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Mung Bean (*Vigna radiata*) Microgreens Filtrate Supplementation in Skim Milk Yoghurt Raised Antioxidant Level

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Abstract
The increasing demand for functional foods supplemented with antioxidants is accelerating the need to enrich skimmed milk yoghurt with mung bean (*Vigna radiata*) microgreens filtrate, which is rich in bioactive compounds. This study aimed to assess the effect of microgreen filtrate concentration on antioxidant levels with consideration to lactic acid and pH level of the yoghurt, as well as to determine the optimal concentration that increases antioxidant activity without reducing physicochemical quality. The research design used a completely randomized design (CRD) with four filtrate concentrations: 0%, 15%, 20%, and 25%. Antioxidant level was tested using the DPPH method, while lactic acid levels were tested using phenolphthalein. Kolmogorov-Smirnov normality test, one-way ANOVA, and Duncan test were used to analyze the antioxidant levels data. Lactic acid levels and pH were analyzed descriptively, referring to SNI 2981:2009. The results showed that 25% filtrate treatment produced the highest antioxidant levels (75.92 ± 5.12), significantly different from other treatments, while lactic acid levels (0.76-0.89%) and pH (4.80-5.17) remained within quality standards. Results indicated that mung bean microgreens filtrate at 25% concentration increased the skim milk yoghurt's antioxidant level without affecting the product's physicochemical quality.

Keywords: Microgreens; functional foods; bioactive compounds; fermentation; healthy lifestyle

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INTRODUCTION

The development of the times and urbanization have changed people's lifestyles, especially in urban areas. Urban communities are increasingly exposed to air pollution from vehicles and cigarette smoke and tend to consume fast food that is low in fibre and nutrients (Ministry of Health Republic Indonesia, 2022). This consumption pattern increases the risk of health problems, such as constipation due to lack of fibre and diarrhoea due to contamination of pathogenic bacteria from unhygienic snacks (Widodo *et al.*, 2019). In addition, low-nutrient foods also increase exposure to free radicals in the body. Free radicals are molecules or atoms with unpaired electrons, so they are highly reactive and can trigger biomolecular damage at the cellular to organ level (Pratama and Busman, 2020; Handayani *et al.*, 2018). If not balanced with adequate antioxidant intake, free radicals trigger a chain reaction that damages cell structure and function, increasing the risk of various degenerative diseases (Widyanto *et al.*, 2020).

One way to reduce the negative impact of free radicals is by consuming functional foods rich in antioxidants. Yoghurt is one of the popular fermented milk products and has long been recognized as a functional food. Yoghurt is produced through milk fermentation by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria that produce lactic acid and beneficial probiotics (Fatmawati *et al.*, 2020). This fermentation results in an increased shelf life and safety of food products, which are also enhanced with health-beneficial components that improve digestive and immune systems (Marco *et al.*, 2017). Consistent with the current healthy food trend, yoghurt development nowadays focuses on natural ingredients containing antioxidants, including microgreens.

Microgreens are the seeds of young plants, harvested between7 and 14 days after sowing, and possess a higher concentration of nutrients and bioactive compounds than adult plants do (Kyriacou *et al.*, 2019). Mung bean (*Vigna radiata*) microgreens contain phenolic compounds, flavonoids, and vitamins C, E, and K, which act as natural antioxidants (Hidayat *et al.*, 2022; Salim, 2021). Antioxidants protect cells from the oxidation of other molecules. Thus, the production of harmful free radicals is inhibited. Findings from empirical research suggest that the antioxidant found in the microgreens of the mung bean are4-6 times higher than those found in the mature plant (Weber, 2017; Bhaswant *et al.*, 2023). Another component found in the mung beans with notable antioxidant properties is its flavonoid content found in the mung beans. These flavonoids can counteract the effect of free radicals and inflammation (Fakhrudin *et al.*, 2020).

