Food Research 8 (Suppl. 4) : 1 - 9 (2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Production of high-protein Indonesian velvet bean (M*ucuna pruriens* l.) flour using response surface methodology and characteristic analysis

[1,*]Astawan, M., [1]Rachmawati, N.A., [1]Kristianto, A., [2]Wresdiyati, T. and [3]Sardjono, R.E.

[1]*Department of Food Science and Technology, Faculty of Agricultural Engineering and Technology, IPB University, Bogor, Indonesia*
[2]*School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia*
[3]*Department of Chemistry, Indonesia University of Education, Bandung, Indonesia*

Article history:
Received: 16 August 2023
Received in Revised: 8 July 2024
Accepted: 13 July 2024
Available Online: 30 August 2024

Keywords:
High protein,
Mucuna pruriens,
Response surface methodology,
Velvet bean

DOI:
https://doi.org/10.26656/fr.2017.8(S4).1

Abstract

Indonesian velvet bean (*Mucuna pruriens* L.) has potential as a plant-based protein source for high-protein food products. Unfortunately, these beans are not widely used due to their high cyanide content. This study aimed to find a new approach to increasing protein content in velvet bean flour by implementing response surface methodology (RSM) and studying its characteristics. A preliminary study showed that white-type velvet bean flour with 24-hr soaking water treatment was chosen as the main treatment for producing high-protein velvet bean flour because it had less than 10 ppm of cyanide (2.85 ppm) and had the highest protein content (32.54%). RSM findings revealed that the optimum process variables were 10% initial substrate concentration, 0.3 mL additional α-amylase enzyme, 95.15°C temperature, pH 5.6, and a liquefaction time of 50.32 mins processing. The enzyme hydrolysis method efficiently decreased non-protein components while increasing protein by 28%, which also affected the improvement of the physicochemical and functional qualities of high-protein velvet bean flour. The high-protein velvet bean flour had a yield of 30.73%, protein content of 41.83%, bulk density of 0.43 g/mL, brownish-white color, and water activity was 0.62. Moreover, water absorption capacity and oil absorption capacity levels were 1.67 mL/g and 1.83 mL/g, respectively. The emulsion capacity and stability were 32 and 56%, respectively. Foam capacity and stability were 64 and 67%, respectively. The optimum gelling concentration was 15%, and the *in vitro* protein digestibility was 79%. To summarize, high-protein velvet bean flour has the potential to be developed as a food product, particularly for sausage, bread, and meat analogs.

1. Introduction

Protein's use in the food industry is expanding. Unfortunately, the food industry continues to rely on soybeans as its primary source of protein, requiring it to import materials from other nations. The high value of soybean imports reached 2.5 million tons in 2021, demonstrating the country's reliance on imported protein sources (Badan Pusat Statistik (BPS), 2020). This condition has a negative impact on the development of Indonesian local food ingredients.

Indonesia has various indigenous beans that are high in protein yet underutilized. Velvet bean (*Mucuna pruriens* L.) is one of these beans. According to Epriliati (2020), the velvet bean contains 22-31% protein (db) and has a nutritional quality comparable to soybean. Furthermore, the crop produces a high yield even in drought and low soil fertility conditions. On average, velvet bean yields 0.5-3.4 tons/ha (Ayerdi-Gotor and Marraccini, 2022). Velvet beans are still underutilized due to the presence of antinutrients (tannin, antitrypsin, phytates, etc.) and cyanide toxin, which is naturally present in the seeds. As a result, a pre-treatment technique, such as soaking, steaming, boiling, autoclaving, germination, or fermentation, is commonly used to lower the cyanide and antinutrient concentration (Epriliati 2020; Das *et al.,* 2022).

This study focused on the production of high-protein velvet bean flour. The response surface methodology (RSM) was used to optimize the production of high-protein velvet bean flour, which allows numerous factors

*Corresponding author.
Email: *astawan@apps.ipb.ac.id*

eISSN: 2550-2166
Published by Rynnye Lyan Resources

and their interactions to be calculated concurrently. Flour -making technology was chosen because it offers several benefits, including easier handling, processing, and storage. High-protein velvet bean flour is planned to be utilized as a strategy to increase protein intake in people's diets. The flour can be used as a substitute for meat-based foods to balance animal and vegetable protein sources. That condition lowers protein-based product prices and increases people's purchasing power for protein-source foods. This research aimed to determine the best pre-treatment method for producing velvet bean flour with a low cyanide content, find the optimum process for producing high-protein velvet bean flour using RSM, and explore its physicochemical and functional properties.

2. Materials and methods

This study was separated into two stages: preliminary research and main research. In the preliminary research, the recommended treatment was determined by taking the two most significant criteria into account: cyanide concentration and the most excellent protein content. RSM then analyzed the velvet bean flour to obtain optimum results with the highest protein content in the main research. The high-protein velvet bean flour was then analyzed for its nutritional content (proximate analysis), physicochemical and functional characteristics, and *in vitro* protein digestibility.

