Characteristics of Mung Bean Sprouted Milk, a good Drink Rich in Antioxidant for consumption during the Covid-19 pandemic

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Abstract

Background: *Mung beans (Vigna radiata) are known to be rich in polyphenols as antioxidants and immune-stimulants. However, high oligosaccharides in it often cause flatulence, so processing is necessary to minimize the levels.*

Objectives*: This study aims to explore the characteristics of Mung bean sprouted milk, which is reflected in the phenolic content, soluble protein, fiber, and viscosity based on germination time and the proportion of sprouts – water.*

Materials and Methods*: The study used a randomized block design, factorial, with the factor of germination time, and the proportion of mung bean sprouts - water. Making Mung bean sprout milk begins with soaking the beans for 7 hours, drained, and placed in a closed container until it germinates. Sprouts were washed and added with water, blended until smooth, filtered, obtained mung bean sprout milk (MungbeS-Milk). Followed by Phenolic Test (Folin-Ciocalteu method), soluble protein (Lowry method), fiber (AOAC, 2005), and viscosity. Data were analyzed using ANOVA, and continued with Duncan's Multiple Range Test (DMRT) if there was significance at the 5% level. Selection of the best formula using the effectiveness index (deGarmo et al., 1984).* ***Result:*** *The duration of germination increased the phenolic content, soluble protein, fiber, and viscosity ($P<0.05$). The best formula was obtained with 10 hours of germination, with the proportion of sprouts -water = 1: 6.*

Conclusion: *The best MungbeS-Milk contained phenolic antioxidants of 836.47 mg GAE/L, soluble protein 30.90%, fiber 4.21%, and viscosity 1, 83 cP, with a pH of 6.64. MungbeS-Milk is suitable as a source of antioxidant drinks during the Covid-19 pandemic.*

Keywords: *Mung bean sprouted milk, phenolic, soluble protein, fiber, viscosity*

BACKGROUND

During the current coronavirus pandemic, improving the immune system is the main health target. It is stated that the optimal work of the immune system is strongly supported by the cellular antioxidant status [1]. Several researchers reported that functional foods rich in antioxidants, apart from being able to overcome oxidative stress, can also improve immune status [2, 3, 4,5].

Regarding the improvement of the immune system, people generally prefer milk consumption to other products. Meanwhile, the fulfillment of milk needs is mostly in the form of animal milk, especially cow's milk and a small portion of goat's milk. The need for milk consumption is increasing, the more

expensive the price, so not all people can reach it. On the other hand, there are some people who cannot accept animal milk products because of the high lactose, it becomes a challenge to innovate to get vegetable milk products rich in antioxidants that have immune-stimulant potential, which of course are cheaper than animal milk. One type of antioxidant-rich beans is Mung beans.

Mung beans *(Vigna radiata)* are known to be rich in protein, oligosaccharides, and polyphenols. Polyphenols are the main contributors as antioxidants, immune-stimulants, and anti-tumors. However, high oligosaccharides often cause flatulence, so processing is necessary to minimize the levels. One way to minimize it by soaking and germination. Meanwhile, Winarsi *et al*. [6] reported that the phenolic content of mung bean-based yogurt that had been soaked for 12 hours increased from 237.830 ± 17.80 to 525.958 ± 48.9 mg GAE/L, but the beany flavor was still felt. Red bean germination that has been carried out by Winarsi *et al*. [7] can remove the beany flavor, reduce the anti-nutrition content, and increase the phenolic antioxidant content [8]. Compounds that are antioxidants are not only phenolic, but fiber are also potential antioxidants. However, it is not known how long mung bean germination has maximum antioxidant activity. Moreover, the proportion of sprouts and water also affects the antioxidant content. This study aimed to determine the effect of germination time and the proportion of sprouts - water on the phenolic content, soluble protein, fiber, and viscosity of mung bean sprouted milk.

Research methods

The research with Randomized Block Design and factorial. The first factor was germination time, while the second factor was the proportion of mung bean sprouts - water, with 3 replications. Germination time (L) consisted of 2 levels, namely: L1 = 5 hours and L2 = 10 hours. Proportion of Mung Bean Sprouts - Water (K), consisting of 4 levels, namely: K1 = 1:3; K2 = 1:6; K3 = 1:8; and K4 = 1:10. In total there are 24 experimental units.

a. Making Mung bean sprout milk

Mung beans are soaked for 7 hours, drained, and placed in a closed container until it germinates. Sprouts were washed and added with water in a ratio according to the specified level, then blended until smooth, filtered, and obtained mung bean sprout milk (MungbeS-Milk).

b. Phenolic Content Test with Folin-Ciocalteu Method [9].