Adding natural agents rich in antioxidants has been widely studied and proven to enhance yoghurt's functional properties. For example, broccoli and moringa extracts added to yoghurt have been shown to increase antioxidant without affecting the viability of lactic acid bacteria (Syaubari *et al.*, 2023; Purbosari *et al.*, 2019). Likewise, studies have shown that adding mung bean extracts improves the microbiological characteristics, acceptability, and chemical properties of yoghurt (Alawiyah *et al.*, 2024). However, the specific use of mung bean microgreen filtrate in yoghurt is rarely studied, especially regarding its effect on the antioxidant level of the final product, with consideration to lactic acid level, and pH.

Using microgreen filtrate in yoghurt production is an innovative method to increase antioxidant levels without affecting the fermentation process. Phenolic in microgreens can react with milk proteins, thereby affecting the activity of lactic acid bacteria (Oh *et al.*, 2016). On the other hand, the metabolism process of lactic acid bacteria in fermentation can convert phenolic compounds to more potent compounds (Rocchetti *et al.*, 2022). Moreover, the bioactive compounds in microgreens are stable at low pH values, which matches the pH of yoghurt (Xiao *et al.*, 2012; Kyriacou *et al.*, 2016). Hence, it is vital to examine the effect of mung bean microgreen filtrate on yoghurt in terms of antioxidant levels, lactic acid levels, and pH.

Physicochemical factors, particularly lactic acid levels and pH are key determinants of yoghurt quality. Yoghurts must comply with SNI 2981:2009, with pH values ranging from 4.6 to 5.4 and lactic acid levels of 0.5 to 2.0%. Other added ingredients, like microgreen filtrate, may affect the factors mentioned. Therefore, an analysis must be conducted to verify whether the yoghurt still meets the quality parameters. Also, the analysis of antioxidant levels is vital for assessing the improvement in yoghurt's functional value added by the filtrate from mung bean microgreen.

Based on the information highlighted above, this research aims to examine whether mung bean (*Vigna radiata*) microgreens filtrate supplementation in skim milk yoghurt can raise antioxidant levels. The results of the research will provide a scientific platform for the design and development of healthier value-adding functional yoghurt

MATERIALS AND METHODS

This experimental research was conducted in the Microbiology Laboratory and Cell and Molecular Biology Laboratory, Faculty of Mathematics and Natural Sciences, Surabaya State University, from January to February 2025. This study used a completely randomized design (CRD) with one factor, namely the concentration of mung bean (*Vigna radiata*) microgreens filtrate consisting of four concentrations: 0% (control), 15%, 20%, and 25% against the volume of skim milk yoghurt. Each treatment in the antioxidant levels test was repeated six times, while duplicates were done for lactic acid levels and pH.

Mung bean seeds first was washed and soaked in water for 8-12 hours before planting. Floating seeds were discarded, while healthy seeds were planted on 2.5 x 2.5 cm rockwool media moistened with water and placed in a tray (Jihni and Hikmawati, 2021). Each rockwool unit had six holes in which theseedsbe positioned. During the growing period, the media was sprayed with water regularly, and the seeds were exposed to sunrays in the morning to ensure adequate light (Sakila and Yastori, 2022). Microgreens were harvested at the age of seven days when height reached around 5-7 cm (Jihni and Hikmawati, 2021). Harvested mung bean (*Vigna radiata*) microgreens was then made into filtrate by pureeing washed fresh microgreens with distilled water at ratio of 1:4 (w/v). The puree was then filtered using a sieve until a clear filtrate was obtained (Masyhura *et al.*, 2021).

Skim milk yoghurt was made from skim milk powder dissolved in distilled water at 1:10 ratio (w/v) (Masyitoh *et al.*, 2018). Skim milk was heated at 90°C for 1 hour and stirred to prevent coagulation of protein (Hasanah and Rosma, 2021). Microgreen filtrate was added to heated skim milk at 3 types of concentration; 15%, 20%, and 25% (Alawiyah *et al.*, 2024). The filtrate was measured for volume, then put into a glass beaker and pasteurized at 75˚C for 15 minutes. Skim milk and pasteurized mung bean (*Vigna radiata*) microgreens filtrate were cooled until 37-45˚C (Hasanah and Rosma, 2021). Commercial yoghurt starter, as much as 5% was inoculated into pasteurized mixture and added 4% sugar (Sari *et al.*, 2024; Zakaria *et al.,* 2023). Mixture was lastly incubated at room temperature, 27-35˚C for 12 hours (Winarsi, 2019).

