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Effect of germination and eggshells nanocalcium fortification on characteristics of germinated mung bean drink (*Vigna radiata*.)

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1 Effect of germination and eggshells nanocalcium fortification on characteristics of germinated
2 mung bean drink (*Vigna radiata*.)

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9 ABSTRACT

10 Germinated mung bean drinks with eggshell nanocalcium fortification are an alternative beverage to
11 replace milk. This study aimed to determine the effect of germination time and eggshells
12 nanocalcium fortification on physicochemical characteristics of the germinated mung bean drink.

13 This study used a Factorial Completely Randomized Design with two treatments factors. The first
14 factor was germination time which consisted of three treatment levels (6, 12 and 18 hours) and the
15 second factor was the type of eggshell nanocalcium which consisted of two type of treatment
16 (chicken and duck eggshell). Each experiment was repeated three times. Observed parameters in
17 this study were chemical characteristics (protein, calcium, vitamin C, and pH) and physical
18 characteristics (viscosity and stability) of germinated mung bean drink. The results showed that the
19 germination time treatment significantly affected the value of protein, calcium, vitamin C, pH,
20 viscosity, and stability of the germinated mung bean drink. Germination increased vitamin C in
21 mung beans with an average value of 8,21-14,66 mg/100 g. Eggshell nanocalcium fortification
22 showed a significant effect on increasing calcium of germinated mung bean drink. The highest
23 calcium content of germinated mung bean drink was found in drink produced from 6 hours of
24 germination with fortification of duck nanocalcium, which was 24.82%.

25 **Key words:** physicochemical, chicken, duck, calcium, vitamin C

Introduction

To meet the calcium needs of the Indonesian people who generally suffer from lactose intolerance and digestive disorders after consuming milk, it is necessary to drink milk substitutes made from plant-based foods that contain high protein and calcium. Mung bean is the third highest source of vegetable protein after soybeans and peanuts with a content of protein around 20-25% (Purwono and Hartono, 2005; Hastuti et al., 2018). One of the superior varieties of mung beans is VIMA-1 (*Vigna sinensis*–Malang). VIMA-1 has a promising market potential due to its high protein content (28.02%), low fat (0.40%), and high starch content (67.62%) (Agency for Research and Development, 2019). However, mung beans have anti-nutritional compounds, compounds found naturally in various types of beans, which can prevent the absorption of nutrients in the body.

One process that can increase the nutrition in beans is germination. Germinated mung beans have improved nutritional, functional and biological properties by changing the content, nutritional composition and bioactive compounds, as well as eliminating anti-nutritional factors in beans (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 shoots in germinated seeds ranges from 2 mm to up to 5 mm (Munarko et al., 2019).

The germination process in mung bean seeds is able to increase the nutritional value by activating enzymes that can reduce or eliminate anti-nutritional factors (Ebert et al. 2017). Phytic acid, the main anti-nutritional substance found in green beans, can be reduced from 12.0 mg/g to 4.03 mg/g through the germination process (Faradilla et al., 2012). Furthermore, germination is able to reduce anti-nutritional substances in germinated bean flour by 66.70% (Nur et al., 2019), so the bioavailability of vitamins and minerals in the flour increase. This is because the germination process can release the bound form of vitamins and minerals into a freer form so that it is more easily digested and absorbed by the human digestive tract. Previous research explained that during

53 germination there is a breakdown of complex molecules into simpler forms which causes
54 germination of green beans to be more easily digested and absorbed by the body (Ghavidel and
55 Prakash, 2007; Masood et al., 2014). In addition, the content of vitamin C increased with the
56 increasing duration time of germination. Ebert et al. (2017) stated that the average vitamin C in
57 germinated bean was 2.7 times higher than that without germination.

58

59 The eggshells of chickens and ducks are household waste that has not been utilized optimally due to
60 the lack of public knowledge about the contents in it. Eggshells consist of 95.1% salt, 3.3% organic
61 matter, and 1.6% water. The main component of inorganic salts in eggshells is dominated by
62 calcium carbonate (CaCO_3) of 98.5% (Nurjayanti et al., 2012). The bioavailability of eggshells is \pm
63 40% (Szeleszczuk et al., 2015) with calcium content in chicken and duck eggshells of 25.73% and
64 23.67%, respectively (Aminah and Meikawati, 2016).

65

66 Calcium contained in eggshells has the potential as an alternative source of calcium that can be
67 fortified in germinated mung bean drinks as a substitute for milk. Calcium is generally available in
68 micro size which is thought to be only able to absorb 50% of the total calcium consumed in its
69 metabolism. The nano form of eggshell calcium can make it easier for calcium to be more soluble
70 and optimally absorbed. According to Widyastuti and Kusuma (2017), nanocalcium is the
71 manufacture of particles with a size of less than 100 nm by changing the nature or function of a
72 material.

73

74 There are two methods in the manufacture of nanoparticles, namely top down (physical method)
75 and bottom up (chemical method). The bottom up method is a method of making nanoparticles by
76 arranging atoms or molecules to form nanometer-sized particles from a solution. In the bottom up
77 method, the formation of nanoparticles has a high degree of cohesion so that it is able to produce a
78 more uniform size. One of the bottom-up methods that is often used is precipitation because this

79 method is very effective in the manufacture of nanoparticles, the process is simple, and requires low
80 costs. The precipitation method is carried out by controlling the solubility of the material in the
81 solution through changes in pH (Suptijah et al., 2012). Therefore, the objective of this study was to
82 investigate the effect of mung bean germination time and nanocalcium fortification of eggshells on
83 the characteristics of germinated mung bean drink (*Vigna radiata*).

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Materials and Methods

86 The materials used in this study were VIMA-1 mung beans, eggshells (chicken and duck), 1N HCL,
87 3N NaOH, aquabidest, and other chemicals for analysis. The tools used were 100 mesh sieve,
88 Beaker glass (Pyrex, Japan), blender (Philips HR 2116, China), Erlenmeyer (Iwaki, Japan), hot
89 plate, muffle furnace (Barnstead FB1410M-26, USA), mortar, electric oven (Memmert, USA),
90 measuring pipette (Iwaki, Japan), pH meter (Oakton do-35613, USA), Spectrophotometer (UV-
91 VISAE S80, China), AAS (Thermo Scientific Nicolet™10, USA), analytical balance (Ohaus
92 AR2140, USA), and a Brookfield viscometer (NDJ-8S, China).

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Research Methods

95 This research was carried out at the Agricultural Product Chemistry Laboratory and Agricultural
96 Product Processing Laboratory, Agricultural Technology Department, Faculty of Agriculture,
97 Sriwijaya University. This research was conducted using a completely randomized factorial design
98 with two treatment factors, germination time of mung bean (A) which consisted of 3 treatment
99 levels (A1 = 6 hours; A2 = 12 hours; and A3 = 18 hours) and the type of eggshell nanocalcium (B)
100 which consisted of 2 treatment (B1 = chicken eggshell, and B2 = duck eggshell). The treatment was
101 repeated 3 times. Mung bean drink without germination and addition of eggshell nanocalcium was a
102 control. Addition of eggshell nanocalcium to all treatments was 20% of the RDI for calcium, which
103 was 1200 mg/day per serving size (240 mL).

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Research Implementation

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Eggshell micro powder preparation (Rahmawati and Nisa, 2015)

Preparation of eggshell micro powder was carried out by preparing 500 g for each chicken and duck eggshells and washed with running water until clean. The eggshells were boiled at 100°C for 3 minutes to kill pathogenic microbes, and then the eggshells were drained. The eggshells were baked for 3 hours at 60°C, and placed at room temperature. The cold eggshells were mashed using a blender at a speed of ± 18.000 rpm for 3 minutes. The eggshell powder was then sieved through a 100 mesh sieve. The finished eggshell powder was put into OPP (Oriented Polystyrene) plastic and stored at 4°C for analysis.

Eggshell nanocalcium powder preparation (Khoerunnisa, 2011)

Eggshell powder was soaked in 1N HCl (1:5) for 48 hours and extracted at 90°C for 1 hour. The extracts were filtered using filter paper to obtain the filtrate and precipitate. The filtrate was precipitated with the addition of 3N NaOH and stirred, and then allowed to stand until a precipitate was formed. The precipitate was then neutralized using aquabidest until the pH was neutral. The solution is separated from the precipitate by pouring it slowly so that the precipitate does not get wasted. The precipitate was dried in oven for 3 hours at a temperature of 105°C, followed by burning in muffle furnace at a temperature of 600°C for 5 hours, and then crushed using a mortar to obtain nanocalcium powder. The nanocalcium powder was vacuum packed and stored at 4°C.

Mung bean germination (Wea et al., 2014)

The mung beans were sorted and washed with running water until clean and then soaked for 6 hours. The mung beans are drained and placed on tray with a damp cloth. The green beans is placed in room temperature and germinated for 6, 12 and 18 hours, where every 6 hours watering is done with water. Germinated mung beans are separated from the skin and taken as much as 100 grams for further processing.

131 **Germinated mung bean drinks preparation (Wea et al., 2014)**

132 The germinated mung beans were blanched by boiling at 80°C for 2 minutes, peeled and then
133 mashed with a blender for 3 minutes, added with water in a ratio of 1: 7.5. The germinated mung
134 bean drinks is filtered and then cooked by adding nanocalcium eggshell and 7% sugar. Cooking is
135 carried out until it boils and stirred for 5 minutes then cooled for 30 minutes.

136

137 **Analysis**

138 The analysis of the germinated mung bean drinks were chemical characteristics: protein content
139 using Lowry method (Harjanto, 2017), calcium content was using AAS method (AOAC, 2005),
140 vitamin C content (Sudarmadji et al. 2007), pH value (Sudarmadji et al., 2014), and physical
141 characteristics: viscosity (Yuwono and Susanto, 1998) and stability (Wibowo et al., 2014).

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Results

144 **Analysis of the characteristics of germinated mung bean drink with nanocalcium fortification**

145 The interaction between germination time and the type of eggshell nanocalcium gave no significant
146 effect on all characteristics of germinated mung bean drink (Table 1). This was because addition of
147 the nanocalcium (CaO) powder of chicken and duck eggshells to the germinated mung bean drinks
148 in the same amount (20% of nutrition facts label for calcium). Moreover, the characteristics of the
149 eggshell nanocalcium (CaO) powder were white color, very fine texture (particle size of CaO in
150 chicken and duck eggshells is 41 .54 nm and 24.90 nm), and neutral pH so that it gave no
151 significant effect on the value of viscosity, stability, protein, pH and vitamin C of germinated mung
152 bean drink (Table 2).

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157 Table 1. Physical and chemical characteristics of germinated mung bean drinks with nanocalcium
158 fortification

Treatments	Parameter					
	Viscosity (mPa.s)	Stability (%)	Protein (%)	pH	Vitamin C (mg/100g)	Calcium (%)
A ₁ B ₁	27,60±0,20 ^a	87,00±1,00 ^a	1,90±0,05 ^a	6,81±0,02 ^a	8,21±1,02 ^a	17,82
A ₁ B ₂	27,13±0,15 ^a	85,67±1,53 ^a	1,81±0,09 ^a	6,77±0,04 ^a	7,62±1,02 ^a	24,82
A ₂ B ₁	26,17±0,29 ^a	73,00±1,00 ^a	1,77±0,11 ^a	6,74±0,04 ^a	11,73±1,01 ^a	16,45
A ₂ B ₂	26,03±0,45 ^a	71,67±1,53 ^a	1,66±0,14 ^a	6,69±0,04 ^a	11,14±1,01 ^a	23,66
A ₃ B ₁	25,53±0,21 ^a	65,67±0,58 ^a	1,56±0,14 ^a	6,63±0,04 ^a	14,66±1,01 ^a	15,66
A ₃ B ₂	24,97±0,76 ^a	64,67±1,53 ^a	1,50±0,18 ^a	6,62±0,01 ^a	13,49±1,02 ^a	22,49
Kontrol	28,80±0,01 ^a	91,33±0,58 ^a	2,01±0,16 ^a	6,81±0,02 ^a	5,28±1,76 ^b	0,00

159 Note: Numbers marked with the same letter notation in the same column indicate not significantly
160 different (5%)
161 Data = mean ± standard deviation
162 Control = germination time of green beans 0 hours without the addition of nanocalcium
163 A₁ = mung bean germination time for 6 hours B₁ = nanocalcium of chicken eggshell
164 A₂ = mung bean germination time for 12 hours B₂ = nanocalcium of duck eggshell
165 A₃ = mung bean germination time for 18 hours
166

167 Table 2. Effect of eggshell nanocalcium fortification on the characteristics of germinated mung
168 bean drinks

Treatments	Parameter				
	Viscosity (mPa.s)	Stability (%)	Protein (%)	pH*	Vitamin C (mg/100g)
B ₁	26,43±0,23 ^a	75,22±0,86 ^a	1,74±0,10 ^a	6,73±0,03 ^a	11,54±1,02 ^a
B ₂	26,04±0,46 ^a	74,00±1,53 ^a	1,66±0,13 ^a	6,69±0,03 ^a	10,75±1,02 ^a

169 Note: Numbers marked with the same letter notation in the same column indicate not significantly
170 different (5%)
171 Data = mean ± standard deviation
172 B₁ = nanocalcium of chicken eggshell
173 B₂ = nanocalcium of duck eggshell
174

175 Germination time was significantly effect the characteristics of germinated mung been drinks
176 (Table 3).

177

178 Table 3. The effect of germination time on the characteristics of germinated mung bean drinks

Treatments	Parameter				
	Viscosity (mPa.s)	Stability (%)	Protein (%)	pH	Vitamin C (mg/100g)
A ₁	27,37±0,49 ^a	65,17±1,26 ^a	1,53±0,07 ^a	6,62±0,03 ^a	7,92±1,02 ^a
A ₂	26,10±0,37 ^b	72,33±1,26 ^b	1,72±0,13 ^{ab}	6,72±0,04 ^b	11,44±1,01 ^b
A ₃	25,25±0,18 ^c	86,33±1,05 ^c	1,85±0,16 ^b	6,79±0,02 ^c	14,08±1,02 ^c

179 Note: Numbers marked with the same letter notation in the same column indicate not significantly
 180 different (5%)

181 Data = mean ± standard deviation

182 A₁ = mung bean germination time for 6 hours

183 A₂ = mung bean germination time for 12 hours

184 A₃ = mung bean germination time for 18 hours

185

186

Discussion

187 Table 1 shows a significant increase in the vitamin C and calcium content of germinated mung bean
 188 drinks to the control. The increase in vitamin C in germinated mung bean drinks in all treatments in
 189 this study was due to the food reserves contained in the cotyledons in the form of starch hydrolyzed
 190 by the amylase enzyme into simple sugars which were then synthesized into vitamin C during the
 191 germination process. The results of this study were in line with the opinion of Ebert et al. (2017)
 192 which stated that the content of vitamin C in the germinated peanut seeds increased by 2.7 times
 193 compared to the ungerminated seed.

194

195 The calcium content of germinated mung bean drinks fortified with nanocalcium of duck eggshell
 196 was higher than the calcium content of germinated mung bean drinks fortified with nanocalcium of
 197 chicken eggshell. This was because the calcium content in duck eggshell nanocalcium (45.50%)
 198 was higher than the calcium level in chicken eggshell nanocalcium (35.50%).

199

200

201

202 **Viscosity**

203 Viscosity is a measure of the size of the friction that occurs in the fluid. The greater the viscosity of
204 a food, the thicker the material, and vice versa (Srihidayati, 2017). The viscosity of germinated
205 mung bean drinks in the control treatment (0 hour germination without nanocalcium fortification)
206 was higher (28.80 ± 0.01 m.Pa.s) compared to that of nanocalcium fortified germinated mung bean
207 drinks for all treatments in this study. This is due to the fact that ungerminated mung bean starch
208 has not been hydrolyzed so that it still has higher starch content than germinated mung bean starch.
209 In the preparation of mung bean drinks, starch will undergo gelatinization in the presence of heat
210 treatment which will cause the starch granules to break and the starch molecules to come out and be
211 released from the granules and then enter the solution system. The results of this study are in line
212 with the results of research conducted by Nurjanati et al. (2018), based on the hedonic test,
213 ungerminated red bean milk (0 hours) has a higher viscosity than germinated red bean milk (8, 16
214 and 24 hours).

