THESIS

PENGARUH GERMINASI DAN FORTIFIKASI NANOKALSIUM CANGKANG TELUR TERHADAP KARAKTERISTIK MINUMAN GERMINASI KACANG HIJAU (*Vigna radiata*.)

THE EFFECT OF EGGSHELLS NANOCALCIUM FORTIFICATION ON THE CHARACTERISTICS OF MUNG BEAN GERMINATED DRINK (*Vigna radiata*.)

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SUMMARY

REVICHA CAHAYA PERTIWI. *The Effect of Eggshells Nano-Calcium Fortification on the Characteristics of Mung Bean Germinated Drink* (*Vigna radiata*.) (Supervised by **NURA MALAHAYATI**).

This study aimed to determine the effect of germination duration and fortification of eggshells nanocalcium on the physicochemical characteristics of mung bean germinated drink. This study used a Factorial Completely Randomized Design (RALF) with two treatment factors, the first factor was germination duration (factor A) which consisted of three treatment levels (6,12 and 18 hours), the second factor was the type of eggshell (factor B) which consisted of two levels of treatment (chicken egg shell and duck egg shell). Each experiment was repeated three times. The parameters observed in this study were chemical characteristics (protein level, calcium level and vitamin C level) and physical characteristics (pH, viscosity and stability) of mung bean germination drink.

The results showed that the germination time treatment significantly affected the levels of protein, calcium, vitamin C, pH value, viscosity and stability of the mung bean germination drink produced. Germination increased the vitamin C level in mung beans with a mean value of 79.77-111.44 mg/100 g. A1B2 (6 hours of germination with fortified duck nanocalcium powder) showed the best result with a pH value of 6.77, a viscosity of 27.13 mPa.s and a stability of 87.00% which was close to the value of mung bean extract water in commercial product. Eggshell nanocalcium fortification showed a significant effect on increasing the calcium level of mung bean germinated drink. The best value on calcium level of mung bean germinated drink was found in A1B2 (6 hours of germination with fortification of duck nanocalcium powder), which was 24.82%.

Keywords: mungbean, germination, nanocalcium

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APPROVAL SHEET

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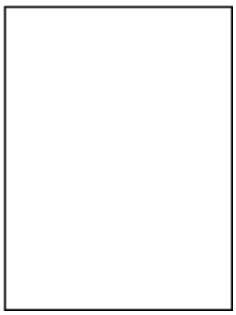

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BIOGRAPHY

REVICHA CAHAYA PERTIWI was borned in Lampung City, Central Lampung on June 19th, 1999. The writer is the second of three children from Mr. Achmad Nuraidy and Mrs. Fatimah.

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Praise and gratitude to Allah SWT for His blessings and grace, the writer was able to complete the thesis entitled "The Effect of Eggshells Nano-Calcium Fortification on the Characteristics of Mung Bean Germinated Drink (Vigna radiata.)". The thesis was submitted to fulfill one of the requirements for obtaining a bachelor's degree in Agricultural Technology at Sriwijaya University.

1. Dean of the Faculty of Agriculture, Sriwijaya University.
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16. Classmate of class 2017 Palembang.

The writer hopes that this thesis can be useful for the readers. The writer realizes that there are many imperfections in the preparation of this thesis, the writer expects criticism and suggestions from the readers. Thank you.

Palembang, September 2021

Writer

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CHAPTER 1
INTRODUCTION

1.1. Background

In Indonesia, mung bean (Vigna radiata) can grow in almost all areas. Mung bean is the third source of protein after soybeans and peanuts with a content of about 20-25%. In each 100 g dry weight, mung bean generally contains 22.20 g protein, fat 1.20 g, carbohydrates 62.30 g, fiber 4.63 g, vitamin C 6.00 mg and calcium 125 mg (DKBM, 2017). One of the leading varieties of mung bean that is known is VIMA-1 (Vigna sinensis–Malang). VIMA-1 has a promising market potential due to its high protein level, low fat and high starch. The content of mung bean of the VIMA-1 variety which consists of 28.02% protein, 0.40% fat and 67.62% starch content on a dry basis (Agency for Research and Development, 2019). The low fat content in it makes mung bean good for health.

Not only the shape of the seeds but also germinating mung bean have increased nutritional, functional and biological characters by changing the content, nutritional composition and bioactive compounds, as well as eliminating anti-nutritional factors in them (Liu et al., 2020). Germination is a condition in which dormant seeds begin to germinate and grow into seedlings under the right growing conditions. The growth of sprout in germinated seeds ranges from 2 mm to up to 5 mm (Munarko et al., 2019) with growth times ranging from 6 to 18 hours.

Germination of mung bean seeds can increase the nutritional value in them by activating enzymes that can reduce or eliminate anti-nutritional factors (Ebert et al., 2017). A common anti-nutrient found in mung bean is phytic acid. Germination was able to reduce the phytic acid content in mung bean from 12.0 mg/g to 4.03 mg/g (Faradilla et al., 2012). Not only that, the content of vitamin C increases with the length of germination. The mean vitamin C in germinated mung seed was 2.7 times higher than without germination. Germination in mung bean produces the ratio of vitamin C is 1.7 times higher than that of vitamin C found in soybean germination (Ebert et al., 2017). The germination process can also increase the bioavailability of proteins and minerals, such as calcium. This happens because the germination process can release the bound form of the

substance in mung bean seeds into a freer form, so that it is more easily digested and absorbed by the human digestive system.

Germinated mung bean have the prospect of being developed into a nutritious and alternative drink for those who are allergic to cow's milk, known as lactose intolerant. Lactose intolerance is a clinical syndrome that occurs due to a person's inability to digest lactose due to a lack or not producing the enzyme lactase in the digestive system (Saputra, 2019).

Chicken and duck egg shells are household waste that has not been utilized optimally due to the lack of public knowledge about the contents in it. Egg shells consist of 95.1% salt, 3.3% organic matter, and 1.6% water. The main component of inorganic salts in egg shells is dominated by calcium carbonate ($CaCO_3$) of 98.5% (Nurjayanti et al., 2012). The bioavailability of egg shells is ±40% (Szeleszczuk et al., 2015) with calcium level in chicken and duck egg shells, which are 25.73% and 23.67%, respectively (Aminah and Meikawati, 2016).

Calcium is an important mineral in the maintenance of bones. Calcium contained in egg shells can be fortified in mung bean germination drinks as an effort to increase calcium consumption from other easily available sources. Calcium is generally available in micro size, which is thought to be absorbed by only 50% of the total calcium consumed in its metabolism. The application of nanocalcium to eggshell powder can help calcium to be easier dissolved and optimally absorbed. According to Widyastuti and Kusuma P. (2017), nanocalcium is a manufacture of particles with a size of less than 100 nm by changing the nature or function of a material.

There are two methods used in the manufacture of nanoparticles, namely top down and bottom up. The top down method (physical method), is a process the manufacture of nano-sized particles is carried out directly or mechanically, while the bottom-up (chemical method) is a method of making nanoparticles carried out by arranging atoms or molecules to form nanometer-sized particles from solution. In the bottom up method, the formation of nanoparticles has a high regularity so that it is able to produce a more uniform pattern size. The precipitation method is part of the bottom up method. This method is done by controlling the solubility of the material in the solution by controlling the temperature, pH and solvent. The precipitation method is very effective in the

manufacture of nanoparticles because of the simple process and low cost (Suptijah et al., 2012).

Berdasarkan informasi di atas, peneliti ingin mengetahui mengenai pengaruh fortifikasi nanokalsium terhadap minuman germinasi kacang hijau (*Vigna radiata*.) yang dihasilkan. Based on the information above, the researchers wanted to know about the effect of nanocalcium fortification on the mung bean (Vigna radiata.) germination drink produced.

1.2. Objective

The objective of this study was to determine the effect of eggshels nanocalcium fortification on the characteristics of mung bean germinated drink (Vigna radiate).

1.3. Hypothesis

It is suspected that the duration of germination and fortification of nanocalcium eggshell significantly affected the physicochemical characteristics of the mung bean germination drink.