Antioxidant levels of yoghurt was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. A 0.2 mL yoghurt sample was mixed with 3.8 mL of 50 µM DPPH solution, vortexed until homogeneous, and incubated in the dark for 30 minutes. Absorbance was measured using a UV-Vis spectrophotometer at the DPPH maximum wavelength (approximately 517 nm). The antioxidant percentage was calculated using the formula (Purwanto *et al.,* 2017):

$$\text{Antioxidant levels (\%)} = \left(\frac{Abs.\ blank - Abs.\ sample}{Abs.\ blank} \times 100\%\right) \quad (1)$$

Note:
Abs. blank = absorbance of 50 µM DPPH solution
Abs. sample = absorbance of yogurt sample

Lactic acid levels was determined by titration analysis. About 20 mL yoghurt sample was mixed with double its volume of CO_2- free distilled water and 2 mL of 1% phenolphthalein indicator. The mixture was titrated with 0.1 N NaOH solution until a stable pink endpoint was reached. Lactic acid levels was calculated using the formula (SNI, 2009):

$$\text{Lactic acid level (\%)} = \frac{V \times N \times 90}{W} \times 100\% \quad (2)$$

Note:
W = Weight of the sample
V = Volume of NaOH solution
N = Normality of NaOH solution
90 = Equivalent Weight of lactic acid

pH of mixture was measured using a pH meter. Approximately 30 mL of homogenized yoghurt sample was measured by immersing the pH electrode (Putri *et al.,* 2023). Data collected were tested for normality using the *Kolmogorov-Smirnov* test, before analysed using one-way analysis of variance (ANOVA). Duncan's multiple range post-hoc test was applied for pairwise comparisons if significant differences was obtained from ANOVA. Data analysis was conducted using SPSS version 23.0 for Windows 11. The lactic acid and pH values were also compared against the standards set by the Indonesian National Standard (SNI) 2981:2009 for yoghurt.

RESULTS

Data on antioxidant levels in skim milk yoghurt with mung bean (*Vigna radiata*) microgreens filtrate are presented in Table 1. Based on the results of the *Kolmogorov-Smirnov* test, it was found that the antioxidant levels data from various treatments were normally distributed (α=0.05). Antioxidant data were then analysed using one-way ANOVA test, resulted in significant difference (α=0.05). Further analysis using Duncan's multiple range test showed significant differences among the treatments. The concentration groups of 0%, 15%, and 20% were not significantly different from each other. However, the 25% concentration was clearly substantially differed from others. Thus, the mung bean microgreen filtrate significantly increases the antioxidant properties of skim milk yoghurt. The highest increases occurred in the presence of 25%.

Table 1. Antioxidant levels in skim milk yoghurt supplemented with mung bean microgreens (*Vigna radiata*) fltrate

Filtrate concentration (%)	Antioxidant activity (%)*
Control (0%)	64,95 ± 4,55[a]
15% concentration	64,97 ± 3,63[a]
20% concentration	65,17 ± 1,63[a]
25% concentration	75,92 ± 5,12[b]

Note: *) Different letters indicate significant differences based on Duncan's test ($p < 0.05$).

According to the Indonesian National Standard (SNI) 2981:2009, high-quality yoghurt should contain 0.5% to 2.0% of lactic acid levels. The level of lactic acid levels in skim milk yoghurt supplemented with mung bean microgreens filtrate is presented in Table 2. Lactic acid levels in skim milk yoghurt supplemented with mung bean microgreens filtrate ranges from 0.76% to 0.89%. The lowest lactic acid levels was observed in the 0% concentration group (0.76%), while the highest was recorded in the 25% concentration group (0.89%). These results indicate that all treatments complied with the quality standards specified by SNI 2981:2009.

