



## Improvement of Functional Properties of Jack Bean (*Canavalia ensiformis*) Flour by Germination and Its Relation to Amino Acids Profile

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### Abstract

Jack bean as a source of vegetable protein had not been popular. Seed germination had been known to improve its nutritional quality, especially protein and amino acid profile. This study determined the effect of germination on the color, beany flavor, protein content, functional properties, and amino acid profile of jack bean flour. A complete randomized design was used for this experiment. Germination was carried out for 0, 24, 48, and 72 hours. The seed (control) and germinated jack bean flours were analyzed for oil absorption, water absorption, emulsifying and foaming capacities, as well as the soluble protein content to determine the best germination time. Furthermore, the amino acid profile of the jack bean flour produced from the best germination time was analyzed. The results of this study indicated that the total and soluble protein of the seed and germinated jack bean seeds for 0, 24, 48, 60, and 72 hours were 23.30 and 5.95; 22.61 and 7.61; 21.18 and 10.68; 23.26 and 10.22; 23.98 and 10.81%, respectively. Germination of jack bean improved the functional properties. A germination time of 72 hours increased the oil capacity, water absorption capacity, foaming capacity and decreased the emulsion capacity significantly. The hydrophilic and hydrophobic amino acids of the germinated jack bean flour increased to 3.21 and 2.12% of the seed flour, respectively. The increase of the foaming capacity was related to the increase in hydrophobic amino acids



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### Keywords

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of germinated jack bean flour compared to seed flours, that were glycine 1.23 and 1.01; alanine 1.29 and 1.01; valine 1.16 and 1.00; leucine 1.84 and 1.09%, respectively. Germination of jack bean for 72 hours increased significantly the essential amino acids, namely: leucine, lysine, and valine.

## Introduction

In Indonesia, there are many local legumes some of which have not been used optimally. The use of these local legumes can replace soybeans, which are still mostly imported. Food products that are imported continuously can certainly be a source of threat to food security.<sup>1</sup> In fact, many research efforts have been made to utilize native Indonesian legumes. However, the results are still not optimal and cannot be implemented to replace soybeans. Therefore, further research is needed to explore the potential of local legumes, such as jack bean seeds which have great potential to be developed in food products.<sup>2</sup> Jack bean is easy to cultivate because it is tolerant to acid dry land and can grow in all types of soil. Jack beans are high in protein which is approximately 29.8 – 32.2%,<sup>3</sup> but has low in vitro protein digestibility of 63.39 to 76.92%, because it contains anti-nutritional compounds like trypsin inhibitor and low content of essential amino acids such as leucine, lysine, and tryptophan.<sup>4</sup>

The quality of protein depends on its anti-nutritional compounds and its essential amino acid content.<sup>5</sup> Anti-nutritional compounds and beany flavor of legumes can be reduced through processing methods, including fermentation, germination, soaking, and cooking.<sup>6</sup> During the germination process, chemical reactions such as hydrolysis, oxidation, and synthesis, as well as protein mobilization, occur. The germination of legume seeds is associated with an increase in the activity of protease enzymes which hydrolyze large molecular weight protein to low molecular weight protein, simple peptides, and free amino acids.<sup>7</sup> Decreasing the molecular weight of protein will increase the solubility of the protein which affects its functional properties.<sup>8</sup> Germination can increase the nutritional value of protein due to increase protein hydrolysis which increases digestibility<sup>9</sup> and reduce trypsin inhibitors.<sup>10</sup>