2.1 Materials

The velvet beans (*Mucuna pruriens* L.) used were white and striped types obtained from Kulonprogo Regency (Yogyakarta) and Ponorogo Regency (East Java), Indonesia.

2.2 Process of making velvet bean flour

Velvet beans were boiled for 30 mins before the skin was peeled (Romulo and Surya 2021). They were then divided into seven groups: raw (P0), without skin (P1), without skin after 30 mins of boiling (P2), without skin after 30 mins of steaming (P3), without skin after 6 hrs of soaking (P4), without skin after 12 hrs of immersion (P5), without skin after 24 hrs of immersion (P6), and germination (P7).

Immersion was carried out using clean water (1:10 w/v) without changing the water, while the germination treatment refers to Mugendi *et al.* (2010). Velvet beans with various treatments were dried by a cabinet dryer (50^{o}C, 24 hrs) and then floured by a disc mill (80 mesh).

2.3 Determination of hydrogen cyanide

Hydrogen cyanide was determined using the alkaline titration method (Association of the Official Analytical Collaboration (AOAC) International, 2005).

2.4 Proximate analysis

The proximate analysis carried out by AOAC International (2012) included the following parameters: moisture by gravimetry, ash by gravimetry, fat by Soxhlet method, protein by Kjeldahl method, and carbohydrate content (by difference).

2.5 The process of making high-protein velvet bean bean flour

The liquefaction process will hydrolyze the chosen velvet bean flour to produce high-protein velvet bean flour. Liquefaction is melting starch gel from high viscosity to lower viscosity by hydrolyzing starch into smaller molecules (oligosaccharides or dextrin) using α-amylase. To begin the manufacturing process, velvet bean flour was dissolved in water at a 10% concentration determined by their viscosity tests. The optimum temperature, pH, and liquefaction time values were obtained from the RSM program. The pH of the treated flour suspension was then elevated for enzyme inactivation before being decreased again to neutralize the color. The suspension was cooled and centrifuged (3000 rpm; 15 mins). After that, the residue was taken to dry using a spray dryer.

2.6 Analysis of physicochemical characteristics

The analysis includes water activity, bulk density, color, and whiteness index. Analysis of bulk density was conducted as described by Adeleke dan Odedeji (2010). The sample was placed in a previously weighed 10 mL measuring cup. The sample was compacted to 10 mL and then weighed. Bulk density was expressed as a weight ratio of sample to volume in g/mL. Meanwhile, the color analysis was performed using chromameter (Konica Minolta CR-310), and the whiteness index could be obtained from the result of the color analysis using the following equation:

$$\text{Whiteness index (\%)} = 100 - [(100 - L)^2 + a^2 + b^2]^{1/2}$$

2.7 Water absorption capacity and oil absorption capacity

As much as 0.5 g sample was mixed with 5 mL of distilled water pH 7.0 (for WAC) or 5 mL of corn oil (for OAC) and stirred for 1 min. After that, it was allowed to stand for 30 mins at 25°C before being centrifuged (3000 rpm; 25 mins). The volume of supernatant is measured, and the retained liquid is represented as milliliters of

https://doi.org/10.26656/fr.2017.8(S4).1

water or oil per g of sample (Adeleke and Odedeji 2010).

2.8 Foaming capacity and stability

Analysis of foaming capacity and stability was done as described by Huda *et al.* (2012). As much as 2 g sample was dissolved in 100 mL of distilled water and stirred with a magnetic stirrer. The solution was adjusted to pH 8 with 2 N of NaOH. The initial volume was recorded, then blended for 2 mins. After that, the foam volume was re-recorded after 30 s and 1 hr. The foam capacity is defined as the ratio of the foam volume after 30 s to the initial volume. Meanwhile, foam stability is defined as the ratio of foam volume after one hr to foam volume after 30 s.

2.9 Emulsifying capacity and stability

The procedure was assessed as described by Adeleke and Odedeji (2010). A sample of 2 g was added to 100 mL with water. After 5 mins, the sample was stirred with a magnetic stirrer. A 25 mL sample was mixed with 25 mL of corn oil. The mixture was blended for 1 min before centrifuging (3000 rpm; 10 mins). The generated emulsion was held at room temperature for some time to monitor the emulsion's stability over time. The emulsion volume was measured at 0.5, 1, 2, 4, and 6 hrs.

2.10 Gel strength

Samples were dissolved in 10 mL of distilled water to obtain solution concentrations of 7.5, 10, 12.5, and 15%. The solution was adjusted to pH 8.0 by adding 2 N of NaOH. The solution was pipetted as much as 3.0 mL into a test tube. The test tube was put in a 100°C water bath for 15 mins. The tube was removed and kept at 4°C for 2 hrs. Gel strength was evaluated qualitatively on a scale of 1–4 (Astawan, Wresdiyati, Subarna *et al*., 2020).