Table 2. Lactic acid levels in skim milk yoghurt with mung bean microgreens (*Vigna radiata*) filtrate

Filtrate concentration (%)	Lactid acid content (%)	SNI 2981:2009
Control (0%)	0,76	
15% Concentration	0,85	0,5-2,0%
20% Concentration	0,87	
25% Concentration	0,89	

According to SNI 2981:2009, the acceptable pH range for yoghurt is between 4.6 and 5.4. The average pH values of skim milk yoghurt before and after fermentation with mung bean microgreen filtrate are presented in Table 3. The pH values of the yoghurt after fermentation ranged from 4.80 to 5.17. The highest pH was observed in the 0% concentration group (5.17), while the lowest was recorded in the 25% concentration group (4.80). These findings indicate that all pH values fall within the acceptable quality standards established by SNI 2981:2009.

Table 3. pH of skim milk yoghurt with mung bean microgreens (*Vigna radiata*) filtrate

Filtrate concentration (%)	pH		pH standard
	Before fermentation	After fermentation	(SNI 2981:2009)
control (0%)	6,17	5,17	
15% concentration	6,18	5,01	4,6-5,4
20% concentration	6,18	4,97	
25% concentration	6,19	4,80	

DISCUSSION

From the results, the addition mung bean (*Vigna radiata*) microgreens filtrate to skim milk yogurt hasa positive effect on antioxidant levels, lactic acid levels, and a decrease in pH, which are interrelated as a result ofbiochemical processes during fermentation. An increase in antioxidant levels, measured by the DPPH method as reported by Nurfadillah *et al.,* (2016), showed that a 25% filtrate concentration produced significantly higher values than other treatments. The mean values of antioxidant levels for the filtrate concentration of0%, 15%, 20%, and 25% treatments, respectively, were 64.95±4.55; 64.97±3.63; 65.17±1.62; and 75.92±5.12, indicating that all values increased with added filtrate concentration.

This directly ties to the increasing bioactive compound content of mung bean microgreens, including phenolics, flavonoids, and vitamin C (Hidayat *et al.,* 2022; Salim, 2021). These compounds can donate electrons or hydrogen to free radicals, thereby protecting theoxidation mechanism by breaking the oxidation chain reaction (Pratiwi, 2023). During fermentation, lactic acid bacteria (LAB), especially *Lactobacillus*, contributed significantly to the formation of bioactive peptides such as Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) with high antioxidant activity (Widodo, 2017; Suhartatik *et al.,* 2023). Additionally, the antioxidant action is supported by the activity of LAB enzymes such as superoxide dismutase (SOD) and catalase, thereby increasing the synergistic effect of yoghurt antioxidants.

Microgreens filtrate not only containsbioactive compounds, but also contains simple carbohydrates such as glucose and sucrose (Yang *et al.,* 2024), which act as additional substrates for LAB. The presence of such substrates enhanceLAB functionin fermenting lactose to form lactic acid (Graham, 2023; Ichimura, 2024). This fermentation process increases H^+ ion concentrations from lactic acid, resulting in a decrease inpH and acidic conditions that are not conducive to pathogenic microbes, while, at the same time,improving the bioavailability and stability of bioactive phytochemicals (Dou *et al.,* 2022; Hakim *et al.,* 2023). As stated by Kårlund *et al.,* (2020), in this acidic enviroment, fermentation favours the release of phenolic and flavonoid compounds from plant materials, thereby making it an antioxidant-rich food. Increased antioxidant levels are significant for health, because unneutralized free radicals can trigger oxidative stress, a risk factor for various degenerative diseases such as cancer, diabetes mellitus, and cardiovascular disease (Phaniendra *et al.,* 2015). Maharani *et al.,* (2021) also emphasized that high antioxidant compound content is essential in reducing the risk of these diseases. Therefore, yoghurt fortification with mung bean microgreens filtrate can increase the functional value of yoghurt as an oxidative stress prevention food.