Flour and protein isolates, especially from soybeans, have been used as ingredients to improve the quality

of food products and as functional ingredients.<sup>11</sup> Germination of soybean seeds has been shown to change the amount and type of amino acids.<sup>12</sup> Amino acid changes during germination are thought to improve the functional properties and nutritional value of proteins. The amounts of hydrophobic and hydrophilic amino acids affect the oil absorption capacity, water absorption capacity, and surfactant properties of proteins such as emulsifying and foaming capacities.<sup>13</sup> Germination of black soybean seeds has been shown to improve functional properties of proteins such as water absorption and foaming capacity but reduced emulsion stability.<sup>14</sup> Germination of jack bean seeds is thought to affect the total protein content, dissolved protein, and functional properties of the protein which is thought to be related to changes in the number and type of amino acids due to germination, however, research on this is scarce. This study determined the effect of germinating jack bean (*Canavalia ensiformis*) seeds for 0 (without germination), 24, 48, and 72 hours on the lightness value, unpleasant flavor, total protein content, dissolved protein, and protein functional properties of jack bean flours (seed and germinated jack bean flour).

## Materials and Methods

### Materials

Jack bean seeds (*Canavalia ensiformis* Dc) were obtained from Nanggulan village, Special Region of Yogyakarta, Indonesia. The chemicals used for the analyses were HCl (Merck, Germany), NaOH (Merck, Germany), H<sub>2</sub>SO<sub>4</sub> (Merck, Germany), aquadest, Phenolphthalein (Merck, Germany), corl oil, NaCl (Merck, Germany), NaOH-Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (Merck, Germany), H<sub>3</sub>BO<sub>3</sub> (Merck, Germany), buffer asetat 0.01M pH 5.9; MeOH (Merck, Germany), and Tetrahydrofuranne (Merck, Germany). The equipment used include the incubator (BL 2555 Kerper Boulevard, Germany), oven Memmert (GmbH+Co Type ULM 500, Germany), vortex (Type 37600 mixer, Thermolyne, USA), magnetic stirrer (IKA-Combimag RCT, Staufen, Germany), High Performance Liquid Chromatography (Colom

C18 4.6x250mm, Germany), cabinet dryer (IL 70 BL 2101, Indonesia), waterbath (Unitronict orbital 6032011, Spain), spectrophotometer UV-VIS (Shimadzu 1240, Japan), and centrifuge (Hettich zentrifugen D-78532, Germany).

#### **Germination of Jack Bean Seeds and Preparation of Jack Bean Flour**

The germination procedure of Kanetro<sup>11</sup> was used. Firstly, sorting was done to obtain the best bean seeds. The seeds (1 kg) were thoroughly washed and soaked in 3 liters of distilled water. After which, germination was carried out for 0 (soaked seeds, without incubation), 24, 48, and 72 hours at room temperature (27°C). Incubation for 72 hours is the maximum duration traditionally applied for the preparation of vegetable based-germinated jack bean seed. This was done in a container covered with a wet cloth to provide high humidity. The germinated jack beans were then dried using a cabinet dryer at 50°C for 24 hours to obtain a moisture content of 7%. The dried seeds were then ground into powder using a dry blender at the highest speed for 5 minutes, and sieved with a 60 mesh sieve size. The resulting flour was packed in a plastic-coated with aluminum foil and stored in an incubator at 5°C until further use.

#### **Lightness Value and Unpleasant Odor Analysis**

Analyses of the level of lightness (L) of the jack bean flours were done using a chromameter. The odor or unpleasant smell was tested using the scoring test method.<sup>15</sup> Panelists for the sensory test were selected using the duo trio test method. Twenty panelists who could distinguish an unpleasant smell from a pleasant smell were used. Odor testing was carried out using a rating questionnaire on an intensity value of 1 to 9. An intensity value of 5 and below was designated as pleasant odor and an intensity value of greater than 5 was designated as an unpleasant odor.