CHAPTER 2
LITERATURE REVIEW

2.1. Mung Bean (*Vigna radiata*.)

Mung bean are one of the mainstay commodities of food crops that are in great demand by the public. Mung bean (Vigna radiata.) belongs to the leguminosae family (Lestari, 2018). Mung beans have a smaller seed size than other legumes. Generally, mung bean seeds are dull or shiny green, some of them are yellow, brown and black (Elygio, 2019). Mung beans are the third source of protein after soybeans and peanuts with a content of about 20-25%, in 100 g dry weight of mung beans there are 22.20 g protein, 1.20 g fat, 62.30 g carbohydrates, 4.63 g fiber (DKBM, 2017). Mung beans have starch that is easily digested so that it can be used in the process of making food. Mung bean starch consisted of 28.80% amylose and 71.20% amylopectin. Mung beans are also composed of 72.80% unsaturated fatty acids and 27.70% saturated fatty acids (Wea et al., 2014). The high level of unsaturated fatty acids in mung beans make these beans good for consumption, especially in maintaining heart health (Triyono et al., 2010).

In addition, mung beans are rich in amino acids in the form of aromatic amino acids, leucine, isoleucine, valine and glutamic acid. Mung beans have a relatively low sulfur amino acid content, thus providing an advantage in calcium retention (Widjajaseputra et al., 2019). Mung beans are also equipped with micronutrients in the form of calcium (125 mg/100g), phosphorus (326 mg/100g), iron (6.70 mg/100g), vitamin B1 (0.64 mg/100g), vitamin A (57 mg/100g). IU/100g) and vitamin C (6.00 mg/100g) (DKBM, 2017).

2.1.1 Kacang Hijau VIMA-1 (Vigna radiata.)

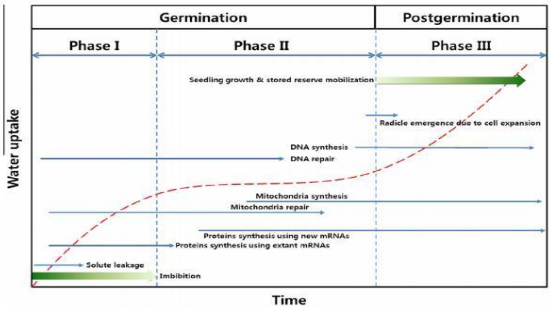
Mung beans VIMA-1 are a variety of mung beans named Vima-1 through SK Number 833/Kpts/SR.120/6/2008 dated 24-6-2008. Mung bean varieties assembled by the Research Institute for Legumes and Tubers This Malang tuber (Balitkabi) was obtained through artificial crosses from the elders' male VC 1973A and female 2750A and systematic selection to obtain the MMC 157d Kp-1 line which has early maturity and powdery mildew resistance. VIMA-1 green beans can be distinguished from other types by the appearance of the seeds which are dull green in color. The advantages of VIMA-1 are that it has a fairly high yield, an early age, and is resistant to powdery mildew. In addition, the nutritional content of mung beans VIMA-1 is higher than mung beans in general. The nutritional composition of VIMA-1 mung beans consisted of protein

content of 28.02% on a dry basis, fat content of 0.40% on a dry basis and starch content of 67.62% on a dry basis. The low fat content in VIMA-1 mung beans make these mung beans good for consumption (Balitbang, 2019).

Source: Badan Litbang (2019)
Figure 2.1. Mung bean seed VIMA-1

2.2. Germination

The germination process is a complex series of morphological and biochemical changes. Germination can be defined as a condition where seeds germinate under the right growth conditions. Germination refers to when the condition sprouts are 2–5 mm long (Munarko et al., 2019). There are three phases in the germination process which can be seen in Figure 2.2.



Source: Bewley (1997)
Figure 2.2. *Physical and Metabolic Events during Germination and Post-Germinative Growth*

The germination phase consists of the imbibition phase, the formation of the buds, and the elongation of the sprouts. The imbibition phase is a condition where the water around the seed environment enters quickly, so that the seeds swell. This process is able to suppress the physical conditions required in the germination process. The second phase, namely the formation of buds. In this phase, the seeds will grow slowly. Seeds will undergo synthesis of various types of proteins, mitochondria, and other compounds that support the germination process, while water absorption at this stage is relatively stable. The third stage is the sprout elongation or post-germination phase which is characterized by the elongation of the sprouts which indicates the process of further sprout growth (Cho and Lim, 2016). The germination process can be seen in Figure 2.3.

Source: Personal Documentation

Figure 2.3. Germination Process

In the germination process, complex compounds are broken down into simple ones (Rachim et al., 2020). According to Widajati (2013), carbohydrates will be converted into dextrins or smaller parts, namely in the form of glucose, fats will be broken down into fatty acids and glycerol, and proteins will be simplified into free amino acids that are utilized by the embryo in its growth. Germination treatment has been shown to be able to reduce levels of anti-nutritional substances by 66.7% (Nur et al., 2019), so that the vitamins and minerals contained inside such as thiamin, vitamin A, phosphorus, calcium, iron will experience an increase in bioavailability. In addition, the content of vitamin C in germinating bean seeds will increase 2.7 times higher than in the form of seeds (Ebert et al., 2017). The germination process is considered as an effective way to improve the nutritional quality of cereals and legumes, because the germinated seeds have better palatability, digestibility and availability of certain nutrients compared to their seed. form.

2.3. Mung Bean Germination Drink

The quality of the mung bean germination drink will be based on the quality of the soybean juice drink. According to Andrestian and Hatimah (2015) the quality requirements of mung bean juice do not exist yet, therefore the quality requirements of mung bean germination drinks refer to the quality requirements of SNI soy juice with SNI 01-3830-1995, as shown in Table 2.1.

Table 2.1. Soy Juice Quality Requirements

No.	Criteria Test	Unit	Milk	D*rink*
1	Condition :	-		Normal
1.1	Smell	-	Normal	Normal
1.2	Taste	-	Normal	Normal
1.3	Color	-	Normal	Normal
2	pH	-	6,5 – 7,0	6,5-7,0
3	Protein	% b/b	Min. 2.0	Min. 1.0
4	Fat	% b/b	Min. 1.0	Min. 0.30
5	Amount of solid	% b/b	Min. 11.50	Min. 11.50
6	Food additives based on			
6.1	Sweeteners			
6.2	Coloration			
6.3	Preservative			
7	Metal Contamination			
7.1	Plumbum (Pb)	mg/kg	Max. 0,2	Max. 0,2
7.2	Copper (Cu)	mg/kg	Max. 2	Max. 2
7.3	Zinc (Zn)	mg/kg	Max. 5	Max. 5
7.4	Tin (Sn)	mg/kg	Max. 40/250	Max.
7.5	Mercury (Hg)	mg/kg	Max. 0,03	40/250
				Max. 0,03
8	Arsenic contamination	mg/kg	Max. 0,1	Max. 0,1
9	Microbial contamination			
9.1	Total plate number	Colony/mL	Max. $2x10^2$	Max. $2x10^2$
9.2	Coli bacteria	APM/mL	Max. 20	Max. 20
9.3	Escherichia Coli	APM/mL	Max. 3	Maks. 3
9.4	Salmonella	-	Negative	Negative
9.5	Staphylococcus aureus	Colony/mL	0	0
9.6	Vibrio sp.	-	Negative	Negative
9.7	Kapang	Colony/mL	Max. 50	Max. 50

Source: BSN (1995)

2.4. Egg Shell

Egg consists of three main components, namely, egg shell or shell (11% of egg weight), egg white (57% of egg weight) and egg yolk (32% of egg weight). The egg shell is the outermost part of the egg which functions to provide protection for the components of the egg contents from physical, chemical and microbiological damage (Hajar et al., 2016). Egg shell has a porous structure with a thickness of 0.2-0.4 mm. The shell of a chicken egg consists of four layers, namely the cuticle, foam layer, mamillary layer and membrane layer (Kumaji, 2019). Egg shells contain calcium and minerals which are composed of a simple protein in the form of albumin with a strong structure. Egg shells contain nearly 95.1% salt, 3.3% organic matter, and 1.6% water. Most of the egg shell content consists of calcium carbonate (CaCO3) which is 98.5% and magnesium carbonate (MgCO3) about 0.85% (Nurjayanti et al., 2012).

Types of egg shells that are commonly consumed by the public are chicken and duck egg shells. There are two kinds of chicken eggs, namely chicken eggs (country) and free-range chicken eggs. Chicken eggs have a brown shell color as shown in Figure 2.4. while the duck egg shell has a blue color as shown in Figure 2.5. The color difference in egg shells is influenced by genetics and the structure of each egg. The brown color of the chicken egg shell is caused by the presence of porphyrin compounds and the blue color in duck egg shells is caused by the pigment biliverdin (Yonata et al., 2017). Calcium levels contained in duck egg shells and chicken egg shells were 25.73% and 23.67%, respectively (Aminah and Meikawati, 2016).

