 76(9), 2493-2499. https://doi.org/10.3168/jds.S0022-0302(93)77584-0

Kok, C.R. and Hutkins, R. (2018). Yogurt and other fermented foods as sources of health-promoting bacteria. *Nutrition Reviews,* 76(S1), 4-15. https://doi.org/10.1093/nutrit/nuy056

Krabi, R.E., Assamoi, A.A., Ehon, F.A. and Niamke, S.L. (2015). Screening of lactic acid bacteria as potential starter for the production of attieke, a fermented cassava food. *Journal of Food Engineering*, 14(1), 21-29.

Laddomada, B., Caretto, S. and Mita, G. (2015). Wheat Bran Phenolic Acids: Bioavailability and Stability in Whole Wheat-Based Foods. *Molecules*, 20(9), 15666-15685. https://doi.org/10.3390/molecules200915666

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

Limon, R.I., Penas, E., Torino, M.I., Martinez-Villaluenga, C., Duenas, M. and Frias, J. (2015). Fermentation enhances the content of bioactive compounds in kidney bean extracts. *Food Chemistry*, 172(1), 343-352. https://doi.org/10.1016/j.foodchem.2014.09.084

Lutgendorff, F., Akkermans, L.M. and Soderholm, J.D. (2008). The role of microbiota and probiotics in stress–induced gastro–intestinal damage. *Current Molecular Medicine*, 8(4), 282-298. https://doi.org/10.2174/156652408784533779

Lutgendorff, F., Nijmeijer, R.M., Sandstrom, P.A., Trulsson, L.M., Magnusson, K.E., Timmerman, H.M., van Minnen, L.P., Rijkers, G.T., Gooszen, H.G., Akkermans, L.M. and Soderholm, J.D. (2009). Probiotics prevent intestinal barrier dysfunction in acute pancreatitis in rats via induction of ileal mucosal glutathione biosynthesis. *PloS One,* 4(2), e4512. https://doi.org/10.1371/journal.pone.0004512

Majesty, J., Argo, B.D. and Nugroho, W.A. (2015). Pengaruh penambahan sukrosa dan lama fermentasi terhadap kadar serat nata dari sari nanas (Nata de Pina). *Jurnal Keteknikan Pertanian Tropis dan Biosistem*, 3(1), 80-85. [In Bahasa Indonesia].

Maynard, C.L., Elson, C.O., Hatton, R.D. and Weaver, C.T. (2012). Reciprocal interactions of the intestinal microbiota and immune system. *Nature,* 489, 231-241. https://doi.org/10.1038/nature11551

Middleton, E., Kandaswamy, C. and Theoharides, T.C. (2000). The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. *Pharmacological Reviews*, 52(4), 673-751.

Mishra, V., Shah, C., Mokashe, N., Chavan, R., Yadav, H. and Prajapati, J. (2015). Probiotics as Potential Antioxidants: A Systematic Review. *Journal of Agricultural and Food Chemistry,* 63(14), 3615–3626. https://doi.org/10.1021/jf506326t

Nurjanati, M., Winarsi, H. and Dwiyanti, H. (2018). Efek lama perkecambahan terhadap sifat sensori dan kadar protein terlarut susu kecambah kacang merah (sukarah) untuk remaja obesitas. *Jurnal Gizi dan Pangan Soedirman*, 2(2), 27-42. https://doi.org/10.20884/1.jgps.2018.2.2.1352. [In Bahasa Indonesia].

Prasetyo, H. (2010). Pengaruh Starter Yoghurt pada Level Tertentu terhadap Karakteristik Yoghurt yang Dihasilkan. Surakarta, Indonesia: Universitas Sebelas Maret. [In Bahasa Indonesia].

Primurdia, E.G. and Kusnadi, J. (2014). Aktivitas antioksidan minuman probiotik sari kurma (*Phoenix Dactilyfera* L.) dengan isolat L. *Plantarum* dan L. *Casei*. *Jurnal Pangan dan Agroindustri*, 2(3), 98-109. [In Bahasa Indonesia].

Polyorach, S., Poungchompu O., Wanapat M., Kang, S. and Cherdthong A. (2016). Optimal cultivation time for yeast and lactic acid bacteria in fermented milk and effects of fermented soybean meal on rumen degradability using nylon bag technique. *Asian Australasian Journal of Animal Sciences,* 29(9), 1273-1279. https://doi.org/10.5713/ajas.15.0798

Poutahidis, T., Kleinewietfeld, M., Smillie, C., Levkovich, T., Perrotta, A., Bhela, S. and Erdman, S.E. (2013). Microbial reprogramming inhibits western diet-associated obesity. *PLoS One,* 8(7), e68596. https://doi.org/10.1371/journal.pone.0068596

Reboredo-Rodríguez, P., Varela-López, A., Forbes-Hernández, T.Y., Gasparrini, M., Afrin, S., Cianciosi, D. and Giampieri, F. (2018). Phenolic compounds isolated from olive oil as nutraceutical tools for the prevention and management of cancer and cardiovascular diseases. *International Journal of Molecular Sciences*, 19(8), 1-21. https://doi.org/10.3390/ijms19082305

Riaz Rajoka, M.S., Shi, J. and Zhu, J. (2017). Capacity of lactic acid bacteria in immunity enhancement and cancer prevention. *Applied Microbiology and Biotechnology,* 101(1), 35-45. https://doi.org/10.1007/s00253-016-8005-7

Sintasari, R.A., Kusnadi, J. and Ningtyas, D.W. (2014). Effect of skimmed milk and sucrose addition towards characteristic probiotic drink of brown rice juice. *Jurnal Pangan dan Agroindustri*, 2(3), 65-75.

