

COWPEA SPROUTED MILK RICH IN PHENOLIC ANTIOXIDANTS, VITAMIN C, PROTEIN, AND DIETARY FIBER AS AN ANTIDIABETIC DRINK

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Abstract

This study aimed to obtain a formula of Cowpea sprouted milk rich in phenolic, vitamin C, protein, and dietary fiber based on the duration of germination. The experimental study used a randomized block design with 0, 8, 10, and 12 hours germination time treatments, with 5 replications. Cowpeas were washed, soaked in warm water at 40°C for 10 hours, then drained in a basket, placed in a damp place, and sprayed with water once every 6 hours to germinate. Sprouts are washed, including the skin, blended with added water 8 times, filtered, so that a smooth liquid is obtained. Added 5% sugar into the smooth liquid, while stirring, is heated until it boils, called Cowpea sprout milk (CowpeaS-Milk). The product was determined for phenolic content (Follin Ciocalteu), vitamin C (Yodometry), fiber (oven), and soluble protein (Lowry). The data were tested using ANOVA, followed by the DMRT if there was a significant level of 5%. The duration of germination increased the levels of phenolic ($P=0.003$), fiber ($P=0.02$), soluble protein ($P=0.05$), and vitamin C ($P=0.05$). The best formula was obtained from CowpeaS-Milk with a germination time of 12 hours, containing phenolic antioxidants 4.67 mgGAE/g, vitamin C 75.8 mg/100g, dietary fiber 1.28%, and soluble protein 33%. Based on its nutritional content, CowpeaS-Milk is useful for people with Diabetes Mellitus.

Keywords: Cowpea sprouted milk, phenolic, fiber, protein, vitamin C

Introduction

The incidence of type-2 diabetes mellitus (T2DM) increases from year to year and is mostly experienced at the age of >30 years (Gómez-Huelgas *et al.*, 2018). There are several triggers for the pathogenesis of T2DM, including inflammation and oxidative stress (Winarsi *et al.*, 2016). It is stated that functional foods rich in antioxidants can overcome oxidative stress, suppress inflammatory markers, and control blood sugar levels (Burkar *et al.*, 2017). One type of antioxidant-rich nut is Cowpeas.

Cowpeas (*Vigna unguiculata*) reported by Thangadurai (2005) contain protein 29.2%, fat 29.2%, fiber 4.9%, ash 3.6%, and energy 1,737 KJ/100 g, as well as minerals K 11.18 mg, Ca

22.63 mg, Na 96.4 mg, Zn 9.5 mg, Fe 4.47 mg, Cu 9.31 mg, Pb 2.24 mg, and P 421.58 mg/100 g. In addition to potential as antioxidants, Cowpeas have various pharmacological effects such as antidiabetic (Ashraduzzaman *et al.*, 2011) and hypocholesterolemic (Weththasinghe *et al.*, 2014). The high Zn content proves that Cowpeas have antioxidant potential (Winarsi *et al.*, 2020), but the acceptance of Cowpea products is limited by the presence of beany flavor and anti-nutrients in it, so it needs pre-processing.

Germination, in addition to minimizing beany flavor, has also been reported to increase its protein and antioxidant content (Winarsi *et al.*, 2020). All of the components that make up peanuts are degraded during germination, so that their glucose, fatty acid, and amino acid content increases. According to Ferdiawan and Nurwantoro (2019), the longer the germination time, the higher the protein content, as well as the phenolic, fiber, and vitamin C levels (Winarsi *et al.*, 2021).

Ferdiawan and Nurwantoro (2019) reported that the highest protein content was found in Cowpeas with 24-hour germination. In the mung bean germination experiment conducted by Zhou (2019), with a time variable of 36, 48, and 72 hours it significantly increased total phenolics. This study was focused on getting sprouts with a length of 0.5-1.5 cm to obtain optimal antioxidant content so that the germination time was adjusted to local conditions.

The problem is, how is the Cowpea sprouted milk formula rich in phenolic antioxidants, fiber, vitamin C, and protein-based on germination time? This study aimed to determine the effect of germination time on the levels of phenolic, fiber, vitamin C, and protein of Cowpea sprouted milk, and to obtain the best formula.