Source: personal documentation(2020)
Figure 2.4. Checken egg shell

Source: personal documentation (2020)
Figure 2.5. Duck egg shell

Egg shells have the potential as a source of calcium that can be used to fulfill the body's mineral needs. One of the efforts that can be done is by fortification. Fortification is the addition of one or more essential nutrients to food, either naturally present in food or not, with the aim of preventing or correcting deficiencies in a nutrient (Arisyi, 2016). Calcium fortified foods play an important role in helping to meet calcium needs to reduce the risk of osteoporosis (Wasilewski et al., 2019). Egg shell fortification in foodstuffs is generally in the form of flour or powder (Safitri et al., 2014) which is done by smoothing the shell first and added during the food or beverage processing. Foods fortified with egg shells have been shown to increase the calcium level in them. Egg shell has a bioavailability of ±40% (Szeleszczuk et al., 2015), where the bioavailability can be categorized as good (Qolis et al., 2020). Safitri et al. (2014), stated that the addition of eggshell flour gave a significant increase in the calcium level of soy milk, which was 35.83±4.35 mg/240 ml.

2.5. Nanocalsium

Nanocalcium is calcium processed with nanotechnology which produces particles measuring 10-1000 nm by changing the characteristic or function of a material (Fatoni et al., 2020). The formation of calcium particles in the form of nano or 10-9m has advantage that the absorption of the material can be carried out optimally by the body so that it is more efficient than in the size normally consumed (First et al., 2019). There are two methods used in the manufacture of nanoparticles, namely top down and bottom up. The top down method (physical method) is the manufacture of nanoparticles that is carried out directly or mechanically, while the bottom up (chemical method) is a method of making nanoparticles by arranging atoms or molecules to form nanometer-sized particles. In the bottom up method, the formation of nanoparticles has high regularity so that it can produce a more uniform pattern size. Some common methods of synthesis nanocalcium contained in the bottom up is heating or thermal, coprecipitation, precipitation, sol gel, microwave, hydrothermal/solvothermal, template synthesis, biomimetic synthesis, supercritical fluid method and ionic liquid synthesis. The precipitation method is carried out by controlling the solubility of the material in solution through changes in pH (Suptijah et al., 2012).

Isolation of calcium from egg shells was carried out starting from the demineralization stage of HCl, namely the process of dissolving minerals contained in the shell, especially CaCO3. The reaction between the CaCO3 content in the shell and the HCl solution produces calcium chloride (CaCl2). CaCl2 is then precipitated using sodium hydroxide (NaOH) so that a precipitate is formed in the form of Ca(OH)2 and salt (NaCl) (Widyastuti and Kusuma P, 2017). NaCl salt as a by-product in the form of a solution is neutralized using distilled water. The Ca(OH)2 precipitate formed is burned in a temperature of 600 so that the final product is formed in the form of calcium oxide (CaO) (Suptijah et al., 2012; Sunardi 2020).

CHAPTER 3
RESEARCH METHODOLOGY

3.1. Time and Place

This study was conducted at the Agricultural Product Chemistry Laboratory and Agricultural Product Technology Laboratory, Agrotechnology Department, Faculty of Agriculture, Sriwijaya University, South Sumatra. This study was conducted from April 2021 to June 2021.

3.2. Tools and Techniques

The tools that used in this study were: 1) AAS (Atomic Absorption Specthophoyometry) (Thermo Scientific Nicolet™ iS™ 10, USA), 2) 100 mesh sieve, 3) plastic basin, 4) Beaker glass (Phyrex, Japan), 5) burette, 6) blender (Philips HR 2116, China) , 7) glass funnel, 8) Erlenmeyer (Iwaki, Japan), 9) Whattman filter paper, 10) stove (Rinnai, China), 11) volumetric flask (Phyrex, Japan), 12) muffle furnace (Barnstead FB1410M-26, USA), 13) mortar, 14) electric oven (Memmert, USA), 15) pot, 16) stirrer, 17) pH meter (Oakton do-35613, USA), 18) dropper, 19) measuring pipette (Iwaki, Japan), 20) UV-VISAE S80 spectrophotometer (A & E LAB , China), 21) test tube, 22) analytical balance (Ohaus AR2140, USA), 23) Brookfield NDJ-8S viscometer (China)

The materials that used in this study were: 1) aquabidest, 2) sulfuric acid (HCL) 1N, 3) 1% starch, 4) biovine serum albumin (BSA), 5) chicken and duck egg shells, 6) mung beans VIMA-1, 7) 0.01N iodine solution, 8) 3N NaOH, 9) Lowry's reagent A, 10) Lowry's reagent B.

3.3. Research Method

This study was conducted using a completely randomized factorial design (RALF), with two treatment factors, namely germination time (A) which consisted of 3 treatment levels and egg shell type (B) which consisted of 2 treatment levels, obtained 6 treatment combinations. Each treatment combination is carried out repeated three times. The treatment can be seen in table 3.1.

Table 3.1. Treatment Factors in Making Mung Bean Germination Drink

Factor A: Germination time (A)	Factor B: Egg shell type
A1: 6 hours	B1: chicken egg shell
A2: 12 hours	B2: duck egg shell
A3: 18 hours	

The germination treatment at 0 hour and without the addition of eggshell nanocalcium in mung bean germination drink was carried out as a control.

Nanocalcium eggshell powder added to the sample was 22% based on the Nutrition Label Number (ALG) for the general category of calcium, i.e. each 1100 mg ingredients

The data obtained are processed using analysis of variance (ANOVA) 5% for chemical and physical characteristics. Treatments that have a significant effect will be further tested using the Honest Significant Difference (HSD) test at 5% level.

3.4. Statistical Analysis

According to Hanafiah (2002), the general model for Completely Randomized Design Factorial (RALF) using two treatment factors are as follows::

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

Description:

Y_{ijk} = observation value

μ = mean value

α_i = mung bean germination time treatment

β_j = effect of egg shell type

$(\alpha\beta)_{ijk}$ = effect of treatment interaction

ε_{ijk} = galat

The results of the measurements that had been carried out were processed using parametric statistical analysis of Completely Randomized Factorial Design (RALF). The variance analysis table can be seen in Table 3.2.

Table 3.2. List of Factorial Completely Randomized Design Variance Analysis (RALF)

Source of variance (SK)	Degree of freedom (db)	Total of squared (JK)	Total of squared Tengah	Fcount	F-table 5%
Treatment (P)	$V_1 = (m.n) - 1$	JKP	JKP/V_1	KTP/KTG	(V_1,V_2)
Factor A	$V_2 = m - 1$	JKA	JKA/V_2	KTA/KTG	(V_2,V_5)
Factor B	$V_3 = n - 1$	JKB	JKB/V_3	KTB/KTG	(V_3,V_5)
Interaction AB	$V_4 = (m-1)(n-1)$	JKAB	$JKAB/V_4$	KTAB/KTG	(V_4,V_5)
Galat	$V_5 = V_6 - V_1$	JKG	JKG/V_5		
Total	$V_6 = (m.n.r) - 1$	JKTotal	JKT/V_6		

Source: Hanafiah (2002)

Description:

m = total of treatment A

n = total of treatment B

r = repetiiton

The significance of each treatment in the analysis of variance was carried out by comparing the Ftable on the 5% test based on the following comparison:

1. If F_{count} is bigger than F_{table} 5%, then it is declared to have a significant effect and marked with *.
2. If F_{count} is bigger than or as big as the F_{table} 5%, then it is declared to have no significant effect and marked with ^{ns}.

If the results of the analysis of variance show that Fcount is bigger than Ftable was then continued with the HSD test (Honest Significant Difference) to find out the mean difference in each experiment. The formula used for the HSD test is:

$$HSD = q\,\alpha\,(p,v)\ x\ Sy$$

$$\bar{S}y_{\alpha} = \sqrt{\frac{KTG}{3xr}} \Longrightarrow \text{for treatment of mung bean germination time}$$

$$\bar{S}y_{\beta} = \sqrt{\frac{KTG}{2xr}} \Longrightarrow \text{for the treatment of egg shell types}$$

$$\bar{S}y_{\alpha\beta} = \sqrt{\frac{KTG}{r}} \Longrightarrow \text{for treatment interactions}$$

Description :

q = the value in the table q at the test level 5%

p = number of treatments tested

v = error of degree freedom

KTG = galat center squared

r = total repetition

α = mung bean germination time treatment

β = egg shell type treatment

α β = effect of treatment interaction

Furthermore, to determine the level of accuracy obtained from the experimental results, according to Gomez and Gomez (1995), it is necessary to test the coefficient of variance. If the coefficient of variance is less than 15%, it means that this research has good accuracy. The value of the coefficient of variance is expressed with the following formula

$$KK = \frac{\sqrt{KTG}}{\bar{X}} \times 100\%$$

Description:

KK = coefficient of variance

KTG = galat center squared

$\bar{X}$ = the mean value of all data

3.5. Procedures

This study consisted of four phases, namely, 1) the phase of making eggshell micro powder, 2) the phase of making eggshell nanocalcium powder, 3) the phase of making mung bean germination, 4) the phase of making mung bean germination drink with nanocalcium fortification.

