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Effect of Pre-Milling Method on Physicochemical and Functional Properties of Velvet Bean (*Mucuna pruriens* L.) Flour

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Abstract Velvet bean (*Mucuna pruriens* L.) is considered an alternative protein source and functional food, but it contains cyanide and antinutritional factors. The purpose of this study was to determine the effect of pre-treatments before the milling step on physicochemical characteristics, functional properties, nutrition value, cyanide content, and antinutritional factor. The six pre-treatments on the beans were raw bean, dehulled bean, dehulled and boiled for 30 minutes, dehulled and steamed for 30 minutes, dehulled and soaked (6, 12, and 24 hours), and germinated bean. Effect of the treatments on proximate, cyanide and total phenolic contents, antioxidant capacity, protein digestibility, and starch digestibility of the velvet bean flour were investigated. The flour with the best treatment according to its protein content and nutritional value was further tested for dietary fiber, minerals (calcium and phosphor), and physical properties. Raw velvet bean contained the highest concentration of cyanide. All treatments reduced cyanide to the recommended safe limit, which is 10 mg per kg of dry weight according to FAO/WHO. The germination process increased phenolic content, while the other treatments decreased it significantly. Decrease of phenolic content followed by an increase in protein digestibility. Soaking dehulled velvet bean for 24 hours was the most effective treatment. The calcium and phosphor content of the flour was 200 and 528 mg/100 g, respectively. The dietary fiber content was 6.7%, while water and oil absorption capacity were 1.67 and 1.3 ml/g, respectively.

Keywords Milling, *Mucuna pruriens*, Pre-treatment, Velvet Bean

1. Introduction

Indonesia has many potential local legumes to be developed as processed food to achieve food diversification. Various local legumes are on par with soybean in terms of nutritional quality. Characterization of raw materials showed that protein content of soybean, mung bean, velvet bean, jack bean, and winged bean flour respectively 37.58, 23.25, 31.44, 31.40, and 41.57 percent [1], [2]. However, this potential has not yet been optimally developed, which resulted in relatively limited utilization. One of the local legumes is velvet bean (*Mucuna pruriens* L.). To date, the utilization of velvet bean remains limited

despite its high potential to be developed, one of them is made into flour. Velvet bean flour can be utilized as both a raw ingredient or partial substitute ingredient in various processed food products due to its high nutritional value [3]–[5]. Based on its dry weight, velvet bean has a nutritional value of about 23-35% protein and 14% crude fat [6], [7]. In addition, velvet bean also has several benefits, such as anticarcinogenic effect, antivirus, antimicrobe, anti-inflammatory, antihypertensive, anti-Parkinson, antioxidant agent, and can prevent neurological disorders [8].

However, same with other legume commodities, velvet bean also has antinutritional and toxic substances. The antinutritional compound in velvet bean includes tannins, digestive enzyme inhibitors (antitrypsin and anti-amylase), phytic acid, and haemagglutinin [9]. Raw velvet bean contains hydrogen cyanide (HCN), a toxic compound. Poisoning of HCN in traditional food products is still considered an error in production, even considered normal in certain cases. This is certainly highly concerning since HCN is a chemical compound that causes poisoning, paralysis, and even death [10]. The pre-treatment in velvet bean processing is expected to increase its nutritional value, maintain its functional properties, and reduce HCN to its safe limit up to a maximum of 10 mg/kg dry weight to fulfill the nutritional and safety aspects of the product. The treatments include dehulling, thermal process (boiling and steaming), soaking, and germination to obtain velvet bean flour with the desired characteristics as previously mentioned [11]–[14].

However, some of the abovementioned pre-treatments have the potential to affect the nutritional and functional components of the velvet bean. Therefore, optimization of pre-treatments before milling to reduce HCN until the allowed threshold but still retains its nutritional compounds that are functional for human health is necessary. The objective of this study was to determine the effects of several pre-treatments of velvet bean before milling on its nutritional value, as well as physicochemical and functional characteristics.

2. Materials and Methods

2.1. Materials

The raw ingredient consisted of velvet bean (*Mucuna pruriens* L.) white variety obtained from Kulonprogo Regency (Special Region of Yogyakarta). The materials used for this study were methanol p.a, DPPH (Sigma), ascorbic acid, multienzyme protease [trypsin (Sigma), chymotrypsin (Merck), and peptidase (Fluka)], α-amylase (Fluka), termamyl (*B. Licheniformis*, Sigma), pepsin (Sigma), pancreatin (Sigma), 3.5-dinitrosalicylic acid, Na-phosphate buffer, standard maltose, standard Ca, standard P, corn oil, and chemical materials for analysis purposes. The equipment consisted of UV-VIS double beam-1800 spectrophotometer (Shimadzu), UV-VIS double beam-2450 spectrophotometer (Shimadzu), atomic absorption spectrometry (Agilent Technologies 200 Series AA model 290 KS), chromameter (CR-300 Minolta), whiteness meter (Kett Electric Laboratory C-100-3), and centrifuge (Eppendorf Centrifuge 5810R), and glass utensils for analysis.