Research Methods

This non-factorial experimental study used a Randomized Block Design, with variable germination times of 0, 8, 10, 12 hours with 5 replications.

a. Cowpea Germination Production (Wea *et al.*, 2014; Winarsi *et al.*, 2020)

Cowpeas are sorted and washed thoroughly, then soaked for 10 hours in warm water (40-45°C), then drained and placed in a humid place, without light. Germination was carried out for 8 hours, 10 hours, and 12 hours. Every 6 hours, water is sprayed over the beans.

b Making Cowpea Sprout Milk (Winarsi *et al.*, 2020 with slight modification)

Cowpea sprouts were washed with clean water without peeling the skin. Cowpea sprouts are blended with water, with the ratio of Cowpea sprouts: water = 1: 8, until a smooth liquid is

obtained. As much as 5% sugar is added to the smooth liquid, cooked while stirring, until it boils, called CowpeaS-Milk.

c. Determination of the phenolic content of CowpeaS-Milk using the Folin-Ciocalteu method (Orak, 2006)

Take 1 ml of CowpeaS-Milk solution put it into a test tube and add 4 ml of distilled water. 0.2 ml of Folin-Ciocalteu reagent was added to the test tube. The mixture was allowed to stand for 5 minutes, then 1 ml of 5% Na₂CO₃ solution was added, and incubated for 1 hour in the dark. The absorbance was read by a spectrophotometer at a wavelength of 747 nm.

d. Determination of dissolved protein levels by the Lowry method (Goretti and Purwanto, 2014)

Lowry A was prepared by mixing Folin Ciocalteu reagent with distilled water (1: 1). Lowry B was prepared by preparing 50 ml of a solution (2% Na₂CO₃ 0.1 N NaOH) + (1% CuSO₄ + 1% Sodium Potassium Tartrate) in water. Take 1 ml of Cowpea sprout milk solution and put it in a test tube. Added 8 ml of Lowry's reagent B, and left for 10 min. Add 1 ml of Lowry's reagent A, shake, and leave for 20 minutes. The absorbance was read at a wavelength of 600 nm.

e. Vitamin C Analysis (Iodometric Method) (Sudarmadji *et al.*, 1997)

Weighed 5 g of CowpeaS-Milk was put into a 100 ml volumetric flask and added with distilled water up to the mark. Filtered using filter paper to separate the filtrate. Take 5 ml of the filtrate using a pipette, and put it into an Erlenmeyer, add 2 ml of 1% starch solution. Titrate with 0.01 N iodine (I₂) until a blue color. The content of vitamin C can be calculated using the formula:

$$\text{Vitamin C content (mg/100g)} = \frac{(\text{Vol I}_2 \times 0,88 \times Fp) \times 100}{W \text{ sampel (g)}}$$

Notes: V I₂ = Volume of iodine (ml); 0.88 = 0.88 mg of vitamin C is equivalent to 1 ml of 0.01 N I₂ solution; Fp = dilution factor; Ws= Sample weight (g)

f. Fiber Content Analysis (Korompot *et al.*, 2018)

Weighed 5 g of CowpeaS-Milk was put into an Erlenmeyer. As much as 50 ml of 0.313 N H₂SO₄ was added, then covered with aluminum foil and boiled for 30 minutes. Add 50 ml of 0.255 N NaOH and cover with aluminum foil, then boil again for 30 minutes. Filtered using filter paper that has been dried, which has been weighed before. The precipitate contained in the filter paper was flushed successively using hot 1.25% H₂SO₄, hot water, and 96% ethanol. The filter paper was put in a petri dish and heated in an oven at 105°C. After the weight of the filter paper is weighed, then the residual weight is calculated, using the final filter paperweight minus the initial filter paperweight, the amount of CowpeaS-milk fiber is obtained. The amount of fiber is calculated using the formula:

$$\text{Fiber content (\%)} = (\text{residual weight (gr)})/(\text{sample weight (gr)}) \times 100\%$$

g. Data analysis

The data obtained was carried out by the One Way ANOVA test, followed by Duncan's Multiple Range Test (DMRT) at a level of 5% if there was significance. To get the best formula, it was determined using the effectiveness index test (DeGarmo *et al.*, 1984).