3.5.1. Eggshell Micro Powder Manufacturing

The process to make eggshell micro powder based on the method mentioned in Rahmawati and Nisa (2015), are:

1. 500 g of chicken and duck egg shells are washed with water until clean.

2. The egg shells are boiled at 100oC for 3 minutes to kill pathogenic microbes, then the shells are drained
3. The egg shells baked in the oven for 3 hours at 60oC, then Egg shells are placed at temperature room.
4. Then cooled egg shells are mashed by blender at a speed of ± 18,000 rpm for 3 minutes until smooth.
5. The egg shell powder is then sieved through a 100 mesh sieve.
6. Eggshell powder is packed in OPP (Oriented Polystyrene) plastic and stored at 4oC for analysis.

3.5.2. Eggshell Nanocalcium Powder Manufacturing

The process of making eggshell nanocalcium powder based on Khoerunnisa's research (2011), are:

1. Eggshell micro powder is soaked in 1N HCl (1:5) solvent for 48 hours.
2. Then extracted at 90oC for 1 hour.
3. The results of the extraction are then filtered with filter paper to obtain the filtrate and precipitate.
4. The obtained filtrate is precipitated with the addition of 3N NaOH and stirred and allowed to stand until a precipitate was formed.
5. The precipitate is then subjected to a neutralization process using aquabidest until the pH is neutral.
6. The solution is separated from the precipitate by pouring it slowly so that the precipitate does not get wasted.
7. The precipitate is ovened for 3 hours at 105oC, then combustion with a muffle furnace at a temperature of 600oC for 5 hours, then smoothing with a mortar to obtain nanocalcium powder.
8. Nanocalcium powder is vacuum packed and stored at 4oC.

3.5.3. Mung Bean Germination

The process of making mung bean sprouts according to Wea et al. (2014), which has been modified, as follows:

1. The mung beans are sorted and washed with running water until clean and then soaked for 6 hours.

2. The mung beans are drained and placed on a winnowing tray with a dampened cloth.
3. Tampah containing mung beans is placed in a humid place (room temperature), then germination is carried out starting at 6 hours, 12 hours and 18 hours, where every 6 hours watering is done with water.
4. Mung beans that have been germinated are then separated from the skin and taken as much as 100 g for further processing.

3.5.4. Mung Bean Germination Drink Manufacturing

The process of making mung bean germination drink according to Wea et al. (2014), which has been modified, as follows:

1. Materials in the form of mung beans that have been germinated are prepared as much as 100 g.
2. The material is blanched by boiling at 80°C for 2 minutes.
3. The ingredients are then blended for 3 minutes with the volume of water added in making this mung bean sprout juice is 1: 7 (100 g of germinated mung beans: 700 mL water).
4. The germinated mung bean juice is then filtered through a tea filter measuring 50 mesh.
5. The juice is cooked by adding 22% egg shell nanocalcium powder and 7% sugar. Cooking is done until boiling and stirring for 5 minutes.
6. The finished mung bean germination drink is cooled for ±30 minutes, then 200mL (80%) is poured into a 250mL bottle and 50mL of water is added.

3.6. Parameters

Parameters observed included chemical characteristics (protein content, vitamin C content, calcium content, pH) and physical characteristics (viscosity and stability).

3.6.1. Protein Level

Determination of protein level using the Lowry method (Harjanto, 2017 which has been modified, as follows:

1. Protein standard solution was prepared, Bovine Serum Albumine (BSA) with various concentration levels from 30-300 g/ml, then each 1 ml was taken and put into a test tube.
2. Added to each tube 8 ml of Lowry B. reagent and leave at temperature room for 10 minutes.

3. Then add 1 ml of Lowry's reagent A, stir and leave for 20 minutes.
4. The absorbance was read at a wavelength of 600 nm using a spectrophotometer.
5. A standard curve on graph paper showing the relationship between absorbance (at the ordinate) and concentration (at the abscissa) is drawn up.

3.6.2. Calcium Level

Determination of calcium levels in samples by AAS (Atomic Absorption Specthophoyometry) at the Chemical Engineering Laboratory of the Sriwijaya Polytechnic using the in-house STD method. The way the analysis works is as follows:

1. The sample is weighed as much as 1 g in a glass beaker then add 50 ml of aquabidest and 5 ml of concentrated sulfuric acid and boil on a hot plate until all parts of the sample are completely dissolved.
2. After about 20 minutes of heating, it is desired to reach temperature room.
3. The solution is put into a 50 ml volumetric flask and aquadest is added up to the mark.
4. Take 5 ml of the solution and put it in a 50 ml volumetric flask up to the mark.
5. The solution was filtered with Whattman filter paper no. 1, the filtrate was then analyzed using AAS (Atomic Absorption Specthophoyometry) with a wave of 422.7 nm.

3.6.3. Vitamin C Level

Measurement of vitamin C was carried out based on Sudarmadji et al. (2007), as follow:

1. A sample of 10 g is put into a 100 mL volumetric flask and aquadest is added to the mark.
2. The filtrate is then separated by filtering and the filtrate is taken as much as 5-25 mL with a pipette.
3. The filtrate is then put into a 125 mL Erlenmeyer and added 2 mL of 1% starch solution.
4. Then titrated with 0.01N iodine standard.
5. The end point of the titration is indicated by the blue color of the iodine-starch.

Calculation of vitamin C levels with standardized iodine solution is 1 mL 0.01N iodine= 0.88 mg ascorbic acid

$$\text{Vitamin C } \frac{\square\square}{100\ \square\ \square\square\square\square\square} = \frac{\square\square\ \square\square\square\ 0,01\ N\ x\ 0,88\ \square\square\ x\ \square P\ x\ 100}{\square\square\square\square\square\ \square\square\square\square\square\square\ (\square)}$$

3.6.4. pH Value

Measurement of pH was carried out using a pH meter based on the method of Sudarmadji et al. (2014), as follows:

1. Before use, the pH-meter electrode was standardized using acid buffer solution (pH 4), neutral buffer (pH 7) and alkaline buffer (pH 10) then cleaned using distilled water and dried.
2. Samples were taken as much as 50 mL.
3. The electrode is dipped into the sample until a stable reading is obtained on the pH meter scale.

3.6.5. Viscosity

Viscosity measurements were carried out using the Brookfield tool (Yuwono and Susanto, 1998) as follows:

1. Viscosity was measured using a brookfield viscometer.
2. The sample is placed in a 250 mL Beaker glass.
3. Prior to the measurement, the spindle was selected by trial and error.
4. A scale reading of more than 100 is selected with a smaller spindle and/or lower speed, while readings below 10 are selected for a larger spindle and/or higher speed.
5. Mung bean germination drink was measured for viscosity.
6. The scale indicated on the tool is read after a certain number of turns.

Calculation: Viscosity (Ns)/m2= Number of readings x calibration factor.

3.6.6. Stability

The stability test of mung bean germination drink was carried out based on Wibowo et al. (2014), as follow:

1. Visual stability test is carried out by distinguishing the separation between the top and bottom.
2. The sample is put into a measuring cup that has the same height in a stationary state during storage in the refrigerator.
3. If a separation line is observed, then its height is measured and calculated as the ratio between the height of the dividing line to the height of the glass filled with mung bean germicidal drink.
4. If the separation line is not observed then the value of the separation index is 1.