215

216 Table 3 shows that the viscosity of the green bean germination drink decreased significantly with
217 the duration of germination. The decrease in the viscosity of the material during germination was
218 caused by the hydrolysis of starch during the germination process by the amylase enzymes. The
219 enzyme is formed at the beginning of germination by giberylic acid (Elobuiké et al., 2021). Starch
220 will be broken down into simple sugars in the form of glucose which is used as energy and needs
221 for seed growth. Another factor that causes a decrease in the viscosity of mung bean germination
222 drink is the increase in vitamin C during the germination process which results in a decrease in pH
223 as shown in Table 3. A decrease in pH results in hydrolysis of glycosidic bonds which causes a
224 decrease in viscosity. The results of this study are in line with the statement of Widjaja et al. (2019),
225 that a solution will decrease its viscosity if its pH decreases.

226

227

228 **Stability**

229 The stability of the products can be seen by the presence or absence of precipitate in the product
230 (Farikha et al., 2013). Germinated mung bean drinks in the control treatment (0 hour germination
231 without the addition of nanocalcium) had a higher stability value (91%) compared to all treatments
232 of germinated mung bean drinks fortified with nanocalcium in this study. This was because
233 germinated mung bean seeds absorb more water than ungerminated mung bean seeds. During
234 germination, bean seeds require optimal environmental conditions that are 50-80% RH (Yuwariah
235 et al., 2015). Water absorption in the germination process is needed to maintain cell turgidity,
236 including cell enlargement, photosynthetic reactions, salt solvents, gases and other substances that
237 are transported between cells in tissues to maintain cell growth (Felania, 2017).

238

239 Table 3 shows that the stability of the germinated mung bean drinks decreased significantly as the
240 duration of germination increased. This was due to an increase in the water content of germinated
241 mung beans along with the length of germination due to the watering the mung bean seeds every 6
242 hours during germination time. The water content of germinated mung bean with a time of 0, 6, 12
243 and 18 hours were 4.32%, 4.99%, 5.42% and 7.22%, respectively. The increase in water content in
244 germinated mung beans resulting germinated mung bean drinks have more water content in turns it
245 affects the stability value of the drinks. In line with the viscosity value of the germinated mung bean
246 drinks, the average value of stability of each treatment decreased with the duration of germination.
247 The decrease in viscosity at germinated mung bean drinks correlated to that in stability.

248

249 **Protein Content**

250 The protein of germinated mung bean drinks in treatment A2 (12 hours of germination time) was
251 not significantly different from that of treatment A1 (6 hours of germination time) and treatment A3
252 (12 hours of germination time). The decreasing of protein content in germinated mung bean drinks
253 was due to the hydrolysis of protein into amino acids that are used for embryo growth. The results

254 of this study were in line with the statement of Masood et al. (2014) which stated a decrease in
255 protein content along with an increase in amino acid content during germination of green beans.
256 This is due to an increase in the activity of the protease enzyme which is an endogenous enzyme
257 that is active during the imbibition process, when green beans are in contact with water. Proteases
258 hydrolyze proteins into peptides and amino acids (Ferdiawan et al., 2019). Free amino acids along
259 with glutamic and aspartic acids (in the form of amides) will be translocated to the embryo in the
260 formation of new structures in line with the ongoing germination stage (Pertiwi et al., 2013).

261

262 **pH value**

263 pH is a parameter used to determine changes in the acidity level of a food product (Widowati et al.,
264 2020). Table 3 shows that the longer the germination time, the lower the pH of the germinated
265 mung bean drinks significantly. According to Wea et al. (2014), in germinated seed plants there was
266 an increase in vitamin synthesis, especially vitamin C (Wea et al., 2014). This situation is in
267 accordance with the vitamin C content in the germinated mung bean drinks in this study (Table 3)
268 so that the pH of the germinated mung bean drinka is low. Based on Indonesian National Standard
269 01-3830-1995, the pH standard for bean drinks is 6.5-7.0. The pH of germinated mung bean drinks
270 with nanocalcium fortification in this study has a value of 6.62-6.79; it has met the pH standard of
271 bean drinks.

272

273 **Vitamin C**

274 Table 3 shows that the vitamin C of the germinated mung bean drinks increased significantly with
275 increasing of germination time. The increase in vitamin C along with germination time is caused by
276 several enzyme systems being active during germination. This happens because of the accumulation
277 of ascorbic acid as a result of biosynthesis. Germination causes reactivation of the enzyme L-
278 Galactono- γ -lactone dehydrogenase which is involved in the oxidation of L-galactono-1,4-lactone
279 to ascorbic acid. The activity of this enzyme increased in parallel to the biosynthesis of ascorbic

280 acid during the seed germination process. Differences in the level of ascorbic acid biosynthesis in
281 germination of mung bean seeds can also be influenced by legume varieties, maturity, climate, light,
282 harvesting and storage methods (Masood et al., 2014). Increasing the content of vitamin C in
283 germination of mung beans can help the solubility of nanocalcium fortification in the germinated
284 mung bean drinks. This is in line with the statement of Yonata et al. (2017), that pH can affect the
285 solubility of minerals. Acidic conditions and small particle size can increase the speed of
286 nanocalcium dissolution from both duck and chicken eggshells, so that the germinated mung bean
287 drinks with eggshells nanocalcium fortification has good digestibility.

288

289 Calcium Content

290 Based on the results of the analysis, calcium content of nanocalcium in chicken and duck eggshells
291 was 35.50% and 45.50%, respectively. Calcium content in eggshells is influenced by the thickness
292 and type of eggshell. Duck eggshells have an average thickness of 0.36 - 0.46 mm (Septiana et al.,
293 2015), while the average thickness of chicken eggshells was 0.33 - 0.35 mm (Azizah et al., 2015).
294 Differences in eggshell thickness can affect the amount of mineral content and organic salts,
295 especially calcium carbonate (CaCO_3) which acts as a source of calcium in eggshells.

296

297 Fortification is a process of increasing the content of essential micronutrients in the form of
298 vitamins and minerals into food, aiming to increase nutrients that do not yet exist or to enrich
299 existing nutrients (Valentina et al., 2014). According to the Indonesian Food and Drug Supervisory
300 Agency (2019), the minimum amount of micronutrient fortification in foodstuffs is 10% of the RDI
301 per serving. In this study, the amount of nanocalcium fortification of chicken and duck eggshells in
302 germinated mung bean drink was 20% of the RDI for calcium per 250 mL serving.

303

304 Calcium content of nongerminated mung bean drinks with nanocalcium fortification of chicken and
305 duck eggshells was 18.97% and 25%, respectively. This showed that the calcium content of

306 germinated mung bean drinks both fortified with nanocalcium chicken and duck eggshells
307 decreased with the increasing of germination time (Table 1). This was in accordance with the
308 statement of Oghbaei and Prakash (2020) that the calcium content decreased significantly in
309 germinated and peeled bean seeds. Peeling can reduce some of the minerals present in the skin of
310 green beans. However, this germination and skin peeling treatment had an impact on increasing the
311 bioavailability of mung beans which contributed to stimulating the reduction of anti-nutritional
312 substances. The percentage of bioavailability in the seeds before germination was 14.91–17.19%
313 and in the germinated beans which were peeled 22.96–25.27%.

314

315 In addition, the decrease in calcium content of germinated mung bean drinks in this study was
316 thought to be due to the treatment with germination time less than 24 hours so that the phytase
317 enzyme had not worked optimally, causing the complex phytic acid that binds to minerals such as
318 calcium could not be completely released. Based on the results of research by Ghavidel and
319 Davoodi (2011), enzyme activity (phytase, amylase and protease) increased significantly at 24, 48,
320 and 72 hours of germination in several types of legumes, one of which was green beans. The
321 maximum enzymatic activity occurred at 72 hours of germination. Although the calcium of
322 germinated mung bean drinks decreased in the 6, 12 and 18 hour germination treatment, but with an
323 increase in vitamin C at the same germination time treatment it could increase the calcium
324 absorption efficiency of the germinated mung bean drinks.

325

Conclusion

326 Nanocalcium fortification of chicken and duck eggshells by 20% of the RDI for calcium per 250
327 mL serving had no significant effect on the physical (viscosity and stability) and chemical (protein,
328 pH, and vitamin C) characteristics of germinated mung bean drinks. Germination of mung beans at
329 different times was significantly increased value of pH, vitamin C, protein, and stability; but
330 significantly decreased value of viscosity. Germinated mung bean drinks are a good carrier for
331 chicken and duck eggshells nanocalcium fortification.

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Table 1. Physical and chemical characteristics of germinated mung bean drinks with nanocalcium fortification

Treatments	Parameter					
	Viscosity (mPa.s)	Stability (%)	Protein (%)	pH	Vitamin C (mg/100g)	Calcium (%)
A ₁ B ₁	27,60±0,20 ^a	87,00±1,00 ^a	1,90±0,05 ^a	6,81±0,02 ^a	8,21±1,02 ^a	17,82
A ₁ B ₂	27,13±0,15 ^a	85,67±1,53 ^a	1,81±0,09 ^a	6,77±0,04 ^a	7,62±1,02 ^a	24,82
A ₂ B ₁	26,17±0,29 ^a	73,00±1,00 ^a	1,77±0,11 ^a	6,74±0,04 ^a	11,73±1,01 ^a	16,45
A ₂ B ₂	26,03±0,45 ^a	71,67±1,53 ^a	1,66±0,14 ^a	6,69±0,04 ^a	11,14±1,01 ^a	23,66
A ₃ B ₁	25,53±0,21 ^a	65,67±0,58 ^a	1,56±0,14 ^a	6,63±0,04 ^a	14,66±1,01 ^a	15,66
A ₃ B ₂	24,97±0,76 ^a	64,67±1,53 ^a	1,50±0,18 ^a	6,62±0,01 ^a	13,49±1,02 ^a	22,49
Kontrol	28,80±0,01 ^a	91,33±0,58 ^a	2,01±0,16 ^a	6,81±0,02 ^a	5,28±1,76 ^b	0,00

Note: Numbers marked with the same letter notation in the same column indicate not significantly different (5%)

Data = mean ± standard deviation

Control = germination time of green beans 0 hours without the addition of nanocalcium

A₁ = mung bean germination time for 6 hours

B₁ = nanocalcium of chicken eggshell

A₂ = mung bean germination time for 12 hours

B₂ = nanocalcium of duck eggshell

A₃ = mung bean germination time for 18 hours

Table 2. Effect of eggshell nanocalcium fortification on the characteristics of germinated mung bean drinks

Treatments	Parameter				
	Viscosity (mPa.s)	Stability (%)	Protein (%)	pH*	Vitamin C (mg/100g)
B ₁	26,43±0,23 ^a	75,22±0,86 ^a	1,74±0,10 ^a	6,73±0,03 ^a	11,54±1,02 ^a
B ₂	26,04±0,46 ^a	74,00±1,53 ^a	1,66±0,13 ^a	6,69±0,03 ^a	10,75±1,02 ^a

Note: Numbers marked with the same letter notation in the same column indicate not significantly different (5%)

Data = mean ± standard deviation

B₁ = nanocalcium of chicken eggshell

B₂ = nanocalcium of duck eggshell

Germination time was significantly effect the characteristics of germinated mung been drinks

Table 3. The effect of germination time on the characteristics of germinated mung bean drinks

Treatments	Parameter				
	Viscosity (mPa.s)	Stability (%)	Protein (%)	pH	Vitamin C (mg/100g)
A ₁	27,37±0,49 ^a	65,17±1,26 ^a	1,53±0,07 ^a	6,62±0,03 ^a	7,92±1,02 ^a
A ₂	26,10±0,37 ^b	72,33±1,26 ^b	1,72±0,13 ^{ab}	6,72±0,04 ^b	11,44±1,01 ^b
A ₃	25,25±0,18 ^c	86,33±1,05 ^c	1,85±0,16 ^b	6,79±0,02 ^c	14,08±1,02 ^c

Note: Numbers marked with the same letter notation in the same column indicate not significantly different (5%)

Data = mean ± standard deviation

A₁ = mung bean germination time for 6 hours

A₂ = mung bean germination time for 12 hours

A₃ = mung bean germination time for 18 hours

**2. Bukti Review dari Reviewer Pertama dan ke Dua
(22 Desember 2022)**

1 Effect of germination and eggshells nanocalcium fortification on characteristics of germinated
2 mungbean drink (*Vigna radiata*.)

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9 **ABSTRACT**

10 Germinated mung bean drinks with eggshell nanocalcium fortification are an alternative beverage to
11 replace milk. This study aimed to determine the effect of germination time and eggshells
12 nanocalcium fortification on physicochemical characteristics of the germinated mung bean drink.

13 This study used a Factorial Completely Randomized Design with two treatment factors. The first
14 factor was germination time which consisted of three treatment levels (6, 12 and 18 hours) and the
15 second factor was the type of eggshell nanocalcium which consisted of two types of treatment
16 (chicken and duck eggshell), plus the control treatment as mung bean drink without germination
17 and addition of eggshell nanocalcium. Each experiment was repeated three times. Observed
18 parameters in this study were chemical characteristics (protein, calcium, vitamin C, and pH) and
19 physical characteristics (viscosity and stability) of germinated mung bean drink. The results showed
20 that the germination time treatment significantly affected the value of protein, calcium, vitamin C,
21 pH, viscosity, and stability of the germinated mung bean drink. Germination increased vitamin C in
22 mung beans with an average value of 8,21-14,66 mg/100 g. Eggshell nanocalcium fortification
23 showed a significant effect on increasing calcium of germinated mung bean drink. The highest
24 calcium content of germinated mung bean drink was found in drink produced from 6 hours of
25 germination with fortification of duck nanocalcium, which was 24.82%.

26 **Keywords:** physicochemical, chicken, duck, calcium, vitamin C

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Introduction

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To meet the calcium needs of the Indonesian people who generally suffer from lactose intolerance and digestive disorders after consuming milk, it is necessary to drink milk substitutes made from plant-based foods that contain high protein and calcium. Mung bean is the third highest source of vegetable protein after soybeans and peanuts with a content of protein around 20-25% (Purwono and Hartono, 2005; Hastuti et al., 2018). One of the superior varieties of mung beans is VIMA-1 (*Vigna sinensis*–Malang). VIMA-1 has a promising market potential due to its high protein content (28.02%), low fat (0.40%), and high starch content (67.62%) (Agency for Research and Development, 2019). However, mungbeans have anti-nutritional compounds, compounds found naturally in various types of beans, which can prevent the absorption of nutrients in the body.

One process that can increase the nutrition in beans is germination. Germinated mung beans have improved nutritional, functional and biological properties by changing the content, nutritional composition and bioactive compounds, as well as eliminating anti-nutritional factors in beans (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 shoots in germinated seeds ranges from 2 mm to up to 5 mm (Munarko et al., 2019).

The germination process in mung bean seeds is able to increase the nutritional value by activating enzymes that can reduce or eliminate anti-nutritional factors (Ebert et al. 2017). Phytic acid, the main anti-nutritional substance found in green beans, can be reduced from 12.0 mg/g to 4.03 mg/g through the germination process (Faradilla et al., 2012). Furthermore, germination is able to reduce anti-nutritional substances in germinated bean flour by 66.70% (Nur et al., 2019), so the bioavailability of vitamins and minerals in the flour increase. This is because the germination process can release the bound form of vitamins and minerals into a freer form so that it is more

53 easily digested and absorbed by the human digestive tract. Previous research explained that during
54 germination there is a breakdown of complex molecules into simpler forms which causes
55 germination of green beans to be more easily digested and absorbed by the human body (Ghavidel
56 and Prakash, 2007; Masood et al., 2014). In addition, the content of vitamin C increased with the
57 increasing duration time of germination. Ebert et al. (2017) stated that the average vitamin C in
58 germinated bean was 2.7 times higher than that without germination.

59

60 The eggshells of chickens and ducks are household waste that has not been utilized optimally due to
61 the lack of public knowledge about the contents in it. Eggshells consist of 95.1% salt, 3.3% organic
62 matter, and 1.6% water. The main component of inorganic salts in eggshells is dominated by
63 calcium carbonate (CaCO₃) of 98.5% (Nurjayanti et al., 2012). The bioavailability of eggshells is ±
64 40% (Szeleszczuk et al., 2015) with calcium content in chicken and duck eggshells of 25.73% and
65 23.67%, respectively (Aminah and Meikawati, 2016).

66

67 Calcium contained in eggshells has the potential as an alternative source of calcium that can be
68 fortified in germinated mung bean drinks as a substitute for milk. Calcium is generally available in
69 micro size which is thought to be only able to absorb 50% of the total calcium consumed in its
70 metabolism. The nano form of eggshell calcium can make it easier for calcium to be more soluble
71 and optimally absorbed. According to Widyastuti and Kusuma (2017), nanocalcium is the
72 manufacture of particles with a size of less than 100 nm by changing the nature or function of a
73 material.