Results and Discussion

1. Effect of Germination Time on Phenolic Levels of CowpeaS-Milk

The duration of germination increased the phenolic content of CowpeaS- milk ($P = 0.003$). The highest phenolic content was found in CowpeaS- milk with a germination time of 12 hours, which was 4.67 mg GAE/g (Figure 1). The results of this study are following the findings of Khang *et al.* (2016) who have germinated white Cowpeas, the phenolic content increased.

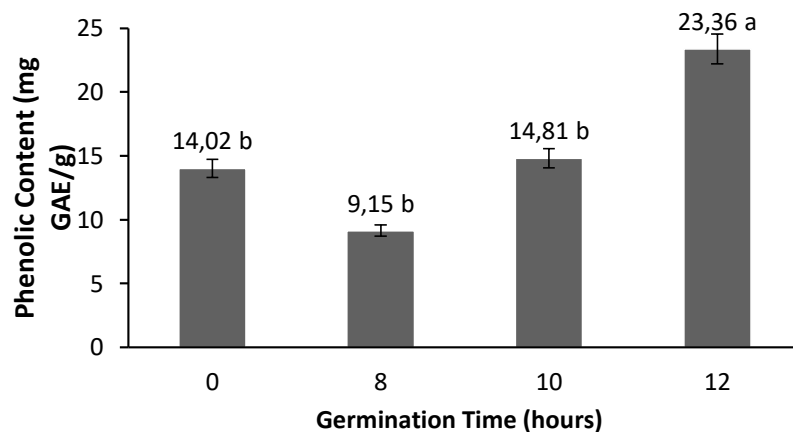


Figure 1. Effect of germination time on the phenolic content of Cowpea sprouted milk.
Note: Numbers followed by the same letter are not significantly different in the 5%.

Germination begins with soaking the beans in water. In this stage, water imbibition occurs into the beans, and activates biosynthetic enzymes, resulting in metabolic products in the form of phenolics and flavonoids (Bewley *et al.*, 2013). During germination, beans undergo an elicitation process that activates the antioxidant defense system of phenolics and flavonoids to protect themselves from disturbances during growth.

The enzyme phenylalanine ammonia-lyase (PAL) catalyzes the elimination of ammonia molecules from cinnamic acid, then produces phenolic compounds as secondary metabolite products (Setyorini and Yusnawan, 2016). Therefore, the phenolic content of Cowpeas increased with the duration of germination.

In this study, the skin of the Cowpea sprouts was not removed but included in the manufacture of CowpeaS-Milk. Christman *et al.* (2018) stated that many phenolic compounds are present in the skin of nuts. Peanut shells contain high levels of phenolics, which act as a protective layer for the cotyledons. Nurjanati *et al.* (2018) stated that the phenolic content of sprouted red bean milk (without skin) was 337 mg GAE/L, lower than that of skinned red bean sprouted milk which was 452 mg GAE/L. Thus the manufacture of whole CowpeaS-Milk (complete with skin) can increase the levels of phenolic which has the potential as an antioxidant and beneficial for health.

2. Effect of Germination Time on Soluble Protein Content of CowpeaS-Milk

The duration of germination increased the soluble protein content of CowpeaS-Milk ($p = 0.05$), with the highest protein content of 33% (Figure 2). The results of this study support the findings Mardiyanto and Sudarwati (2015) who have germinated soybeans so that the dissolved protein content increased at 48 hours of germination because the water that was imbibed into the beans simultaneously included oxygen and became a means of transporting food reserves from the storage area to the embryo axis. In such conditions, the enzymes in it are activated for the synthesis of compounds needed for the formation of new cells, including protein compounds. During germination, proteins are hydrolyzed by protease enzymes into amino acids with smaller molecular weights and are water-soluble (Afify *et al.*, 2011). Therefore, the duration of germination plays a role in increasing the soluble protein content of CowpeaS-Milk.