Stabilitas visual = $\frac{h}{\square} \times 100\%$

Description:

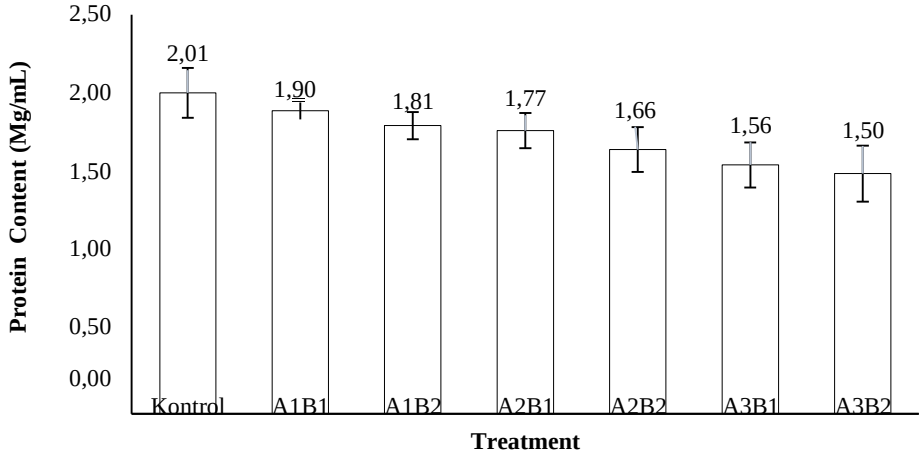
h = the height between the boundary of the deposit to the bottom

H = total height of the bottle

CHAPTER 4
RESULTS AND DISCUSSION

4.1. Protein Level

Protein analysis in samples using the Lowry method. The working principle of this method was the reaction between protein and phosphotungstate-phosphomolybdic acid which gave a blue color depending on the content of tryptophan and tyrosine residues. The Lowry method was chosen because it has a sensitivity 100 times higher than the Biuret method (Purwanto, 2014). Based on the results of the analysis, the protein value of mung bean germination drink with nanocalcium fortification ranged from 1.50±0.18 mg/mL to 1.90±0.05 mg/mL, while the protein value of the germination drink in the control treatment was 2.01±0.16mg/mL. The highest protein value was found in the A1B1 treatment (6 hours of germination time with the addition of chicken calcium nano powder) and the lowest protein value was found in the A3B2 treatment (18 hours of germination time with the addition of duck nanocalcium powder). The mean protein value of green bean germination drink with nanocalcium fortification can be seen in Figure 4.1.



Description : Control =germinasi duration 0 hour, no addition of nanocalsium powder A_1 = germination duration 6 hours B_1 = chicken nanocalsium powder A_2 = germination duration 12 hours B_2 =duck nanocalsium powder A_3 = germination duration 18 hours

Figure 4.1. Mean Protein Level of Mung Bean Germination Drink with Nanocalcium Fortification

The results of the analysis of variance showed that the treatment with long germination time (factor A) had a significant effect on the protein value produced, while the type of nanocalcium powder added (factor B) and the two treatments had no significant effect on the protein value of the drink. germination of the resulting mung beans. HSD test results level 5% effect of germination time on the protein value of mung bean germination drink can be seen in Table 4.1.

Table 4.1. Vakue test HSD 5% Effect of Germination Duration on Protein Value Mung Bean Germination Drink

Treatment	Protein (mg/mL)*
A3 (germination duration 18hours)	1,53 ±0,07[a]
A2 (germination duration 12hours)	1,72±0,13[ab]
A1 (germination duration 6 hours)	1,85±0,16[b]

Description : Numbers marked with the same letter notation in the same column indicate that they were not significally different (5%)
*Data = rerata ± deviation standart

The protein content in mung bean drink in the control treatment had a bigger value than the mung bean germination drink with nanocalcium fortification. Fortification of chicken and duck nanocalcium powder in mung bean germination drink did not contribute to protein, because the powder component was pure calcium in nano form. This indicates that the difference in the protein value of the drink was influenced by the presence of germination. In Table 4.1. It is known that the protein value of treatment A3 (germination duration 18 hours) was significantly different from treatment A1 (6 hours germination duration), but not significantly different from A2 (12 hours germination duration). The decrease in protein content in germination drinks was due to the reshuffling of complex molecules into simpler forms during germination, where protein was broken down into amino acids which were used in embryo growth. In line with the statement of Masood et al. (2014), that the decrease in protein content coincided with an increase in the amino acid content during germination. This was due to an increase in the activity of the protease enzyme which was initiated by the imbibition process in mung beans. Water imbibition triggers active endogenous enzymes, one of which is protease. Proteases hydrolyze proteins into peptides and amino acids (Ferdiawan et al., 2019). Free amino acids together with glutamic and aspartic acids (in the form of

amides) will be translocated in the embryo in the formation of new structures in line with the ongoing germination stage (Pertiwi et al., 2013).

4.2. Calcium Level

Analysis of calcium levels aimed to determine the amount of calcium in nanocalcium powder and the effect between before and after fortification of nanocalcium on mung bean germination drink. Analysis of calcium levels in samples using the Atomic Absorption Spectrophotometry (AAS) method with the principle of absorption of light with a certain wavelength (Azis et al., 2018).

The manufacture of nanocalcium powder from chicken and duck egg shells in addition to mung bean germination drink was carried out using the bottom up method (chemical method). The bottom up method commonly used in the manufacture of nanoparticles was precipitation. There are four processes in the precipitation method, namely dissolving the calcium component of eggshell using an acid solvent (HCl), precipitation using NaOH solution, neutralization using distilled water and burning Ca(OH)2 crystals (Suptijah et al., 2012; Sunardi et al., 2020).

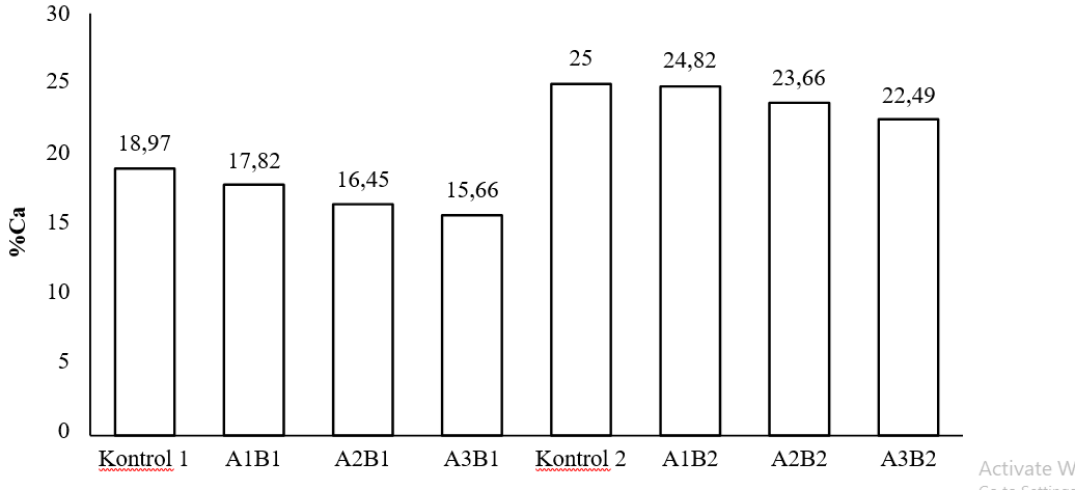
Isolation of calcium from egg shells was carried out using HCl which was also known as demineralization. Demineralization is the process of dissolving minerals contained in eggs, especially CaCO3 minerals. Soaking the sample in HCL solvent causes the egg shell matrix to expand, making it easier for the solvent to enter the matrix, causing calcium to be more easily released from the egg shells of broiler chickens and ducks (Suptijah et al., 2012). At this stage, foam will usually form in the solution, due to the production of CO2 and H2O on the surface of the solution. The reaction between the CaCO3 content in egg shells and HCl solution produces calcium chloride (CaCl2). The formed calcium chloride (CaCl2) was then precipitated with NaOH to produce a precipitate in the form of calcium hydroxide (Ca(OH)2) and salt (NaCl). The precipitate was separated by rinsing using aquabidest until neutral pH. The last stage in the manufacture of calcium was a calcination process or combustion at a temperature of 600ºC, aiming to remove water, CO2 and other organic compounds that are still bound to the precipitate into the final product in the form of calcium oxide (CaO) (Handayani and Syahputra, 2017).

Nanocalcium egg shells produced from chicken and ducks had yields of 11.30% and 13.50%, respectively, from 50 g of calcium micropowder used. Nanocalcium powder, both chicken and duck egg shells have a white color with a smooth texture. The white powder color was obtained from the presence of HCL immersion and heating treatment. The value of the whiteness of the eggshell flour increased as the pH value of the soaking solution decreased. Porphyrin compounds in race egg shells and biliveridine will decomposes and then dissolves optimally (pH 3.0 – 4.0) (Yonata et al., 2017), while the high temperature treatment on egg shells caused the loss of organic compounds such as fat, protein, carbon and others (Hanura et al., 2017).