74

75 There are two methods in the manufacture of nanoparticles, namely top down (physical method)
76 and bottom up (chemical method). The bottom up method is a method of making nanoparticles by
77 arranging atoms or molecules to form nanometer-sized particles from a solution. In the bottom up
78 method, the formation of nanoparticles has a high degree of cohesion so that it is able to produce a

79 more uniform size. One of the bottom-up methods that is often used is precipitation because this
80 method is very effective in the manufacture of nanoparticles, the process is simple, and requires low
81 costs. The precipitation method is carried out by controlling the solubility of the material in the
82 solution through changes in pH (Suptijah et al., 2012). Therefore, the objective of this study was to
83 investigate the effect of mung bean germination time and nanocalcium fortification of eggshells on
84 the characteristics of germinated mung bean drink (*Vigna radiata*).

85 **What were the hypothesis of the research?**

86 **Material and Methods**

87 The materials used in this study were VIMA-1 mung beans, eggshells (chicken and duck), 1N HCL,
88 3N NaOH, aquabidest, and other chemicals for analysis. The tools used were 100 mesh sieve,
89 Beaker glass (Phyrex, Japan), blender (Philips HR 2116, China), Erlenmeyer (Iwaki, Japan), hot
90 plate, muffle furnace (Barnstead FB1410M-26, USA), mortar, electric oven (Mettler, USA),
91 measuring pipette (Iwaki, Japan), pH meter (Oakton do-35613, USA), Spectrophotometer (UV-
92 VISAE S80, China), AAS (Thermo Scientific NicoletTM10, USA), analytical balance (Ohaus
93 AR2140, USA), and a Brookfield viscometer (NDJ-8S, China).

94

95 **Research Methods**

96 This research was carried out at the Agricultural Product Chemistry Laboratory and Agricultural
97 Product Processing Laboratory, Faculty of Agriculture, Sriwijaya University, Indonesia(-
98 3.2196118175204167, 104.64789811916464). This research was conducted using a completely
99 randomized factorial design with two treatment factors, germination time of mung bean (A) which
100 consisted of 3 treatment levels (A1 = 6 hours; A2 = 12 hours; and A3 = 18 hours) and the type of
101 eggshell nanocalcium (B) which consisted of 2 treatment (B1 = chicken eggshell, and B2 = duck
102 eggshell). The treatment was repeated 3 times. Mung bean drink without germination and addition
103 of eggshell nanocalcium was a control. Addition of eggshell nanocalcium to all treatments was 20%
104 of the RDI for calcium, which was 1200 mg/day per serving size (240 mL).

105

106

Research Implementation

107 Eggshell micro powder preparation (Rahmawati and Nisa, 2015)

108 Preparation of eggshell micro powder was carried out by preparing 500 g for each chicken and duck
109 eggshells and washed with running water until clean. The eggshells were boiled at 100°C for 3
110 minutes to kill pathogenic microbes, and then the eggshells were drained. The eggshells were baked
111 for 3 hours at 60°C, and placed at room temperature. The cold eggshells were mashed using a
112 blender at a speed of ± 18.000 rpm for 3 minutes. The eggshell powder was then sieved through a
113 100 mesh sieve. The finished eggshell powder was put into OPP (Oriented Polystyrene) plastic and
114 stored at 4°C for analysis.

115

116 Eggshell nanocalcium powder preparation (Khoerunnisa, 2011)

117 Eggshell powder was soaked in 1N HCl (1:5) for 48 hours and extracted at 90°C for 1 hour. The
118 extracts were filtered using filter paper to obtain the filtrate and precipitate. The filtrate was
119 precipitated with the addition of 3N NaOH and stirred, and then allowed to stand until a precipitate
120 was formed. The precipitate was then neutralized using aquabidest until the pH was neutral. The
121 solution is separated from the precipitate by pouring it slowly so that the precipitate does not get
122 wasted. The precipitate was dried in oven for 3 hours at a temperature of 105°C, followed by
123 burning in muffle furnace at a temperature of 600°C for 5 hours, and then crushed using a mortar to
124 obtain nanocalcium powder. The nanocalcium powder was vacuum packed and stored at 4°C.

125

126 Mung bean germination (Wea et al., 2014)

127 The mung beans were sorted and washed with running water until clean and then soaked for 6
128 hours. The mung beans are drained and placed on tray with a damp cloth. The green beans
129 were placed in room temperature and germinated for 6, 12 and 18 hours, where every 6

130 hours watering is done with water. Germinated mung beans are separated from the skin and taken as
131 much as 100 grams for further processing.

132 **Germinated mungbean drinks preparation (Wea et al., 2014)**

133 The germinated mung beans were blanched by boiling at 80°C for 2 minutes, peeled and then
134 mashed with a blender for 3 minutes, added with water in a ratio of 1: 7.5. The germinated mung
135 bean drinks was filtered and then cooked by adding nanocalcium eggshell and 7% sugar. Cooking
136 was carried out until it boils and stirred for 5 minutes and finally cooled for 30 minutes.

137

138 **Analysis**

139 The analysis of the germinated mung bean drinks were chemical characteristics: protein content
140 using Lowry method (Harjanto, 2017), calcium content was using AAS method (AOAC, 2005),
141 vitamin C content (Sudarmadji et al. 2007), pH value (Sudarmadji et al., 2014), and physical
142 characteristics: viscosity (Yuwono and Susanto, 1998) and stability (Wibowo et al., 2014).

143 How was done the statistical analysis? Was done test Dunnett to compare treatments with control?

144

145

Results

146 **Analysis of the characteristics of germinated mung bean drink with nanocalcium fortification**

147 The interaction between germination time and the type of eggshell nanocalcium was no significant
148 effect on all characteristics of germinated mung bean drink, excepted for Vitamin C and calcium
149 that were higher in all treatments compared to control (Table 1). The non-significance among the
150 treatments was attributed to addition of the nanocalcium (CaO) powder of chicken and duck
151 eggshells to the germinated mung bean drinks in the same amount (20% of nutrition facts label for
152 calcium). Moreover, the characteristics of the eggshell nanocalcium (CaO) powder were white
153 color, very fine texture (particle size of CaO in chicken and duck eggshells is 41.54 nm and 24.90
154 nm, respectively), and neutral pH so that it takes no significant effect on the value of viscosity,
155 stability, protein, pH and vitamin C of germinated mung bean drink (Table 2).

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158

159

160 Table 1. Physical and chemical characteristics of germinated mung bean drinks with nanocalcium
161 fortification

Treatments	Parameter					
	Viscosity (mPa.s)	Stability (%)	Protein (%)	pH	Vitamin C (mg/100g)	Calcium (%)
A ₁ B ₁	27,60±0,20 ^a	87,00±1,00 ^a	1,90±0,05 ^a	6,81±0,02 ^a	8,21±1,02 ^a	17,82
A ₁ B ₂	27,13±0,15 ^a	85,67±1,53 ^a	1,81±0,09 ^a	6,77±0,04 ^a	7,62±1,02 ^a	24,82
A ₂ B ₁	26,17±0,29 ^a	73,00±1,00 ^a	1,77±0,11 ^a	6,74±0,04 ^a	11,73±1,01 ^a	16,45
A ₂ B ₂	26,03±0,45 ^a	71,67±1,53 ^a	1,66±0,14 ^a	6,69±0,04 ^a	11,14±1,01 ^a	23,66
A ₃ B ₁	25,53±0,21 ^a	65,67±0,58 ^a	1,56±0,14 ^a	6,63±0,04 ^a	14,66±1,01 ^a	15,66
A ₃ B ₂	24,97±0,76 ^a	64,67±1,53 ^a	1,50±0,18 ^a	6,62±0,01 ^a	13,49±1,02 ^a	22,49
Control	28,80±0,01 ^a	91,33±0,58 ^a	2,01±0,16 ^a	6,81±0,02 ^a	5,28±1,76 ^b	0,00

162 Note: Numbers marked with the same letter notation in the same column indicate not significantly
163 different (5%) write the significant letter in the calcium column.

164 Data = mean ± standard deviation

165 Control = germination time of green beans 0 hours without the addition of nanocalcium

166 A₁ = mung bean germination time for 6 hours

B₁ = nanocalcium of chicken eggshell

167 A₂ = mung bean germination time for 12 hours

B₂ = nanocalcium of duck eggshell

168 A₃ = mung bean germination time for 18 hours

169

170 Table 2. Effect of eggshell nanocalcium fortification on the characteristics of germinated mung bean
171 drinks Why the data of calcium is no showing in this Table?

Treatments	Parameter				
	Viscosity (mPa.s)	Stability (%)	Protein (%)	pH*	Vitamin C (mg/100g)
B ₁	26,43±0,23 ^a	75,22±0,86 ^a	1,74±0,10 ^a	6,73±0,03 ^a	11,54±1,02 ^a
B ₂	26,04±0,46 ^a	74,00±1,53 ^a	1,66±0,13 ^a	6,69±0,03 ^a	10,75±1,02 ^a

172 Note: Numbers marked with the same letter notation in the same column indicate not significantly
173 different (5%)

174 Data = mean ± standard deviation

175 B₁ = nanocalcium of chicken eggshell
 176 B₂ = nanocalcium of duck eggshell
 177

178 Germination time had significantly effect on the characteristics of germinated mung been drinks
 179 (Table 3). Write the results of the characterisitics analysed (Viscosity, Stability, Protein, pH and
 180 Vitamin C).
 181

182 Table 3. The effect of germination time on the characteristics of germinated mung bean drinks

Treatment		Parameter				Vitamin C (mg/100g)
s	Viscosity (mPa.s)	Stability (%)	Protein (%)	pH		
A ₁	27,37±0, 49 ^a	65,17±1, 26 ^a	1,53±0, 07 ^a	6,62±0, 03 ^a	7,92±1,02 ^a	
A ₂	26,10±0, 37 ^b	72,33±1, 26 ^b	1,72±0, 13 ^{ab}	6,72±0, 04 ^b	11,44±1,01 ^b	
A ₃	25,25±0,18 ^c	86,33±1,05 ^c	1,85±0,16 ^b	6,79±0,02 ^c	14,08±1,02 ^c	

183 Note: Numbers marked with the same letter notation in the same column indicate not significantly
 184 different (5%)

185 Data = mean ± standard deviation

186 A₁ = mung bean germination time for 6 hours

187 A₂ = mung bean germination time for 12 hours

188 A₃ = mung bean germination time for 18 hours
 189

190 Discussion

191 Table 1 shows a significant increase in the vitamin C and calcium content of germinated mung bean
 192 drinks to the control. The increase in vitamin C in germinated mung bean drinks in all treatments in
 193 this study was due to the food reserves contained in the cotyledons in the form of starch hydrolyzed
 194 by the amylase enzyme into simple sugars which were then synthesized into vitamin C during the
 195 germination process. The results of this study were in line with the opinion of Ebert et al. (2017)

196 which stated that the content of vitamin C in the germinated peanut seeds increased by 2.7 times
197 compared to the ungerminated seed.

198

199 The calcium content of germinated mung bean drinks fortified with nanocalcium of duck eggshell
200 was higher than the calcium content of germinated mung bean drinks fortified with nanocalcium of
201 chicken eggshell (Show this result in the Table 2). This was because the calcium content in duck
202 eggshell nanocalcium (45.50%) was higher than the calcium level in chicken eggshell nanocalcium
203 (35.50%).

204

205

206

207 **Viscosity**

208 Viscosity is a measure of the size of the friction that occurs in the fluid. The greater the viscosity of
209 a food, the thicker the material, and vice versa (What is better in this case? High or low viscosity?
210 (Srihidayati, 2017). The viscosity of germinated mung bean drinks in the control treatment (0 hour
211 germination without nanocalcium fortification) was higher (28.80 ± 0.01 m.Pa.s) compared to that of
212 nanocalcium fortified germinated mung bean drinks for all treatments in this study. This is due to
213 the fact that ungerminated mung bean starch has not been hydrolyzed so that it still has higher
214 starch content than germinated mung bean starch. In the preparation of mung bean drinks, starch
215 will undergo gelatinization in the presence of heat treatment which will cause the starch granules to
216 break and the starch molecules to come out and be released from the granules and then enter the
217 solution system. The results of this study are in line with the results of research conducted by
218 Nurjanati et al. (2018), based on the hedonic test, ungerminated red bean milk (0 hours) has a higher
219 viscosity than germinated red bean milk (8, 16 and 24 hours).

220

221 Table 3 shows that the viscosity of the green bean germination drink decreased significantly with
222 the duration of germination. The decrease in the viscosity of the material during germination was
223 caused by the hydrolysis of starch during the germination process by the amylase enzymes. The
224 enzyme is formed at the beginning of germination by gibberellic acid (Elobuiké et al., 2021). Starch
225 will be broken down into simple sugars in the form of glucose which is used as energy and needs
226 for seed growth. Another factor that causes a decrease in the viscosity of mung bean germination
227 drink is the increase in vitamin C during the germination process which results in a decrease in pH
228 as shown in Table 3. A decrease in pH results in hydrolysis of glycosidic bonds which causes a
229 decrease in viscosity. The results of this study are in line with the statement of Widjaja et al. (2019),
230 that a solution will decrease its viscosity if its pH decreases.

231

232

233 **Stability**

234 The stability of the products can be seen by the presence or absence of precipitate in the product
235 (Farikha et al., 2013). What means high or low values of stability? Is high value better or no for
236 mung bean drink? Germinated mung bean drinks in the control treatment (0 hour germination
237 without the addition of nanocalcium) had a higher stability value (91%) compared to all treatments
238 of germinated mung bean drinks fortified with nanocalcium in this study. This was because
239 germinated mung bean seeds absorb more water than ungerminated mung bean seeds. During
240 germination, bean seeds require optimal environmental conditions that are 50-80% RH (Yuwariah
241 et al., 2015). Water absorption in the germination process is needed to maintain cell turgidity,
242 including cell enlargement, photosynthetic reactions, salt solvents, gases and other substances that
243 are transported between cells in tissues to maintain cell growth (Felania, 2017).

244

245 Table 3 shows that the stability of the germinated mung bean drinks decreased significantly as the
246 duration of germination increased. This was due to an increase in the water content of germinated

247 mung beans along with the length of germination due to the watering the mung bean seeds every 6
248 hours during germination time. The water content of germinated mung bean with a time of 0, 6, 12
249 and 18 hours were 4.32%, 4.99%, 5.42% and 7.22%, respectively. The increase in water content in
250 germinated mung beans resulting germinated mung bean drinks have more water content in turns it
251 affects the stability value of the drinks. In line with the viscosity value of the germinated mung bean
252 drinks, the average value of stability of each treatment decreased with the duration of germination.
253 The decrease in viscosity at germinated mung bean drinks correlated negatively to that in stability.

254

255 **Protein Content**

256 The protein of germinated mung bean drinks in treatment A3(18hours of germination time) was
257 significantly higher from that of treatment A1 (6 hours of germination time) but without differ from
258 that A2(12 hours of germination time). The decreasing of protein content in germinated mung bean
259 drinks was due to the hydrolysis of protein into amino acids that are used for embryo growth. The
260 results of this study were in line with the statement of Masood et al. (2014) which stated a decrease
261 in protein content along with an increase in amino acid content during germination of green beans.
262 This is due to an increase in the activity of the protease enzyme which is an endogenous enzyme
263 that is active during the imbibition process, when green beans are in contact with water. Proteases
264 hydrolyze proteins into peptides and amino acids (Ferdiawan et al., 2019). Free amino acids along
265 with glutamic and aspartic acids (in the form of amides) will be translocated to the embryo in the
266 formation of new structures in line with the ongoing germination stage (Pertiwi et al., 2013).

267

268 **pH value**

269 pH is a parameter used to determine changes in the acidity level of a food product What is the
270 adequate pH for mung beans drink? (Widowati et al., 2020). Table 3 shows that the longer the
271 germination time, the lower the pH of the germinated mung bean drinks was recorded significantly.
272 According to Wea et al. (2014), in germinated seed plants there was an increase in vitamin

273 synthesis, especially vitamin C (Wea et al., 2014). This situation is in accordance with the vitamin
274 C content in the germinated mung bean drinks in this study (Table 3) so that the pH of the
275 germinated mung bean drink is low. Based on Indonesian National Standard 01-3830-1995, the pH
276 standard for bean drinks is 6.5-7.0. The pH of germinated mung bean drinks with nanocalcium
277 fortification in this study has a value of 6.62-6.79; it has met the pH standard of bean drinks.