#### **Chemical Analysis (Moisture Content, Protein Content, and Protein Solubility):**

Moisture content analysis was conducted using the oven heating method, and protein content was done using the Kjeldahl micro method.<sup>16</sup> The protein solubility was tested according to the method of Okezie and Bello.<sup>17</sup> One point five (0.5) grams of samples were dispersed into 50 ml of distilled water and stirred for 30 minutes. After which it

was centrifuged at 4500 rpm for 30 minutes. The supernatant was analyzed by using the Kjeldahl micro method.<sup>16</sup>

#### **Water and Oil Absorption Capacity**

The sample (0.2 g) was mixed with 10 mL of water or corn oil in a centrifuge tube. It was thoroughly mixed by vortexing at high speed for 1 minute. It was then left at room temperature for 30 minutes and centrifuged again at 4500 rpm for 10 minutes. The volume of water or oil that was free after centrifugation was measured. The amount of oil or water absorbed (total minus free) was multiplied by its density to convert it into grams. Absorption capacity was expressed as the grams of oil or water absorbed (or retained) per gram of sample (as in basis). The density of oil (0.92 g/mL) was determined, and water was assumed to have a density of 1 g/cm<sup>3</sup>.<sup>17</sup>

#### **Foaming**

One point five (1.5) grams of sample was blended with 40 mL distilled water at high speed for 2 minutes and transferred into a 100 mL graduated cylinder. The blender jar was washed with 10 mL distilled water which was then added gently to the graduated cylinder. Volumes were recorded before and after whipping, and the percentage volume increase due to whipping was calculated as volume before whipping minus volume after whipping divided by volume before whipping.<sup>17</sup>

#### **Emulsion Capacity**

The sample (0.5 g) was blended with 17 mL of 3% NaCl solution at high speed for 30 seconds. While blending, 15 mL of corn oil was added. Blending was continued for an additional 30 seconds and transferred into a 50 mL graduated centrifuge tube. It was then kept in a water bath at 80°C for 7.5 minutes and centrifuged again at 4500 rpm for 20 minutes. The volume of corn oil separated from each sample after centrifugation was measured. Emulsion capacity was expressed as the amount of oil emulsified per gram of sample.<sup>17</sup>

#### **Analysis of Amino Acid Profile**

Sample preparation was done by adding 0.37 g of protein isolate to 10 mL of 6 N HCl, and hydrolyzed by autoclaving at 110 oC for 12 hours. After that, the samples were cooled and neutralized by adding 6 N NaOH. Two-point five (2.5) mL of 40% Pb-

acetate and 1 mL of 15% oxalate acid were added and injected with 50 mL aqua bidestilata (aquades with two cycles distillation process). Three (3) mL of the solution was filtered through a millex 0.45  $\mu\text{m}$ , and then mixed with OPA solution for 3 minutes. After which 30  $\mu\text{L}$  sample solution was injected into HPLC.<sup>18</sup> Preparation of amino acids standard solution for HPLC analysis was done by mixing 50  $\mu\text{L}$  of 2.5 ppm amino acid standard solution to 950  $\mu\text{L}$  OPA solution for 3 minutes. The 30  $\mu\text{L}$  standard solution was injected into the HPLC machine. The conditions for the HPLC analysis were Column: Eurosper 100-5 C18, 250 x 4.6 mm with precolumn

P/N: 1115Y535; Eluen: A = 0.01 M Acetic buffer pH5.9; Eluen B = MeOH: 0.01 M Acetic buffer pH5.9: THF 80:15:5;  $\lambda$  Fluorescence: Ext = 340 nm; Em = 450 nm.

#### Statistical Analysis

All data were analyzed using one-way ANOVA of SPSS (Statistical Package for Social Science) software version 22.0 (SPSS Inc., Chicago, IL, U.S.A). Comparison of means among samples was done using Duncan's multiple range tests at a significant level of  $p < 0.05$ .