Based on the results of the analysis, the calcium level of the egg shells of chickens and ducks were 35.50% and 45.50%. This indicates that the calcium level of the duck egg shell nanocalcium powder was higher than that of the chicken egg shell nanocalcium powder. The percentage of calcium in each egg shell was influenced by differences in the thickness of the type of egg shell. Duck egg shells have average thickness of 0.36 to 0.46 mm (Septiana et al., 2015), while the average thickness of broiler egg shells is 0.33 – 0.35 mm (Azizah et al., 2015). Differences in egg shell thickness can affect the amount of mineral content and organic salts, especially calcium carbonate (CaCO3) which acts as a source of calcium in egg shells.

The amount of fortification of nanocalcium powder in chicken and duck egg shells in mung bean germination drink was 484mg or equivalent to 22% ALG (1100 mg/each day) each 250mL. Fortification is the process of increasing the content of essential micronutrients in the form of vitamins and minerals into food with the aim of increasing nutrients that do not yet exist or enriching existing nutrients (Valentina et al., 2014). According to the Food and Drug Supervisory Agency (2019), the minimum amount of micronutrient fortification in foodstuffs is 10% ALG each serving. If the RDA for calcium is 1100 mg/day, then with consuming 4 bottles and a half (serving size 250mL each bottle) of mung bean germination drink can meet the daily RDA for calcium. Fortification of food products is a solution to meet the need for calcium that is not sourced from one ingredient, thereby preventing mineral deficiencies.

The results of the analysis of calcium levels of mung bean germination drink with nanocalcium fortification, ranging from 15.66% to 24.82%. The highest calcium content value was found in the A1B2 treatment, which was 24.82% and the lowest calcium level value was found in the A3B1 treatment, which was 15.66%. The value of calcium levels in mung bean germination drink with fortification nanocalcium can be seen in Figure 4.2.



Description : Control 1 = Germination duration 0 hour, addition of chicken nanocalcium powder Control 2 = germination duration 0 hour, addition of duck nanocalcium powder A_1= germination duration 6 hours B_1 =chicken nanocalcium powder A_2 = germination duration 12 hours B_2 = egg nanocalcium powder A_3 = germination duration 18 hours

Figure 4.2. Mean Calcium Level of Mung Bean Germination Drink with Nanocalcium Fortification

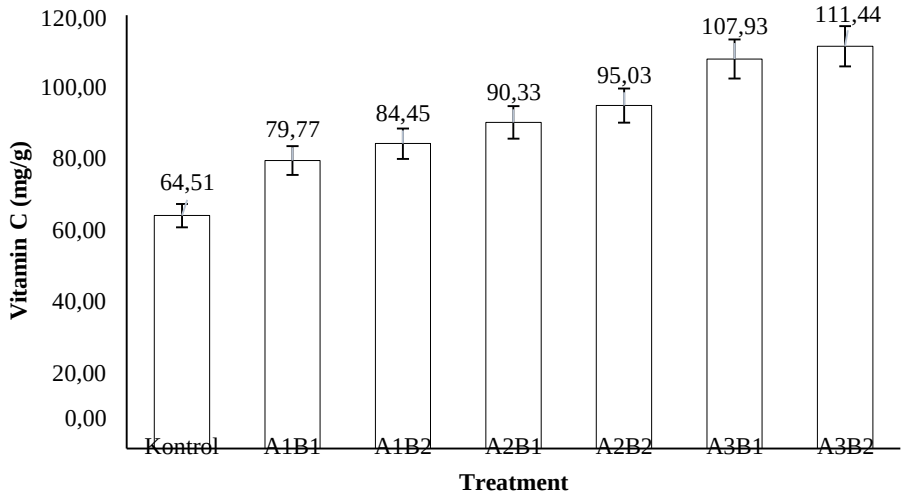
The control treatment was data on the germination duration of mung beans with the addition of chicken and duck nanocalcium powder. The existence of control data aimed to compare specifically the effect of fortification of nanocalcium species on the calcium content of the germination drink produced. Based on Image 4.2. It is known that the calcium content of mung bean germination drink at 0 hours from the treatment of chickens and ducks had a higher value than the germination treatment of 6 to 18 hours.

Calcium levels in mung bean germination drinks generally decrease with the length of germination. This is in accordance with the statement of Oghbaei and Prakash (2020), that the calcium content decreased significantly in germinated and peeled peanut seeds. Peeling can reduce some of the minerals in the shell of mung beans. However, this germination and skin peeling treatment had an impact on increasing the bioavailability of mung bean seeds which contributed to stimulating the decrease of anti-inflammatory substances. nutrition. The percentage of bioavailability in peanut seeds before germination was 14.91–17.19% and in shelled germination beans it was 22.96–25.27% (Oghbaei and Prakash, 2020). The decrease in calcium content is thought to be due to the treatment of germination time with a not too far range so that some enzymes have not yet formed work optimally. In accordance with the statement of Ghavidel and Davoodi (2011), that in the first 24 hours of germination the enzymatic activity was relatively less, but there was an increase after 48 hours and 72 hours in all samples. The phytic acid in the mung bean germination treatment may decrease during germination, but it is not significant due to the less optimal work of the phytase enzyme, so that the complex phytic acid that binds to minerals such as phosphorus and calcium cannot be completely released.

The lack of calcium in mung bean germination was overcome by fortification of nanocalcium eggshell powder. Based on the analysis, fortification had a significant effect on increasing calcium content in green bean germination drinks.

4.3. Vitamin C Level

Based on the results of the analysis, the value of vitamin C levels of mung bean germination drink with nanocalcium fortification ranged from 79.77±2.03 mg/100g to 111.44±5.38 mg/100g, while the value of vitamin C germination drink without treatment was 64. ,51±2.03 mg/100g. The value of vitamin C generally increased from 0 hours of germination duration, A1B1 to A3B2 treatments. The highest value of vitamin C was found in the A3B2 treatment (germination duration of 18 hours with addition of duck nanocalcium powder) and the lowest value of vitamin C was found in the A1B1 treatment (6 hours of germination duration with the addition of chicken calcium nanopowder). The value of the mean value vitamin C content of mung bean germination drink with nanocalcium fortification can be seen in Figure 4.3.



Description : Control = Germination duration 0 hour, without addition of nanocalcium powder A_1 = germination duration 6 hours B_1 = chicken nanocalcium powder A_2 = germination duration 12 hours B_2 = duck nanocalcium powder A_3 = germination duration 18 hours

Figure 4.3. Mean Vitamin C Content of Mung Bean Germination Drink with Nanocalcium Fortification

The results of the analysis of variance showed that the length of germination duration (factor A) had a significant effect on the value of vitamin C produced, while the type of nanocalcium powder added (factor B) and the two factors (A and B) had a no significant effect to the vitamin C value of the mung bean germination drink produced. It can be seen that the increase in the value of vitamin C in the drink was caused by the germination treatment.

The results of the HSD test at 5% level, the effect of germination time on the viscosity value of mung bean germination drink can be seen in Table 4.2.

Table 4.2. HSD Test Value 5% Effect of Germination Duration on Vitamin C Level Value of Mung Bean Germination Drink

Treatment	Vitamin C (mg/mL)*
A1(Germinayion duration 6hours)	82,11±2,78[a]
A2(Germination duration 12hours)	92,68±4,44[b]
A3 (Germination duration 18hours)	109,68±5,37[c]

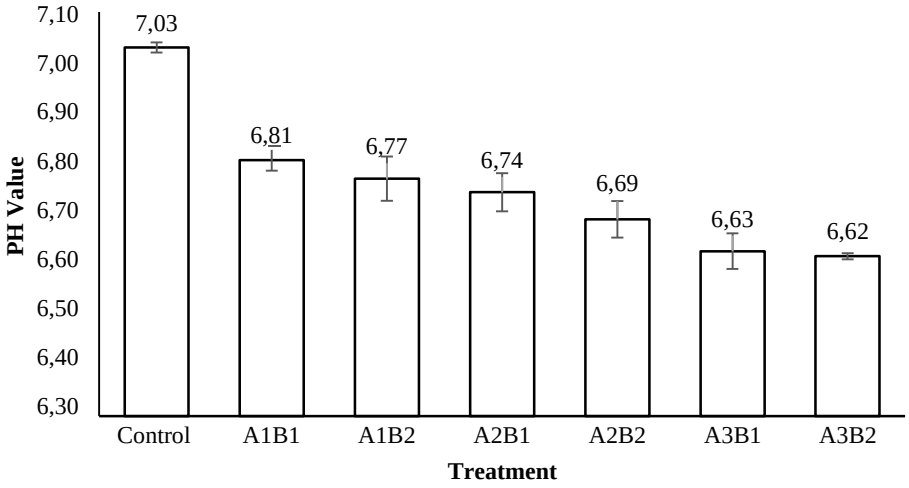
Description : Numbers marked with the same letter notation in the same column indicate that they were not significantly different (5%)
*Data = rerata ± deviation standart