2.2. Pre-treatment before Milling

The pre-treatments done to velvet bean before milling were whole raw bean (RB), dehulled (DB), dehulled and proceeded with boiling for 30 minutes (DBO), dehulled and proceeded with steaming (T: 100^{o}C) for 30 minutes (DST), dehulled and proceeded with soaking for 6 hours (DS6), dehulled and proceeded with soaking for 12 hours (DS12), dehulled and proceeded with soaking for 24 hours (DS24), and germinated bean (GB). The soaking was done using distilled water (1:5 b/v) without changing the water. The germination treatment referred to Mugendi et al. [15], specifically, the velvet bean was sterilized and soaked in 70% ethanol for 1 minute. The beans were then soaked in water (1:10 b/v) for 12 hours at room temperature. Then, the beans were drained and spread on wet cotton in a certain container. The germination was done in a dark room for three days at 25^{o}C and 80% relative humidity, then froze to stop the germination process before milling step. Then the pre-treated beans were dried for 12 hours at 50^{o}C, then milled with a pin disc mill and sieved with an 80-mesh sieve, and the 8 treatments of velvet bean flours were obtained.

2.3. Yield and Chemical Composition Analysis (Proximate)

The yield was calculated based on a percentage comparison between the weight of the resulted velvet bean flour and the weight of the raw velvet bean that was used as the raw ingredient. The proximate analysis of velvet bean flour was done to determine the moisture content (AOAC 925.10) and ash content (AOAC 923.03) with the gravimetry method, protein content (AOAC 950.48) with the Kjeldahl method, and fat content (AOAC 948.22) with the Soxhlet method [16]. The carbohydrate total was determined by difference.

2.4. HCN and Total Phenolic Content Analysis

The hydrogen cyanide (HCN) content in the sample was determined using the alkaline titration method [17]. A Kjeldahl flask was filled with 20 g of sample and 200 ml of water, then the solution was distilled after 2 hours. The distillate was collected and distilled to a specific volume in a flask containing 20 ml of a 2.5% NaOH solution. Before titrating with 0.02 M $AgNO_3$, 8 ml of 6 M NH_4OH and 2 ml of 5% KI solution were added to the distillate. HCN was calculated as followed:

$$HCN\ (mg) = (ml\ of\ 0.02\ M\ AgNO_3) / 1.08 \quad (1)$$

The total phenolic content was tested with the method explained by Doss et al. [18] with slight modification. A sample of 5 g was extracted using methanol at a 1:6 (w/v) ratio. The mixture was then macerated at room temperature for three hours using stirrer. The mixture obtained was then further macerated and stored for 12 hours in the dark. The supernatant and precipitate were then separated using filter paper. The supernatant was subsequently removed by vacuum evaporation using a rotary evaporator at 40°C. 1 mL of the extract was pipetted into a test tube with 5 mL of Folin-Ciocalteu reagent (1:1 with water). The solution was left at room temperature for 5 minutes. 4 mL of 7.5% (w/v) sodium carbonate solution was added to the sample solution and vortexed. The test tube was then stored in the dark room for 2 hours and the absorbance was measured at a wavelength of 765 nm. The total phenolic content was determined by a standard curve from several concentrations of gallic acid in mg gallic acid/g extract unit.

2.5. Antioxidant Capacity Analysis

The antioxidant capacity of velvet bean flour was done with the DPPH method. The objective of this analysis was to determine the inhibition effect of the antioxidant compound in the sample against DPPH free radicals by observing the color change after incubation. The color change was measured by measuring the absorbance using a spectrophotometer at 517 nm wavelength. The reference standard was the ascorbic acid standard [19].

2.6. Protein Digestibility Analysis

The protein digestibility was observed in vitro with multienzyme protease following the method by Hsu et al. [20]. The multienzyme solution consisted of a mixture of 1.6 mg trypsin, 3.1 mg chymotrypsin, and 1.3 mg peptidase per ml of distilled water and adjusted to pH 8.00 using 0.1 N NaOH or 0.1 N HCl. A total of 25 ml of sample suspension (6.25 mg protein/ml) was adjusted to pH 8.00. Then the sample was put in a 37°C water bath for 5 minutes while stirring. A total of 2.5 ml of the multienzyme solution was added (the time the enzyme was added was recorded as zero time) into the sample suspension while still stirring. The pH value of the sample suspension was recorded at the 10th minute. Protein digestibility is expressed by the following formula:

$$Y = 210.464 - 18.103x \quad (2)$$

Y = Protein digestibility
x = pH at the 10th minute

2.7. Starch Digestibility Analysis

The starch digestibility was analyzed in vitro and referred to Anderson et al. [21] method. A total of 1 g of sample was added to 100 ml of distilled water and then heated in a water bath until it reached 90°C, then cooled and 5 ml of phosphate buffer pH 7 and 3 ml of distilled water were added to 2 ml of the sample solution. Each sample was made twice, one of which was used as a blank. Samples were incubated at 37°C for 15 minutes. Sample solution and blank were added to 5 ml of α-amylase enzyme solution (1 mg/ml in phosphate buffer pH 7). The two tubes were incubated again for 30 minutes, then transferred to a test tube containing 2 ml of DNS solution (dinitrosalicylic acid). The solution was heated in boiling water for 12 minutes, then immediately cooled under running water. A total of 10 ml of distilled water was then added, then stirred until homogeneous with a vortex. The absorbance of the sample and blank solutions was then measured with a UV-Vis spectrophotometer at a wavelength of 520 nm. The higher the measured maltose, indicates higher the digestibility of the starch sample. The in vitro starch digestibility is stated as a relative value to pure starch.