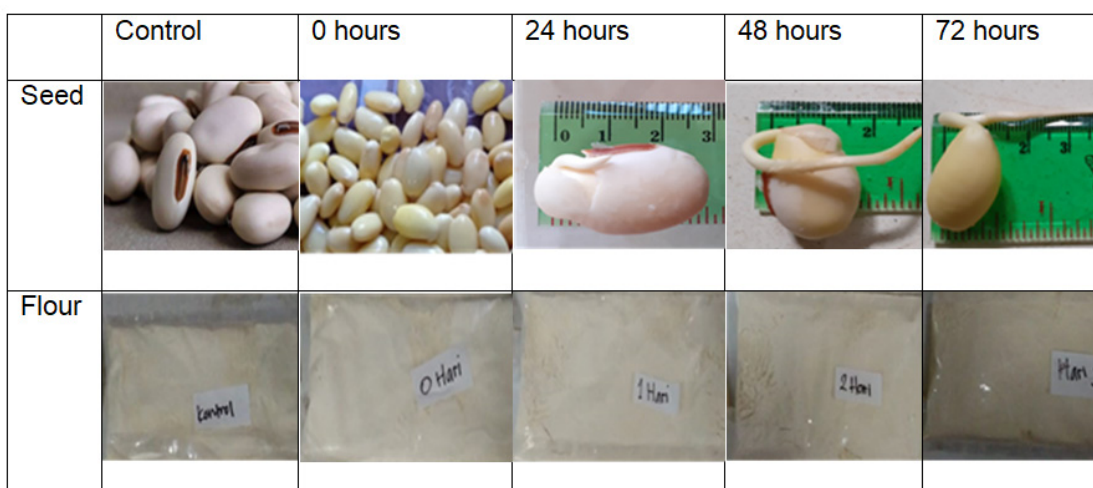


Fig. 1: Seed and flour of control and germinated jack bean

## Results and Discussion

### Lightness Value and Unpleasant Odor Analysis

Table 1 shows that germination time had a significant effect ( $p < 0.05$ ) on the lightness and odor of jack bean flours. Pictorial representation of the change in the intensity of lightness is shown in Figure 1. The lightness value decreased with prolong germination time. The change in lightness level is probably related to the browning reaction occurring during germination and during the process of making flour. Germination increased the activity of the polyphenol oxidase enzyme<sup>19</sup> and content of polyphenol compounds.<sup>20, 21</sup> During germination there is a breakdown of starch and protein into simpler molecular weight compounds, namely sugars and amino acids which can increase the non-enzymatic browning correction of Maillard during the process of germination which could end up in the flour.

Germination is known to increase reducing sugar levels increasing Maillard Reaction.<sup>22</sup>

The unpleasant odor is caused by the oxidation reaction of unsaturated fatty acids which is catalyzed by the enzyme lipoxygenase.<sup>23,24</sup> Table 1 shows that, the unpleasant odor decreased with longer germination of jack bean seeds for up to 72 hours. Although in the initial period of germination the intensity of unpleasant odors increased (from 0 to 48 hours), the intensity of unpleasant odors decreased at 72 hours. This is consistent with the study of mung bean seed germination, which showed that lipoxygenase activity increased at the beginning of 48 hours of germination and then decreased sharply from 96 hours to 192 hours of germination.<sup>25</sup> The odor intensity of the 72 hours germinated seed flour was significantly lower ( $p < 0.05$ ) than that of

jack bean flour (control), thus germinated jack bean flour has the potential to be used in food processing products because it may not affect the flavor of the final product.

**Table 1: Lightness value and unpleasant odor intensity of control (seed) and jack bean flours**

Germination time	Lightness colour (L)	Unpleasant odor intensity (scores)
Seed flour (Control)	95.66 ±0.05a	5.34 ± 1.11a
0 hours	95.22 ±0.01a	4.32 ± 0.95c
24 hours	94.54 ±0.03b	4.52 ± 0.77bc
48 hours	94.05 ±0.04bc	4.75 ± 0.56b
72 hours	93.45 ±0.08c	4.46 ± 0.91c

\*Mean with different superscript in the column indicates significant difference at  $p < 0.05$ .