278

279 **Vitamin C**

280 Table 3 shows that the vitamin C of the germinated mung bean drinks increased significantly with
281 increasing of germination time. The increase in vitamin C along with germination time is caused by
282 several enzyme systems being active during germination. This happens because of the accumulation
283 of ascorbic acid as a result of biosynthesis. Germination causes reactivation of the enzyme L-
284 Galactono- γ -lactone dehydrogenase which is involved in the oxidation of L-galactono-1,4-lactone
285 to ascorbic acid. The activity of this enzyme increased in parallel to the biosynthesis of ascorbic
286 acid during the seed germination process (citation). Differences in the level of ascorbic acid
287 biosynthesis in germination of mung bean seeds can also be influenced by legume varieties,
288 maturity, climate, light, harvesting and storage methods (Masood et al., 2014). Increasing the
289 content of vitamin C in germination of mung beans can help the solubility of nanocalcium
290 fortification in the germinated mung bean drinks. This is in line with the statement of Yonata et al.
291 (2017), that pH can affect the solubility of minerals. Acidic conditions and small particle size can
292 increase the speed of nanocalcium dissolution from both duck and chicken eggshells, so that the
293 germinated mung bean drinks with eggshells nanocalcium fortification has good digestibility.

294

295 **Calcium Content**

296 Based on the results of the analysis, calcium content of nanocalcium in chicken and duck eggshells
297 was 35.50% and 45.50%, respectively. Calcium content in eggshells is influenced by the thickness

298 and type of eggshell. Duck eggshells have an average thickness of 0.36 - 0.46 mm (Septiana et al.,
299 2015), while the average thickness of chicken eggshells was 0.33 - 0.35 mm (Azizah et al., 2015).
300 Differences in eggshell thickness can affect the amount of mineral content and organic salts,
301 especially calcium carbonate (CaCO₃) which acts as a source of calcium in eggshells.

302

303 Fortification is a process of increasing the content of essential micronutrients in the form of
304 vitamins and minerals into food, aiming to increase nutrients that do not yet exist or to enrich
305 existing nutrients (Valentina et al., 2014). According to the Indonesian Food and Drug Supervisory
306 Agency (2019), the minimum amount of micronutrient fortification in foodstuffs is 10% of the RDI
307 per serving. In this study, the amount of nanocalcium fortification of chicken and duck eggshells in
308 germinated mung bean drink was 20% of the RDI for calcium per 250 mL serving.

309

310 Calcium content of nongerminated mung bean drinks with nanocalcium fortification of chicken and
311 duck eggshells was 18.97% and 25%, respectively. This showed that the calcium content of
312 germinated mung bean drinks both fortified with nanocalcium chicken and duck eggshells
313 decreased with the increasing of germination time (Table 1). This was in accordance with the
314 statement of Oghbaei and Prakash (2020) that the calcium content decreased significantly in
315 germinated peeled bean seeds. Peeling can reduce some of the minerals present in the skin of green
316 beans. However, this germination and skin peeling treatment had an impact on increasing the
317 bioavailability of mung beans which contributed to stimulating the reduction of anti-nutritional
318 substances. The percentage of bioavailability in the seeds before germination was 14.91–17.19%
319 and in the germinated beans which were peeled 22.96–25.27%.

320

321 In addition, the decrease in calcium content of germinated mung bean drinks in this study was
322 thought to be due to the treatment with germination time less than 24 hours so that the phytase
323 enzyme had not worked optimally, causing the complex phytic acid that binds to minerals such as

324 calcium could not be completely released. Based on the results of research by Ghavidel and
325 Davoodi (2011), enzyme activity (phytase, amylase and protease) increased significantly at 24, 48,
326 and 72 hours of germination in several types of legumes, one of which was green beans. The
327 maximum enzymatic activity occurred at 72 hours of germination. Although the calcium of
328 germinated mung bean drinks decreased in the 6, 12 and 18 hour germination treatment, but with an
329 increase in vitamin C at the same germination time treatment it could increase the calcium
330 absorption efficiency of the germinated mung bean drinks.

331 **Conclusion**

332 Nanocalcium fortification of chicken and duck eggshells by 20% of the RDI for calcium per 250
333 mL serving had no significant effect on the physical (viscosity and stability) and chemical (protein,
334 pH, and vitamin C) characteristics of germinated mung bean drinks. Germination of mung beans at
335 different times was significantly increased value of pH, vitamin C, protein, and stability; but
336 significantly decreased value of viscosity. Germinated mung bean drinks are a good carrier for
337 chicken and duck eggshells nanocalcium fortification.

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Effect of germination and eggshells nanocalcium fortification on characteristics of germinated mung bean drink (*Vigna radiata*.)

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ABSTRACT

Germinated mung bean drinks with eggshell nanocalcium fortification are an alternative beverage to replace milk. This study aimed to determine the effect of germination time and eggshells nanocalcium fortification on physicochemical characteristics of the germinated mung bean drink. This study used a Factorial Completely Randomized Design with two treatments factors. The first factor was germination time which consisted of three treatment levels (6, 12 and 18 hours) and the second factor was the type of eggshell nanocalcium which consisted of two type of treatment (chicken and duck eggshell), plus the control treatment as mung bean drink without germination and addition of eggshell nanocalcium. Each experiment was repeated three times. Observed parameters in this study were chemical characteristics (protein, calcium, vitamin C, and pH) and physical characteristics (viscosity and stability) of germinated mung bean drink. The results showed that the germination time treatment significantly affected the value of protein, calcium, vitamin C, pH, viscosity, and stability of the germinated mung bean drink. Germination increased vitamin C in mung beans with an average value of 8,21-14,66 mg/100 g. Eggshell nanocalcium fortification showed a significant effect on increasing calcium of germinated mung bean drink. Germinated mung bean drink produced from 6 hours of germination and fortification of duck nanocalcium had the highest calcium content with value of 24.82%.

Key words: Physicochemical, Chicken, Duck, Calcium, Vitamin C

INTRODUCTION

To meet the calcium needs of the Indonesian people who generally suffer from lactose intolerance and digestive disorders after consuming milk, it is necessary to drink milk substitutes made from plant-based foods that contain high protein and calcium. Mung bean is the third highest source of vegetable protein after soybeans and peanuts with a content of protein around 20-25% (Purwono and Hartono, 2005; Hastuti et al., 2018). One of the superior varieties of mung beans is VIMA-1 (*Vigna sinensis*–Malang). VIMA-1 has a promising market potential due to its high protein content (28.02%), low fat (0.40%), and high starch content (67.62%) (Agency for Research and Development, 2019). However, mung beans have anti-nutritional compounds, compounds found naturally in various types of beans, which can prevent the absorption of nutrients in the body.

One process that can increase the nutrition in beans is germination. Germinated mung beans have improved nutritional, functional and biological properties by changing the content, nutritional composition and bioactive compounds, as well as eliminating anti-nutritional factors in beans (Liu et al., 2020). Germination is a condition in which dormant seeds begin to germinate and grow into seedlings under the right growing conditions. Germination in brown rice refers to the point where the growth of a radicle ranges from 2 mm to 5 mm, and pre-germination brown rice is the stage with an expanded radicle exposed approximately 0.5–1 mm (Watanabe et al., 2004). Moreover, Palmiano and Juliano (1972) stated that the optimal germination period was 18 to 24 hours.

The germination process in mung bean seeds is able to increase the nutritional value by activating enzymes that can reduce or eliminate anti-nutritional factors (Ebert et al. 2017). Phytic acid, the main anti-nutritional substance

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found in green beans, can be reduced from 12.0 mg/g to 4.03 mg/g through the germination process (Faradilla et al., 2012). Furthermore, germination is able to reduce anti-nutritional substances in germinated bean flour by 66.70% (Nur et al., 2019), so the bioavailability of vitamins and minerals in the flour increase. This is because the germination process can release the bound form of vitamins and minerals into a freer form so that it is more easily digested and absorbed by the human digestive tract. Previous research explained that during germination there is a breakdown of complex molecules into simpler forms which causes germination of green beans to be more easily digested and absorbed by the human body (Ghavidel and Prakash, 2007; Masood et al., 2014). In addition, the content of vitamin C increased with the increasing duration time of germination. Ebert et al. (2017) stated that the average vitamin C in germinated bean was 2.7 times higher than that without germination.

The eggshells of chickens and ducks are household waste that has not been utilized optimally due to the lack of public knowledge about the contents in it. Eggshells consist of 95.1% salt, 3.3% organic matter, and 1.6% water. The main component of inorganic salts in eggshells is dominated by calcium carbonate (CaCO₃) of 98.5% (Nurjayanti et al., 2012). The bioavailability of eggshells is \pm 40% (Szeleszczuk et al., 2015) with calcium content in chicken and duck eggshells of 25.73% and 23.67%, respectively (Aminah and Meikawati, 2016).

Calcium contained in eggshells has the potential as an alternative source of calcium that can be fortified in germinated mung bean drinks as a substitute for milk. Calcium is generally available in micro size which is thought to be only able to absorb 50% of the total calcium consumed in its metabolism. The nano form of eggshell calcium can make it easier for calcium to be more soluble and optimally absorbed. According to Widyastuti and Kusuma (2017), nanocalcium is the manufacture of particles with a size of less than 100 nm by changing the nature or function of a material.

There are two methods in the manufacture of nanoparticles, namely top down (physical method) and bottom up (chemical method). The bottom up method is a method of making nanoparticles by arranging atoms or molecules to form nanometer-sized particles from a solution. In the bottom up method, the formation of nanoparticles has a high degree of cohesion so that it is able to produce a more uniform size. One of the bottom-up methods that is often used is precipitation because this method is very effective in the manufacture of nanoparticles, the process is simple, and requires low costs. The precipitation method is carried out by controlling the solubility of the material in the solution

through changes in pH (Suptijah et al., 2012). Therefore, the objective of this study was to investigate the effect of mung bean germination time and nanocalcium fortification of eggshells on the characteristics of germinated mung bean drink (*Vigna radiata*).

The hypothesis of this study was it is suspected that germination time and type of eggshell nanocalcium fortification has a significant effect on the physico-chemical characteristics of the resulting germinated mung bean drink.

MATERIAL AND METHODS

The materials used in this study were VIMA-1 mung beans, eggshells (chicken and duck), 1N HCL, 3N NaOH, aquabidest, and other chemicals for analysis. The tools used were 100 mesh sieve, Beaker glass (Pyrex, Japan), blender (Philips HR 2116, China), Erlenmeyer (Iwaki, Japan), hot plate, muffle furnace (Barnstead FB1410M-26, USA), mortar, electric oven (Mettler, USA), measuring pipette (Iwaki, Japan), pH meter (Oakton do-35613, USA), Spectrophotometer (UV-VISAE S80, China), AAS (Thermo Scientific NicoletTM10, USA), analytical balance (Ohaus AR2140, USA), and a Brookfield viscometer (NDJ-8S, China).

RESEARCH METHODS

This research was carried out at the Agricultural Product Chemistry Laboratory and Agricultural Product Processing Laboratory, Faculty of Agriculture, Sriwijaya University, South Sumatera, Indonesia (-3.2196118175204167, 104.64789811916464). This research was conducted using a completely randomized factorial design with two treatment factors, germination time of mung bean (A) which consisted of 3 treatment levels (A1 = 6 hours; A2 = 12 hours; and A3 = 18 hours) and the type of eggshell nanocalcium (B) which consisted of 2 treatment (B1 = chicken eggshell, and B2 = duck eggshell). The treatment was repeated 3 times. Mung bean drink without germination and addition of eggshell nanocalcium was a control. Addition of eggshell nanocalcium to all treatments was 20% of the Recommended Dietary Intake for calcium, which was 1200 mg/day per serving size (240 mL).

RESEARCH IMPLEMENTATION

Eggshell micro powder preparation (Rahmawati and Nisa, 2015)

Preparation of eggshell micro powder was carried out by preparing 500 g for each chicken and duck eggshells and

washed with running water until clean. The eggshells were boiled at 100°C for 3 minutes to kill pathogenic microbes, and then the eggshells were drained. The eggshells were baked for 3 hours at 60°C, and placed at room temperature. The cold eggshells were mashed using a blender at a speed of ± 18.000 rpm for 3 minutes. The eggshell powder was then sieved through a 100 mesh sieve. The finished eggshell powder was put into OPP (Oriented Polystyrene) plastic and stored at 4°C for analysis.

Eggshell nanocalcium powder preparation (Khoerunnisa, 2011)

Eggshell powder was soaked in 1N HCl (1:5) for 48 hours and extracted at 90°C for 1 hour. The extracts were filtered using filter paper to obtain the filtrate and precipitate. The filtrate was precipitated with the addition of 3N NaOH and stirred, and then allowed to stand until a precipitate was formed. The precipitate was then neutralized using aquabidest until the pH was neutral. The solution is separated from the precipitate by pouring it slowly so that the precipitate does not get wasted. The precipitate was dried in oven for 3 hours at a temperature of 105°C, followed by burning in muffle furnace at a temperature of 600°C for 5 hours, and then crushed using a mortar to obtain nanocalcium powder. The nanocalcium powder was vacuum packed and stored at 40°C.

Mung bean germination (Wea et al., 2014)

The mung beans were sorted and washed with running water until clean and then soaked for 6 hours. The mung beans are drained and placed on tray with a damp cloth. The green beans is placed in room temperature and germinated for 6, 12 and 18 hours, where every 6 hours watering is done with water. Germinated mung beans are separated from the skin and taken as much as 100 grams for further processing.

Germinated mung bean drinks preparation (Wea et al., 2014)

The germinated mung beans were blanched by boiling at 80°C for 2 minutes, peeled and then mashed with a blender for 3 minutes, added with water in a ratio of 1: 7.5. The germinated mung bean drinks is filtered and then cooked by adding nanocalcium eggshell and 7% sugar. Cooking is carried out until it boils and stirred for 5 minutes then cooled for 30 minutes.

Analysis

The analysis of the germinated mung bean drinks were chemical characteristics: protein content using Lowry method (Harjanto, 2017), calcium content was using AAS method (AOAC, 2005), vitamin C content (Sudarmadji et al. 2007), pH value (Sudarmadji et al., 2014),

and physical characteristics: viscosity (Yuwono and Susanto, 1998) and stability (Wibowo et al., 2014).

Statistical analysis

All analyses were performed in triplicates. The data were subjected to the analysis of variance followed by Fisher's least significant difference (LSD) test to compare among treatment means and control. Differences were considered at a significant level of 95% ($p < 0.05$) by using SPSS v.19 software.

RESULTS

Analysis of the characteristics of germinated mung bean drink with nanocalcium fortification

The interaction between germination time and the type of eggshell nanocalcium no significant effect on all characteristics of germinated mung bean drink, excepted for Vitamin C and calcium that were higher in all treatments compared to control (Table 1). The non-significance among the treatments was attributed to addition of the nanocalcium (CaO) powder of chicken and duck eggshells to the germinated mung bean drinks in the same amount (20% of nutrition facts label for calcium). Moreover, the characteristics of the eggshell nanocalcium (CaO) powder were white color, very fine texture (particle size of CaO in chicken and duck eggshells is 41.54 nm and 24.90 nm respectively), and neutral pH so that it take to the no significant effect on the value of viscosity, stability, protein, pH and vitamin C of germinated mung bean drink (Table 2).

Germination time was significantly effect the characteristics of germinated mung been drinks (Table 3).

DISCUSSION

Table 1 shows a significant increase in the vitamin C and calcium content of germinated mung bean drinks to the control. The increase in vitamin C in germinated mung bean drinks in all treatments in this study was due to the food reserves contained in the cotyledons in the form of starch hydrolyzed by the amylase enzyme into simple sugars which were then synthesized into vitamin C during the germination process. The results of this study were in line with the opinion of Ebert et al. (2017) which stated that the content of vitamin C in the germinated peanut seeds increased by 2.7 times compared to the ungerminated seed.