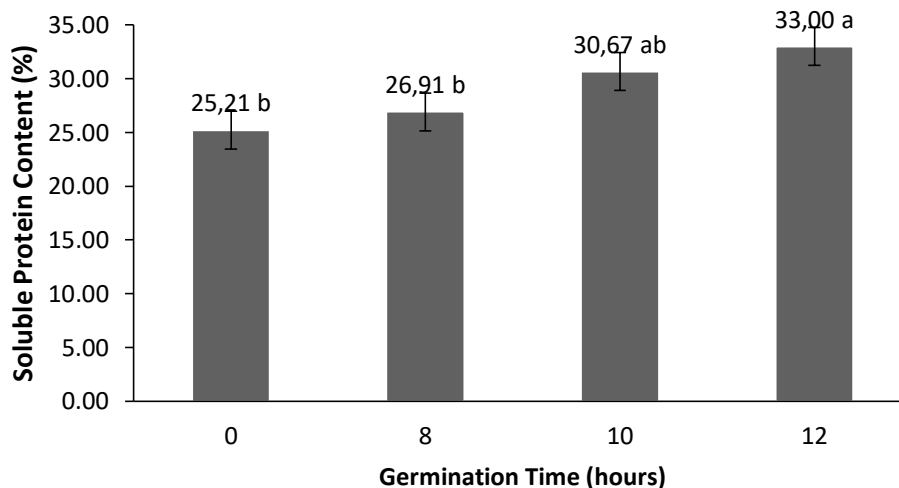


Figure 2. Effect of germination time on the soluble protein content of CowpeaS-Milk.
Note: Numbers followed by the same letter are not significantly different in the 5%.

Soluble protein is more easily extracted in water, so its levels in CowpeaS-Milk increase. Soluble protein is part of the total protein contained in foodstuffs that can be dissolved in water. It has a chain of less than 10 amino acids, so it is more easily digested by the human intestine (Winarsi *et al.*, 2021).

c. Effect of Germination Time on Fiber Content of CowpeaS-Milk

The duration of germination increased the fiber content of CowpeaS-Milk ($P=0.02$), with the highest fiber content found in the 12-hour germination treatment, namely 1.28%, while the lowest was found in the 8-hour germination treatment (0.91%) (Table 1). The results of this study are in line with the findings of Megat *et al.* (2016), that there was a significant increase in dietary fiber in the germination of legumes, kidney beans, green beans, and soybeans. The increased fiber content in sprouts occurs due to the process of structural carbohydrate synthesis, namely the formation of new cellulose and hemicellulose which are the largest components of cell walls (Shah *et al.*, 2011). Cellulose and hemicellulose are fiber compounds.

Table 1. Fiber content of CowpeaS-Milk based on germination time

Germination times (hours)	Dietary fiber (%)	P value
0	0,59±0,38 ^a	0,02
8	0,91±0,03 ^{ab}	
10	1,08±0,11 ^b	
12	1,28±0,11 ^b	

Notes: Numbers followed by the same letter indicate that there is no significant difference in the 5%; n=3

In this study, the Cowpea sprouts were included in the manufacture of CowpeaS-Milk, because the Cowpea epidermis contains a lot of fiber such as cellulose. The addition of soybean husk can increase the fiber and protein content so that it can be used as a quality food product. This is in line with the research conducted by Safitri *et al.* (2017), the highest fiber content is found in Cowpea milk from Cowpea with skin, compared to Cowpea milk without skin. Another study also conducted by Khalid and Elhardallou (2016), the manufacture of flour made from Cowpeas with skin produces higher fiber compared to flour made from Cowpeas without skin.

d. Effect of Germination Time on Vitamin C Levels of CowpeaS-Milk

There was a significant effect of germination time on the increase in vitamin C levels of CowpeaS-Milk ($P=0.05$), with the highest levels found in milk with a germination time of 12 hours, namely 75.8 mg/100g, and the lowest at 8 hours of germination (64.08 mg/100g) (Table 2).

Table 2. Vitamin C Levels of Cowpea Sprout Milk by Germination Time

Germination Time (Hour)	Vitamin C (mg/100g)	P-value
0	46,52±8,8 ^a	0,05
8	64,08±10,5 ^{ab}	
10	67,38±13,6 ^{ab}	
12	75,80±9,07 ^b	

Notes: Numbers followed by the same letter indicate no significant difference in the 5%; n=3.