Siregar, M.N.H., Lilik, E.R. and Djalal, R. (2014). Pengaruh penambahan berbagai konsentrasi kultur dan lama pemeraman pada suhu ruang terhadap pH, viskositas, kadar keasaman, dan Total Plate Count (TPC) set yoghurt. Malang, Indonesia: Universitas Brawijaya.

Sokrab, A.M., Ahmed, I.A.M. and Babiker, E.E. (2012). Effect of germination on antinutritional factors, total, and extractable minerals of high and low phytate corn (*Zea mays* L.) genotypes. *Journal of the Saudi Society of Agricultural Sciences*, 11(2), 123-128. https://doi.org/10.1016/j.jssas.2012.02.002

Talwalkar, A. and Kailasapathy, K. (2003). Metabolic and biochemical responses of probiotic bacteria to oxygen. *Journal of Dairy Science*, 86(8), 2537-2546. https://doi.org/10.3168/jds.S0022-0302(03)73848-X

Towaha, J., Anggraini, D.A.E. and Rubiyo. (2012). Keragaman mutu biji kakao dan produk turunannya pada berbagai tingkat fermentasi: studi kasus di Tabanan, Bali. *Pelita Perkebunan*, 28(3), 166-183. [In Bahasa Indonesia].

Turpin, W., Renaud, C., Avallone, S., Hammoumi, A., Guyot, J.-P. and Humblot, C. (2016). PCR of crtNM combined with analytical biochemistry: An efficient way to identify carotenoid producing lactic acid bacteria. *Systematic and Applied Microbiology,* 39 (2), 115-121. https://doi.org/10.1016/j.syapm.2015.12.003

Winarsi, H., Agustia, F.C., Ramadhan, G.R., Zaki, I., Putri, W.A.K., Sulistyaning, A.R., Farida, F. and Sari, H.P. (2021). Polyphenol rich mung bean *(Vigna radiata)* yogurt for obesity prevention. *Food Research*, 5(2), 136-143. https://doi.org/10.26656/fr.2017.5(2).500

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., Sasongko, N.D., Purwanto, A. and Nuraeni, I. (2013). Ekstrak daun kapulaga menurunkan indeks atherogenik dan kadar gula darah tikus diabetes induksi Alloxan. *Agritech*, 33(3), 273-280. [In Bahasa Indonesia].

Winarsi, H., Septiana, A.T., Kartini. and Hanifah, I.N. (2019). Fermentation of lactic acid bacteria improving phenolic and fibre content of red kidney bean sprouts milk (*Phaseolus Vulgaris* L.) yogurt, a functional drink for obesity. *Jurnal Gizi dan Pangan Soedirman*, 3(1), 64-75. https://doi.org/10.20884/1.jgps.2019.3.1.1526

Winarsi, H., Septiana, A.T. and Roselia, A. (2020). Amelioration abdominal circumference, blood pressure, and body mass index women with metabolic syndrome using red kidney bean sprout milk yogurt. *Jurnal Gizi dan Pangan Soedirman*, 4 (2), 173-188.

Winarsi, H., Wijayanti, S.P.M. and Purwanto, A. (2012). Soy germ protein with or without-Zn improve plasma lipid profile in metabolic syndrome women. *Hayati Journal of Biosciences,* 19(1), 25-30. https://doi.org/10.4308/hjb.19.1.25

Worku, A. and Sahu, O. (2017). Significance of fermentation process on biochemical properties of *Phaseolus vulgaris* (red beans). *Biotechnology Reports*, 16, 5-11. https://doi.org/10.1016/j.btre.2017.09.001

Yes'se. (2014). Perubahan kandungan tokoferol dan total fenol selama proses fermentasi spontan dan fermentasi menggunakan penambahan bakteri asam laktat *(Lactobacillus plantarum)* pada biji kakao (*Theobroma cacao* L.). Semarang, Indonesia: Universitas Katolik Soegijapranata. [In Bahasa Indonesia].

Zubaidah, E., Saparianti, E. and Hindrawan, J. (2012). Studi aktivitas antioksidan pada bekatul dan susu skim terfermentasi probiotik (*Lactobacillus plantarum* B2 dan *Lactobacillus acidophillus*). *Jurnal Teknologi Pertanian*, 13(2), 111-118. [In Bahasa Indonesia].

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