The calcium content of germinated mung bean drinks fortified with nanocalcium of duck eggshell was higher than the calcium content of germinated mung bean drinks fortified with nanocalcium of chicken eggshell. This was

Table 1: Physical and chemical characteristics of germinated mung bean drinks with nanocalcium fortification

Treatments	Parameter					
	Viscosity (mPa.s)	Stability (%)	Protein (%)	pH	Vitamin C (mg/100g)	Calcium (%)
A ₁ B ₁	27.60 ± 0.20 ^a	87.00 ± 1.00 ^a	1.90 ± 0.05 ^a	6.81 ± 0.02 ^a	8.21 ± 1.02 ^a	17.82
A ₁ B ₂	27.13 ± 0.15 ^a	85.67 ± 1.53 ^a	1.81 ± 0.09 ^a	6.77 ± 0.04 ^a	7.62 ± 1.02 ^a	24.82
A ₂ B ₁	26.17 ± 0.29 ^a	73.00 ± 1.00 ^a	1.77 ± 0.11 ^a	6.74 ± 0.04 ^a	11.73 ± 1.01 ^a	16.45
A ₂ B ₂	26.03 ± 0.45 ^a	71.67 ± 1.53 ^a	1.66 ± 0.14 ^a	6.69 ± 0.04 ^a	11.14 ± 1.01 ^a	23.66
A ₃ B ₁	25.53 ± 0.21 ^a	65.67 ± 0.58 ^a	1.56 ± 0.14 ^a	6.63 ± 0.04 ^a	14.66 ± 1.01 ^a	15.66
A ₃ B ₂	24.97 ± 0.76 ^a	64.67 ± 1.53 ^a	1.50 ± 0.18 ^a	6.62 ± 0.01 ^a	13.49 ± 1.02 ^a	22.49
Control	28.80 ± 0.01 ^a	91.33 ± 0.58 ^a	2.01 ± 0.16 ^a	6.81 ± 0.02 ^a	5.28 ± 1.76 ^b	nd

Numbers marked with the same letter notation in the same column indicate not significantly different (5%)

Data = mean ± standard deviation

Control = germination time of green beans 0 hours without the addition of nanocalcium

A₁ = mung bean germination time for 6 hours B₁ = nanocalcium of chicken eggshell

A₂ = mung bean germination time for 12 hours B₂ = nanocalcium of duck eggshell

A₃ = mung bean germination time for 18 hours

Table 2: Effect of eggshell nanocalcium fortification on the characteristics of germinated mung bean drinks

Treatments	Parameter				
	Viscosity (mPa.s)	Stability (%)	Protein (%)	pH*	Vitamin C (mg/100g)
B ₁	26.43 ± 0.23 ^a	75.22 ± 0.86 ^a	1.74 ± 0.10 ^a	6.73 ± 0.03 ^a	11.54 ± 1.02 ^a
B ₂	26.04 ± 0.46 ^a	74.00 ± 1.53 ^a	1.66 ± 0.13 ^a	6.69 ± 0.03 ^a	10.75 ± 1.02 ^a

Numbers marked with the same letter notation in the same column indicate not significantly different (5%)

Data = mean ± standard deviation

B₁ = nanocalcium of chicken eggshell

B₂ = nanocalcium of duck eggshell

Table 3: The effect of germination time on the characteristics of germinated mung bean drinks

Treatments	Parameter				
	Viscosity (mPa.s)	Stability (%)	Protein (%)	pH	Vitamin C (mg/100g)
A ₁	27.37 ± 0.49 ^a	86.33 ± 1.05 ^c	1.85 ± 0.16 ^b	6.79 ± 0.02 ^c	7.92 ± 1.02 ^a
A ₂	26.10 ± 0.37 ^b	72.33 ± 1.26 ^b	1.72 ± 0.13 ^{ab}	6.72 ± 0.04 ^b	11.44 ± 1.01 ^b
A ₃	25.25 ± 0.18 ^c	65.17 ± 1.25 ^a	1.53 ± 0.07 ^a	6.62 ± 0.03 ^a	14.08 ± 1.02 ^c

Numbers marked with the same letter notation in the same column indicate not significantly different (5%)

Data = mean ± standard deviation

A₁ = mung bean germination time for 6 hours

A₂ = mung bean germination time for 12 hours

A₃ = mung bean germination time for 18 hours

because the calcium content in duck eggshell nanocalcium (45.50%) was higher than the calcium level in chicken eggshell nanocalcium (35.50%).

VISCOSITY

Viscosity is a measure of the size of the friction that occurs in the fluid. The greater the viscosity of a food, the thicker the material, and vice versa (Srihidayati, 2017). The viscosity of germinated mung bean drinks in the control treatment (0 hour germination without nanocalcium fortification) was higher (28.80±0.01 m.Pa.s) compared to that of nanocalcium fortified germinated mung bean drinks for all treatments in this study. This is due to the fact that ungerminated mung bean starch has not been hydrolyzed so that it still has higher starch content than germinated mung bean starch. In the preparation of mung bean drinks, starch will undergo gelatinization in the presence of heat treatment which will cause the starch granules to break and

the starch molecules to come out and be released from the granules and then enter the solution system. The results of this study are in line with the results of research conducted by Nurjanati et al. (2018), based on the hedonic test, ungerminated red bean milk (0 hours) has a higher viscosity than germinated red bean milk (8, 16 and 24 hours).

Table 3 shows that the viscosity of the green bean germination drink decreased significantly with the duration of germination. The decrease in the viscosity of the material during germination was caused by the hydrolysis of starch during the germination process by the amylase enzymes. The enzyme is formed at the beginning of germination by giberylic acid (Elobuikie et al., 2021). Starch will be broken down into simple sugars in the form of glucose which is used as energy and needs for seed growth. Another factor that causes a decrease in the viscosity of mung bean germination drink is the increase in vitamin C during the germination process which results in a decrease in pH as shown in Table 3. A decrease in pH

results in hydrolysis of glycosidic bonds which causes a decrease in viscosity. The results of this study are in line with the statement of Widjaja et al. (2019), that a solution will decrease its viscosity if its pH decreases.

STABILITY

The stability of the products can be seen by the presence or absence of precipitate in the product (Farikha et al., 2013). Germinated mung bean drinks in the control treatment (0 hour germination without the addition of nanocalcium) had a higher stability value (91%) compared to all treatments of germinated mung bean drinks fortified with nanocalcium in this study. This was because germinated mung bean seeds absorb more water than ungerminated mung bean seeds. During germination, bean seeds require optimal environmental conditions that are 50-80% RH (Yuwariah et al., 2015). Water absorption in the germination process is needed to maintain cell turgidity, including cell enlargement, photosynthetic reactions, salt solvents, gases and other substances that are transported between cells in tissues to maintain cell growth (Felania, 2017).

Table 3 shows that the stability of the germinated mung bean drinks decreased significantly as the duration of germination increased. This was due to an increase in the water content of germinated mung beans along with the length of germination due to the watering the mung bean seeds every 6 hours during germination time. The water content of germinated mung bean with a time of 0, 6, 12 and 18 hours were 4.32%, 4.99%, 5.42% and 7.22%, respectively. The increase in water content in germinated mung beans resulting germinated mung bean drinks have more water content in turns it affects the stability value of the drinks. In line with the viscosity value of the germinated mung bean drinks, the average value of stability of each treatment decreased with the duration of germination. The decrease in viscosity at germinated mung bean drinks correlated to that in stability.

PROTEIN CONTENT

The protein of germinated mung bean drinks in treatment A2 (12 hours of germination time) was not significantly different from that of treatment A1 (6 hours of germination time) and treatment A3 (12 hours of germination time). The decreasing of protein content in germinated mung bean drinks was due to the hydrolysis of protein into amino acids that are used for embryo growth. The results of this study were in line with the statement of Masood et al. (2014) which stated a decrease in protein content along with an increase in amino acid content during germination of green beans. This is due to an increase in the activity of

the protease enzyme which is an endogenous enzyme that is active during the imbibition process, when green beans are in contact with water. Proteases hydrolyze proteins into peptides and amino acids (Ferdiawan et al., 2019). Free amino acids along with glutamic and aspartic acids (in the form of amides) will be translocated to the embryo in the formation of new structures in line with the ongoing germination stage (Pertivi et al., 2013).

pH VALUE

pH is a parameter used to determine changes in the acidity level of a food product (Widowati et al., 2020). Table 3 shows that the longer the germination time, the lower the pH of the germinated mung bean drinks significantly. According to Wea et al. (2014), in germinated seed plants there was an increase in vitamin synthesis, especially vitamin C (Wea et al., 2014). This situation is in accordance with the vitamin C content in the germinated mung bean drinks in this study (Table 3) so that the pH of the germinated mung bean drink is low. Based on Indonesian National Standard 01-3830-1995, the pH standard for bean drinks is 6.5-7.0. The pH of germinated mung bean drinks with nanocalcium fortification in this study has a value of 6.62-6.79; it has met the pH standard of bean drinks.

VITAMIN C

Table 3 shows that the vitamin C of the germinated mung bean drinks increased significantly with increasing of germination time. 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 causes reactivation of the enzyme L-Galactono- γ -lactone dehydrogenase which is involved in the 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 germination of mung beans can help the solubility of nanocalcium fortification in the germinated mung bean drinks. This is in line with the statement of Yonata et al. (2017), that pH can affect the solubility of minerals. Acidic conditions and small particle size can increase the speed of nanocalcium dissolution from both duck and chicken eggshells, so that the germinated mung bean drinks with eggshells nanocalcium fortification has good digestibility.

Calcium content

Based on the results of the analysis, calcium content of nanocalcium in chicken and duck eggshells was 35.50% and 45.50%, respectively. Calcium content in eggshells is influenced by the thickness and type of eggshell. Duck eggshells have an average thickness of 0.36 - 0.46 mm (Septiana et al., 2015), while the average thickness of chicken eggshells was 0.33 - 0.35 mm (Azizah et al., 2015). Differences in eggshell thickness can affect the amount of mineral content and organic salts, especially calcium carbonate (CaCO₃) which acts as a source of calcium in eggshells.

Fortification is a process of increasing the content of essential micronutrients in the form of vitamins and minerals into food, aiming to increase nutrients that do not yet exist or to enrich existing nutrients (Valentina et al., 2014). According to the Indonesian Food and Drug Supervisory Agency (2019), the minimum amount of micronutrient fortification in foodstuffs is 10% of the RDI per serving. In this study, the amount of nanocalcium fortification of chicken and duck eggshells in germinated mung bean drink was 20% of the RDI for calcium per 250 mL serving.

Calcium content of nongerminated mung bean drinks with nanocalcium fortification of chicken and duck eggshells was 18.97% and 25%, respectively. This showed that the calcium content of germinated mung bean drinks both fortified with nanocalcium chicken and duck eggshells decreased with the increasing of germination time (Table 1). This was in accordance with the statement of Oghbaei and Prakash (2020) that the calcium content decreased significantly in germinated and peeled bean seeds. Peeling can reduce some of the minerals present in the skin of green beans. However, this germination and skin peeling treatment had an impact on increasing the bioavailability of mung beans which contributed to stimulating the reduction of anti-nutritional substances. The percentage of bioavailability in the seeds before germination was 14.91–17.19% and in the germinated beans which were peeled 22.96–25.27%.

In addition, the decrease in calcium content of germinated mung bean drinks in this study was thought to be due to the treatment with germination time less than 24 hours so that the phytase enzyme had not worked optimally, causing the complex phytic acid that binds to minerals such as calcium could not be completely released. Based on the results of research by Ghavidel and Davoodi (2011), enzyme activity (phytase, amylase and protease) increased significantly at 24, 48, and 72 hours of germination in several types of legumes, one of which was green beans. The maximum enzymatic activity occurred at 72 hours of germination. Although the calcium of germinated mung bean drinks

decreased in the 6, 12 and 18 hour germination treatment, but with an increase in vitamin C at the same germination time treatment it could increase the calcium absorption efficiency of the germinated mung bean drinks.

CONCLUSION

Nanocalcium fortification of chicken and duck eggshells by 20% of the RDI for calcium per 250 mL serving had no significant effect on the physical (viscosity and stability) and chemical (protein, pH, and vitamin C) characteristics of germinated mung bean drinks. Germination of mung beans at different times was significantly increased value of pH, vitamin C, protein, and stability; but significantly decreased value of viscosity. Germinated mung bean drinks are a good carrier for chicken and duck eggshells nanocalcium fortification.

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Author Queries???

AQ1: Kindly check the reference

AQ2: Kindly cite the reference Azis, Putri, Aprilia, Ayuliasari, Hartini, Putra. 2018 in the text part

AQ3: Kindly provide in English language

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**3. Bukti Comment untuk Reviewer
dan Artikel yang di Resubmit
(2 Januari 2023)**

REVISION

No.	Queries	Comment
AQ 1	: The references have already checked	
AQ 2	: Azis, M. Y. et al., 2018. Eksplorasi Kadar Kalsium (Ca) dalam Limbah Cangkang Kulit Telur Bebek dan Burung Puyuh Menggunakan Metode Titrasi dan AAS. <i>Al-Kimiya</i> , 5(2), Pp. 74-77.	The reference cited in line number 65
AQ 3	: The references have already provided in English language	
AQ 4	: Badan Penelitian dan Pengembangan (BaLitbang). 2019. <i>Balai Penelitian Tanaman Aneka Kacang dan Umbi</i> . (Online). http://www.litbang.pertanian.go.id/varietas/546/ . Diakses pada 18 Maret 2021.	The reference cited in line number 34
AQ 5	: Badan Pengawas Obat dan Makanan Republik Indonesia. 2019. Peraturan Badan Pengawas Obat dan Makanan Nomor 22 Tahun 2019 tentang Informasi Nilai Gizi Pada Label Pangan Olahan. Jakarta: BPOM.	The reference cited in line number 112
AQ 6	: Badan Standarisasi Nasional.1995. <i>Susu Kedelai</i> . Sni 01-3830-1995. Badan Standarisai Nasional. Jakarta: BSN.	The reference cited in line number 278
AQ 7	: Daftar Komposisi Bahan Makanan (DKBM). 2017. <i>Tabel Komposisi Pangan Indonesia</i> . Jakarta: PT. Elex Media Komputindo.	The reference cited in line number 32
AQ 8	: Handayani, L. dan Syahputra, F., 2017. Isolasi dan Karakterisasi Nanokalsium dari Cangkang Tiram (<i>Crassostrea gigas</i>). <i>JPHP</i> , 20(3), pp. 515-523.	The reference cited in line number 84
AQ 9	: Hanura, A. B., Trilaksani, W. dan Suptijah, P., 2017. Karakterisasi Nanohidroksiapatit Tulang Tuna Thunnus Sp. Sebagai Sediaan Biomaterial. <i>Jurnal Ilmu Dan Teknologi Kelautan Tropis</i> , 9(2), Pp. 619-629.	The reference cited in line number 84
AQ 10	: Author initial: NM, TWW. MIS, RCP	
AQ 11	: Purwanto, M. G. M., 2014. Perbandingan Analisa Kadar Protein Terlarut dengan Berbagai Metode Spektroskopi UV-Visible. <i>Jurnal Ilmiah Sains dan Teknologi</i> , 7(2), pp. 64-71.	The reference cited in line number 149
AQ 12	: Sunardi, S., Krismawati, E. D. dan Mahayana, A., 2020. Sintesis dan Karakterisasi Nanokalsium Oksida dari Cangkang Telur. <i>ALCHEMY Jurnal Penelitian Kimia</i> , 16(2), pp. 250-259.	The reference cited in line number 85
AQ13	: Received: September 26, 2022 Accepted: January 13, 2023.	
AQ14	: Research Article	

1 Effect of germination and eggshells nanocalcium fortification on characteristics of germinated
2 mung bean drink (*Vigna radiata*.)

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9 ABSTRACT

10 Germinated mung bean drinks with eggshell nanocalcium fortification are an alternative beverage to
11 replace milk. This study aimed to determine the effect of germination time and eggshells
12 nanocalcium fortification on physicochemical characteristics of the germinated mung bean drink.

13 This study used a Factorial Completely Randomized Design with two treatments factors. The first
14 factor was germination time which consisted of three treatment levels (6, 12 and 18 hours) and the
15 second factor was the type of eggshell nanocalcium which consisted of two type of treatment
16 (chicken and duck eggshell), plus the control treatment as mung bean drink without germination
17 and addition of eggshell nanocalcium. Each experiment was repeated three times. Observed
18 parameters in this study were chemical characteristics (protein, calcium, vitamin C, and pH) and
19 physical characteristics (viscosity and stability) of germinated mung bean drink. The results showed
20 that the germination time treatment significantly affected the value of protein, calcium, vitamin C,
21 pH, viscosity, and stability of the germinated mung bean drink. Germination increased vitamin C in
22 mung beans with an average value of 8,21-14,66 mg/100 g. Eggshell nanocalcium fortification
23 showed a significant effect on increasing calcium of germinated mung bean drink. Germinated
24 mung bean drink produced from 6 hours of germination and fortification of duck nanocalcium had
25 the highest calcium content with value of 24.82%.