2.11 In vitro protein digestibility analysis

Analysis was done according to Hsu *et al*. (1977) procedure. The samples were suspended in distilled water at a concentration of 6.25 mg protein/mL. A 25 mL sample solution was put in a small beaker glass, and the pH was adjusted to 8.0. The sample was then placed in a 37°C water bath and agitated for 5 mins. While stirring in the 37°C water bath, 2.5 mL of the multienzymes solution was added to the sample suspension (once the enzyme was added, the time was recorded as time zero). The pH of the sample suspension was measured precisely at the tenth min. The protein digestibility equation was stated as,

$Y = 210.464 - 18.103x$; Y: protein digestibility and x: pH at 10^{th} min

2.12 Experimental design

The preliminary treatment design for cyanide reduction used a completely randomized design (CRD). The RSM program optimization with the Box Behnken design is carried out to get high-protein velvet bean flour. The fixed variables are the substrate concentration, which will be determined by evaluating the viscosity of velvet bean flour using a Brookfield Viscometer, and the amount of enzyme added, which will be determined by the α-amylase enzyme activity test. The process components included in the changing variables were temperature, pH, and liquefaction time, which will become RSM input and be analyzed for their effect on the response. The temperature variable was set at 90–100°C, the pH variable at 5–6, and the time variable at 20–60 mins.

2.13 Statistical analysis

The data were analyzed using the One-Way-ANOVA (Analysis of Variance) with a confidence level of 95% ($\alpha = 0.05$) and continued with Duncan's further test to determine whether there is a significant difference between treatments. The main research data obtained using the response surface methodology (RSM) were analyzed using the Design Expert® program. The significance level for the model was set at 1% mode, and the confidence interval in the verification stage was set at 99%.

3. Results and discussion

3.1 Hydrogen cyanide content as safety limit factor

Natural cyanogenic glucoside compounds found in velvet beans can degrade into cyanide and cause poisoning. As a result, its presence in food materials should be maintained to a minimum level. HCN content in raw velvet beans ranges between 12–17 mg/kg, depending on environmental variables and variety (Nwaoguikpe *et al*. 2011). Based on the ANOVA results, the treatment significantly lowered ($p<0.05$) the level of HCN (Table 1).

The data showed that HCN levels decreased significantly in the soaking and germination treatments compared to boiling and steaming. Soaking and germination were shown to lower HCN levels in velvet bean flour by more than 85% in white and striped velvet bean varieties. According to Nwaoguikpe *et al*. (2011), combined processes like soaking and boiling had a highly significant detoxifying effect, which could reduce up to 56.34%.

The increase in hydrolysis reaction caused the decrease in HCN compounds from boiling and steaming

https://doi.org/10.26656/fr.2017.8(S4).1

treatments. The hydrolyzed HCN dissolves in water and is carried along with water vapor and condensate (Bekhit *et al.,* 2018). Meanwhile, a hydrolysis reaction occurs in the soaking process when the water penetrates the beans (Epriliati 2020). The hydrolysis reaction was catalyzed by endogenous enzymes that cause cyanogenic glucoside compounds to be converted into water-soluble HCN (Betancur-Ancona 2008). The germination process accelerates the activity of hydrolytic enzymes, particularly glucosidase, which results in the hydrolysis of the cyanogenic glucoside into HCN, a volatile compound at room temperature (Bekhit *et al.,* 2018).

Table 1. Levels of cyanide acid (HCN) in velvet bean flour.

Treatments	HCN Levels (mg/kg db) in velvet bean	
	White type	Striped type
Raw	19.88±1.83^{d}	20.11±0.08^{c}
Seed coat removed	19.02±0.91^{d}	2.95±0.18^{b}
Seed coat removed + boiled 30 mins	13.83±3.30^{c}	2.32±0.90^{ab}
Seed coat removed + steamed 30 mins	8.40±0.86^{b}	1.83±0.05^{ab}
Seed coat removed + soaked 6 hrs	2.92±0.76^{a}	1.50±0.04^{a}
Seed coat removed + soaked 12 hrs	2.76±0.29^{a}	1.66±0.03^{ab}
Seed coat removed + soaked 24 hrs	2.85±0.36^{a}	1.64±0.06^{ab}
Germinated	2.87±0.25^{a}	1.62±0.03^{ab}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

3.2 Protein content in velvet bean flour

The increased protein content in velvet bean flour to produce high-protein flour makes protein the essential nutrient that must be maintained and improved. The ANOVA results showed that the treatment significantly ($p<0.05$) increased the protein content (Table 2). The 24-hr seed soaking treatment resulted in the highest increase in protein content, which was 4.12% in the white variety and 3.36% in the striped variety. These findings were in contrast to the findings of Nwaoguikpe *et al*. (2011) and Mang *et al.* (2015).

Nwaoguikpe *et al.* (2011) found that denaturation and dissolution of nitrogen components during boiling caused a reduction in protein content during the soaking and boiling processes. According to Akinmutimi and Ukpabi (2008), boiling for 30 mins could maintain protein levels in velvet beans. However, boiling for 60 and 90 mins resulted in a significant decrease in protein levels.