The results of this study support the findings of Masood *et al.* (2014) that vitamin C levels increased in sprouted beans, due to the activity of the enzyme L-Galactono-gamma-lactone dehydrogenase catalyzing the oxidation of L-galactono-1, 4-lactone to ascorbic acid, or vitamin C. The enzyme L-Galactono-gamma- lactone dehydrogenase is a key enzyme in ascorbic acid biosynthesis; the longer germination the enzyme activity increased significantly so that the ascorbic acid level increased. Germination time of 12 hours showed vitamin C content of CowpeaS-Milk was 75.80 mg/100g, higher than other germination.

Vitamin C is one of the nutrients needed by the body. This vitamin is usually found in fruits and vegetables. The vitamin C content of un-germinated Cowpea seeds was 34.44 mg/100 g of material (Tresina and Mohan, 2011), but the length of germination significantly increased the vitamin C content of CowpeaS-Milk.

e. The best CowpeaS-Milk formula

The selection of the best formula was based on the phenolic content, soluble protein, fiber, and vitamin C of CowpeaS-Milk, determined using the effectiveness index (DeGarmo *et al.*, 1984). Variables are sorted by priority and contribution to the importance of each variable, with a relative value of 0 – 1. The weighting for phenolic is given a value of 1, soluble protein 0.9, fiber 0.8, and vitamin C 0.7. The highest productivity value was found in the CowpeaS-Milk formula with 12 hours of germination, which contained phenolic antioxidants of 4.67 mgGAE/100g, soluble protein 33%, fiber 1.28%, and vitamin C 75.8 mg/100 g.

Phenolic compounds are antioxidants that have antibacterial, antiviral, and anti-inflammatory activities (Hossain and Uddin, 2020). Phenolics also work as alpha-amylase and

alpha-glucosidase inhibitors which slow down the digestion of carbohydrates, thereby reducing the rise in blood sugar (Asgar, 2013). Polyphenols, especially flavonoids, tannins, and phenolic acids can inhibit glucosidase and amylase enzymes for the digestion of carbohydrates into glucose (Lin *et al.*, 2016). In other words, phenolic compounds can control the digestion of carbohydrates. In addition to modulating carbohydrate digestion, phenolics also improve conditions of hyperglycemia, dyslipidemia, and insulin resistance, thereby restoring pancreatic beta-cell function, and reducing oxidative stress. In the long term, phenolic compounds can prevent the development of complications of diabetes, such as cardiovascular disease, neuropathy, nephropathy, and retinopathy. Thus, CowpeaS-Milk with high phenolic content is beneficial for diabetics.

Proteins are polymers of amino acids linked by peptide bonds. The main function of protein is to form new tissue, maintain existing tissue, and as a regulator of metabolic processes. Protein has thermogenic properties, providing a longer feeling of fullness, eliminating the urge to eat again, and suppressing subsequent energy intake, thereby controlling blood sugar levels. Rosenvinge Skov *et al.* (1999) reported that the high-protein diet group produced a longer feeling of fullness than the low-protein diet. Therefore, protein-rich CowpeaS-Milk is suitable for consumption by diabetics.

CowpeaS-Milk also contains dietary fiber (1.28%). One of the health effects of fiber-rich functional food is that it can prevent the development of diabetes and hypercholesterolemia. The nature of fiber can increase digesta viscosity, and inhibit the absorption of sugar and cholesterol (Li *et al.*, 2019). Thus, CowpeaS-Milk with its fiber content has the potential to control blood sugar and cholesterol levels, making it suitable for people with diabetes and dyslipidemia.

Vitamin C as an antioxidant helps in reducing the production of free radicals (Christie-David *et al.*, 2015). Vitamin C can also increase insulin sensitivity and insulin modulation in the process of non-oxidative glucose metabolism. Vitamin C can help increase the mass of pancreatic beta cells, thereby stimulating the production of more insulin (Utami *et al.*, 2015). Therefore, CowpeaS-Milk which is known to be rich in vitamin C is beneficial for people with diabetes mellitus.