26 **Key words:** physicochemical, chicken, duck, calcium, vitamin C

Introduction

To meet the calcium needs of the Indonesian people who generally suffer from lactose intolerance and digestive disorders after consuming milk, it is necessary to drink milk substitutes made from plant-based foods that contain high protein and calcium. Mung bean is the third highest source of vegetable protein after soybeans and peanuts with a content of protein around 20-25% (Purwono and Hartono, 2005; Food Composition List, 2017; Hastuti et al., 2018). One of the superior varieties of mung beans is VIMA-1 (*Vigna sinensis*–Malang). VIMA-1 has a promising market potential due to its high protein content (28.02%), low fat (0.40%), and high starch content (67.62%) (Agency for Research and Development, 2019). However, mung beans have anti-nutritional compounds, compounds found naturally in various types of beans, which can prevent the absorption of nutrients in the body.

One process that can increase the nutrition in beans is germination. Germinated mung beans have improved nutritional, functional and biological properties by changing the content, nutritional composition and bioactive compounds, as well as eliminating anti-nutritional factors in beans (Liu et al., 2020). Germination is a condition in which dormant seeds begin to germinate and grow into seedlings under the right growing conditions. Germination in brown rice refers to the point where the growth of a radicle ranges from 2 mm to 5 mm, and pre-germination brown rice is the stage with an expanded a radicle exposed approximately 0.5–1 mm (Watanabe et al., 2004). Moreover, Palmiano and Juliano (1972) stated that the optimal germination period was 18 to 24 hours.

The germination process in mung bean seeds is able to increase the nutritional value by activating enzymes that can reduce or eliminate anti-nutritional factors (Ebert et al. 2017). Phytic acid, the main anti-nutritional substance found in green beans, can be reduced from 12.0 mg/g to 4.03 mg/g through the germination process (Faradilla et al., 2012). Furthermore, germination is able to reduce anti-nutritional substances in germinated bean flour by 66.70% (Nur et al., 2019), so the

53 bioavailability of vitamins and minerals in the flour increase. This is because the germination
54 process can release the bound form of vitamins and minerals into a freer form so that it is more
55 easily digested and absorbed by the human digestive tract. Previous research explained that during
56 germination there is a breakdown of complex molecules into simpler forms which causes
57 germination of green beans to be more easily digested and absorbed by the human body (Ghavidel
58 and Prakash, 2007; Masood et al., 2014). In addition, the content of vitamin C increased with the
59 increasing duration time of germination. Ebert et al. (2017) stated that the average vitamin C in
60 germinated bean was 2.7 times higher than that without germination.

61

62 The eggshells of chickens and ducks are household waste that has not been utilized optimally due to
63 the lack of public knowledge about the contents in it. Eggshells consist of 95.1% salt, 3.3% organic
64 matter, and 1.6% water. The main component of inorganic salts in eggshells is dominated by
65 calcium carbonate (CaCO_3) of 98.5% (Nurjayanti et al., 2012; Azis et al., 2018). The bioavailability
66 of eggshells is $\pm 40\%$ (Szeleszczuk et al., 2015) with calcium content in chicken and duck eggshells
67 of 25.73% and 23.67%, respectively (Aminah and Meikawati, 2016).

68

69 Calcium contained in eggshells has the potential as an alternative source of calcium that can be
70 fortified in germinated mung bean drinks as a substitute for milk. Calcium is generally available in
71 micro size which is thought to be only able to absorb 50% of the total calcium consumed in its
72 metabolism. The nano form of eggshell calcium can make it easier for calcium to be more soluble
73 and optimally absorbed. According to Widyastuti and Kusuma (2017), nanocalcium is the
74 manufacture of particles with a size of less than 100 nm by changing the nature or function of a
75 material.

76

77 There are two methods in the manufacture of nanoparticles, namely top down (physical method)
78 and bottom up (chemical method). The bottom up method is a method of making nanoparticles by

79 arranging atoms or molecules to form nanometer-sized particles from a solution. In the bottom up
80 method, the formation of nanoparticles has a high degree of cohesion so that it is able to produce a
81 more uniform size. One of the bottom-up methods that is often used is precipitation because this
82 method is very effective in the manufacture of nanoparticles, the process is simple, and requires low
83 costs. The precipitation method is carried out by controlling the solubility of the material in the
84 solution through changes in pH (Suptijah et al., 2012; Handayani and Syahputra, 2017; Hanura et
85 al., 2017; Sunardi et al, 2020). Therefore, the objective of this study was to investigate the effect of
86 mung bean germination time and nanocalcium fortification of eggshells on the characteristics of
87 germinated mung bean drink (*Vigna radiata*).

88

89 The hypothesis of this study was it is suspected that germination time and type of eggshell
90 nanocalcium fortification has a significant effect on the physico-chemical characteristics of the
91 resulting germinated mung bean drink.

92

93

Material and Methods

94 The materials used in this study were VIMA-1 mung beans, eggshells (chicken and duck), 1N HCL,
95 3N NaOH, aquabidest, and other chemicals for analysis. The tools used were 100 mesh sieve,
96 Beaker glass (Phyrex, Japan), blender (Philips HR 2116, China), Erlenmeyer (Iwaki, Japan), hot
97 plate, muffle furnace (Barnstead FB1410M-26, USA), mortar, electric oven (Mettler, USA),
98 measuring pipette (Iwaki, Japan), pH meter (Oakton do-35613, USA), Spectrophotometer (UV-
99 VISAE S80, China), AAS (Thermo Scientific NicoletTM10, USA), analytical balance (Ohaus
100 AR2140, USA), and a Brookfield viscometer (NDJ-8S, China).

101

102

Research Methods

103 This research was carried out at the Agricultural Product Chemistry Laboratory and Agricultural
104 Product Processing Laboratory, Faculty of Agriculture, Sriwijaya University, South Sumatera,

105 Indonesia (-3.2196118175204167, 104.64789811916464). This research was conducted using a
106 completely randomized factorial design with two treatment factors, germination time of mung bean
107 (A) which consisted of 3 treatment levels (A1 = 6 hours; A2 = 12 hours; and A3 = 18 hours) and the
108 type of eggshell nanocalcium (B) which consisted of 2 treatment (B1 = chicken eggshell, and B2 =
109 duck eggshell). The treatment was repeated 3 times. Mung bean drink without germination and
110 addition of eggshell nanocalcium was a control. Addition of eggshell nanocalcium to all treatments
111 was 20% of the Recommended Dietary Intake for calcium, which was 1200 mg/day per serving size
112 (240 mL) (Food and Drug Supervisory Agency of the Republic of Indonesia, 2019).

113

114

Research Implementation

Eggshell micro powder preparation (Rahmawati and Nisa, 2015)

116 Preparation of eggshell micro powder was carried out by preparing 500 g for each chicken and duck
117 eggshells and washed with running water until clean. The eggshells were boiled at 100°C for 3
118 minutes to kill pathogenic microbes, and then the eggshells were drained. The eggshells were baked
119 for 3 hours at 60°C, and placed at room temperature. The cold eggshells were mashed using a
120 blender at a speed of ± 18.000 rpm for 3 minutes. The eggshell powder was then sieved through a
121 100 mesh sieve. The finished eggshell powder was put into OPP (Oriented Polystyrene) plastic and
122 stored at 4°C for analysis.

123

Eggshell nanocalcium powder preparation (Khoerunnisa, 2011)

125 Eggshell powder was soaked in 1N HCl (1:5) for 48 hours and extracted at 90°C for 1 hour. The
126 extracts were filtered using filter paper to obtain the filtrate and precipitate. The filtrate was
127 precipitated with the addition of 3N NaOH and stirred, and then allowed to stand until a precipitate
128 was formed. The precipitate was then neutralized using aquabidest until the pH was neutral. The
129 solution is separated from the precipitate by pouring it slowly so that the precipitate does not get
130 wasted. The precipitate was dried in oven for 3 hours at a temperature of 105°C, followed by

131 burning in muffle furnace at a temperature of 600°C for 5 hours, and then crushed using a mortar to
132 obtain nanocalcium powder. The nanocalcium powder was vacuum packed and stored at 4°C.

133

134 **Mung bean germination (Wea et al., 2014)**

135 The mung beans were sorted and washed with running water until clean and then soaked for 6
136 hours. The mung beans are drained and placed on tray with a damp cloth. The green beans is placed
137 in room temperature and germinated for 6, 12 and 18 hours, where every 6 hours watering is done
138 with water. Germinated mung beans are separated from the skin and taken as much as 100 grams
139 for further processing.

140

141 **Germinated mung bean drinks preparation (Wea et al., 2014)**

142 The germinated mung beans were blanched by boiling at 80°C for 2 minutes, peeled and then
143 mashed with a blender for 3 minutes, added with water in a ratio of 1: 7.5. The germinated mung
144 bean drinks is filtered and then cooked by adding nanocalcium eggshell and 7% sugar. Cooking is
145 carried out until it boils and stirred for 5 minutes then cooled for 30 minutes.

146

147 **Analysis**

148 The analysis of the germinated mung bean drinks were chemical characteristics: protein content
149 using Lowry method (Purwanto, 2014; Harjanto, 2017), calcium content was using AAS method
150 (AOAC, 2005), vitamin C content (Sudarmadji et al. 2007), pH value (Sudarmadji et al., 2014), and
151 physical characteristics: viscosity (Yuwono and Susanto, 1998) and stability (Wibowo et al., 2014).

152

153 **Statistical analysis**

154 All analyses were performed in triplicates. The data were subjected to the analysis of variance
155 followed by Fisher's least significant difference (LSD) test to compare among treatment means and

156 control. Differences were considered at a significant level of 95% ($p < 0.05$) by using SPSS v.19
 157 software.

158 **Results**

159 **Analysis of the characteristics of germinated mung bean drink with nanocalcium fortification**

160 The interaction between germination time and the type of eggshell nanocalcium no significant
 161 effect on all characteristics of germinated mung bean drink, excepted for Vitamin C and calcium
 162 that were higher in all treatments compared to control (Table 1). The non-significance among the
 163 treatments was attributed to addition of the nanocalcium (CaO) powder of chicken and duck
 164 eggshells to the germinated mung bean drinks in the same amount (20% of nutrition facts label for
 165 calcium). Moreover, the characteristics of the eggshell nanocalcium (CaO) powder were white
 166 color, very fine texture (particle size of CaO in chicken and duck eggshells is 41.54 nm and 24.90
 167 nm respectively), and neutral pH so that it take to the no significant effect on the value of viscosity,
 168 stability, protein, pH and vitamin C of germinated mung bean drink (Table 2).

169

170 Table 1. Physical and chemical characteristics of germinated mung bean drinks with nanocalcium
 171 fortification

Treatments	Parameter					
	Viscosity (mPa.s)	Stability (%)	Protein (%)	pH	Vitamin C (mg/100g)	Calcium (%)
A ₁ B ₁	27.60±0.20 ^a	87.00±1.00 ^a	1.90±0.05 ^a	6.81±0.02 ^a	8.21±1.02 ^a	17.82
A ₁ B ₂	27.13±0.15 ^a	85.67±1.53 ^a	1.81±0.09 ^a	6.77±0.04 ^a	7.62±1.02 ^a	24.82
A ₂ B ₁	26.17±0.29 ^a	73.00±1.00 ^a	1.77±0.11 ^a	6.74±0.04 ^a	11.73±1.01 ^a	16.45
A ₂ B ₂	26.03±0.45 ^a	71.67±1.53 ^a	1.66±0.14 ^a	6.69±0.04 ^a	11.14±1.01 ^a	23.66
A ₃ B ₁	25.53±0.21 ^a	65.67±0.58 ^a	1.56±0.14 ^a	6.63±0.04 ^a	14.66±1.01 ^a	15.66
A ₃ B ₂	24.97±0.76 ^a	64.67±1.53 ^a	1.50±0.18 ^a	6.62±0.01 ^a	13.49±1.02 ^a	22.49
Control	28.80±0.01 ^a	91.33±0.58 ^a	2.01±0.16 ^a	6.81±0.02 ^a	5.28±1.76 ^b	nd

172 Note: Numbers marked with the same letter notation in the same column indicate not significantly
 173 different (5%)

174 Data = mean ± standard deviation

175 Control = germination time of green beans 0 hours without the addition of nanocalcium
 176 A₁ = mung bean germination time for 6 hours B₁ = nanocalcium of chicken eggshell
 177 A₂ = mung bean germination time for 12 hours B₂ = nanocalcium of duck eggshell
 178 A₃ = mung bean germination time for 18 hours
 179

180 Table 2. Effect of eggshell nanocalcium fortification on the characteristics of germinated mung
 181 bean drinks

Treatments	Parameter				
	Viscosity (mPa.s)	Stability (%)	Protein (%)	pH*	Vitamin C (mg/100g)
B ₁	26.43±0.23 ^a	75.22±0.86 ^a	1.74±0.10 ^a	6.73±0.03 ^a	11.54±1.02 ^a
B ₂	26.04±0.46 ^a	74.00±1.53 ^a	1.66±0.13 ^a	6.69±0.03 ^a	10.75±1.02 ^a

182 Note: Numbers marked with the same letter notation in the same column indicate not significantly
 183 different (5%)
 184 Data = mean ± standard deviation
 185 B₁ = nanocalcium of chicken eggshell
 186 B₂ = nanocalcium of duck eggshell
 187

188 Germination time was significantly effect the characteristics of germinated mung been drinks
 189 (Table 3).
 190

191 Table 3.The effect of germination time on the characteristics of germinated mung bean drinks

Treatments	Parameter				
	Viscosity (mPa.s)	Stability (%)	Protein (%)	pH	Vitamin C (mg/100g)
A ₁	27.37±0.49 ^a	86.33±1.05 ^c	1.85±0.16 ^b	6.79±0.02 ^c	7.92±1.02 ^a
A ₂	26.10±0.37 ^b	72.33±1.26 ^b	1.72±0.13 ^{ab}	6.72±0.04 ^b	11.44±1.01 ^b
A ₃	25.25±0.18 ^c	65.17±1.25 ^a	1.53±0.07 ^a	6.62±0.03 ^a	14.08±1.02 ^c

192 Note: Numbers marked with the same letter notation in the same column indicate not significantly
 193 different (5%)
 194 Data = mean ± standard deviation
 195 A₁ = mung bean germination time for 6 hours
 196 A₂ = mung bean germination time for 12 hours
 197 A₃ = mung bean germination time for 18 hours
 198

199

Discussion

200 Table 1 shows a significant increase in the vitamin C and calcium content of germinated mung bean
201 drinks to the control. The increase in vitamin C in germinated mung bean drinks in all treatments in
202 this study was due to the food reserves contained in the cotyledons in the form of starch hydrolyzed
203 by the amylase enzyme into simple sugars which were then synthesized into vitamin C during the
204 germination process. The results of this study were in line with the opinion of Ebert et al. (2017)
205 which stated that the content of vitamin C in the germinated peanut seeds increased by 2.7 times
206 compared to the ungerminated seed.

207

208 The calcium content of germinated mung bean drinks fortified with nanocalcium of duck eggshell
209 was higher than the calcium content of germinated mung bean drinks fortified with nanocalcium of
210 chicken eggshell. This was because the calcium content in duck eggshell nanocalcium (45.50%)
211 was higher than the calcium level in chicken eggshell nanocalcium (35.50%).

212

213 Viscosity

214 Viscosity is a measure of the size of the friction that occurs in the fluid. The greater the viscosity of
215 a food, the thicker the material, and vice versa (Srihidayati, 2017). The viscosity of germinated
216 mung bean drinks in the control treatment (0 hour germination without nanocalcium fortification)
217 was higher (28.80 ± 0.01 m.Pa.s) compared to that of nanocalcium fortified germinated mung bean
218 drinks for all treatments in this study. This is due to the fact that ungerminated mung bean starch
219 has not been hydrolyzed so that it still has higher starch content than germinated mung bean starch.
220 In the preparation of mung bean drinks, starch will undergo gelatinization in the presence of heat
221 treatment which will cause the starch granules to break and the starch molecules to come out and be
222 released from the granules and then enter the solution system. The results of this study are in line
223 with the results of research conducted by Nurjanati et al. (2018), based on the hedonic test,

224 ungerminated red bean milk (0 hours) has a higher viscosity than germinated red bean milk (8, 16
225 and 24 hours).