Chemical Analysis (Moisture Content, Protein Content, and Protein Solubility). Table 2 shows that, the longer the germination of a jack bean, the lower the moisture content. The difference in water content is probably due to differences in the chemical composition of the germinated seeds. The presence of water in food can be expressed as moisture content and water activity which are influenced by the chemical composition of the material.<sup>26</sup> The presence of starch granules in the material affects

the water resistance during drying.<sup>27</sup> The decrease in moisture content during germination may be due to changes in the texture of the seeds which became softer due to soaking, resulting in a decrease in the soluble fiber.<sup>28</sup> Also, it resulted in the structure of the seeds being more hollow, making it easier for water to evaporate on the sprouts during drying. Germination of wheat germ for 168 hours resulted in a decrease of around 50% of its dietary fiber.<sup>28</sup>

**Table 2: Moisture content, protein content and protein solubility of seed and germinated jack bean flour**

Parameter	Control (Seed)	Germination Time			
		0 hour	24 hour	48 hour	72 hour
Moisture (%)	13.31±0.06 <sup>d</sup>	7.12±0.03 <sup>b</sup>	7.20±0.01 <sup>bc</sup>	7.31±0.01 <sup>c</sup>	6.86±0.12 <sup>a</sup>
Crude protein (%)	23.30±0.01 <sup>b</sup>	22.61±0.51 <sup>b</sup>	21.18±0.13 <sup>a</sup>	23.26±0.03 <sup>b</sup>	23.98±0.79 <sup>c</sup>
Protein solubility (%)	5.95±0.226 <sup>a</sup>	7.61±0.42 <sup>ab</sup>	10.68±1.04 <sup>bc</sup>	10.22±1.94 <sup>bc</sup>	10.81±1.91 <sup>c</sup>

\*Mean with different superscript in the column indicates significant difference at  $p < 0.05$ .

Germination of jack beans significantly affected ( $p < 0.05$ ) protein content levels, as shown in Table 2. Protein content decreased slightly at the beginning of germination until 24 hours, then consistently increased until 72 hours of germination. During germination, there is a mobilization of food reserves which involves the process of degradation and compilation of compounds needed for the development of seeds into new plants.<sup>7</sup> Increased protein levels during germination also occurred

during germination of Horse gram (*Macrotyloma uniflorum* (Lam.) for 18 hours.<sup>6</sup> Carbohydrates are the main source of energy in the early stages of germination, whereas protein will be used in the late stages of embryonic development, therefore, the increase in protein levels after germination is caused by a faster decrease in the percentage of carbohydrates and fats than protein. Protein synthesis and degradation of legume components during germination can increase protein content.<sup>29</sup>

Table 2 shows that during germination for up to 72 hours, there was a significant increase in protein solubility ( $p < 0.05$ ). This is consistent with previous research on soybean germination which showed increased protein solubility levels as indicated by an increase in free amino acid levels.<sup>12</sup> During germination, there is an increase in the activity of the protease enzyme which hydrolyzes protein.<sup>30</sup> Protein hydrolysis results in short peptide chains or a reduction in molecular weight so that protein solubility will increase.<sup>7</sup> According to Coimbra and Jorge<sup>31</sup> protein, hydrolysis affects hydrogen bonding and hydrophilic interactions related to the solubility of proteins. Germination is done by immersing the seeds in water which causes the protein structure to be detached, thereby increasing protein solubility<sup>32</sup>

The solubility properties of proteins are closely related to the functional properties of proteins<sup>8</sup>

### Functional Properties of Seed and Germinated Jack Bean Flour

Oil absorption is an important property in food formulations because it can improve the flavor and mouthfeel of food.<sup>33</sup> The absorption capacity of oil is influenced by the number of hydrophobic amino acids or the number of non-polar amino acids that make up the protein.<sup>5,34</sup> The ability of jack bean flour to bind oil with various germination time treatments can be seen in Table 3. Germination significantly increased ( $p < 0.05$ ) the oil absorption capacity of the jack bean flours. Jack bean flour, germinated for 72 hours, had the highest oil absorption capacity.