Even though the protein content of velvet bean flour after liquefaction fell short of the target (60%), according to the nutritional comparison claim issued by Badan Pengawas Obat dan Makanan (BPOM) (2022), a product can be claimed as high-protein if it contains 25% more protein than the original product. Based on the verification results, the chosen process design had a protein content of 41.41%, which was 28.52% greater than the protein in the original product (32.33%).

Table 2. Protein content of velvet bean flour.

Treatments	Protein content (% db) in velvet bean	
	White type	Striped type
Raw	29.51±0.11^{a}	26.23±0.20^{a}
Seed coat removed	31.20±0,20^{b}	29.08±0.16^{bcd}
Seed coat removed + boiled 30 mins	32.03±0.30^{c}	29.43±0.23^{cde}
Seed coat removed + steamed 30 mins	31.58±0.22^{bc}	28.21±0.68^{b}
Seed coat removed + soaked 6 hrs	31.80±0.28^{bc}	29.18±0.08^{bcd}
Seed coat removed + soaked 12 hrs	31.57±0.51^{bc}	28.46±0.12^{bc}
Seed coat removed + soaked 24 hrs	32.54±0.25^{c}	30.09±0.09^{de}
Germinated	31.01±0.17^{b}	30.30±0.28^{e}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

3.3 Selected treatment

The results of cyanide analysis on velvet bean flour with various treatments offer various potential treatment methods that can reduce HCN to its safety standard limit (10 mg/kg material). Therefore, selecting raw materials such as high-protein flour is based on the protein content. According to the results, the white-type velvet bean flour with 24-hr immersion was chosen as the main treatment for producing high-protein velvet bean flour.

3.4 Determination of substrate concentration and enzyme dose

Substrate concentration significantly impacts determining the initial reaction of the α-amylase. This study dissolved velvet bean flour in water and concentrated it at 10, 15, 20, and 30%. Rapid Visco Analyzer analyzed the velvet bean flour suspension to determine the gelatinization profile. According to the data, the initial temperature of velvet bean flour required to achieve gelatinization was 84°C for seven mins. This information is necessary for the α-amylase to liquefy or degrade starch molecules, as the enzyme works optimally after the flour has gelatinized.

Table 3 shows the viscosity measurement at various velvet bean flour concentrations. The 15, 20, and 30% concentrations showed viscosity too thick to fit the

https://doi.org/10.26656/fr.2017.8(S4).1

specifications of the expected flour suspension for the following process. The gel was physically formed at a 10% concentration but could still flow and had a viscosity of 205 cP. Suspension 10% was the best suspension for high-protein flour production.

The activity of the termamyl α-amylase enzyme was tested using the optimal standard conditions for α-amylase. According to Liu *et al*. (2014), the optimal temperature for amylase enzyme activity was 95°C, and the optimal pH was 5.2. The activity of α-amylase (*Bacillus licheniformis*) was 5441.8399 IU/mL. If the required enzyme dose to hydrolyze complex starch is 100 units/g, then 15 g of the sample requires 1500 units, equivalent to 0.3 mL of the α-amylase enzyme.

Table 3. Protein content of velvet bean flour.

Suspension concentration	Viscosity (cP)
10%	205
15%	12375
20%	>2 million
30%	>2 million

3.5 Optimization of protein content with response surface methodology

This study showed that temperature, pH, and processing time contribute to process optimization. The RSM used in this study was the Box Behnken model, which was used to estimate the best point in a sensitive range, such as the properties of enzymes that are extremely sensitive and cannot function outside of their optimum environmental conditions. The response used in this study was the protein level response, which was consistent with the study's purpose to increase the protein content of velvet bean flour.

Because it had the broadest range out of all three variables, liquefaction time (20–60 mins) was known to increase the protein content significantly. The longer the liquefaction process, the more carbohydrate components are degraded, which will increase the protein content. However, excessive processing time might result in protein degradation. Temperature and pH also impacted the protein level; in other words, they affected the ability of enzymes to hydrolyze carbohydrates.

The RSM contour plot graphs illustrate how variable interaction affected the response value at the protein level (Figure 1). The different hues in the contour plot graph expressed protein level response values. The blue hue or low area indicated the most deficient protein content response, 26.22%. Meanwhile, the highest protein level indicated by the red or high area was 40.51%.

The response of protein levels in the 26.22–40.51% range was optimized with a target value of 60%. The optimization target value that can be obtained is known as desirability, represented by a value ranging from 0–1: the greater the desirability value, the more likely the process to achieve the desired response variable. The chosen methods comprised 95.15°C, a pH value of 5.6, and a processing time of 50.62 mins. The selected processes were expected to have a protein content of 41.44%. Based on the protein content target of 60%, the chosen method had a desirability value of 0.69 or would generate a product with characteristics that match 69% of the target.