2.8. Dietary Fiber and Mineral Analysis

The total dietary fiber, soluble dietary fiber, and insoluble dietary fiber (AOAC 991.43) were determined using the enzymatic-gravimetry method [22]. Sample was subjected to gelatinize using a thermally stable-amylase, then digested by protease and amyloglucosidase enzymes to remove protein and starch in the sample. The insoluble dietary fiber was determined by weighing the dried residue after the enzymatic treatment. The total dietary fiber was obtained on a different aliquot by weighing the dry precipitate that resulted from the addition of ethyl alcohol to the enzyme-treated sample. The soluble dietary fiber was calculated by the difference of the total and insoluble dietary fiber fractions.

The analyzed minerals consisted of calcium (AOAC 985.35) and phosphor (AOAC 965.17) content which were measured with atomic absorption spectrometry and UV-Vis spectrophotometer [23]. For calcium analysis, a total of 2.5 g of sample was ashed using the dry ashing method and then ash solution was prepared. 1, 2, or 5 ml of ash solution was pipetted into a 50 ml volumetric flask, 2.5 ml of lanthanum chloride solution was added, then adjusted with distilled water. The AAS instrument with an acetylene-air flame was set at a wavelength of 422.7 nm. The absorbance of the sample and standard was determined using an atomic absorption spectrophotometer. Calcium concentration was calculated based on the standard curve that has been obtained.

For phosphor analysis, phosphorus stock solution was prepared by weighing 1.917 g of KH_2PO_4 and dissolved with distilled water and diluted to 1 liter. 1 ml of solution is equivalent to 0.1 mg P_2O_5. A total of 1-3 g of the sample was ashed and ash solution was made. 1-2 ml of

the ash solution and 25-30 ml of distilled water were added into a 50 ml measuring flask, then being neutralized with a few drops of ammonia solution (1:8 v/v), and added with one drop of nitric acid (1:2 v/v). 12.5 ml of molybdovanadate reagent was added to the solution and diluted with distilled water up to the tera mark. After 10 minutes, the absorbance of the solution was measured at a wavelength of 470 nm using a UV-Vis spectrophotometer. Phosphor concentration was calculated based on the standard curve of phosphorus stock.

2.9. Analysis of Flour Physical and Functional Properties

2.9.1. Color and Whiteness Index

The measurement was done by referring to the method by Mugendi et al. [15]. The measured sample was measured on L, a, and b value scales. The L value indicates the lightness parameter which ranges from 0 (black) to 100 (white). The notation a indicates the reflective light which results in chromatic red-green hue with +a value (positive) from a-100 for red hue and -a value (negative) from 0-(-80) for green hue. Notation b states the chromatic mixture of blue-yellow hue with +b (positive) value from 0-70 for yellow hue and -b (negative) from 0-(-70) for blue hue. The measurement of the whiteness index was done by using a whiteness meter. The sample was placed in a container to be measured. The whiteness index and sequence number of measurements will appear in the equipment. The sample whiteness index is stated as the relative whiteness index to the whiteness index standard of $BaSO_4$, which is 110.8.

2.9.2. Bulk Density

Bulk density was determined by referring to the method by Okezie and Bello [24]. A measurement cup of 10 mL was weighed and a 10 mL sample was filled. The measurement cup that was filled with the sample was weighed again and the subtraction of sample weight shows the weight of the sample per 10 mL. Bulk density is stated in g/mL or g/cm^3 unit.

2.9.3. Water and Oil Absorption Capacity

The measurement of water and oil absorption capacity was conducted by referring to Mugendi et al. [15] method. A sample of 0.5 g was added with 5 mL of pH 7 distilled water (for water absorption capacity test) or 5 mL of corn oil (for oil absorption capacity test), then stirred for 1 minute and set for 30 minutes at 25°C. The mixture was centrifuged at 3000 rpm for 25 minutes. The free liquid volume is measured and the retained liquid is stated as mL of water or oil per gram of sample.

2.10. Statistical Analysis

The result from the study was analyzed using One-Way ANOVA and proceeded with the Duncan post-hoc test at a 95% confidence level to determine the effects of pre-treatments on the established parameters. The analysis was performed using SPSS 20 software.

3. Results and Discussion

3.1. Yield of Velvet Bean Flour

Velvet bean consists of a 95% edible portion of the whole raw bean. If the efficiency of the milling process was ideal, the result would be flour with a non-significant weight difference from the weight of the edible portion. The yield percentage in the production of velvet bean flour is shown in Table 1. The study showed that germination treatment resulted in a relatively lower flour yield (40.48%). This was due to the water imbibition process into the bean during the germination, causing the granules to swell and form large cavities between cells, allowing for easier release of the water during drying [25], resulting in more water disappears after the drying and milling process [26]. The raw bean had the highest yield since it did not experience loss in other components, such as the seed coat from the skin dehulling process. Boiling and soaking can also reduce flour yield due to the loss of water-soluble nutritional components during the process [27], [28]. Meanwhile steaming can also reduce the flour yield due to the fact that velvet bean structure changes as a result of steam tempering. After absorbing a lot of water vapor, the bean became compact and rigid, which may have also caused protein-protein, protein-starch, starch-starch, and other components to interact more tighly. The high temperature also led to protein denaturation, which altered the water binding properties of starches and proteins. Consequently, the bean were difficult to grind, resulted in the decrease of flour yield [29].