**Table 2: Moisture content, protein content and protein solubility of seed and germinated jack bean flour**

Parameter	Control (Seed)	Germination Time			
		0 hour	24 hour	48 hour	72 hour
Moisture (%)	13.31±0.06 <sup>d</sup>	7.12±0.03 <sup>b</sup>	7.20±0.01 <sup>bc</sup>	7.31±0.01 <sup>c</sup>	6.86±0.12 <sup>a</sup>
Crude protein (%)	23.30±0.01 <sup>b</sup>	22.61±0.51 <sup>b</sup>	21.18±0.13 <sup>a</sup>	23.26±0.03 <sup>b</sup>	23.98±0.79 <sup>c</sup>
Protein solubility (%)	5.95±0.226 <sup>a</sup>	7.61±0.42 <sup>ab</sup>	10.68±1.04 <sup>bc</sup>	10.22±1.94 <sup>bc</sup>	10.81±1.91 <sup>c</sup>

\*Mean with different superscript in the column indicates significant difference at  $p < 0.05$ .

**Table 3: Functional properties of seed and germinated jack bean flour**

Parameter	Control (Seed)	Germination Time			
		0 hour	24 hour	48 hour	72 hour
Oil absorption (%)	1.81±0.004 <sup>a</sup>	1.82±0.011 <sup>ab</sup>	1.84±0.008 <sup>b</sup>	1.86±0.014 <sup>c</sup>	1.89±0.001 <sup>d</sup>
Water absorption (%)	1.21±0.022 <sup>ab</sup>	1.55±0.008 <sup>c</sup>	1.15±0.017 <sup>a</sup>	1.38±0.017 <sup>ab</sup>	1.82±0.079 <sup>d</sup>
Foaming (%)	3.30±0.354 <sup>c</sup>	2.00±0.071 <sup>b</sup>	1.25±0.283 <sup>a</sup>	3.35±0.071 <sup>c</sup>	4.15±0.071 <sup>d</sup>
Emulsion (%)	14.75±0.354 <sup>c</sup>	13.25±0.071 <sup>a</sup>	13.80±0.283 <sup>b</sup>	14.45±0.071 <sup>c</sup>	13.15±0.071 <sup>a</sup>

\*Mean with different superscript in the column indicates significant difference at  $p < 0.05$ .

Higher protein levels will lead to higher oil absorption.<sup>35</sup> The hydrophobic nature of protein plays a major role in the process of absorption of oil,<sup>13</sup> the ability of foodstuffs to bind water and oil is inseparable from the involvement of proteins which are caused by the presence of hydrophilic (easy to absorb water) and lipophilic (easy to absorb oil)

groups.<sup>36</sup> Hydrophobic amino acids such as leucine, isoleucine, and alanine will affect the absorption capacity of oil. The more hydrophobic an amino acid, the greater the oil absorption capacity.<sup>13</sup> This is evidenced in Table 4 which shows an increase in hydrophobic amino acids in jack beans germinated for 72 hours compared to control jack beans, namely

glycine 1.23 and 1.01; alanine 1.29 and 1.01; valine 1.16 and 1.00; leucine 1.84 and 1.09%, respectively. The water absorption capacity is an important functional property for predicting the use of water in the process of mixing flour with water.<sup>13</sup> The water absorption capacity is influenced by the chemical composition of the ingredients, especially protein and starch. The higher the number of hydrophilic amino acids that make up protein and the higher the starch content, the higher the water absorption capacity.<sup>5</sup> Germination jack bean has an effect on the water absorption capacity which decreased at the beginning of germination, and then increased significantly ( $p < 0.05$ ) until 72 hours of germination as shown in Table 3. This suggests that the increase

in protein content during germination to 72 hours as presented in Table 2 may play a role in increasing the water absorption capacity. The decrease in water absorption capacity at the beginning of germination is probably due to the decrease in protein molecular weight due to hydrolysis during germination for up to 48 hours. The increase in water absorption capacity after 48 hours of germination is probably due to an increase in the number of hydrophilic amino acids, especially histidine, serine, and lysine as shown in Table 4. The more polar amino acids are present, the higher the water absorption capacity.<sup>37</sup> This is consistent with the germination of black soybean seeds which is known to increase water absorption capacity.<sup>14</sup>