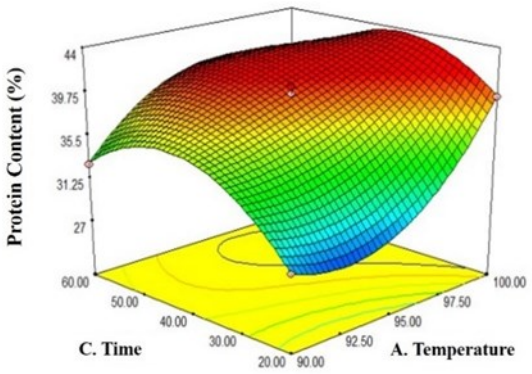

Figure 1. Contour plot and 3-D graph of protein content at pH 5.50.

3.6 Nutrient content and velvet bean flour yield

According to the research, there were specific differences in the nutritional composition of velvet bean flour and high-protein velvet bean flour. The differences in nutritional content were suspected to be caused by changes in nutrient proportion throughout the liquefaction process. Table 4 compares velvet bean flour's nutritional composition and yield with high-protein velvet bean flour.

Table 4. Comparison of nutritional content and yield of velvet bean flour and high protein velvet bean flour.

Parameter	Velvet bean flour	High protein velvet bean Flour
Ash (%db)	1.66 ± 0.06	3.02±0.01
Fat (%db)	5.34 ± 0.06	4.64±0.18
Protein (%db)	32.54 ± 0.25	41.83±0.71
Carbohydrate (%db)	60.45 ±0.38	49.51±0.96
Yield (%)	54.04 ±3.13	30.73±0.60

Compared to regular velvet bean flour, the protein content of high-protein velvet bean flour increased by 9.28 g/100 g while the carbohydrate content decreased by 10.94 g/100 g. The liquefaction method used to produce high-protein flour caused this condition. Protein and other nutritional compounds rise proportionally as

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

https://doi.org/10.26656/fr.2017.8(S4).1

carbohydrate component degrades.

3.7 Physicochemical characteristics of high-protein velvet bean flour

Bulk density is one of the flour characteristics. The produced high-protein flour (Table 5) had a lower bulk density than the bulk density of velvet bean flour reported by Mang *et al*. (2015). However, compared with soybean flour and soybean protein isolate, which had bulk densities of 0.34 and 0.39 g/mL, respectively (Astawan, Wresdiyati, Yoshari *et al.,* 2020; Astawan, Wresdiyati, Purnomo *et al.,* 2020), high-protein velvet bean flour had a higher bulk density. Flour with a higher bulk density will be more efficient in storing and packing.

Besides aroma and texture, color is one of food's most critical visual attributes. The results showed that the high-protein velvet bean flour color was not too dark and tended to be light (Table 5). This condition might broaden the utilization of high-protein velvet bean flour in various food products. The flour had a medium brightness level, as indicated by the L value of 50.04. The notation "a" is a chromatic hue mixture of red and green, whereas the notation "b" is a chromatic hue mixture of yellow and blue. When the a and b values from this study were plotted on a color graph, it could be seen that the sample had brownish-white color.

Table 5. Physicochemical characteristics of high protein velvet bean flour.

Parameter	Results
Bulk Density (g/mL)	0.43±0.00
Colour	
L	50.04±0.50
a	+1.89±0.02
b	+5.24±0.04
Whiteness index (%)	44.48±0.45
Water activity (a_w)	0.62±0.00
Protein digestibility (%)	78.58±0.18

The color of high-protein velvet bean flour, which tended to be brownish-white, was caused by non-enzymatic browning reactions during drying pre-treatment and chemical reactions during the liquefaction process. The liquefaction process breaks down carbohydrates into simpler components such as simple sugars and dextrins. These simple sugars, particularly reducing sugars, can react with proteins and cause a non-enzymatic browning reaction.

Water activity (a_w) is the amount of free water present in foods and is used to determine food safety related to chemical, microbiological, and enzymatic damages in food. The water activity of high-protein velvet bean flour was 0.62, as shown in Table 5. Based on a_w value, the chances that might occur are caused by microbiological (mold) and chemical (non-enzymatic browning reactions and lipid oxidation) factors.

3.8 Functional characteristics of high-protein velvet bean flour

Water absorption of protein is an essential functional attribute in food processing since it determines the product's juiciness and mouthfeel. According to the data (Table 6), the water absorption value of high-protein velvet bean flour (1.77 g water/g sample) was lower than soybean flour (2.24 g water/g sample) but higher than velvet bean flour (1.50 g water/g sample), germinated soybean flour (1.27 g water/g sample), and soy protein isolate (1.57 g water/g sample) (Adebowale *et al*., 2005; Astawan and Hazmi 2016; Astawan, Wresdiyati, Subarna *et al.,* 2020). Based on the high water absorption value, high-protein velvet bean flour can be applied as an ingredient in sausages and bakeries.