Based on Table 4.2. value of vitamin C content in A1 treatment (germination duration 6 hours) was significantly different from A2 (12 hours germination duration) and A3 (germination duration 18 hours). The increase in vitamin C along with germination time is caused by several enzyme systems being active during germination. This happens because of the accumulation of ascorbic acid as a result of biosynthesis. Germination cause reactivation of an enzyme (L-Galactono-γ-lactone dehydrogenase) involved in oxidation of L-galactono-1,4-lactone to ascorbic acid. The activity of this enzyme increased in parallel to the biosynthesis of ascorbic acid during the seed germination process. Differences in the level of ascorbic acid biosynthesis in germination of mung bean seeds can also be influenced by legume varieties, maturity, climate, light, harvesting and storage methods (Masood et al., 2014). Increasing the content of vitamin C in mung bean germination can help the solubility of nanocalcium fortification in drink. This is in line with the statement of Yonata et al. (2017), that pH can affect the solubility of minerals. The acidic conditions and the small particle size can increase the speed of dissolving nanocalcium from both duck and chicken shells, so that the resulting germination drink with fortified nanocalcium had good digestibility.

4.4. pH Value

Measurement of pH is a parameter used to determine changes in the acidity level of a food product (Widowati et al., 2020). Based on the analysis of the mung bean germination drink, the control treatment (germination duration 0 hour without the addition of nanocalcium powder) had a pH value of 7.03±0.01, while the mean pH value of mung bean germination drink with nanocalcium fortification ranged from 6.62±0.01 to 6.81±0.02. The highest pH value was found in the A1B1 treatment (germination duration 6 hours with the addition of

chicken nanocalcium powder). The lowest pH value was found in the A3B2 treatment (germination duration of 18 hours with the addition of duck calcium nano powder). Mean pH value of mung bean germination drink with fortification nanocalcium can be seen in Figure 4.4.



Description: Control = Germination duration 0 hour, without addition of nanocalcium powder A_1 = Germination duration 6 hours B_1 = chickn nanocalcium powder A_2 = Germination duration 12 hours B_2 = duck nanocalcium powder A_3 = Germination duration 18 hours

Figure 4.4. Mean pH Value of Mung Bean Germination Drink with Nanocalcium Fortification

Based on the results of the analysis of variance, the length of germination duration (factor A) had a significant effect on the resulting pH value, while the type of calcium nanopowder added (factor B) and the two factors (A and B) had no significant effect to the pH value of the mung bean germination drink produced. Fortification of 22% nanocalcium from the Nutritional Adequacy Ratio (RDA) did not affect the pH of the germination drink because the nanocalcium obtained had a neutral pH, so it did not have a significant effect on the pH of the mung bean germination drink.

The results of the HSD test at 5% level, the effect of germination time on the pH of the mung bean germination drink can be seen in Table 4.3.

Table 4.3. HSD Test Value 5% Effect of Germination Time on the pH Value of Drinks Mung Bean Germination

Treatment	pH Value *
A3(Gemination duration 18 hours)	6,62±0,03[a]
A2(Germination duration 12 hours)	6,72±0,04[b]
A1(Germination duration 6 hours)	6,79±0,02[c]

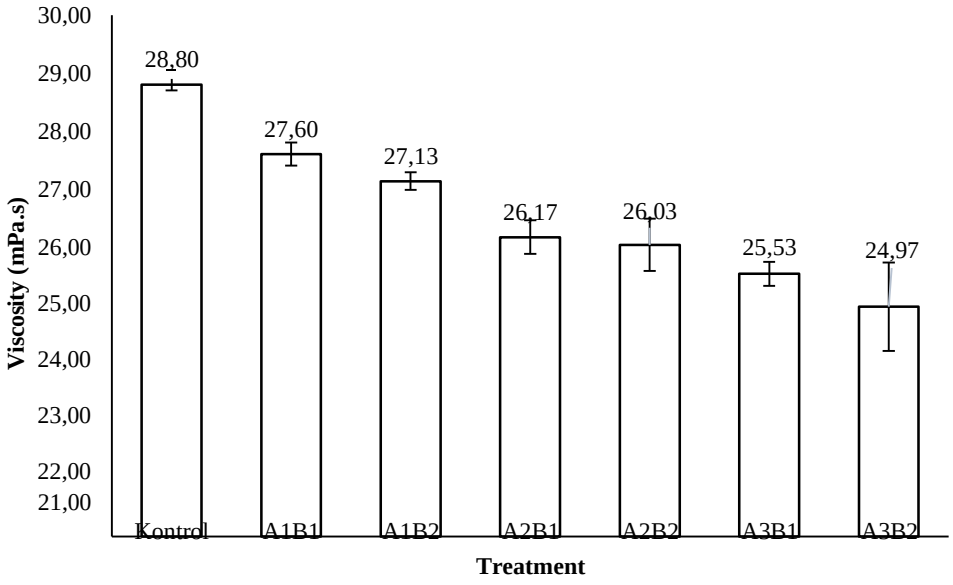
Description : Numbers marked with the same letter notation in the same column indicate that they are not significantly different (5%)
*Data = rerata ± deviation standart

Table 4.3. showed that the pH value of treatment A3 (germination duration 18 hours) was significantly different from treatment A2 (12 hours germination duration) and A1 (6 hours germination duration). The duration of germination can produce the pH value of the mung bean germination drink being more acidic or it can be said that the pH was getting lower. At a certain age from the growth of germinated seeds, there is an increase in the ability to synthesize vitamins, especially vitamin C (Wea et al., 2014). This was in accordance with the results of the Vitamin C parameters, that the content of vitamin C in mung bean germination drinks had increased, causing the pH of the resulting drink to become acidic. Based on SNI 01-3830-1995, the pH standard which refers to soy juice drinks was 6.5-7.0. Mung bean germination drink with nanocalcium fortification had a pH value of 6.62 - 6.81. It can be interpreted that the pH of the mung bean germination drink with nanocalcium fortification had met the quality of the SNI drink.

4.5. Viscosity

Viscosity is a measure of the viscosity of a fluid which states the size of the friction that occurs in the sample. The bigger the viscosity value of a food ingredient, the thicker the material, and vice versa (Srihidayati, 2010). Based on the results of the analysis, the mung bean germination drink in the control treatment (germination duration 0 hour, without the addition of nanocalcium powder) had a viscosity value of 28.80±0.10 mPa.s. Meanwhile, the mean viscosity value of mung bean germination drink started from 24.97±0.76 mPa.s up to 27.60±0.20 mPa.s. The highest viscosity value was found in the A1B1 treatment (6 hours of germination duration with the addition of chicken nanocalcium powder). The lowest viscosity value was found in the A3B2 treatment

(germination duration of 18 hours with addition of duck nanocalcium powder). Viscosity values in mung bean germination drinks A1B1 and A1B2 treatments were in the range of viscosity values for commercial mung bean juice drinks obtained from measurements, namely 26.90-27.66 mPa.s. The viscosity of the mung bean germination drink in the control treatment had a higher viscosity than the germinated mung bean drink. This was because mung beans without germination (0 hours) have high starch content, starch will undergo gelatinization in the presence of heat treatment. When the starch solution reaches the gelatinization temperature, the starch granules break and the starch molecules come out and were released from the granules and entered the solution system (Nurjanati et al., 2018), so the resulting viscosity was higher. The mean viscosity value of mung bean germination drink with nanocalcium fortification can be seen in Figure 4.5.



Description : Control = Germination duration 0 hour, without addition of nanocalcium powder A_1 = Germination duration 6 hours B_1 = chicken nanocalcium powder A_2 = Germination duration 12 hours B_2 = duck nanocalcium powder A_3 = Germination duration 18 hours

Figure 4.5. Mean Viscosity Value of Mung Bean Germination Drink with Nanocalcium Fortification

Based on the results of analysis of variance it showed that the treatment of germination duration (factor A) had a significant effect on the viscosity value produced, while the treatment of the type of nanocalcium powder added (factor B) and the treatment of both factors (A and B) gave no significant effect on the viscosity value of the mung bean germination drink produced.

The results of the HSD test at 5% level, the effect of germination duration on the viscosity value of mung bean germination drink can be seen in Table 4.4.