Weighed 5 g of the sample, added 20 ml of distilled water and then filtered using filter paper, taken 1 ml of the filtrate plus 4 ml of distilled water, added 0.2 ml of Folin-Ciocalteu reagent which was diluted with distilled water in a ratio of 1: 1, shaken. Let stand for 5 min plus 1 mL of 5% Na2CO3 solution and vortex. Set aside for 1 hour in the dark. The absorbance was read at a wavelength of 747 nm.

c. Determination of dissolved protein levels by the Lowry method [10]

The first is the preparation of a standard curve solution first. Prepared 6 test tubes. Put into the test tube: 0 ; 0.1 ; 0.2 ; 0.4 ; 0.6 ; 0.8 and 1.0 ml of BSA standard protein and added with distilled water to a volume of 1 ml, plus 8 ml of Lowry B reagent in each tube, mixed and left for 10 min. Then 1 ml of Lowry's reagent A was added and left for 20 min until a blue color was formed. Then the absorbance was measured at a wavelength of 600 nm and a standard curve was made. Then the determination of the sample. Pipette exactly 1 ml of the milk sample into the test tube then add 8 ml of Lowry B reagent in each tube, mix and leave for 10 min. Then 1 ml of Lowry A reagent was added and left for 20 min until a blue color was formed and the absorbance was measured at a wavelength of 600 nm.

d. Determination of crude fiber content [11].

A sample of 5 g was weighed and put into an erlenmeyer. A 50 ml of 1.25% H2SO4 was added, then the erlenmeyer was closed using aluminum foil and heated at 80°C for 30 min using a water bath, then 50 ml of 3.25% NaOH was added and heated at 80°C for 30 min using a water bath, and cooled at room temperature. Filtered using filter paper that has been weighed before. The filter paper was flushed using 3.25% H2SO4 which had been heated for 15 min then flushed with 96% ethanol which had been heated for 15 min as much as 20 ml, after that it was watered again using 20 ml of heated distilled water. The filter paper was put in a porcelain dish and heated in the oven for 12 hr. After that the weight of the filter paper was weighed and the residual weight was calculated, by means of the final filter paper weight minus the initial filter paper weight, then the amount of mung bean sprouted milk fiber was obtained. The amount of fiber is calculated using the formula:

% Fiber content= (Residue Weight)/(Sample Weight) x100%

e. Viscosity Measurement

Viscosity measurement can be done with a viscometer [12]. The viscosity value is expressed in centipoise (cP). The sample is put into a plastic beaker to the mark and stirred first, a pen with the appropriate size is inserted into the sample whose thickness will be measured, then the stirring rod is adjusted and started from low speed to high speed (up to 1500 revolutions/min). The stirrer is turned off from the motor (without removing the stirrer from the sample), the relationship of the spindle to the rotational speed of the viscosity determination factor is determined.

$$\text{Viscosity (cP)} = \frac{\rho\ sample\ x\ t\ sampel\ x\ \eta\ water\ x\ dilution factor}{\rho\ water\ x\ t\ water}$$

$$\rho \text{ sample} = \frac{m' - m}{v}$$

Note: m, empty pycnometer mass (g); m`, pycnometer mass + sample (g); v, pycnometer volume (ml); η, water viscosity (0.1 cP); ρ, density (g/ml) (ρ water = 1 g/ml); t, time (s)

f. Data analysis

Data were analyzed using Analysis of Variance (ANOVA), if there was a significant effect, Duncan's Multiple Range Test (DMRT) was conducted at the 5% level. Selection of the best formula using the effectiveness index [13].

RESULTS

The test results of total phenol content, soluble protein, crude fiber, and viscosity of MungbeS-Milk can be seen in Table 1.