Oil absorption is essential in various food systems, including emulsified foods. The range of oil absorption of high-protein velvet bean flour (Table 6) was higher (1.83 mL oil/g sample) than soybean flour (0.95 mL oil/g solid) and soybean protein isolate (1.19 mL oil/g sample) reported by Astawan and Hazmi (2016) and Astawan, Wresdiyati, Subarna *et al*. (2020). Based on the oil absorption value, high-protein velvet bean flour has the potential for application to retain flavor, boost palatability, and extend shelf-life.

Based on this study result, the emulsion capacity of high protein velvet bean flour was 32%. (Table 6). This value was less than 56–90% emulsion capacity of six velvet bean cultivars reported by Adebowale *et al.* (2005). However, according to Astawan and Hazmi (2016), it was still greater than soybean flour (1.25%) and germinated soybean flour (12.50%). The high-protein flour emulsion stability curve (Figure 2) showed that high-protein flour had a less stable emulsion

Table 6. Functional characteristics of high protein velvet bean flour.

Parameter	Results
Water absorption capacity (g water/ g sample)	1.77±0.05
Oil absorption capacity (mL oil/ g sample)	1.83±0.03
Gel strength	
7.5% (b/v)	0 (gel not formed)
10% (b/v)	1 (weak gel)
12.5% (b/v)	1 (weak gel)
15% (b/v)	3 (strong gel)
Emulsion capacity (%)	32.00±0.019
Foaming capacity (%)	63.80±0.60

https://doi.org/10.26656/fr.2017.8(S4).1

capacity. The emulsion stability decreased linearly from 0 to 120 mins. However, after 120 to 360 mins, the emulsion stabilized.

The emulsion capacity and stability were determined by the hydrophilic and lipophilic bond stability in a protein. The low capacity and stability in emulsion could be caused by heating at 95.15°C during the liquefaction process, which caused the protein denaturation. According to Epriliati (2020), heating can increase water and oil absorption capacity while decreasing emulsion and foaming capacity but has no effect on foam stability.

The foam-forming ability is essential in whipped cream products. The foaming capacity of high-protein velvet bean flour (Table 6) was still within the value range of velvet bean flour foaming capacity (60.9–84.9%). Meanwhile, stability was 47.2–75%, depending on the variety and processing process (Mang *et al.* 2015). The foam stability at pH 8.0 was 66.80% (Figure 3), which was lower than the foam stability at pH 7.0

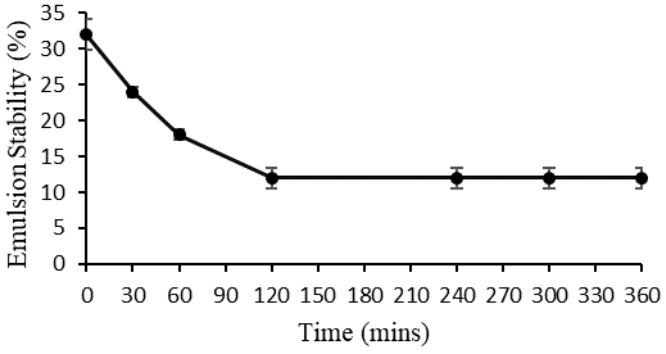

Figure 2. Emulsion stability (%) of high-protein velvet bean flour.

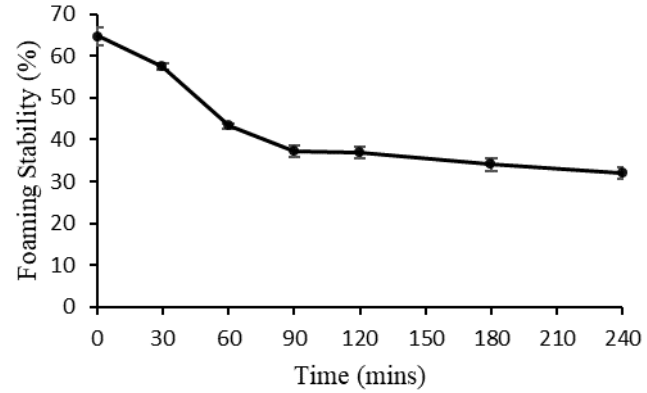

Figure 3. Foaming stability (%) of high-protein velvet bean flour.

(93.75%) (Mugendi *et al.*, 2010). Alternative methods can be implemented to enhance the foaming capacity and stability. This can be accomplished by increasing the protein solubility to pH 10.0. (Mugendi *et al.* 2010).

Gelation is a rheological characteristic associated with a protein molecule's capability to absorb water from its surrounding environment. At 10% concentration, high protein flour began forming a gel with the weak gel dislodged when the tube was tilted. However, at 15% concentration, the gel appearance was solid and did not dislodge when the tube was tilted (Table 6). The gelling characteristics depend on the ratios of components such as protein, fat, and carbohydrate. The high protein content of velvet bean flour causes gel formation, which becomes stronger at a lower concentration. These findings may be used for food products that require strong gel, such as soups, sauces, and meat products.