226

227 Table 3 shows that the viscosity of the green bean germination drink decreased significantly with
228 the duration of germination. The decrease in the viscosity of the material during germination was
229 caused by the hydrolysis of starch during the germination process by the amylase enzymes. The
230 enzyme is formed at the beginning of germination by giberylic acid (Elobuiké et al., 2021). Starch
231 will be broken down into simple sugars in the form of glucose which is used as energy and needs
232 for seed growth. Another factor that causes a decrease in the viscosity of mung bean germination
233 drink is the increase in vitamin C during the germination process which results in a decrease in pH
234 as shown in Table 3. A decrease in pH results in hydrolysis of glycosidic bonds which causes a
235 decrease in viscosity. The results of this study are in line with the statement of Widjaja et al. (2019),
236 that a solution will decrease its viscosity if its pH decreases.

237

238 **Stability**

239 The stability of the products can be seen by the presence or absence of precipitate in the product
240 (Farikha et al., 2013). Germinated mung bean drinks in the control treatment (0 hour germination
241 without the addition of nanocalcium) had a higher stability value (91%) compared to all treatments
242 of germinated mung bean drinks fortified with nanocalcium in this study. This was because
243 germinated mung bean seeds absorb more water than ungerminated mung bean seeds. During
244 germination, bean seeds require optimal environmental conditions that are 50-80% RH (Yuwariah
245 et al., 2015). Water absorption in the germination process is needed to maintain cell turgidity,
246 including cell enlargement, photosynthetic reactions, salt solvents, gases and other substances that
247 are transported between cells in tissues to maintain cell growth (Felania, 2017).

248

249 Table 3 shows that the stability of the germinated mung bean drinks decreased significantly as the
250 duration of germination increased. This was due to an increase in the water content of germinated
251 mung beans along with the length of germination due to the watering the mung bean seeds every 6
252 hours during germination time. The water content of germinated mung bean with a time of 0, 6, 12
253 and 18 hours were 4.32%, 4.99%, 5.42% and 7.22%, respectively. The increase in water content in
254 germinated mung beans resulting germinated mung bean drinks have more water content in turns it
255 affects the stability value of the drinks. In line with the viscosity value of the germinated mung bean
256 drinks, the average value of stability of each treatment decreased with the duration of germination.
257 The decrease in viscosity at germinated mung bean drinks correlated to that in stability.

258

259 **Protein Content**

260 The protein of germinated mung bean drinks in treatment A2 (12 hours of germination time) was
261 not significantly different from that of treatment A1 (6 hours of germination time) and treatment A3
262 (12 hours of germination time). The decreasing of protein content in germinated mung bean drinks
263 was due to the hydrolysis of protein into amino acids that are used for embryo growth. The results
264 of this study were in line with the statement of Masood et al. (2014) which stated a decrease in
265 protein content along with an increase in amino acid content during germination of green beans.
266 This is due to an increase in the activity of the protease enzyme which is an endogenous enzyme
267 that is active during the imbibition process, when green beans are in contact with water. Proteases
268 hydrolyze proteins into peptides and amino acids (Ferdiawan et al., 2019). Free amino acids along
269 with glutamic and aspartic acids (in the form of amides) will be translocated to the embryo in the
270 formation of new structures in line with the ongoing germination stage (Pertiwi et al., 2013).

271

272 **pH value**

273 pH is a parameter used to determine changes in the acidity level of a food product (Widowati et al.,
274 2020). Table 3 shows that the longer the germination time, the lower the pH of the germinated

275 mung bean drinks significantly. According to Wea et al. (2014), in germinated seed plants there was
276 an increase in vitamin synthesis, especially vitamin C (Wea et al., 2014). This situation is in
277 accordance with the vitamin C content in the germinated mung bean drinks in this study (Table 3)
278 so that the pH of the germinated mung bean drinka is low. Based on National Standardization
279 Agency (1995) SNI 01-3830-1995, the pH standard for bean drinks is 6.5-7.0. The pH of
280 germinated mung bean drinks with nanocalcium fortification in this study has a value of 6.62-6.79;
281 it has met the pH standard of bean drinks.

282

283 **Vitamin C**

284 Table 3 shows that the vitamin C of the germinated mung bean drinks increased significantly with
285 increasing of germination time. The increase in vitamin C along with germination time is caused by
286 several enzyme systems being active during germination. This happens because of the accumulation
287 of ascorbic acid as a result of biosynthesis. Germination causes reactivation of the enzyme L-
288 Galactono- γ -lactone dehydrogenase which is involved in the oxidation of L-galactono-1,4-lactone
289 to ascorbic acid. The activity of this enzyme increased in parallel to the biosynthesis of ascorbic
290 acid during the seed germination process. Differences in the level of ascorbic acid biosynthesis in
291 germination of mung bean seeds can also be influenced by legume varieties, maturity, climate, light,
292 harvesting and storage methods (Masood et al., 2014). Increasing the content of vitamin C in
293 germination of mung beans can help the solubility of nanocalcium fortification in the germinated
294 mung bean drinks. This is in line with the statement of Yonata et al. (2017), that pH can affect the
295 solubility of minerals. Acidic conditions and small particle size can increase the speed of
296 nanocalcium dissolution from both duck and chicken eggshells, so that the germinated mung bean
297 drinks with eggshells nanocalcium fortification has good digestibility.

298

299

300 Calcium Content

301 Based on the results of the analysis, calcium content of nanocalcium in chicken and duck eggshells
302 was 35.50% and 45.50%, respectively. Calcium content in eggshells is influenced by the thickness
303 and type of eggshell. Duck eggshells have an average thickness of 0.36 - 0.46 mm (Septiana et al.,
304 2015), while the average thickness of chicken eggshells was 0.33 - 0.35 mm (Azizah et al., 2015).
305 Differences in eggshell thickness can affect the amount of mineral content and organic salts,
306 especially calcium carbonate (CaCO₃) which acts as a source of calcium in eggshells.

307

308 Fortification is a process of increasing the content of essential micronutrients in the form of
309 vitamins and minerals into food, aiming to increase nutrients that do not yet exist or to enrich
310 existing nutrients (Valentina et al., 2014). According to the Indonesian Food and Drug Supervisory
311 Agency (2019), the minimum amount of micronutrient fortification in foodstuffs is 10% of the RDI
312 per serving. In this study, the amount of nanocalcium fortification of chicken and duck eggshells in
313 germinated mung bean drink was 20% of the RDI for calcium per 250 mL serving.

314

315 Calcium content of nongerminated mung bean drinks with nanocalcium fortification of chicken and
316 duck eggshells was 18.97% and 25%, respectively. This showed that the calcium content of
317 germinated mung bean drinks both fortified with nanocalcium chicken and duck eggshells
318 decreased with the increasing of germination time (Table 1). This was in accordance with the
319 statement of Oghbaei and Prakash (2020) that the calcium content decreased significantly in
320 germinated and peeled bean seeds. Peeling can reduce some of the minerals present in the skin of
321 green beans. However, this germination and skin peeling treatment had an impact on increasing the
322 bioavailability of mung beans which contributed to stimulating the reduction of anti-nutritional
323 substances. The percentage of bioavailability in the seeds before germination was 14.91–17.19%
324 and in the germinated beans which were peeled 22.96–25.27%.

325

326 In addition, the decrease in calcium content of germinated mung bean drinks in this study was
327 thought to be due to the treatment with germination time less than 24 hours so that the phytase
328 enzyme had not worked optimally, causing the complex phytic acid that binds to minerals such as
329 calcium could not be completely released. Based on the results of research by Ghavidel and
330 Davoodi (2011), enzyme activity (phytase, amylase and protease) increased significantly at 24, 48,
331 and 72 hours of germination in several types of legumes, one of which was green beans. The
332 maximum enzymatic activity occurred at 72 hours of germination. Although the calcium of
333 germinated mung bean drinks decreased in the 6, 12 and 18 hour germination treatment, but with an
334 increase in vitamin C at the same germination time treatment it could increase the calcium
335 absorption efficiency of the germinated mung bean drinks.

336

337

Conclusion

338 Nanocalcium fortification of chicken and duck eggshells by 20% of the RDI for calcium per 250
339 mL serving had no significant effect on the physical (viscosity and stability) and chemical (protein,
340 pH, and vitamin C) characteristics of germinated mung bean drinks. Germination of mung beans at
341 different times was significantly increased value of pH, vitamin C, protein, and stability; but
342 significantly decreased value of viscosity. Germinated mung bean drinks are a good carrier for
343 chicken and duck eggshells nanocalcium fortification.

344

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**4. Bukti Decision Letter untuk Article Accepted
(13 Januari 2023)**

January 13, 2023

Dear Nura Malahayati

I am pleased to inform you that your manuscript titled as "Effect of germination and eggshells nanocalcium fortification on characteristics of germinated mung bean drink (*Vigna radiata*.)" (Manuscript Number: EJFA-2022-09-277 was accepted for publication in the Emirates Journal of Food and Agriculture.

As we declared in "Instructions for Authors", you need to contribute to Emirates Journal of Food and Agriculture for Publication Fee (APC).

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**5. Bukti Artikel Published Online
(8 April 2023)**

RESEARCH ARTICLE

Effect of germination and eggshells nanocalcium fortification on characteristics of germinated mung bean drink (*Vigna radiata*.)

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ABSTRACT

Germinated mung bean drinks with eggshell nanocalcium fortification are an alternative beverage to replace milk. This study aimed to determine the effect of germination time and eggshells nanocalcium fortification on physicochemical characteristics of the germinated mung bean drink. This study used a Factorial Completely Randomized Design with two treatments factors. The first factor was germination time which consisted of three treatment levels (6, 12 and 18 hours) and the second factor was the type of eggshell nanocalcium which consisted of two type of treatment (chicken and duck eggshell), plus the control treatment as mung bean drink without germination and addition of eggshell nanocalcium. Each experiment was repeated three times. Observed parameters in this study were chemical characteristics (protein, calcium, vitamin C, and pH) and physical characteristics (viscosity and stability) of germinated mung bean drink. The results showed that the germination time treatment significantly affected the value of protein, calcium, vitamin C, pH, viscosity, and stability of the germinated mung bean drink. Germination increased vitamin C in mung beans with an average value of 8,21-14,66 mg/100 g. Eggshell nanocalcium fortification showed a significant effect on increasing calcium of germinated mung bean drink. Germinated mung bean drink produced from 6 hours of germination and fortification of duck nanocalcium had the highest calcium content with value of 24.82%.

Key words: Physicochemical, Chicken, Duck, Calcium, Vitamin C

INTRODUCTION

To meet the calcium needs of the Indonesian people who generally suffer from lactose intolerance and digestive disorders after consuming milk, it is necessary to drink milk substitutes made from plant-based foods that contain high protein and calcium. Mung bean is the third highest source of vegetable protein after soybeans and peanuts with a content of protein around 20-25% (Purwono and Hartono, 2005; Hastuti et al., 2018). One of the superior varieties of mung beans is VIMA-1 (*Vigna sinensis*–Malang). VIMA-1 has a promising market potential due to its high protein content (28.02%), low fat (0.40%), and high starch content (67.62%) (Agency for Research and Development, 2019). However, mung beans have anti-nutritional compounds, compounds found naturally in various types of beans, which can prevent the absorption of nutrients in the body.

One process that can increase the nutrition in beans is germination. Germinated mung beans have improved nutritional, functional and biological properties by changing the content, nutritional composition and bioactive compounds, as well as eliminating anti-nutritional factors in beans (Liu et al., 2020). Germination is a condition in which dormant seeds begin to germinate and grow into seedlings under the right growing conditions. Germination in brown rice refers to the point where the growth of a radicle ranges from 2 mm to 5 mm, and pre-germination brown rice is the stage with an expanded radicle exposed approximately 0.5–1 mm (Watanabe et al., 2004). Moreover, Palmiano and Juliano (1972) stated that the optimal germination period was 18 to 24 hours.

The germination process in mung bean seeds is able to increase the nutritional value by activating enzymes that can reduce or eliminate anti-nutritional factors (Ebert et al. 2017). Phytic acid, the main anti-nutritional substance

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found in green beans, can be reduced from 12.0 mg/g to 4.03 mg/g through the germination process (Faradilla et al., 2012). Furthermore, germination is able to reduce anti-nutritional substances in germinated bean flour by 66.70% (Nur et al., 2019), so the bioavailability of vitamins and minerals in the flour increase. This is because the germination process can release the bound form of vitamins and minerals into a freer form so that it is more easily digested and absorbed by the human digestive tract. Previous research explained that during germination there is a breakdown of complex molecules into simpler forms which causes germination of green beans to be more easily digested and absorbed by the human body (Ghavidel and Prakash, 2007; Masood et al., 2014). In addition, the content of vitamin C increased with the increasing duration time of germination. Ebert et al. (2017) stated that the average vitamin C in germinated bean was 2.7 times higher than that without germination.

The eggshells of chickens and ducks are household waste that has not been utilized optimally due to the lack of public knowledge about the contents in it. Eggshells consist of 95.1% salt, 3.3% organic matter, and 1.6% water. The main component of inorganic salts in eggshells is dominated by calcium carbonate (CaCO₃) of 98.5% (Nurjayanti et al., 2012). The bioavailability of eggshells is \pm 40% (Szeleszczuk et al., 2015) with calcium content in chicken and duck eggshells of 25.73% and 23.67%, respectively (Aminah and Meikawati, 2016).

Calcium contained in eggshells has the potential as an alternative source of calcium that can be fortified in germinated mung bean drinks as a substitute for milk. Calcium is generally available in micro size which is thought to be only able to absorb 50% of the total calcium consumed in its metabolism. The nano form of eggshell calcium can make it easier for calcium to be more soluble and optimally absorbed. According to Widyastuti and Kusuma (2017), nanocalcium is the manufacture of particles with a size of less than 100 nm by changing the nature or function of a material.

There are two methods in the manufacture of nanoparticles, namely top down (physical method) and bottom up (chemical method). The bottom up method is a method of making nanoparticles by arranging atoms or molecules to form nanometer-sized particles from a solution. In the bottom up method, the formation of nanoparticles has a high degree of cohesion so that it is able to produce a more uniform size. One of the bottom-up methods that is often used is precipitation because this method is very effective in the manufacture of nanoparticles, the process is simple, and requires low costs. The precipitation method is carried out by controlling the solubility of the material in the solution

through changes in pH (Suptijah et al., 2012). Therefore, the objective of this study was to investigate the effect of mung bean germination time and nanocalcium fortification of eggshells on the characteristics of germinated mung bean drink (*Vigna radiata*).

The hypothesis of this study was it is suspected that germination time and type of eggshell nanocalcium fortification has a significant effect on the physico-chemical characteristics of the resulting germinated mung bean drink.

MATERIAL AND METHODS

The materials used in this study were VIMA-1 mung beans, eggshells (chicken and duck), 1N HCL, 3N NaOH, aquabidest, and other chemicals for analysis. The tools used were 100 mesh sieve, Beaker glass (Pyrex, Japan), blender (Philips HR 2116, China), Erlenmeyer (Iwaki, Japan), hot plate, muffle furnace (Barnstead FB1410M-26, USA), mortar, electric oven (Mettler, USA), measuring pipette (Iwaki, Japan), pH meter (Oakton do-35613, USA), Spectrophotometer (UV-VISAE S80, China), AAS (Thermo Scientific NicoletTM10, USA), analytical balance (Ohaus AR2140, USA), and a Brookfield viscometer (NDJ-8S, China).

RESEARCH METHODS

This research was carried out at the Agricultural Product Chemistry Laboratory and Agricultural Product Processing Laboratory, Faculty of Agriculture, Sriwijaya University, South Sumatera, Indonesia (-3.2196118175204167, 104.64789811916464). This research was conducted using a completely randomized factorial design with two treatment factors, germination time of mung bean (A) which consisted of 3 treatment levels (A1 = 6 hours; A2 = 12 hours; and A3 = 18 hours) and the type of eggshell nanocalcium (B) which consisted of 2 treatment (B1 = chicken eggshell, and B2 = duck eggshell). The treatment was repeated 3 times. Mung bean drink without germination and addition of eggshell nanocalcium was a control. Addition of eggshell nanocalcium to all treatments was 20% of the Recommended Dietary Intake for calcium, which was 1200 mg/day per serving size (240 mL).