Table 1. Content of phenolic antioxidants, soluble protein, crude fiber, and viscosity of MungbeS-Milk

Komponen	Kombinasi perlakuan							
	L1K1	L1K2	L1K3	L1K4	L2K1	L2K2	L2K3	L2K4
Total fenol mgGAE/L)	1643,3a	1069,7b	929,5c	392,1f	1738,5a	836,5cd	752,9de	653,2e
Protein terlarut (%)	30,98b	29,35d	25,23e	24,38f	37,23a	30,90b	30,35bc	29,65cd
Serat kasar (%)	5,26b	4,22c	4,26c	4,13c	5,63a	4,21c	3,65d	3,46d
Viskositas (CP)	19c	2,083f	21,83b	6e	18,08c	1,83f	27,83a	14,33d

Note: Numbers followed by different letters indicate that there is a significant difference at the 5% level. L1KI = combination of germination proportion : water = 1: 3 with 5 hours germination time; L1K2 = combination of germination proportion : water=1 : 6 with 5 hours germination time; L1K3 = combination of sprout proportions : water = 1: 8 with 5 hours germination time; L1K4 = combination of sprout proportions: water = 1: 10 with 5 hours of germination time; L2K1 = combination of sprout proportions : water = 1: 3 with 10 hours of germination time; L2K2 = combination of germination proportion : water = 1: 6 with germination time of 10 hours; L2K3 = combination of sprout proportions: water = 1: 8 with 10 hours of germination time; L2K4 = combination of sprout proportions: water = 1: 10 with 10 hours germination time.

a. Effect of germination time and proportion of sprouts - water on total phenolic content of mung bean sprouted milk

Phenolics are one of the antioxidant components in nuts. During the germination process, phenolic compounds and other antioxidant components can be formed, so as to increase the phenolic content of green beans [14]. Germination time increased the phenolic content of MungbeS-Milk ($P<0.05$) (Table 1). In accordance with the findings of Winarsi *et al*. [15], that germination not only reduces the content of anti-nutrients, but also increase amino acids, fiber, and other bioactive components, including phenolic compounds. Paixa˜o *et al*. [16] stated that the content of phenolic compounds is closely related to its antioxidant activity. Lushaini *et al*. [17] added that the greater the phenolic content of the kedadai leaves,

the higher the antioxidant activities. The high phenolic content of MungbeS-Milk may have high antioxidant activity.

The proportion of sprout-water reduced the total phenolic content of MungbeS-Milk ($P<0.05$), as well as the combination of germination time and the proportion of sprouts - water, also decreased the phenolic content ($P<0.05$). Phenolic compounds are found in mung beans or their sprouts, therefore the higher the proportion of water, the lower the phenolic content.

b. Effect of germination time and proportion of sprouts - water on soluble protein content of mung bean sprouted milk

Soluble protein is an oligopeptide or amino acid that is easily absorbed by the digestive system. Mentioned that soluble protein is an oligopeptide containing less than 10 amino acids, and has properties that are easily absorbed [18]. Protein quality is determined by its high digestibility and amino acid composition, especially essential amino acids [19, 20]. Mung beans are known as a source of nutrients, especially vegetable protein. The amino acid content of mung bean seeds is quite complete consisting of essential amino acids, namely isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, as well as non-essential amino acids such as alanine, arginine, aspartic acid, glutamic acid, glycine, and tyrosine.

Mung bean protein is quite high (20-25%) [21]. The germination time increased the dissolved protein content ($P=0.00$) (Table 1). This is in accordance with the findings of Shah *et al*. [22] and Wongsiri *et al*. [23] which stated that during germination there was a breakdown of peptide bonds by protease enzymes to produce amino acids. The longer the germination time, the higher the dissolved protein content, because the activity of the protease enzyme was higher, so that the protein degraded into oligopeptides with more short chain amino acids.

The greater the proportion of water in the manufacture of MungbeS-Milk, the lower the dissolved protein content ($P<0.05$). According to Picauly *et al*.[24] the addition of more water followed by a heating process (in processing) allows some proteins to be denatured, as a result the structure of the protein molecules changes and decreases their solubility properties. According to Naga *et al*. [25] denaturation of proteins occurs at different temperatures depending on the nature of the protein, but generally proteins are denatured at a temperature of 70°C. In this study, MungbeS-Milk was cooked at a temperature of 70-80^{o}C, so that there was a possibility of protein being denatured and reducing the dissolved protein content.

c. Effect of combination treatment of germination time and proportion of sprouts - water on crude fiber content of mung bean sprouted milk

Fiber is a non-nutritive substance, there are two types of fiber, namely dietary fiber and crude fiber. Dietary fiber is only found in plant foods and the levels vary depending on the type of material. Dietary fiber can be divided into two types according to its solubility in water, namely soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). Fibers that are included in the water-soluble dietary fiber group are pectin, mucilage and gum. Fibers that are not soluble in water are cellulose, hemicellulose and lignin [26].