**Table 4: Amino acid profile of seed and 72 hours germinated jack bean flour (%)**

No.	Composition	Control (seed)	Germinated (72 hours)
<b>A. Hydrophilic amino acids</b>			
1	Aspartic Acid	2.41± 0.00*	2.55± 0.00*
2	Glutamic Acid	2.10± 0.14*	2.90± 0.00*
3	Asparagine	0.71± 0.01*	0.76± 0.00*
4	Histidine + L-serine	1.49± 0,08*	2.12± 0.00*
5	Threonine	2.21± 0.01*	2.05± 0.00*
6	Glutamine	1.01± 0.01*	0.17± 0.00*
7	Arginine	1.41± 0.01*	1.41± 0.01*
7	Tyrosine	0.94± 0.02*	0.30± 0.00*
8	Lysine	3.00± 0.00*	3.51± 0.00*
<b>Total A</b>		<b>15.28</b>	<b>15.77</b>
<b>B. Hydrophilic amino acids</b>			
9	Alanine	1.01± 0.00*	1.29± 0.00*
10	Glycine	1.01± 0.00*	1.23± 0.00*
11	Tryptophan + Methionine	0.94± 0.04*	0.34± 0.00*
12	Valine	1.00± 0.00*	1.16± 0.00*
13	Phenilalanine	1.97± 0.02*	1.27± 0.00*
14	Isoleucine	1.01± 0.00*	1.07± 0.00*
15	Leucine	1.09± 0.01*	1.84± 0.00*
<b>Total B</b>		<b>8.03</b>	<b>8.20</b>
<b>Total A and B</b>		<b>23.31</b>	<b>23.97</b>

Note: \* significant difference at  $p < 0.05$ .

### Foaming

Foam is a gas dispersion system in a continuous phase in the form of a liquid.<sup>38</sup> Protein is a foam-

forming agent surfactant because of its ability to form a film in the water interface plane. The film protein can adsorb air during shaking or blowing air.<sup>39</sup>

The surfactant properties of proteins are related to the amphiphilic properties of proteins, which have hydrophobic residues that can bind compounds other than water, and hydrophilic which can interact with parts of the water.<sup>40</sup> The foaming capacity during the germination of jack beans for up to 72 hours was significantly increased ( $p < 0.05$ ) as shown in Table 3. This is consistent with the germination of black soybean seeds which is known to increase the foaming capacity.<sup>14</sup> Germination of sorghum seeds is also known to increase the foaming capacity of sorghum flour.<sup>30</sup> The increase in the foaming capacity was supported by an increase in hydrophobic amino acids in 72 hours germinated jack beans compared to jack bean seeds as shown in Table 4.

### Emulsion

Emulsion capacity states the emulsifiable weight of oil per gram of protein or the maximum volume of oil that a protein solution can emulsify.<sup>41</sup> Table 3 shows that the germination of jack beans has a significant effect ( $p < 0.05$ ) on the emulsifying capacity, which increased slightly in the initial stage of germination up to 48 hours, although not significantly different from the emulsifying capacity of jack bean seed flours. This is in accordance with the research in peanut germination which can improve emulsifying capacity.<sup>42</sup> Germination of jack bean for up to 72 hours was found to significantly reduce ( $p < 0.05$ ) emulsion capacity compared to 48 hours of germination. This was supported by an increase in hydrophilic and hydrophobic amino acids in 72 hours germinated jack beans compared to jack bean seeds which were 3.21% and 2.12%, respectively, as shown in Table 4. The increase in hydrophilic amino acids that was higher than the increase in hydrophobic amino acids caused disruption of the hydrophilic and hydrophobic balance in 72 hours germinated jack beans. The emulsifying capacity was influenced by hydrophilic and hydrophobic equilibrium which is related to the ability of the protein to reduce the tension between the oil-water surfaces. The emulsification properties of green bean protein are lower than soybeans because the number of hydrophobic amino acids in green bean protein is higher than in soybeans.<sup>13</sup>