On the other hand, the addition of filtrate also has an impact on increasing lactic acid levels. The results showed that the average lactic acid levels in the 0%, 15%, 20%, and 25% treatments were 0.76%, 0.85%, 0.87%, and 0.89%, respectively, which were still within SNI 2981:2009, at 0.5-2.0%.

According to Hikmah *et al.,* (2020), the increase in lactic acid levels resulted from LAB activity that utilized lactose and simple sugars in the microgreens filtrate into lactic acid as the final product of metabolism. Increased LAB activity also affects the acidity of yoghurt, supporting the stability of texture and flavor typical of fermented products (Suciati dan Safitri, 2021). In addition to supporting desirable acidity, standardized lactic acid levels are beneficial for digestive health, inhibiting the growth of gut pathogens, and improving the balance of gut microflora (Nughoro *et al.,* 2023; Junita and Mustakim, 2024). However, high level of it can lead to an overly sour taste, an increased risk of digestive disorders, and even potential lactate accumulation in the body that can cause fatigue and metabolic disorders (Zanza *et al.,* 2022). Therefore, keeping lactic acid levels within the standard range is key to product quality and safety.

Furthermore, the decrease in pH also aligns with the results of increasing lactic acid levels. The mean pH of yoghurt after fermentation for 0%, 15%, 20%, and 25% treatments was 5.17, 5.01, 4.97, and 4.80, respectively, still within the pH range stipulated by SNI 2981:2009, which stipulates a pH range of 4.6-5.4. This decrease in pH occurs because LAB ferment lactose into lactic acid using ß-galactosidase and lactate dehydrogenase (LDH) enzymes that break down lactose into glucose and galactose before being converted into lactic acid (Innocente *et al.*, 2016; Lan *et al.*, 2016). A stable decrease in pH indicates that fermentation is running optimally and produces safe and good quality yoghurt (Sari and Herawati, 2024). An optimal pH can be achieved, ensuring harmony in yoghurt flavor, product stability, and safety for consumption. On the contrary, lowpH may pose health risk due to gastric problem. In contrast, a higher pH reduces stabilityand increases the risk of contamination. Therefore, pH measurement plays a critical role in the processing of fortified yoghurt (Harianingsih *et al*., 2017).

The result obtained in the current reasearch is supported by Cahyanti *et al.,* (2023) research report, which shows that the addition of active substances such as vegetable filtrate can result in increased levels of antioxidants and lactic acid and reduced pH. A similar result was observed in the research by Pratiwi *et al.,* (2018) on soybean kefir fortified with red dragon fruit extract, where an increase inactive substances results in a similar effect on the parameters. Consequently, the result showed that the addition of mung bean microgreens filtrate can result in enhanced LAB fermentation activity and reduce pH level. The supplementation can also result in an improved production level of active substances. This will result in the production of a nutritious, health-giving yoghurt.

It can be inferred that mung bean (*Vigna radiata*) microgreens filtrate enhances the antioxidant and lactic acid components of the skimmed milk yoghurt products by reducing the pH values within the standard range, thereby supporting their functional properties. The observations made in this study support the use of microgreens filtrate as a functional component that can enhance the health-promoting properties of yoghurt.

CONCLUSION

Mung bean (*Vigna radiata*) microgreen filtrate to skim milk yoghurt impacted the product's functional and physicochemical quality. The increase in filtrate concentration was directly proportional to the increase in antioxidant levels of 25%, which produced the highest antioxidant levels of 75.92%. Lactic acid levels increased from 0.76% to 0.89%, while pH decreased but remained within the range of yoghurt quality standards according to SNI 2981:2009. Mung bean microgreen filtrates, especially at a concentration of 25%, increased the functional value of skim milk yoghurt without reducing its quality.

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CONFLICT OF INTEREST

There is no conflict of interest

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