Table 1. Effect of pre-milling treatments on yield of velvet bean flour

Treatments	Yield (%)
Raw bean (RB)	76.10
Dehulled bean (DB)	66.35
Dehulled bean + boiled 30 minutes (DBO)	61.79
Dehulled bean + steamed 30 minutes (DST)	63.80
Dehulled bean + soaked 6 hours (DS6)	62.30
Dehulled bean + soaked 12 hours (DS12)	62.04
Dehulled bean + soaked 24 hours (DS24)	54.04
Germinated bean (GB)	40.48

Table 2. Chemical composition of velvet bean flour with various pre-milling treatments

Treatments	Moisture (%wb)	Ash (%db)	Fat (%db)	Protein (%db)	Carbohydrate (%db)
RB	7.48 ± 0.21^{a}	3.66 ± 0.01^{f}	4.61 ± 0.05^{a}	29.50 ± 0.11^{a}	62.22 ± 0.14^{c}
DB	8.89 ± 0.11^{b}	3.24 ± 0.03^{e}	5.49 ± 0.05^{d}	31.20 ± 0.20^{b}	60.07 ± 0.22^{a}
DBO	11.30 ± 0.16^{e}	2.79 ± 0.04^{d}	5.31 ± 0.04^{c}	32.03 ± 0.30^{bc}	59.88 ± 0.23^{a}
DST	9.24 ± 0.06^{bc}	3.25 ± 0.02^{e}	5.52 ± 0.06^{d}	31.58 ± 0.22^{b}	59.66 ± 0.30^{a}
DS6	9.83 ± 0.01^{d}	2.07 ± 0.01^{c}	4.98 ± 0.01^{b}	31.80 ± 0.28^{bc}	61.15 ± 0.28^{b}
DS12	9.73 ± 0.16^{d}	1.87 ± 0.01^{b}	5.25 ± 0.07^{c}	31.57 ± 0.51^{b}	61.31 ± 0.42^{bc}
DS24	9.41 ± 0.18^{cd}	1.66 ± 0.06^{a}	5.34 ± 0.06^{cd}	32.54 ± 0.25^{c}	60.45 ± 0.38^{ab}
GB	7.07 ± 0.04^{a}	3.57 ± 0.01^{f}	4.81 ± 0.06^{b}	31.03 ± 0.17^{b}	60.61 ± 0.25^{ab}

*Mean on the same column followed by different letters showed significant difference ($p<0.05$). RB= Raw bean; DB= Dehulled bean; DBO= Dehulled bean + boiled 30 min; DST= Dehulled bean + steamed 30 min; DS6= Dehulled bean + soaked 6 hr; DS12= Dehulled bean + soaked 12 hr; DS24= Dehulled bean + soaked 24 hr; GB= Germinated bean.

3.2. Chemical Composition (Proximate)

The proximate analysis of velvet bean flour from various pre-treatments before milling is shown in Table 2. Table 2 showed that the pre-treatment before milling resulted in a significant variation of chemical composition result ($p<0.05$). The 30 minutes boiling treatment resulted in flour with the highest moisture content, followed by dehulled with soaking, dehulled with steaming, dehulled bean, and lastly raw and germination treatment. The high moisture content in 30 minutes boiling treatment was caused by the absorbed water due to the cell tissue disintegration during the cooking process [30]. The soaking treatment also resulted in higher moisture content than raw, dehulled, and germination treatment due to the long contact time between the water and the bean, which resulted in water being absorbed into the bean [31], [32]. The velvet bean flour with germination treatment showed a relatively low moisture content. During the germination process, water diffusion occurs causing swelling of the granules and tends to form large cavities between cells, allowing for easier release of the water during drying process [33]. In addition, the water absorption is suspected to be insignificant due to the thick and hard structure of the velvet bean hull, which results in lower permeability. This causes the water stored in the bean after the germination process to become relatively low.

In the ash content parameter, it was shown that the pre-treatments (dehulling, boiling, steaming, and soaking) could reduce the ash content significantly compared to the raw bean, which showed that dehulled with soaking for 24 h treatment had the lowest ash content, followed by dehulled with soaking for 12 h and 6 h, dehulled with boiling, dehulled bean, and lastly raw and germination treatment ($p<0.05$). According to Rousseau et al. [34], the mineral content in a food product cannot be damaged by heat, light, oxidizing agent, and extreme pH, but it is mostly caused by leaching or physical separation. The minerals in the bean and legumes are heavily concentrated on the skin and epidermis layer [35]–[37]. Meanwhile, the germination process resulted in flour with insignificant ash content to its raw bean. This was due to the low minerals being utilized for metabolism during the germination process, which resulted in an insignificant change in ash content, even an increase in ash content [38], [39]. Technically, germination treatment had an increase of ash content after 12 h soaking pre-treatment before germination process. The increase of ash content is caused by the decrease of other micro nutritional components during germination, such as fat and carbohydrate [40]. Some studies also reported that ash content can increase

due to phytase activity which hydrolyzed the bond between the proteins, enzymes and minerals, to release the minerals during germination [41]–[43].

