Morphological characteristics, chemical and amino acids composition of flours from velvet beans tempe (*Mucuna pruriens*), an indigenous legumes from Yogyakarta

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Abstract. Velvet beans (*Mucuna pruriens*) an under-utilized legume which grown predominantly in tropical area, including in Indonesia. The morphological characteristics, chemical and amino acid composition of seeds, fermented velvet beans (Benguk tempeh), and Benguk tempeh flours from the legumes *Mucuna pruriens* was evaluated to determine their potential practical applications. The seeds of velvet beans contains 12.73±0.10, 4.34±0.33, 25.49±0.06, 3.66±0.03, 3.90±0.06, and 53.79±0.26 (% wb) of moisture, fat, crude protein, ash, crude fiber and carbohydrate, respectively. The mineral content of velvet seeds was 76.38±8.77, 6.64±0.67, and 3.08±0.12 mg 100 g⁻¹ of calcium, iron, and zinc, respectively. Fermentation process on velvet beans into tempeh brings nutritional changes including the breakdown of certain nutrition content and reduction of anti-nutritional compounds. The velvet beans tempeh contains 54.76±0.24, 2.40±0.16, 12.84±0.56, 1.02±0.13, 3.66±0.49, and 28.99±0.60 (% wb) moisture, fat, crude protein, ash, crude fiber and carbohydrate, respectively. The mineral content of velvet beans tempeh was 55.49±13.91, 6.56±0.39 and 2.60±0.33 mg 100 g⁻¹ of calcium, iron, and zinc, respectively. The flours were prepared by steam blanching, oven-drying (60°C) for 7h and milling. The flours contains 13.13±0.52, 6.78±0.72, 25.29±0.41, 0.76±0.04, 4.34±0.32, and 54.06±0.47 (% wb) of moisture, fat, crude protein, ash, crude fiber and carbohydrate, respectively. The mineral content of flour was 29.43±0.00, 5.87±1.36 and 2.56±0.04 mg 100 g⁻¹ of calcium, iron, and zinc, respectively. The essential amino acids profile of total flour proteins compared favourably with the FAO/WHO reference pattern except for deficiency of sulfo amino acids (methionine and sistin) with concentration of 9.72 mg g⁻¹ crude protein. The flours have potential as flour alternatives for food product development.

1. Introduction

The prevalence of protein malnutrition in developing countries is recognized. Demand of animal protein such as meat, milk and poultry in developing country still inadequate and expensive. Thus, the intensive research about legumes has begun to be explored as protein alternatives for making nutritional ingredients. The ideal diet consists of animal foods and plant foods which appropriate to ensure adequate amounts of protein intake and protein quality with adequate consumption of dietary fiber [1]. In general, source of protein derived from plant protein contribute 65% and animal protein contribute 35% [2]. The right combination and amount of plant protein and animal protein is needed to meet balance of amino acids in the body [3]. One serving size of legumes (90 grams) provide 7-8 gram of protein or 15% of Recommendation Dietary Allowance (RDA) of protein intake for adults with weights 70 kgs [4].
Currently, tempe is one of traditional food in Indonesia which popular in several countries because its nutritional content and bioactive compounds. Tempe usually made from soybeans (Glycine max) with fermentation process using culture of Rhizopus sp. (Rhizopus oryzae or Rhizopus oligosporus). Making soybeans tempe is directly also increase demand of soybeans as raw ingredient for production. Based on data from Central Statistic Agency, in 2017 the total supply of soybeans was 2.45 million tons, which used as food ingredients amounted to 84.6%.

Tempe become the highest consumption level amount 44% of processed soybeans products, then followed by tofu 32.7% and 23.3% in the form of other product such as tauco, oncom and soy sauce [5]. However, soybeans are not commodity originating from Indonesia but from Sub-tropical countries. Thus, the production of soybeans in Indonesia still lower than in Sub-tropical countries such as United States, Brazil, Argentina, China, India, and Paraguay which contributed 92.04% to average production of soybean in global which amounted 271.02 millions tons [6].