RESEARCH IMPLEMENTATION

Eggshell micro powder preparation (Rahmawati and Nisa, 2015)

Preparation of eggshell micro powder was carried out by preparing 500 g for each chicken and duck eggshells and

washed with running water until clean. The eggshells were boiled at 100°C for 3 minutes to kill pathogenic microbes, and then the eggshells were drained. The eggshells were baked for 3 hours at 60°C, and placed at room temperature. The cold eggshells were mashed using a blender at a speed of ± 18.000 rpm for 3 minutes. The eggshell powder was then sieved through a 100 mesh sieve. The finished eggshell powder was put into OPP (Oriented Polystyrene) plastic and stored at 4°C for analysis.

Eggshell nanocalcium powder preparation (Khoerunnisa, 2011)

Eggshell powder was soaked in 1N HCl (1:5) for 48 hours and extracted at 90°C for 1 hour. The extracts were filtered using filter paper to obtain the filtrate and precipitate. The filtrate was precipitated with the addition of 3N NaOH and stirred, and then allowed to stand until a precipitate was formed. The precipitate was then neutralized using aquabidest until the pH was neutral. The solution is separated from the precipitate by pouring it slowly so that the precipitate does not get wasted. The precipitate was dried in oven for 3 hours at a temperature of 105°C, followed by burning in muffle furnace at a temperature of 600°C for 5 hours, and then crushed using a mortar to obtain nanocalcium powder. The nanocalcium powder was vacuum packed and stored at 40°C.

Mung bean germination (Wea et al., 2014)

The mung beans were sorted and washed with running water until clean and then soaked for 6 hours. The mung beans are drained and placed on tray with a damp cloth. The green beans is placed in room temperature and germinated for 6, 12 and 18 hours, where every 6 hours watering is done with water. Germinated mung beans are separated from the skin and taken as much as 100 grams for further processing.

Germinated mung bean drinks preparation (Wea et al., 2014)

The germinated mung beans were blanched by boiling at 80°C for 2 minutes, peeled and then mashed with a blender for 3 minutes, added with water in a ratio of 1: 7.5. The germinated mung bean drinks is filtered and then cooked by adding nanocalcium eggshell and 7% sugar. Cooking is carried out until it boils and stirred for 5 minutes then cooled for 30 minutes.

Analysis

The analysis of the germinated mung bean drinks were chemical characteristics: protein content using Lowry method (Harjanto, 2017), calcium content was using AAS method (AOAC, 2005), vitamin C content (Sudarmadji et al. 2007), pH value (Sudarmadji et al., 2014),

and physical characteristics: viscosity (Yuwono and Susanto, 1998) and stability (Wibowo et al., 2014).

Statistical analysis

All analyses were performed in triplicates. The data were subjected to the analysis of variance followed by Fisher's least significant difference (LSD) test to compare among treatment means and control. Differences were considered at a significant level of 95% ($p < 0.05$) by using SPSS v.19 software.

RESULTS

Analysis of the characteristics of germinated mung bean drink with nanocalcium fortification

The interaction between germination time and the type of eggshell nanocalcium no significant effect on all characteristics of germinated mung bean drink, excepted for Vitamin C and calcium that were higher in all treatments compared to control (Table 1). The non-significance among the treatments was attributed to addition of the nanocalcium (CaO) powder of chicken and duck eggshells to the germinated mung bean drinks in the same amount (20% of nutrition facts label for calcium). Moreover, the characteristics of the eggshell nanocalcium (CaO) powder were white color, very fine texture (particle size of CaO in chicken and duck eggshells is 41.54 nm and 24.90 nm respectively), and neutral pH so that it take to the no significant effect on the value of viscosity, stability, protein, pH and vitamin C of germinated mung bean drink (Table 2).

Germination time was significantly effect the characteristics of germinated mung bean drinks (Table 3).

DISCUSSION

Table 1 shows a significant increase in the vitamin C and calcium content of germinated mung bean drinks to the control. The increase in vitamin C in germinated mung bean drinks in all treatments in this study was due to the food reserves contained in the cotyledons in the form of starch hydrolyzed by the amylase enzyme into simple sugars which were then synthesized into vitamin C during the germination process. The results of this study were in line with the opinion of Ebert et al. (2017) which stated that the content of vitamin C in the germinated peanut seeds increased by 2.7 times compared to the ungerminated seed.

The calcium content of germinated mung bean drinks fortified with nanocalcium of duck eggshell was higher than the calcium content of germinated mung bean drinks fortified with nanocalcium of chicken eggshell. This was

Table 1: Physical and chemical characteristics of germinated mung bean drinks with nanocalcium fortification

Treatments	Parameter					
	Viscosity (mPa.s)	Stability (%)	Protein (%)	pH	Vitamin C (mg/100g)	Calcium (%)
A ₁ B ₁	27.60 ± 0.20 ^a	87.00 ± 1.00 ^a	1.90 ± 0.05 ^a	6.81 ± 0.02 ^a	8.21 ± 1.02 ^a	17.82
A ₁ B ₂	27.13 ± 0.15 ^a	85.67 ± 1.53 ^a	1.81 ± 0.09 ^a	6.77 ± 0.04 ^a	7.62 ± 1.02 ^a	24.82
A ₂ B ₁	26.17 ± 0.29 ^a	73.00 ± 1.00 ^a	1.77 ± 0.11 ^a	6.74 ± 0.04 ^a	11.73 ± 1.01 ^a	16.45
A ₂ B ₂	26.03 ± 0.45 ^a	71.67 ± 1.53 ^a	1.66 ± 0.14 ^a	6.69 ± 0.04 ^a	11.14 ± 1.01 ^a	23.66
A ₃ B ₁	25.53 ± 0.21 ^a	65.67 ± 0.58 ^a	1.56 ± 0.14 ^a	6.63 ± 0.04 ^a	14.66 ± 1.01 ^a	15.66
A ₃ B ₂	24.97 ± 0.76 ^a	64.67 ± 1.53 ^a	1.50 ± 0.18 ^a	6.62 ± 0.01 ^a	13.49 ± 1.02 ^a	22.49
Control	28.80 ± 0.01 ^a	91.33 ± 0.58 ^a	2.01 ± 0.16 ^a	6.81 ± 0.02 ^a	5.28 ± 1.76 ^b	nd

Numbers marked with the same letter notation in the same column indicate not significantly different (5%)

Data = mean ± standard deviation

Control = germination time of green beans 0 hours without the addition of nanocalcium

A₁ = mung bean germination time for 6 hours B₁ = nanocalcium of chicken eggshell

A₂ = mung bean germination time for 12 hours B₂ = nanocalcium of duck eggshell

A₃ = mung bean germination time for 18 hours

Table 2: Effect of eggshell nanocalcium fortification on the characteristics of germinated mung bean drinks

Treatments	Parameter				
	Viscosity (mPa.s)	Stability (%)	Protein (%)	pH*	Vitamin C (mg/100g)
B ₁	26.43 ± 0.23 ^a	75.22 ± 0.86 ^a	1.74 ± 0.10 ^a	6.73 ± 0.03 ^a	11.54 ± 1.02 ^a
B ₂	26.04 ± 0.46 ^a	74.00 ± 1.53 ^a	1.66 ± 0.13 ^a	6.69 ± 0.03 ^a	10.75 ± 1.02 ^a

Numbers marked with the same letter notation in the same column indicate not significantly different (5%)

Data = mean ± standard deviation

B₁ = nanocalcium of chicken eggshell

B₂ = nanocalcium of duck eggshell

Table 3: The effect of germination time on the characteristics of germinated mung bean drinks

Treatments	Parameter				
	Viscosity (mPa.s)	Stability (%)	Protein (%)	pH	Vitamin C (mg/100g)
A ₁	27.37 ± 0.49 ^a	86.33 ± 1.05 ^c	1.85 ± 0.16 ^b	6.79 ± 0.02 ^c	7.92 ± 1.02 ^a
A ₂	26.10 ± 0.37 ^b	72.33 ± 1.26 ^b	1.72 ± 0.13 ^{ab}	6.72 ± 0.04 ^b	11.44 ± 1.01 ^b
A ₃	25.25 ± 0.18 ^c	65.17 ± 1.25 ^a	1.53 ± 0.07 ^a	6.62 ± 0.03 ^a	14.08 ± 1.02 ^c

Numbers marked with the same letter notation in the same column indicate not significantly different (5%)

Data = mean ± standard deviation

A₁ = mung bean germination time for 6 hours

A₂ = mung bean germination time for 12 hours

A₃ = mung bean germination time for 18 hours

because the calcium content in duck eggshell nanocalcium (45.50%) was higher than the calcium level in chicken eggshell nanocalcium (35.50%).

VISCOSITY

Viscosity is a measure of the size of the friction that occurs in the fluid. The greater the viscosity of a food, the thicker the material, and vice versa (Srihidayati, 2017). The viscosity of germinated mung bean drinks in the control treatment (0 hour germination without nanocalcium fortification) was higher (28.80±0.01 m.Pa.s) compared to that of nanocalcium fortified germinated mung bean drinks for all treatments in this study. This is due to the fact that ungerminated mung bean starch has not been hydrolyzed so that it still has higher starch content than germinated mung bean starch. In the preparation of mung bean drinks, starch will undergo gelatinization in the presence of heat treatment which will cause the starch granules to break and

the starch molecules to come out and be released from the granules and then enter the solution system. The results of this study are in line with the results of research conducted by Nurjanati et al. (2018), based on the hedonic test, ungerminated red bean milk (0 hours) has a higher viscosity than germinated red bean milk (8, 16 and 24 hours).

Table 3 shows that the viscosity of the green bean germination drink decreased significantly with the duration of germination. The decrease in the viscosity of the material during germination was caused by the hydrolysis of starch during the germination process by the amylase enzymes. The enzyme is formed at the beginning of germination by giberylic acid (Elobuikie et al., 2021). Starch will be broken down into simple sugars in the form of glucose which is used as energy and needs for seed growth. Another factor that causes a decrease in the viscosity of mung bean germination drink is the increase in vitamin C during the germination process which results in a decrease in pH as shown in Table 3. A decrease in pH

results in hydrolysis of glycosidic bonds which causes a decrease in viscosity. The results of this study are in line with the statement of Widjaja *et al.* (2019), that a solution will decrease its viscosity if its pH decreases.

STABILITY

The stability of the products can be seen by the presence or absence of precipitate in the product (Farikha *et al.*, 2013). Germinated mung bean drinks in the control treatment (0 hour germination without the addition of nanocalcium) had a higher stability value (91%) compared to all treatments of germinated mung bean drinks fortified with nanocalcium in this study. This was because germinated mung bean seeds absorb more water than ungerminated mung bean seeds. During germination, bean seeds require optimal environmental conditions that are 50-80% RH (Yuwariah *et al.*, 2015). Water absorption in the germination process is needed to maintain cell turgidity, including cell enlargement, photosynthetic reactions, salt solvents, gases and other substances that are transported between cells in tissues to maintain cell growth (Felania, 2017).

Table 3 shows that the stability of the germinated mung bean drinks decreased significantly as the duration of germination increased. This was due to an increase in the water content of germinated mung beans along with the length of germination due to the watering the mung bean seeds every 6 hours during germination time. The water content of germinated mung bean with a time of 0, 6, 12 and 18 hours were 4.32%, 4.99%, 5.42% and 7.22%, respectively. The increase in water content in germinated mung beans resulting germinated mung bean drinks have more water content in turns it affects the stability value of the drinks. In line with the viscosity value of the germinated mung bean drinks, the average value of stability of each treatment decreased with the duration of germination. The decrease in viscosity at germinated mung bean drinks correlated to that in stability.

PROTEIN CONTENT

The protein of germinated mung bean drinks in treatment A2 (12 hours of germination time) was not significantly different from that of treatment A1 (6 hours of germination time) and treatment A3 (12 hours of germination time). The decreasing of protein content in germinated mung bean drinks was due to the hydrolysis of protein into amino acids that are used for embryo growth. The results of this study were in line with the statement of Masood *et al.* (2014) which stated a decrease in protein content along with an increase in amino acid content during germination of green beans. This is due to an increase in the activity of

the protease enzyme which is an endogenous enzyme that is active during the imbibition process, when green beans are in contact with water. Proteases hydrolyze proteins into peptides and amino acids (Ferdianawan *et al.*, 2019). Free amino acids along with glutamic and aspartic acids (in the form of amides) will be translocated to the embryo in the formation of new structures in line with the ongoing germination stage (Pertwi *et al.*, 2013).

pH VALUE

pH is a parameter used to determine changes in the acidity level of a food product (Widowati *et al.*, 2020). Table 3 shows that the longer the germination time, the lower the pH of the germinated mung bean drinks significantly. According to Wea *et al.* (2014), in germinated seed plants there was an increase in vitamin synthesis, especially vitamin C (Wea *et al.*, 2014). This situation is in accordance with the vitamin C content in the germinated mung bean drinks in this study (Table 3) so that the pH of the germinated mung bean drink is low. Based on Indonesian National Standard 01-3830-1995, the pH standard for bean drinks is 6.5-7.0. The pH of germinated mung bean drinks with nanocalcium fortification in this study has a value of 6.62-6.79; it has met the pH standard of bean drinks.

VITAMIN C

Table 3 shows that the vitamin C of the germinated mung bean drinks increased significantly with increasing of germination time. 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 causes reactivation of the enzyme L-Galactono- γ -lactone dehydrogenase which is involved in the 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 germination of mung beans can help the solubility of nanocalcium fortification in the germinated mung bean drinks. This is in line with the statement of Yonata *et al.* (2017), that pH can affect the solubility of minerals. Acidic conditions and small particle size can increase the speed of nanocalcium dissolution from both duck and chicken eggshells, so that the germinated mung bean drinks with eggshells nanocalcium fortification has good digestibility.

Calcium content

Based on the results of the analysis, calcium content of nanocalcium in chicken and duck eggshells was 35.50% and 45.50%, respectively. Calcium content in eggshells is influenced by the thickness and type of eggshell. Duck eggshells have an average thickness of 0.36 - 0.46 mm (Septiana et al., 2015), while the average thickness of chicken eggshells was 0.33 - 0.35 mm (Azizah et al., 2015). Differences in eggshell thickness can affect the amount of mineral content and organic salts, especially calcium carbonate (CaCO₃) which acts as a source of calcium in eggshells.

Fortification is a process of increasing the content of essential micronutrients in the form of vitamins and minerals into food, aiming to increase nutrients that do not yet exist or to enrich existing nutrients (Valentina et al., 2014). According to the Indonesian Food and Drug Supervisory Agency (2019), the minimum amount of micronutrient fortification in foodstuffs is 10% of the RDI per serving. In this study, the amount of nanocalcium fortification of chicken and duck eggshells in germinated mung bean drink was 20% of the RDI for calcium per 250 mL serving.

Calcium content of nongerminated mung bean drinks with nanocalcium fortification of chicken and duck eggshells was 18.97% and 25%, respectively. This showed that the calcium content of germinated mung bean drinks both fortified with nanocalcium chicken and duck eggshells decreased with the increasing of germination time (Table 1). This was in accordance with the statement of Oghbaei and Prakash (2020) that the calcium content decreased significantly in germinated and peeled bean seeds. Peeling can reduce some of the minerals present in the skin of green beans. However, this germination and skin peeling treatment had an impact on increasing the bioavailability of mung beans which contributed to stimulating the reduction of anti-nutritional substances. The percentage of bioavailability in the seeds before germination was 14.91–17.19% and in the germinated beans which were peeled 22.96–25.27%.

In addition, the decrease in calcium content of germinated mung bean drinks in this study was thought to be due to the treatment with germination time less than 24 hours so that the phytase enzyme had not worked optimally, causing the complex phytic acid that binds to minerals such as calcium could not be completely released. Based on the results of research by Ghavidel and Davoodi (2011), enzyme activity (phytase, amylase and protease) increased significantly at 24, 48, and 72 hours of germination in several types of legumes, one of which was green beans. The maximum enzymatic activity occurred at 72 hours of germination. Although the calcium of germinated mung bean drinks

decreased in the 6, 12 and 18 hour germination treatment, but with an increase in vitamin C at the same germination time treatment it could increase the calcium absorption efficiency of the germinated mung bean drinks.

CONCLUSION

Nanocalcium fortification of chicken and duck eggshells by 20% of the RDI for calcium per 250 mL serving had no significant effect on the physical (viscosity and stability) and chemical (protein, pH, and vitamin C) characteristics of germinated mung bean drinks. Germination of mung beans at different times was significantly increased value of pH, vitamin C, protein, and stability; but significantly decreased value of viscosity. Germinated mung bean drinks are a good carrier for chicken and duck eggshells nanocalcium fortification.

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