The pre-treatment before milling also significantly affected the fat content of velvet bean flour ($p<0.05$). All dehulling treatments before milling significantly increased fat content of the flour compared to raw bean due to the removal of hull portion and concentration of endosperm, as fat is characteristically present in the cotyledon fraction of seeds [44]–[46]. While, the germination process resulted in flour with lower fat content compared to boiling, steaming, and soaking but significantly higher than raw bean. The lower fat content of germinated bean was due to the increase of lipase enzyme activity as a way to provide energy for sprouting [38], while the germinated bean still had higher fat content compared to raw bean due to non-conversion of free fatty acids to carbohydrates which may lead to increase in fat composition [47]. This agrees with Echendu et al. [48] and Kayembe et al. [46]'s report on ground beans and soybean.

The study results also showed the increase in protein in flour due to the pre-treatment before milling ($p<0.05$). Dehulling, thermal process, and soaking can also increase the protein content due to the degradation of other components on the bean during treatment. According to Akinmutimi and Ukpabi [49], boiling for 30 minutes could retain the protein content, while boiling for more than 60 minutes could significantly reduce the protein content [11]. Germination can increase the protein content of the flour due to the degradation of other components such as fat and carbohydrate into energy for new protein synthesis [50].

The pre-treatment was also shown to significantly reduce the carbohydrate content ($p<0.05$). During the germination treatment, the α-amylase enzyme activity increases and it contributes to carbohydrate hydrolysis to be used as the main source of energy in the initial stage of sprouting [51]. Meanwhile, dehulling, thermal process and soaking can reduce the carbohydrate content due to the loss of oligosaccharide from the hull and water-soluble oligosaccharide during the treatment [52].

3.3. Hydrogen Cyanide and Total Phenolic Content

Hydrogen cyanide (HCN) is one of the toxic compounds that is naturally occurring in legumes. Polyphenol compound is commonly found in plants and reported to possess health benefits as well as antinutritional compounds due to its ability to reduce the nutritional value of certain food materials [53]. The analysis of variance results showed that the pre-treatments before milling significantly affected ($p<0.05$) the HCN and total phenolic content. Table 3 showed that whole bean of velvet bean flour (RB) and dehulled (DB) treatments had no significant difference in terms of HCN content. However, HCN content experienced a significant decline in dehulled and boiled (DBO), steamed (DST), or soaked (DS6-DS24) treatments. This indicates that the cyanogenic glycosides in velvet bean that was used in this study were heavily concentrated in the cotyledon, not on the skin layer. HCN has a boiling point of 26.5^{o}C and is heavily soluble in water and alcohol. Therefore, thermal process, soaking, and rinsing treatments were able to reduce HCN content [9].

Table 3. HCN and total phenolic content of velvet bean flour with various pre-milling treatments

Treatments	HCN Content (mg/kg dry basis)	Total Phenolic Content (g GAE/100 g dry basis)
RB	19.88 ± 1.83^{d}	1.95 ± 0.01^{g}
DB	19.02 ± 0.91^{d}	1.47 ± 0.00^{f}
DBO	13.83 ± 3.30^{c}	1.38 ± 0.01^{d}
DST	8.40 ± 0.86^{b}	1.45 ± 0.01^{e}
DS6	2.92 ± 0.76^{a}	1.12 ± 0.01^{c}
DS12	2.76 ± 0.29^{a}	1.10 ± 0.01^{b}
DS24	2.85 ± 0.36^{a}	0.87 ± 0.01^{a}
GB	2.87 ± 0.25^{a}	2.55 ± 0.01^{h}

*Mean on the same column followed by different letters showed a significant difference ($p<0.05$). RB= Raw bean; DB= Dehulled bean; DBO= Dehulled bean + boiled 30 min; DST= Dehulled bean + steamed 30 min; DS6= Dehulled bean + soaked 6 hr; DS12= Dehulled bean + soaked 12 hr; DS24= Dehulled bean + soaked 24 hr; GB= Germinated bean.

The boiling and steaming treatments accelerate the hydrolysis reaction of cyanogenic glycosides due to the presence of heat energy, which might be hydrolyzed to acetone cyanohydrin with the assistance of β-glycosidase enzyme and then changed to free HCN which is then dissolved into the cooking water, water vapor, and condensate [54]. During the soaking process, the hydrolysis reaction occurs when the water migrates into the bean kernel. The hydrolysis reaction is catalyzed by an endogenous enzyme (β-glycosidases or α-hydroxynitrile lyases) and caused the cyanogenic glycoside to be hydrolyzed and resulting in water-soluble HCN [55]. During the germination, the increase of hydrolytic enzyme, including glycosidase, thus caused the cyanogenic glycoside to be hydrolyzed and the HCN becomes easily evaporated at room temperature [56], [57]. Ramli et al. [58] stated that processing method such as soaking, germination, boiling, autoclave, fermentation, and genetic modification can eliminate most of the toxic effects and antinutritional compounds from the legumes. Fukuba et al. [59] reported that soaking, boiling, steaming, and microwave exposure had reduced total cyanide content by about 5-54%, 44-72%, 22-78%, and 44-72%, respectively. Meanwhile, Padmaja [60] stated that oven drying, sun drying, peeling/dehulling, and fermentation could reduce total cyanide content by about 80-85%, 58-77%, 50%, and 69-91%, respectively.