Table 4.4. HSD Test Value 5% Effect of Germination Time on Viscosity Value Mung Bean Germination Drink

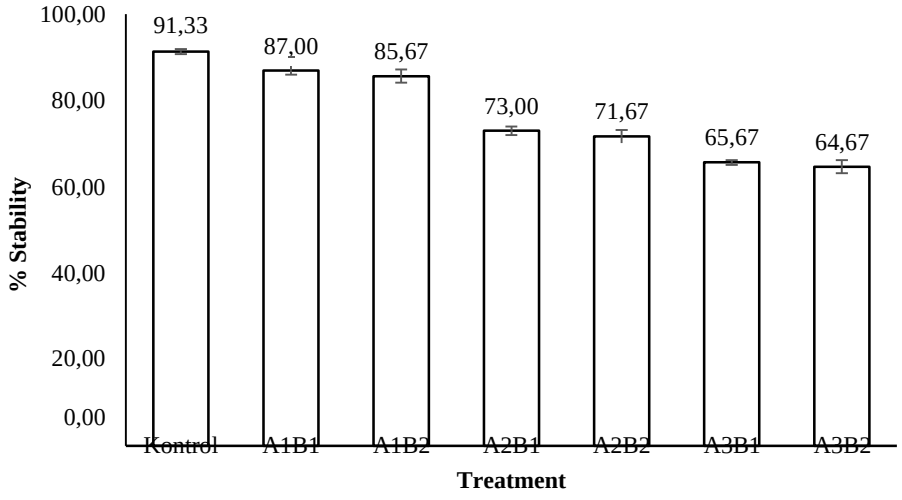
Treatment	Viscosity (mPa.s)*
A3(germination duration 18 hours)	25,25±0,18[a]
A2(germination duration 12 hours)	26,10±0,37[b]
A1(germination duration 6 hours)	27,37±0,49[c]

Description : Numbers marked with the same letter notation in the same column indicate that they were not significantly different (5%)
*Data = rerata ± deviation standart

Table 4.4. showed that the viscosity value of treatment A3 (germination time 18 hours) was significantly different from treatment A2 (12 hours germination duration) and A1 (6 hours germination time). Decrease in the viscosity of the material at the time of germination caused by the hydrolysis of starch during the germination process by the α-amylase and β-amylase. The enzyme was formed at the beginning of germination by giberylic acid (Elobuike et al., 2021). Starch will be broken down into simple sugars in the form of glucose which was used as energy and needs for seed growth. Based on the analyzed parameters of vitamin C and pH, the germination process resulted in an acidic condition. In line with the statement of Widjaja et al. (2019), that the viscosity of a solution decreases if the pH decreases. The decrease in pH caused hydrolysis of the glycosidic bond which results in a decrease in the viscosity of the drink.

4.6. Stability

The stability of the juice can be seen by the presence or absence of deposits in the product (Farikha et al., 2013). The mean stability value of mung bean germination drink with nanocalcium fortification ranged from 64.67±1.53% to with 87.00±1.00%. The highest stability was found in the A1B1 treatment (6 hours of germination duration with the addition of chicken nanocalcium powder). The lowest stability value was found in the A3B2 treatment (germination duration of 18 hours with the addition of duck nanocalcium powder). The stability value of mung bean germination drinks treated with A1B1 (6 hours of germination time with the addition of chicken nanocalcium powder) and A1B2 (6 hours of germination duration with the addition of duck nanocalcium powder) were in the range of stability values of commercial mung bean juice drinks obtained from measurements, ie 85.00-95.00%, while the stability value in other treatments was below the stability of commercial mung bean drink. The stability of commercial mung bean drinks was generally more stable due to the addition of stabilizers in it. The mean stability value of mung bean germination drink with nanocalcium fortification can be see on figure 4.6.



Description : Control = Germination duration 0 hour, without addition of nanocalcium powder A_1 = germination duration 6 hours B_1 = chicken nanocalcium powder A_2 = germination duration 12 hours B_2 = duck nanocalcium powder A_3 = germination duration 18 hours

Figure 4.6. Mean Stability of Mung Bean Germination Drink with Nanocalcium Fortification

In Figure 4.6. It was found that there was a significant decrease in the stability value of the drink with the duration of germination. The results of the

analysis of variance showed that the long germination treatment (factor A) gave a significant effect on the resulting stability value, while the type of nanocalcium powder added (factor B) and the two treatment factors (factors A and B) had no significant effect on the stability value of mung bean germination drink. The mung bean germination drink in the control treatment (0 hours without the addition of powder) had a higher stability value (91%) compared to the nanocalcium fortified mung bean germination drink. This was because the germinated mung bean seeds absorb more water than the ungerminated bean seeds. During germination, peanut seeds require optimal environmental conditions, namely 50-80% RH (Yuwariah et al., 2015). Water absorption in the germination process was needed to maintain cell turgidity, including cell enlargement, photosynthetic reactions, salt solvents, gases and other substances that were transported between cells in tissues to maintain cell growth (Felania, 2017).

The results of the HSD test at 5% level, the effect of germination time on the stability value of mung bean germination drink can be seen in Table 4.5.

Table 4.5. HSD Test Value 5% Effect of Germination Time on Beverage Stability Value Mung Bean Germination

Treatment	% Stability*
A3	65,17±1,26[a]
A2	72,33±1,26[b]
A1	86,33±1,05[c]

Description : Numbers marked with the same letter notation in the same column indicate that they wee not significantly different (5%)
*Data = rerata ± deviation standart

Table 4.5. showed that the stability value of treatment A1 (germination duration 6 hours) was significantly different from treatments A2 (12 hours germination duration) and A3 (germination duration 18 hours). The stability value of mung bean germination drink decreased significantly with the duration of germination. Apart from the effect of water absorption, when germination there was an increase in the crude fiber content. The increase in fiber in germination of mung beans was influenced by the synthesis of structural carbohydrates such as cellulose and hemicellulose which were the largest components of cell wall formation in germinated mung beans (Syed, 2011). In line with the mean value of

each treatment decreased with germination duration. The decrease in viscosity at germination of mung beans correlated that the resulting stability also decreased.

4.7. Best Treatment

The best treatment for mung bean germination drink with nanocalcium fortification was selected based on chemical parameters such as protein content, calcium content, vitamin C, and pH which were close to commercial mung bean juice drinks and firisk parameters in the form of viscosity and stability which were close to commercial mung bean juice drinks.

Table 4.6. Best Parameters Summary

Sample	pH	Viscosity (mPa.s)	Stability (%)	Protein (%b/b)	Vitamin C (mg/100g)	Calcium (%)
Commercial Product	6,50-7,00	26,90-27,66	84,00-90,00	Min 1,00	Tidak Ada	Tidak ada
A1B1	**6,81**	**27,60**	**87,00**	1,90	79,77	17,82
A1B2	**6,77**	**27,13**	**85,67**	1,81	84,45	24,82
A2B1	**6,74**	26,17	73,00	1,77	90,33	16,45
A2B2	**6,69**	26,03	71,67	1,66	95,03	23,66
A3B1	**6,63**	25,53	65,67	1,56	107,93	15,66
A3B2	**6,62**	24,97	64,67	1,50	111,44	22,49

Description : values in bold are close to commercial products

Based on table 4.6. The best treatment for mung bean germination drink samples with nanocalcium fortification was sample A1B2 (germination duration 6 hours and fortification of duck eggshell nanocalcium). This was because sample A1B2 had a pH value, viscosity and stability that was close to the value of commercial mung bean drinks, with the highest calcium content.

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CHAPTER 5
CONCLUSIONS AND SUGGESTIONS

5.1. Conclusions

The conclusions of this research are as follows:

1. The duration of germination significantly affected the levels of protein, calcium, vitamin C, pH, viscosity and stability.
2. Germination can increase the vitamin C level in mung beans with an mean value of 79.77-111.44 mg/100g.
3. The best treatment for germination drink with nanocalcium fortification was found in sample A1B2 (6 hours of germination with fortified duck nanocalcium powder), with a pH value of 6.77, a viscosity of 27.13 mPa.s and a stability of 87.00% which was close to the value of a commercial mung beans juice.
4. Nanocalcium fortification of eggshell had a significant effect on increasing the calcium content of mung bean germination drink.
5. The best treatment for the calcium level of mung bean germination drink was found in sample A1B2 (6 hours of germination with fortification of duck nanocalcium powder), which was 24.82%.

5.2. Suggestions

Suggestions for further research is that it is necessary to re-analyze the bioavailability of minerals in germination of mung beans and variations in the treatment of mung bean types to further enrich the information provided.

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