Conclusion

The duration of germination increased the levels of phenolic, soluble protein, fiber, and vitamin C of CowpeaS-Milk. The best CowpeaS-Milk formula is made from Cowpeas which are germinated for 12 hours, which contains phenolic antioxidants of 4.67 mgGAE/g, 33% soluble protein, 1.28% dietary fiber, and 75.8 mg/100 g vitamin C. Based on its nutritional content, CowpeaS-Milk is suitable for improving the condition of diabetes mellitus.

Acknowledgment

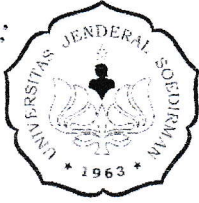
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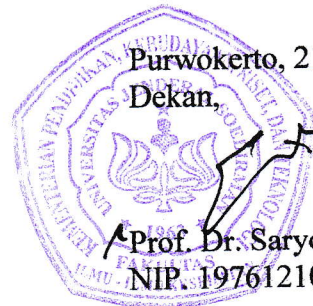
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Berdasarkan Surat permohonan dari Prof. Dr. Ir Hery Winarsi, MS perihal permohonan Surat Tugas sebagai Penyaji, maka Dekan Fakultas Ilmu-ilmu Kesehatan Universitas Jenderal Soedirman memberikan tugas kepada :

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1	Prof. Dr. Ir Hery Winarsi, MS	195703011985032001	Pembina Utama Muda (IVc)
2	Dra. Erminawati, M.Sc, Ph.D	195702151981112001	Pembina (IV/a)
3	Farah F Hapsari	-	-

untuk menjadi Penyaji pada kegiatan Seminar The 4th International Conference Of Health Sciences (ICHS) di Purwokerto tanggal 22-23 September 2021

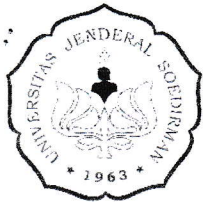
Surat tugas ini dibuat untuk dapat digunakan sebagaimana mestinya.



Purwokerto, 21 September 2021

Dekan,

Prof. Dr. Saryono, S.Kp, M.Kes
NIP. 197612102002121001



KEMENTERIAN PENDIDIKAN, KEBUDAYAAN,
RISET, DAN TEKNOLOGI

UNIVERSITAS JENDERAL SOEDIRMAN
FAKULTAS ILMU-ILMU KESEHATAN

Jalan dr. Soeparno, Grendeng Purwokerto 53122
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SURAT TUGAS
Nomor : 781/UN23.13/DL.17/2021

Berdasarkan Surat permohonan dari Prof. Dr. Ir Hery Winarsi, MS perihal permohonan Surat Tugas sebagai Penyaji, maka Dekan Fakultas Ilmu-ilmu Kesehatan Universitas Jenderal Soedirman memberikan tugas kepada :

No	Nama	NIP	Pangkat/Gol
1	Prof. Dr. Ir Hery Winarsi, MS	195703011985032001	Pembina Utama Muda (IVc)
2	Dra. Erminawati, M.Sc, Ph.D	195702151981112001	Pembina (IV/a)
3	Gumintang Ratna Ramadhan, S.TP., M.Si.	198905032019032016	Penata Muda/IIIb
4	Elsya M Gumelar	-	-
5	Lavia ASS Kencana	-	-

untuk menjadi Penyaji pada kegiatan Seminar International Symposium On Food And Agro-Biodiversity (ISFA) 2021 di Semarang tanggal 15 September 2021

Surat tugas ini dibuat untuk dapat digunakan sebagaimana mestinya.

Purwokerto, 14 September 2021
Dekan,

Prof. Dr. Saryono, S.Kp, M.Kes
NIP. 197612102002121001



CERTIFICATE

No. 5793/UN7.5.5.2/TU/2021.



Hereby proudly presented to

Prof. Dr. Ir. Hery Winarsi, MS

*as a presenter in the 3rd International Symposium on Food and
Agro-biodiversity (the 3rd ISFA) on 14-15 September 2021*



Professor Bambang Waluyo Hadi Eko Prasetyono

Faculty Dean of Animal and Agricultural Sciences
Universitas Diponegoro



Dr Setya Budi Muhammad Abduh

Chairperson of ISFA 2021