3.9 In vitro protein digestibility of high-protein flour

The protein content in the high-protein flour in this study was 78.58% (Table 5). *In vitro* protein digestibility of high-protein velvet bean flour was similar to other legumes, such as germinated cowpea (77.2%), germinated lentil (78.8%), and germinated chickpea (77.6%). (Ghavidel and Prakash 2007). This was due to the varying types and concentrations of antinutrient compounds found in each component. Furthermore, protein digestion is affected by the processing procedure.

Compared to soybeans and their product derivatives, high-protein velvet bean flour had lower digestibility. Soybean had *in vivo* digestibility of 99% (Astawan *et al.*, 2022), and tempe had *in vivo* digestibility of 99% (Maskar *et al.* 2015). However, compared to the commercial soy protein isolate, which had *in vitro* protein digestibility of 76% (Astawan, Wresdiyati, Yoshari *et al*., 2020), high-protein velvet bean flour had better protein digestibility.

The heating process during liquefaction caused protein denaturation, enabling the enzymes to hydrolyse protein into smaller molecules more readily absorbed by the body. Due to the relatively high protein digestibility of high-protein velvet bean flour, it is suggested that it has superior protein quality and can be used as a plant-based protein source to promote protein consumption.

4. Conclusion

The velvet bean's white type was chosen as the raw material for creating high-protein flour after soaking the seeds for 24 hrs. Response surface methodology (RSM) optimization revealed that 95.15°C, pH 5.6, and a liquefaction time of 50.63 mins were the best conditions for producing high-protein velvet bean flour. The optimization process resulted in a 28.5% increase in protein content, improved physicochemical qualities, and functional qualities of high-protein velvet bean flour. Protein digestibility *in vitro* was relatively high (78.58%), adding value to high-protein velvet bean flour as a plant-based protein source. High-protein velvet bean

https://doi.org/10.26656/fr.2017.8(S4).1

flour has the potential to be developed as a raw material or as a partial substitute in food products such as sausages, bakery, and meat substitutes.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors are very grateful for financial support from the Directorate General of Higher Education, Research, and Technology; Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia through the "Penelitian Dasar Kompetitif Nasional" scheme, the fiscal year 2023 under Made Astawan.

References

Adebowale, A.Y., Adeyemi, A.I. and Oshodi, A.A. (2005). Functional and physicochemical properties of flours of six *Mucuna* species. *African Journal of Biotechnology*, 4, 1461–1468.

Adeleke, R.O. and Odedeji, J.O. (2010). Functional properties of wheat and sweet potato flour blends. *Pakistan Journal of Nutrition*, 9, 535–538. https://doi.org/10.3923/pjn.2010.535.538

Akinmutimi, A.H. and Ukpabi, U.H. (2008). Effect of cooking periods on the nutrient composition of velvet beans (*Mucuna pruriens*). *Agricultural Journal*, 3, 204-207.

Astawan, M., Wresdiyati, T., Subarna, Rokaesih and Yoshari, R.M. (2020). Functional properties of tempe protein isolates derived from germinated and non-germinated soybeans. *IOP Conference Series: Earth and Environmental Science,* 443*,* 012001. https://doi.org/10.1088/1755-1315/443/1/012001

Astawan, M., Annisa, A.S.N., Wresdiyati, T., Sahrial and Mursyid (2022). Equivalence Test on the Protein Content and Quality of Transgenic and Non-Transgenic Soybean Flour. *Current Nutrition and Food Science*, 10, 240-249. https://doi.org/10.12944/CRNFSJ.10.1.19

Astawan, M. and Hazmi, K. (2016). Karakteristik fisikokimia tepung kecambah kedelai. *Jurnal Pangan*, 25, 105-112. [In Bahasa Indonesia].

Astawan, M., Wresdiyati, T., Purnomo, E.H. and Purwanto, A. (2020). Equivalence test between the physicochemical properties of transgenic and non-transgenic soy flour. *Journal of Nutritional Science and Vitaminology*, 66(Supplement), S286-S294. https://doi.org/10.3177/jnsv.66.S286

Association of the Official Analytical Collaboration (AOAC) International. (2005). Official Method of Analysis. Washington, D.C., USA: AOAC International.

Association of the Official Analytical Collaboration (AOAC) International. (2012). Official Method of Analysis. Washington, D.C., USA: AOAC International.

Astawan, M., Wresdiyati, T., Yoshari, R.M., Rachmawati, N.A. and Fadilla, R. (2020). The Physicochemical Properties of Tempe Protein Isolated from Germinated and Non-Germinated Soybeans. *Journal of Nutritional Science and Vitaminology*, 66(Supplement), S215-S221. https://doi.org/10.3177/jnsv.66.S215

Ayerdi-Gotor, A. and Marraccini, E. (2022). Innovative Pulses for Western European Temperate Regions: A Review. *Agronomy*, 12(1), 170. https://doi.org/10.3390/agronomy12010170

Badan Pengawas Obat dan Makanan (BPOM). (2022). Pengawasan Klaim Pada Label dan Iklan Pangan. Jakarta: BPOM. [In Bahasa Indonesia].