The pre-treatments before milling also significantly affect the total phenolic content of the flour. Dehulling, thermal process, and soaking significantly affect in reduction of the total phenolic content, while germination increased it. The dehulled velvet bean flour in all treatments had lower total phenolic content compared with raw and germinated velvet bean. This was due to the skin layer which contains phenolic content, especially tannin in high concentrations [12]. The soaking and boiling treatments were known to reduce the total free phenol in velvet bean by 47 and 48.07% [9], [11]. The phenolic compound can also be reduced due to soaking due to its water-soluble properties. The longer the soaking time, the more dissolved the phenolic compound [58]. Table 3 shows a higher decrease in total phenolic content along with the increase in the soaking time.

In thermal processes such as boiling and evaporation, heat exposure influences the presence of the phenolic compound. The dehulling and cooking process at a high temperature can reduce the total phenol and flavonoid in legumes [61]. Meanwhile, the total phenolic content in germination treatment increased. This is due to the increase in endogenous enzyme activity for the synthesis of phenolic compounds [62]. The phenolic compound is one of the ingredients used for lignin production in cell walls [63]. The increase in total phenol content during the germination process is also found in mung beans, white cowpeas, soybeans, peanuts, black beans, and adzuki beans [64].

3.4. Antioxidant Capacity

The analysis of variance showed that pre-treatment before milling had a significant effect ($p<0.05$) on velvet bean flour antioxidant capacity. The antioxidant capacity analysis was observed based on the antioxidant capacity percentage and ascorbic acid equivalent antioxidant capacity from velvet bean flour as shown in Table 4. Table 4 shows that the antioxidant capacity of velvet bean flour experiences a decline due to the pre-treatments. One of the compounds which have antioxidant properties in whole velvet bean is tannin. Tannin belongs to the polyphenol group and has been reported to act as an antioxidant. This compound is majorly concentrated in the skin layer of the velvet bean [13], therefore, the dehulled velvet bean flour had a lower antioxidant capacity compared with the whole velvet bean.

Table 4. Antioxidant capacity of velvet bean flour with various pre-milling treatments

Treatments	Antioxidant Capacity (%)	AEAC (mg/L)
RB	89.38 ± 0.57^{f}	240.17 ± 1.52^{e}
DB	86.56 ± 0.44^{c}	232.67 ± 1.18^{c}
DBO	72.58 ± 1.21^{a}	195.38 ± 3.22^{a}
DST	88.05 ± 0.30^{d}	236.63 ± 0.80^{d}
DS6	77.89 ± 0.30^{b}	209.54 ± 0.80^{b}
DS12	77.58 ± 0.39^{b}	208.71 ± 1.05^{b}
DS24	77.97 ± 0.60^{b}	209.75 ± 1.59^{b}
GB	88.59 ± 0.40^{c}	238.08 ± 1.08^{de}

*Mean on the same column followed by different letters showed a significant difference ($p<0.05$). RB= Raw bean; DB= Dehulled bean; DBO= Dehulled bean + boiled 30 min; DST= Dehulled bean + steamed 30 min; DS6= Dehulled bean + soaked 6 hr; DS12= Dehulled bean + soaked 12 hr; DS24= Dehulled bean + soaked 24 hr; GB= Germinated bean.

The following treatments on dehulled velvet bean such as heating and soaking also further decreased its antioxidant capacity. Heating affects the chemical reactivity and tannin polymerization, while the decrease in antioxidant activity during soaking occurs due to the dissolved tannin with the soaking water [65]. This was in line with the decrease in the total phenolic content of flour from the treatment (Table 3). Antioxidant capacity is also affected by the phenolic components in food material [66]. However, the increase in total phenol content during germination was not in line with the antioxidant capacity. The antioxidant capacity of velvet bean flour with germination treatment was lower compared with the raw velvet bean. This was caused by the phenolic decomposition or inactivation of phenylalanine ammonia-lyase as the key enzyme in phenolic compound biosynthesis that is involved in the germination process [67].

3.5. In Vitro Protein and Starch Digestibility

The protein nutritional value is not solely relied on the high or low level of protein contained within the food material, but also on the availability of whether the protein can be utilized by the body or not, which is known as protein digestibility [68]. The starch digestibility is also needed to be analyzed to determine the carbohydrate digestibility of velvet bean flour. The analysis of variance results showed that the pre-treatments before milling had significant effects ($p<0.05$) on the protein and starch digestibility of velvet bean flour. The result of velvet bean flour protein and starch digestibility analysis are shown in Table 5. Table 5 shows that the protein digestibility of velvet bean flour significantly increased due to dehulling, boiling, steaming, and soaking treatments. This was caused by the reduction in antinutritional components, such as trypsin inhibitor, phytic acid, and phenolic compounds, both simple phenolic compounds, and polyphenols such as tannin [69]. The water that is absorbed into the kernel through the soaking and hydrothermal process results in endogenous enzyme activation which can break down the antinutritional compounds in seeds and legumes [9]. The hydrothermal processes such as further boiling and steaming result in protein denaturation which will open the three-dimension structure of protein molecule, which makes it easier to be hydrolyzed by protease enzyme [70]. Das et al. [71] reported that soaking was able to reduce trypsin inhibitor by 0.5–50% while soaking followed by boiling or cooking was able to increase the percentage decline further to 45–92%. The boiling process had significant effects on the increase of protein digestibility compared to steaming. This is suspected since not all part of the food material is in direct contact with the hot steam during the steaming process.

