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Functional Properties of Winged Bean (*Psophocarpus tetragonolobus* L.) Seed Protein Concentrate

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Abstract: Protein is a food component that has functional properties that affect the properties of food products. Usually, protein was used in the form of protein concentrate mainly soy protein concentrate. This research was aimed to compare the chemical composition and functional properties of full-fat winged bean flour, defatted winged bean flour, and winged bean protein concentrate. Winged bean protein concentrate were prepared by isoelectric precipitation, whereas defatted winged bean flour was dispersed aquadest, the dispersion was centrifuged, supernatant was adjusted to pH 3,6 (isoelectric point of winged bean protein), precipitated protein was removed by centrifugation, washed, neutralized to pH 6.0 and dried in a hot air cabinet drier at 50^{o}C for 6 h. The results showed that full-fat, defatted, and winged bean protein concentrate had different proximate composition and functional properties. Winged bean protein concentrate had better functional properties than the full-fat and defatted winged bean flour. Winged bean protein concentrate are potential to be used in foods that require high emulsion stability, foaming capacity, and foaming stability.
Keywords: Full-fat winged bean flour, defatted winged bean flour, winged bean protein concentrate, functional properties.

Introduction

Winged bean is a leguminous plant that has been utilized in almost all parts of the plant. Fruit, leaves, flowers, bulbs, and winged bean seeds can be consumed both as a vegetable and snack. Winged bean seeds have high protein content that reaches 33.38%[1].

One way to utilize winged bean seed is to process into protein concentrate. Protein concentrate can be made from legumes such as soybeans, mung bean, winged bean, cowpea, and pigeon pea, and seeds that contains oils such as sunflower seeds, sesame seeds, cotton seeds, as well as from dairy products like whey. However, just soybeans are manufactured commercially as protein concentrates and isolates.

Although there are many research on protein concentrate from nuts or seeds such as Bambara groundnut protein concentrate [2], wood apple seed [3], kenaf seed [4], peanut [5], chickpea [6], cashew [7] and African yam bean, lima, and pigeon pea [8], and many more, but winged bean protein concentrate are rarely studied.

Therefore, it is important to study on the functional properties of winged bean protein concentrate as well as their chemical composition.

Experimental

Material

Winged bean seed was obtaned from a local market in Mataram, Lombok, Indonesia. All chemical reagents were obtained from Merck.

Seed preparation

The seeds were manually cleaned to remove broken seed, small branch, dust and other foreign matters. About 10 kg of winged bean were placed in an airtight plastic container at room temperature until used.

Preparation of full-fat winged bean flour

The winged bean (1 kg) seed was soaked in tap water (2 L) at seed to water ratio of 1:2 for 12 h at room temperature. After soaking, the seeds were removed from water and manually dehulled. Dehulled seed were dried in a hot air cabinet drier at 50^{o}C for 11 h and ground in a laboratory blender. The ground materials were passed through sieve in 80 mesh size screen to obtain full-fat winged bean flour.

Preparation of defatted winged bean flour

Full-fat flour (100 g) was defatted with hexane (500 ml) at flour to hexane ratio 1:5 for 2 h. Fat extraction was repeated again once with hexane solvent for 2 h (total 4 h). Residual hexane in flour was air-dried overnight under room temperature for 24 h and passes through a 80-mesh sieve to obtain a uniform defatted flour. Defatted winged bean flour was packed in PE plastic and placed in an airtight plastic container until used and analysis furthermore.

Preparation of winged bean protein fractions

Winged bean protein fractions were made by isoelectric precipitation [9] with a little modification. Finely ground defatted winged bean flour was dispersed in 1:10 aquadest (w/v) and stirred for 1 h at ambient temperature. The dispersion of defatted winged bean flour was centrifuged (4000 rpm for 15 min at 20^{o}C), supernatant was taken and residue was removed. Supernatant was adjusted to pH 3.6 (isoelectric point of winged bean protein) with 1 N HCl. The precipitated protein was removed by centrifugation (4000 rpm for 15 min at 20^{o}C), supernatant was removed, precipitated protein was washed, neutralized to pH 6.0 and dried in a hot air cabinet drier at 50^{o}C for 6 h.