Badan Pusat Statistik (BPS). (2020). Statistical Yearbook of Indonesia 2020. Jakartam Indonesia: BPS [In Bahasa Indonesia].

Bekhit, A.E.D.A., Shavandi, A., Jodjaja, T., Birch, J., Teh, S., Ahmed, I.A.M., Al-Juhaimi, F.Y., Saeedi, P. and Bekhit, A.A. (2018). Flaxseed: Composition, detoxification, utilization, and opportunities. *Biocatalysis and Agricultural Biotechnology*, 13, 129-152. https://doi.org/10.1016/j.bcab.2017.11.017

Betancur-Ancona, D., Gallegos-Tintoré, S., Delgado-Herrera, A., Pérez-Flores, V., Castellanos Ruelas, A. and Chel-Guerrero, L. (2008). Some physicochemical and antinutritional properties of raw flours and protein isolates from *Mucuna pruriens* (velvet bean) and *Canavalia ensiformis* (jack bean). *International Journal of Food Science and Technology*, 43(5), 816-823. https://doi.org/10.1111/j.1365-2621.2007.01521.x

Das, G., Sharma, A. and Sarkar, P.K. (2022). Conventional and emerging processing techniques for the post-harvest reduction of antinutrients in edible legumes. *Applied Food Research*, 2(1), 100112. https://doi.org/10.1016/j.afres.2022.100112

Epriliati, I. (2020). Minimum water consumption method screening of velvet bean (*Mucuna s*p.) processings to produce functional food ingredients. *Journal of Functional Food and Nutraceutical*, 2(1), 1-28. https://doi.org/10.33555/jffn.v2i1.34

Ghavidel, R.A. and Prakash, J. (2007). The impact of germination and dehulling on nutrients, antinutrients,

in vitro iron and calcium bioavailability, and in vitro starch and protein digestibility of some legume seeds. *LWT,* 40(7), 1292–1299. https://doi.org/10.1016/j.lwt.2006.08.002

Hsu, H.W., Vavak, D.L., Satterlee, L.D. and Miller, G.A. (1977). A multienzyme technique for estimating protein digestibility. *Journal of Food Science,* 42(5), 1269-1273. https://doi.org/10.1111/j.1365-2621.1977.tb14476.x

Huda, N., Santana, P., Abdullah, R. and Yang, T.A. (2012). Effect of different dryoprotectant on functional properties of threadfin bream surimi powder. *Journal of Fisheries and Aquatic Science*, 7, 215–223. https://doi.org/10.3923/jfas.2012.215.223

Liu, Y., Fan, S., Liu, X., Zhang, Z., Wang, J., Wang, Z. and Lu, F. (2014). A highly active alpha-amylase from *Bacillus licheniformis*: directed evolution, enzyme characterization, and structural analysis. *Journal of Microbiology and Biotechnology*, 24, 898 -904. https://doi.org/10.4014/jmb.1402.02004

Mang, D.Y., Abdou, A.B., Njintang, N.Y., Djiogue, E.J.M., Bernard, C., Scher, J. and Mbofung, M.C. (2015). Effect of dehulling and boiling on the physicochemical functional and pasting properties of two varieties of Mucuna bean (*Mucuna pruriens* L.) flours. *Journal of Food Measurement and Characterization,* 9, 435–447. https://doi.org/10.1007/s11694-015-9251-6

Maskar, D.H., Hardinsyah, H., Damayanthi, E., Astawan, M. and Wresdiyati, T. (2015). Evaluasi kesepadanan mutu gizi tempe kedelai pangan rekayasa genetik (PRG) dan non-PRG serta dampak konsumsinya pada tikus percobaan. *Indonesian Journal of Nutrition and Food*, 10, 207-216. [In Bahasa Indonesia].

Mugendi, J.B., Njagi, E.M., Kuria, E.N., Mwasaru, M.A., Mureithi, J.G. and Apostolides, Z. (2010). Effect of processing technique on the nutritional composition and anti-nutrient content of mucuna bean (*Mucuna pruriens* L.). *African Journal of Food Science,* 4(4), 156-166.

Nwaoguikpe, R.N., Braide, W. and Ujowundu, C.O. (2011). The effects of processing on the proximate and phytochemical compositions of *Mucuna pruriens* seeds (velvet beans). *Pakistan Journal of Nutrition*, 10(10), 947-951. https://doi.org/10.3923/pjn.2011.947.951

Romulo, A. and Surya, R. (2021). Tempe: A traditional fermented food of Indonesia and its health benefits. *International Journal of Food Science*, 26, 100413. https://doi.org/10.1016/j.ijgfs.2021.100413

https://doi.org/10.26656/fr.2017.8(S4).1