Several studies reported the increase in protein digestibility due to germination treatment on the broad bean, common bean, jack bean, lablab bean, velvet bean, pigeon pea, sesame protein, and soybean tempe protein isolate due to the activation of proteolytic enzyme [71]–[75]. However, this study showed that there was a decrease in protein digestibility of germination-treated velvet bean flour. This could be caused by the different types and phenolic compound content which was synthesized during the germination. According to Liu and Ning [76], a phenolic compound with a lipophilic substituent shows higher binding to protein compared with a phenolic compound without the mentioned substituent. In return, this affects protein digestibility.

Table 5. Effect of pre-milling treatments on protein and starch digestibility of velvet bean flour

Treatments	Protein Digestibility (%)	Starch Digestibility (%)
RB	75.69 ± 0.52^{b}	39.00 ± 0.25^{c}
DB	77.90 ± 0.37^{c}	43.38 ± 0.63^{d}
DBO	81.62 ± 1.87^{d}	30.07 ± 1.47^{a}
DST	78.13 ± 0.46^{c}	34.77 ± 2.12^{b}
DS6	82.16 ± 0.17^{e}	45.25 ± 0.13^{e}
DS12	81.75 ± 0.29^{d}	47.42 ± 0.26^{f}
DS24	82.20 ± 0.24^{e}	48.34 ± 0.09^{g}
GB	69.62 ± 0.29^{a}	48.37 ± 0.64^{g}

*Mean on the same column followed by different letters showed a significant difference ($p<0.05$). RB= Raw bean; DB= Dehulled bean; DBO= Dehulled bean + boiled 30 min; DST= Dehulled bean + steamed 30 min; DS6= Dehulled bean + soaked 6 hr; DS12= Dehulled bean + soaked 12 hr; DS24= Dehulled bean + soaked 24 hr; GB= Germinated bean.

In the starch digestibility parameter, it can be observed that germination, dehulling, and soaking treatments had significant effects on the increase of starch digestibility, but on the contrary, dehulling treatment followed by boiling and steaming could decrease the starch digestibility. Starch digestibility is not only influenced by the presence of phenolic compounds that can prevent the activity of amylase activity, but also influenced by the starch characteristic of velvet bean, such as starch physical properties, protein-starch interaction, food physical form, the integrity of starch-cellular components, and types of starch contained in a certain material [77], [78].

The dehulling and soaking treatments without heating process resulted in the loss of some antinutritional compounds which increased the α-amylase activity. A similar result was also reported by Ghavidel and Prakash [79] who showed that there was an increase of starch digestibility values in green gram, cowpea, lentil, and chickpea from 18.9–25.5% in their raw form to 55.2–64.7% in dehulled form, while Rehman [80] reported that soaking could increase the starch digestibility in black gram and chickpea from 37.4–39% to 45.7–46.8%. Apart from being able to decrease the antinutritional compounds, germination is also capable to increase the starch digestibility due to the increased activity of endogenous amylase in the bean, thus the hydrolyzed starch molecule becomes more simplified and easier to digest [81].

The decrease in starch digestibility from the heat treatment in this study was suspected due to the relatively complex crystalline structure of the velvet bean. Velvet bean has a harder texture compared with other legumes, resulted in lower starch digestibility. According to Ancona et al. [82] study, it showed that corn starch has starch digestibility of 92%, lima bean 84%, and velvet bean 78%. A relatively short heating time (30 minutes) was suspected to cause the gelatinization process to be done partially. The tough cell and tissue structure inhibit the starch granules to swell and dissolve, thus it reduces the hydrolysis rate [83]. Moreover, partially cooking could promote the interaction of protein, thus decreased the starch digestibility due to the interaction between protein and starch which could form a larger aggregate and inhibit the gelatinization of starch granule compared to the native ones [84]. In addition, the decrease in starch digestibility also shows that the velvet bean has higher ratios in SDS (slowly digestible starch) and RS (resistant starch) levels compared to RDS (rapidly digestible starch) after being cooked [85].

3.6. Physicochemical Characteristics and Functional Properties of Velvet bean Flour

Velvet bean with the skin dehulling process followed by soaking for 24 hours was the flour with the best treatment in this study according to its protein content and nutritional value. This study reported that the treatments could significantly increase the flour protein content and had better nutritional value due to having the lowest antinutritional components. The analysis result of physicochemical characteristics and functional properties, as well as mineral content (calcium and phosphor) of the best velvet bean flour can be seen in Table 6. Velvet bean flour that was obtained from the skin dehulling process followed by soaking for 24 hours treatment contained crude protein of 32.54% (Table 2), which can be classified as a high protein flour and has the potential to be used as the main ingredient for concentrate or protein isolate production. The selected treatment also contained total dietary fiber which was equivalent to avocado 6.72% and higher if compared with other fruits such as mango, apple, banana, and pear, as well as several cooked vegetables and legumes such as broccoli (4.66%), corn (4.25%), lima bean (5.23%), peas (3.54%), cowpeas (4.53%), and lentils (5.86%) [86]. Dietary fiber has been widely reported for its health benefits. Dietary fiber has the benefits of reducing blood cholesterol; reducing the risks of diabetes, cardiovascular disease, and colon cancer; binding water in the feces so the feces become softer, and shortening the residence time of feces in the digestive tract; as well as providing fulfilling effect [87]–[90].