Velvet beans (Mucuna pruriens) is an under-utilized legume which grown predominantly in tropical area, including in Yogyakarta, Indonesia. The seeds of velvet beans (Mucuna pruriens) contains high protein, carbohydrate and fiber. Velvet beans (Mucuna pruriens) is type legume which can grow in dry and infertile lands, has high nutritional content, the price relatively cheap, and planted locally in Indonesia so there is no need import from other countries. Velvet beans (Mucuna pruriens), like other legumes, generally contains anti-nutritional compounds such as phytate, polyphenol, protease inhibitors, and aromatic amino acids which cause biological and physiological effect such as decrease of protein digestibility and inhibit the growth of animal [7].

Several processing techniques such as soaking, boiling, roasting, fermentation, and germination has been used to remove anti-nutritional compounds and improve nutritional value of legumes [8]. Tempe was fermented using Rhizopus sp [9]. Some processing techniques in making tempe from velvet beans (Mucuna pruriens) consist of soaking, boiling, draining, peeling, and fermentation. The shelf life of fermented velvet beans (Benguk tempe) is 24 hours after fermentation process. The functional properties of seeds, fermented velvet beans (Benguk tempe), and Benguk tempeh flours from the legumes Mucuna pruriens was evaluated to determine their potential practical applications such as flour alternatives which rich of nutrition content for food product development.

2. Materials and methods

2.1. Materials
The sample of velvet beans (Mucuna pruriens) was taken from local farmers in Yogyakarta, Indonesia. The seeds were dried in room temperature, removed the immature seeds and unwanted materials (stones), then stored in plastic container. Several seeds were milled using grinder and sieved to pass through a 100 mesh sieve, then stored in plastic container covered with aluminium foil at 4°C for analyze.

2.2. Process of making fermented velvet beans (Benguk’s tempe)
The process of making Benguk tempe was adapted from local society in Yogyakarta, Indonesia with several modifications. First, the seeds were washed and boiled with adding ash to decrease hidrogen cyanide content and got tender during 30 minutes (90-95°C). After that, the swollen seeds were soaked in water during 3 × 24 h with changes of water every 8 hours. The seeds were peeled and sliced into small size. Then, the sliced seed were washed and boiled again 30 minutes (90-95°C), cooled, and inoculated with 0.2% inoculum Rhizopus sp., wrapped with polypropilene plastics and fermented during 2 × 24 h at 29-35°C.

2.3. Process of making flour from Benguk’s tempe
The Benguk’s tempe were sliced into small size then steam blanched during 5 minutes. Then, Benguk’s tempe were oven-dried 7 hours (60°C). After oven dried, benguk’s tempe were milled using blender and
sieved to pass through a 60 mesh sieve, then stored in plastic container covered with aluminium foil at 4°C for analyse

2.4. Proximate analysis
Proximate analysis of seeds, fermented velvet beans (Benguk tempeh), and Benguk tempeh flours from the legumes Mucuna pruriens for moisture, ash, fat, crude protein, and crude fibre were determined by the standard methods already described in the AOAC [10]. Nitrogen was determined by micro-Kjehldahl method the percentage nitrogen was converted to protein by multiplying conversion factor 6.25. The carbohydrate content was determined as the weight difference using moisture, crude protein, fat and ash content data.

2.5. Mineral analysis
Mineral analysis of seeds, fermented velvet beans (Benguk tempeh), and Benguk tempeh flours from the legumes Mucuna pruriens for Ca, Fe, and Zn were determined by the standard methods already described in the AOAC [10]. Minerals were analyzed by dry ashing the samples and wet digestion with a mixture of nitric, sulphuric and hydrochloric acids, using atomic absorption spectro-photometer (AAS).