Crude fiber content of MungbeS-Milk increased with the length of germination time ($P<0.05$) (Table 1). The increase in crude fiber content also increases the availability of MungbeS-Milk dietary fiber. It happened due to changes in carbohydrate-forming components, such as cellulose and hemicellulose which are the largest components of cell walls [22]. The cellulose and hemicellulose content are hydrolyzed into reducing sugars [27]. Megat *et al*.[28] confirmed that the dietary fiber content of legumes, kidney beans, green beans, soybeans increased after the germination process. The increase in dietary fiber content is directly proportional to the increase in crude fiber content, because dietary fiber is part of crude fiber. Crude fiber is part of food that cannot be hydrolyzed using sulfuric acid and sodium hydroxide, while dietary fiber is part of food that cannot be hydrolyzed by digestive enzymes. The more the proportion of water (the less the proportion of mung bean sprouts), the lower the dietary fiber content of MungbeS-Milk ($P<0.05$), because it comes from mung bean sprouts.

d. Effect of Germination Time and Proportion of Sprouts - Water on Viscosity of Mung Bean Sprouted Milk

Viscosity is a measure that states the consistency of a liquid or fluid. Viscosity is a fluid property that is closely related to its resistance to flow. Viscosity determines the speed at which a liquid flows. The calculation of viscosity is seen from the numbers listed on the viscometer. The results of viscosity measurements in this study can be seen in Table 1.

The duration of germination did not change the viscosity of MungbeS-Milk ($P>0.05$), while the proportion of sprouts - water decreased the viscosity of MungbeS-Milk ($P<0.05$). Shofi [29] stated that in the germination process water imbibition occurs into the mung bean seeds, so that the water content in mung bean sprouts is higher than that of un-germinated mung bean seeds. The greater the proportion of water, the lower the consistency of the MungbeS-Milk. According to Picauly *et al*. [24] the addition of water that is increasingly used to extract soybeans causes the viscosity of soy milk to decrease, so that the viscosity of milk also decreases. The consistency of an ingredient affects the taste of a food product. The addition of too much water causes the MungbeS-Milk to be too runny and the taste is not strong enough, but the addition of too little water can complicate the filtering process of the milk. In addition, the combination of treatment between germination time and the proportion of sprouts - water decreased the viscosity of MungbeS-Milk.

e. The selected of Mung beans sprout milk formula

By using the effectiveness index, the selected formula is MungbeS-Milk with 10 hr of germination and the proportion of sprouts - water = 1: 6. The best MungbeS-Milk is a healthty drink in the form of a slightly thick greenish liquid, contains phenolic antioxidants of 836.47 mg GAE/L, dissolved protein 30.90%, 4.21% fiber, and 1.83 cP viscosity. Some experts believe that antioxidant-rich food products have the potential to improve immune status [30, 31].

The Covid-19 pandemic as it is today, has affected many people, ranging from asymptomatic, to shortness of breath, and even death. It was stated that patients with Covid-19 had low immune status indicated by high markers of inflammation. Winarsi *et al*. [4] have proven that antioxidant-rich products can improve immunity by suppressing the production of IL-6 and CRP, both of which are products of activated immune cells.

MungbeS-Milk's high protein is easily digested and absorbed by the intestines, and replaces damaged tissue cells. High fiber content, in addition to improving the digestive system, also has the potential as an antioxidant. The liquid form of MungbeS-Milk is a bit thick also makes it easier to drink. Therefore, the MungbeS-Milk which is rich in phenolic antioxidants, is suitable for consumption to maintain immunity during the Covid-19 pandemic.

CONCLUSION

Time of germination increased phenolic content, soluble protein, and fiber, but did not change the viscosity of MungbeS-Milk. The higher the proportion of water used for making MungbeS-Milk, the lower the phenolic content, dissolved protein, fiber, and viscosity. The selected of MungbeS-Milk was greenish white slightly thick, obtained with 10 hours of germination, proportion of sprouts-water = 1: 6, containing phenolic of 836.47 mg GAE/L, soluble protein 30.90%, fiber 4.21%, and viscosity 1 ,83 cP. This antioxidant-rich MungbeS-Milk is a good drink for daily consumption to maintain immunity during the Covid-19 pandemic.

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