Table 6. Physicochemical and functional properties of velvet bean flour with the best treatment

Parameters	Value
Total dietary fiber (%)	6.75 ± 0.28
Soluble dietary fiber (%)	4.75 ± 0.14
Insoluble dietary fiber (%)	2.00 ± 0.13
Mineral	
Calcium (mg/100 g)	201.86 ± 2.24
Phosphor (mg/100 g)	527.77 ± 13.23
Color Value	
L	54.90 ± 0.09
a	+2.02 ± 0.03
b	+6.16 ± 0.04
Whiteness index (%)	51.74 ± 0.05
Bulk density (g/mL)	0.51 ± 0.00
Water absorption capacity (mL/g)	1.67 ± 0.05
Oil absorption capacity (mL/g)	1.28 ± 0.10

Not only limited to dietary fiber, but the velvet bean also has a good source of calcium and phosphor. The analysis result showed that the calcium content of velvet bean flour was higher compared with other legume-based flours, such as cowpea, mung bean, chickpea, peas, peanuts, red bean, and soybean [91]. Meanwhile, the phosphor content in this study was slightly lower compared with the result reported by Kala and Mohan

[92], but higher compared with a study by Balogun and Olatidoye [93].

Velvet bean flour had a bulk density of 0.51 g/mL. This value was following the study result of Adebowale et al. [94] which reported that velvet bean flour had a bulk density of 0.42–0.61 g/cm^3 for full-fat flour and 0.72–0.88 g/cm^3 for defatted flour. This shows that the bulk density value is affected by its fat content. Soaking treatment can also affect the bulk density. Soaking can increase the water content of the bean and loosen the bean structure, which results in softer flour particles [32]. The calculation of bulk density can be used as a reference in the utilization of flour as a food ingredient. Flour with high bulk density can be utilized in food formulation that is easy to be dispersed, such as infant food. In addition, flour with high bulk density can also be applied for bakery and pastry products since it can increase fat absorption. Meanwhile, flour with low bulk density is suitable to be utilized as weaning food, since it does not possess viscous texture and high viscosity [14].

The water absorption capacity (WAC) and oil absorption capacity (OAC) show the ability of a certain material to absorb water and oil. These capacities affect the easiness of flour homogenization when mixed with water or oil during the formulation of a food product. The study result showed that the oil and water absorption capacity of velvet bean flour was 1.67 mL/g and 1.28 mL/g, as of velvet bean is categorized as having high oil and water absorption capacity. A study by Adebowale et al. [94] reported that WAC and OAC values of full-fat velvet bean flour were 1.20 mL/g and 2.40 mL/g. The difference in score was caused by the difference in chemical composition (protein content) and component polarity properties of food material [15]. The high water absorption capacity of the flour can be utilized for product formulation, namely soups, gravies, doughs, and baked products, while flour with high oil absorption capacity can be utilized as a flavor retainer in various food products [94].

Based on the subjective visualization, velvet bean flour with the best treatment in this study had white-brownish color. The study showed that the flour had a whiteness index of 51.74%. Meanwhile, the color analysis with chromameter showed that the flour had an L value of 54.90, a value of +2.02, and b value of +6.16. The white-brownish color of the flour could be due to the non-enzymatic browning during the drying process from the reducing sugar within the velvet bean [95]. Antarlina et al. [32] reported that soaking treatment could affect the whiteness index of sorghum flour. Soaking is also able to inhibit the enzymatic browning reaction caused by the endogenic enzymes in the bean, which is known as phenolase enzyme. Soaking also can trigger the spontaneous fermentation process and stimulate the growth of bacteria, including lactic acid and proteolytic bacteria. The brief fermentation can also increase the flour whiteness index due to the decrease in fiber content. Lactic acid bacteria (LAB) are essential for the degradation of fiber and polysaccharides during fermentation. It is utilized for metabolic processes such as growth, energy, and others [96].

4. Conclusions

The pre-treatments before milling significantly affected the yield, chemical composition, hydrogen cyanide, total phenol content, antioxidant capacity, protein digestibility, and starch digestibility. Dehulling treatment followed by soaking treatment for 24 hours was the best treatment that could increase the nutritional value, maintain the functional properties, and reduce HCN to a safe limit, which is 10 mg per kg of dry weight according to FAO/WHO. Velvet bean flour with the mentioned treatments had a protein content of 32.54%, total dietary fiber of 6.7%, calcium and phosphor of 202 and 528 mg/100g, the whiteness of 51.74%, bulk density of 0.51 mg/L, as well as water and oil absorption of 1.67 and 1.3 mL/g. Velvet bean flour has the potential to be developed as a raw ingredient or partial substitution in food products.

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