2.6. Amino acid analysis
Amino acid analysis was determined using High Performance Liquid Chromatography (HPLC). Samples were freeze-dried and then hydrolysed for 24 h at 110°C in 6 M HCl. The amino acid analysis was performed using an automated precolumn derivatization with O-phthaldialdehyde (OPA) using reverse-phase HPLC. The amino acids content of Benguk tempe flour were compared with the FAO/WHO (1985) reference pattern [11].

2.7. Statistical analysis
The seeds, fermented velvet beans (Benguk tempeh), and Benguk tempeh flours from the legumes Mucuna pruriens results in this study are expressed as mean ± standard deviation. Differences in the mean values were determined at p value < 0.05 and determined by independent sample t-test to compare between the mean values of velvet beans and soybeans using SPSS 2016.

3. Results and discussion

3.1. Morphological characteristic of seeds, fermented velvet beans (Benguk tempeh), and Benguk tempeh flours
In order to compare the morphological characteristic of velvet beans, soybeans were used as as comparison (control). Characteristics of velvet bean seeds, soybean seeds, Benguk tempe, soybeans tempe, Benguk tempe after milled process, and Benguk tempe flour shown in Figure 1.

Figure 1. Characteristic of (a) velvet bean seeds, (b) soybean seeds, (c) Benguk tempe, (d) soybean tempe, (e) Benguk tempe after milled process, (f) Benguk tempe flour (60 mesh).
Based morphological characteristic which shown in figure 1, velvet beans (*Mucuna pruriens*) were light brown in colour, while soybeans (*Gycine max*) were yellow in colour. The average weight of velvet bean seeds (0.95±0.04 gram) was higher than soybean seeds (0.24±0.07 gram). The relatives hardness of velvet bean seeds was higher than soybean seeds. Velvet bean seeds may therefore require a longer time to cook and this could also influence the preference of consumers. Those figures indicate that velvet beans are larger and bulkier than soybeans.

pH value from soaking water of velvet beans (4.37±0.05) was lower than soybean (5.44±0.4). Benguk tempe and soybeans tempe were firmly bound by micelium and formed a sliceable compact white cake. As the result of fermentation process, the inner color of velvet beans became darker than soybean. Both of Benguk tempe and soybeans tempe were developed a pleasant smell like slightly acidic at the time 48 hour fermentation.

Benguk tempe after milled process formed rough granules. Thus, it need sieved to pass through a 60 mesh sieve to become soft granules as usual flour. Benguk tempe flour were light brown in colour. The granule and capability to mix with other food ingredients of Benguk tempe flour were similar with wheat flour. Thus, Benguk tempe flour have potential as flour alternatives for food product development such as cookies, biscuits, cereal flakes, etc.

3.2. Proximate and mineral composition of velvet beans (*Mucuna pruriens*)

The means value of proximate, crude fiber and mineral composition of velvet beans seeds compared with soybean seeds are shown in Table 1. The ash, crude protein, fat and crude fiber of velvet bean seeds are lower than soybean seeds. But, the moisture and carbohydrate content of velvet bean seeds is higher than soybean seeds. The carbohydrate content of velvet beans is twofold higher than soybean seeds. There is significant difference between all proximate composition and crude fiber content among velvet bean seeds and soybean seeds (p<0.05).

| No. | Chemical analysis | Velvet beans (*Mucuna pruriens*) | Soybeans (*Gycine max*) |
|-----|-------------------|---------------------------------|------------------------|
| 1   | Moisture (% wb)   | 12.73±0.10                      | 9.71±0.04              |
| 2   | Ash (% wb)        | 4.19±0.03                       | 5.66±0.16              |
| 3   | Crude protein (% wb) | 4.97±0.38                   | 20.30±0.12             |
| 4   | Fat (% wb)        | 29.21±0.06                      | 42.55±0.16             |
| 5   | Carbohydrate (% wb) | 61.63±0.41                   | 31.50±0.20             |
| 6   | Crude fiber (% wb) | 4.46±0.07                      | 9.61±0.03              |
| 7   | Ca (mg/100 g)     | 76.38±8.77                      | 182.96±17.07           |
| 8   | Fe (mg/100 g)     | 6.64±0.67                       | 12.96±0.90             |
| 9   | Zn (mg/100 g)     | 3.08±0.12                       | 2.96±0.17              |

Results are expressed as means determinations ± SD.