### Amino Acid Profile of Seeds and Germinated Jack Bean Flour

Based on the results from Table 1, Table 2, and Table 3, it was established that 72 hours of germination can

increase the total protein content, dissolved protein, and increase the foaming capacity. For this reason, 72 hours of germinated jack bean flour was chosen for amino acid profile analysis and compared to jack bean seed flour. The amino acid profile of seed and 72 hours germinated jack bean flour is presented in Table 4.

Based on statistical analysis, it is known that 72 hours of germination provided a significant difference in amino acids between seed flour and germinated jack bean flour. The amount of hydrophilic and hydrophobic amino acids of germinated jack bean flour increased by 3.21 and 2.12%, respectively for jack bean flour. This occurred because the germination process requires amino acids for the growth of shoots, thus during germination some amino acid levels change significantly compared to jack bean seeds. Soy germination has also been known to cause changes in the number and type of amino acids.<sup>12</sup> Changes in the number and type of amino acids affect the functional properties of seed flour compared to germinated jack bean as discussed in the section on the description of functional properties including oil absorption capacity, water absorption capacity, foaming capacity and emulsifying capacity of seeds and germinated jack beans (Table 3). The increase in the foaming capacity of germinated jack bean flour for 72 hours compared to seed flour was supported by a significant increase in several hydrophobic amino acids in germinated and non-germinated seed flour, namely glycine 1.23 and 1.01; alanine 1.29 and 1.01; valine 1.16 and 1.00; and leucine 1.84 and 1.09%, respectively.

Table 4 also showed that 72 hours germination of jack beans increased essential amino acids, namely leucine, lysine, and valine, while the other essential amino acids decreased. Research in green bean showed germination resulted in an increase in essential amino acids.<sup>43</sup> Germination of soybean seeds is also known to increase amino acids such as alanine, leucine, lysine, and phenylalanine.<sup>12</sup> Increased level of leucine increases the nutritional value of protein, as well as the potential of jack bean protein as a functional food to prevent diabetes. Leucine is the main amino acid that can increase insulin.<sup>44</sup> The increase of the foaming capacity, essential amino acids and insulin stimulating amino acids in 72 hours germinated jack bean flour



indicates that, germinated flour has the potential to be used as an additive to improve food product systems that require film formation to trap gas, while increasing the nutritional value potential as functional food to prevent diabetes. Products that require protein with good foaming functional properties include whipped topping and cake.<sup>45</sup>

### Conclusion

Germination of jack bean for 0, 24, 48, and 72 hours has a significant effect on moisture content, total protein content, dissolved protein, water absorption capacity, oil absorption, foaming, and emulsification. The protein content of 72 hours of germinated jack beans flour was higher than the rest of the flours. Germination of jack beans for up to 72 hours increased the foaming capacity supported by an increase in hydrophobic amino acids. The amino acid levels of the 72 hours germinated jack bean flour were significantly different from jack bean seeds. The difference in functional properties of seed flour and germinated jack bean flour is related to differences in the amino acid profile of seeds and germination of jack beans. It is hoped that 72 hours

of germinated jack bean flour can be used as a food additive that requires film formation to trap gas, as well as potential as an ingredient for increasing protein nutrition and functional food. Therefore, further research is needed on the application of the germinated jack bean flour in the food system.

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### Conflict of Interest

All authors declare no conflict of interest.

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