Means followed by different superscript in each row indicates significant differences at P < 0.05.

Ca = calcium
Fe = iron
Zn = zinc

Minerals are a component of micro nutrients which have important for the body. The mineral content can be drawn from the ash content of a food ingredient [12]. Minerals consist of two groups, namely macro-minerals and micro-minerals. Macro-minerals are minerals that the body needs in amounts of more than 100 mg a day, whereas micro minerals are needed less than 100 mg a day [13]. Macro-minerals consist of sodium, chlorine, calcium, phosphorus, magnesium, and sulfur. Micro-minerals consist of iron, iodine, manganese, copper, zinc, cobalt, and fluorine [14].

Calcium, iron, and zinc are mineral which have beneficial function on growth. Tabel 1 shows calcium and iron of velvet bean seeds are lower than velvet bean seeds. Besides that, the zinc content of velvet bean seeds is slightly higher than soybean seeds. There is no significant difference between zinc content among velvet bean seeds and soybean seeds (p>0.05).
3.3. Proximate and mineral composition of fermented velvet beans (Benguk’s tempe)

Fermentation brings numerous biochemical, nutritional and organoleptic changes in the raw materials including the breakdown of certain constituents. The changes of proximate and mineral composition in velvet bean tempe and soybean tempe during fermentation process are presented in Table 2. The moisture, crude protein, fat and crude fiber of velvet bean tempe are lower than soybean tempe. But, the ash and carbohydrate content of velvet bean tempe is higher than soybean tempe. The carbohydrate content of velvet beans is twofold higher than soybean tempe. There is no significant difference between protein content among velvet bean tempe and soybean tempe (p>0.05).

| No. | Chemical analysis         | Benguk tempe          | Soybeans tempe       |
|-----|---------------------------|-----------------------|----------------------|
| 1   | Moisture (% wb)           | 54.76±0.24            | 64.49±1.30           |
| 2   | Ash (% wb)                | 2.25±0.28             | 0.39±0.12            |
| 3   | Crude protein (% wb)      | 5.31±0.34             | 22.29±1.17           |
| 4   | Fat (% wb)                | 28.37±1.23            | 41.75±0.38           |
| 5   | Carbohydrate (% wb)       | 64.07±1.86            | 35.57±1.67           |
| 6   | Crude fiber (% wb)        | 8.90±1.08             | 12.42±0.92           |
| 7   | Ca (mg/100 g)             | 55.49±13.91           | 190.08±0.59          |
| 8   | Fe (mg/100 g)             | 6.56±0.39             | 6.46±0.27            |
| 9   | Zn (mg/100 g)             | 2.60±0.33             | 3.00±0.11            |

Results expressed as means determinations ± SD. Means followed by different superscript in each row indicates significant differences at P < 0.05. Ca = calcium, Fe = iron, Zn = zinc.

3.4. Proximate and mineral composition of flour from Benguk’s tempe

The means value of proximate, crude fiber and mineral composition of benguk tempe flour compared with commercial wheat flour are shown in Table 3. The crude protein and fat of Benguk tempe flour are higher than commercial wheat flour. But, the carbohydrate content of benguk flour is lower than commercial wheat flour.

| No. | Chemical analysis         | Benguk tempe flour    | Commercial wheat flour |
|-----|---------------------------|-----------------------|------------------------|
| 1   | Moisture (% wb)           | 13.13±0.52            | 11.80±                  |
| 2   | Ash (% wb)                | 0.87±0.04             | 1.13±                   |
| 3   | Crude protein (% wb)      | 7.80±0.48             | 1.13±                   |
| 4   | Fat (% wb)                | 29.11±0.47            | 10.20±                  |
| 5   | Carbohydrate (% wb)       | 62.22±0.05            | 87.53±                  |
| 6   | Crude fiber (% wb)        | 4.76±0.02             |                        |
| 7   | Ca (mg/100 g)             | 23.49±0.00            | 24.94±                  |
| 8   | Fe (mg/100 g)             | 5.87±1.36             | 1.47±                   |
| 9   | Zn (mg/100 g)             | 2.60±0.04             | 3.17±                   |

Results expressed as means determinations ± SD. Means followed by different superscript in each row indicates significant differences at P < 0.05. Ca = calcium, Fe = iron, Zn = zinc.

*Data not available
There is significant difference between crude protein, fat, and carbohydrate among Benguk tempe flour and commercial wheat flour (p < 0.05). Benguk tempe flour contain sources of protein, iron and zinc. Thus, benguk tempe flour could be one of alternatives ingredients in food product development with better nutrition content.

3.5. Amino acids composition, essential amino acids score, and limiting amino acids of Benguk tempe flour

Amino acids are important components for humans. Amino acids are divided into essential and non-essential amino acids. Essential amino acids are amino acids which cannot be synthesized by body and must be fulfilled from food consumed daily. Essential amino acids consist of histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Non-essential amino acids are amino acids which can be synthesized by body, including alanine, arginine, cysteine, glutamine, glycine, taurine [15]. Amino acids are important to analyze because they describe the quality of a food protein [16]. The amino acids composition, essential amino acids score, and limiting amino acids of Benguk tempe flour are shown in Table 4.

Table 4. Amino acids composition, essential amino acids score, and limiting amino acids of Benguk tempe flour (mg g⁻¹ crude protein).

| No | Amino acids            | Result (mg g⁻¹ crude protein) | Requirement of amino acid among school aged children (FAO 1985) | Amino acid score |
|----|------------------------|-------------------------------|-----------------------------------------------------------------|------------------|
| 1  | Aspartic acid          | 73.59                         |                                                                 |                  |
| 2  | Threonine              | 25.51                         | 28                                                              | 83.99            |
| 3  | Serine                 | 21.24                         |                                                                 |                  |
| 4  | Glutamic acid          | 77.89                         |                                                                 |                  |
| 5  | Glycine                | 27.82                         |                                                                 |                  |
| 6  | Alanine                | 26.05                         |                                                                 |                  |
| 7  | Cystine                | 9.72                          | 22                                                              | 43.68            |
| 8  | Methionine             |                               |                                                                 |                  |
| 9  | Valine                 | 35.40                         | 25                                                              | 100+             |
| 10 | Isoleucine             | 33.12                         | 28                                                              | 100+             |
| 11 | Leucine                | 47.54                         | 44                                                              | 100+             |
| 12 | Tyrosine               | 54.12                         | 22                                                              | 100+             |
| 13 | Phenylalanine          |                               |                                                                 |                  |
| 14 | Histidine              | 14.92                         |                                                                 |                  |
| 15 | Lysine                 | 41.98                         | 44                                                              | 95.41            |
| 16 | Arginine               | 39.71                         |                                                                 |                  |
| 17 | Proline                | 31.11                         |                                                                 |                  |
| 18 | Tryptophan             | 5.31                          | 9                                                               | 59.01            |

Results are expressed as means determinations ± SD.

Essential amino acids.

Among amino acids in legumes, usually lysine constitutes the highest, while sulphur-amino acids are limiting [17]. The amino acids composition of Benguk tempe flour revealed that the protein of this flour contained adequate levels of essential amino acid for as compared with the FAO/WHO (1985), except for the sulphur-containing amino acids (cystine and methionine), which are considered to be the most limiting amino acids in this flour.

4. Conclusion

Therefore, velvet beans, fermented velvet beans (Benguk tempe), and Benguk tempe flour represents potential food source. Benguk tempe flour could be an alternatives flour for food product development
because contains high protein and source of calcium. The preliminary study needs to be followed both of in vitro and in vivo studies.

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