Analysis of the chemical composition

Moisture content (drying at 105^{o}C to constant weight), protein (total nitrogen, calculating by multiplying the protein with 6.25), lipids (soxhlet method), ash (calcinations of sample in oven at 550^{o}C) and total carbohydrate (by difference) were determine according to AOAC standard methods [10]. All proximate analysis was determined on the full-fat flour, defatted flour and winged bean protein concentrate.

Functional properties of winged bean

The following functional properties of the winged bean proteins were carried out.

Water holding capacity [11]

One gram of sample was weighed into a pre-weighed 15 ml centrifuge tubes. For each sample, 10 ml of distilled water was added and mixed using a vortex at the highest speed for 2 min. After the mixture was thoroughly wetted, samples were allowed to stand at room temperature for 30 min, then centrifuged at 3000 rpm for 25 min at 20^{o}C. The supernatant was decanted and the centrifuge tube containing sediment was weighed. Water holding capacity (grams of water per gram of protein) was calculated as WHC = $(W_2\text{-}W_1)/W_0$, where W_0 is the weight of the dry sample (g), W_1 is the weight of the tube plus the dry sample (g), and W_2 is the weight of the tube plus the sediment (g). Triplicate samples were analyzed for each flour (full-fat and defatted) and protein concentrate.

Oil binding capacity [12]

One gram (W_0) of sample was weighed into pre-weighed 15 ml centrifuge tubes and thoroughly mixed with 10 ml (V_1) of soy oil using a vortex mixer. Samples were allowed to stand for 30 min. The protein–oil mixture was centrifuged at 3000 rpm for 20 min at 20°C. Immediately after centrifugation, the supernatant was carefully poured into a 10 ml graduated cylinder, and the volume was recorded (V_2). Fat absorption capacity (milliliter of oil per gram of protein) was calculated as FAC = $(V_1\text{-}V_2)/W_0$. Triplicate samples were analyzed for each flour (full-fat and defatted) and protein concentrate.

Foaming capacity and foaming stability [13]

Concentration of 1% flour (1 g sample in 100 ml aquades) were prepared and adjusted to pH 7.4 with 1.0 N NaOH or 1.0 N HCl. Volumes of 100 ml of sample suspension was blended for 3 min using a high-speed blender, poured into 250 ml graduated cylinders, and the volume of foam were immediately recorded at 0, 15, 30, 45, and 60 min at ambient temperature. Triplicate samples were analyzed for each flour (full-fat and defatted) and protein concentrate. FC and FS were calculated using the following equation:

Foaming capacity (FC) (%)

$$= \frac{\text{(Volume after whipping - Volume before whipping)}}{\text{Volume before whipping}} \times 100\%$$

Foaming stability (FS) (%)

$$\frac{\text{(Volume after whipping at time - Volume before whipping)}}{\text{Volume before whipping}} \times 100\%$$

Emulsifiying activity index (EAI) and emulsion stability index (ESI) [14]

Flour or concentrate (0,1 g) was dissolved in 10 ml of aquades; 6 ml of protein solution (1%) were transferred (using pipette) and 1 ml of soy oil was added followed by homogenization at speed 1 for 1 min. Emulsion immediately mix with 3 ml of 0.3% SDS solution. Take emulsion (using pipette) from bottom of the container at 0 and 10 min after homogenization. The absorbance of the diluted solution was measured at 500 nm using spectrophotometer. Triplicate samples were analyzed for each flour (full-fat and defatted) and protein concentrate. *Emulsifiying activity index* (EAI) dan *Emulsion stability index* (ESI) were calculated using the following equation:

Emulsifiying activity index (EAI) (m^2/g):

$$\frac{2 \times 2{,}303 \times A_0}{0{,}25 \times \text{protein weight } (g)}$$

Emulsion stability index (ESI) (min)

$$\frac{A_{10} \times \Delta t}{\Delta A}$$

Where A_0 is absorbance at 0 min after homogenization; A_{10} is absorbance at 10 min after homogenization; Δt = 10 min; and $\Delta A = A_0\text{-}A_{10}$

Statistical Analysis

The data obtained from the study were analyzed by using analysis of variance (ANOVA), if the results of the analysis show that the treatment are significantly different that will be a further test using Duncan's Multiple Range Test (DMRT) at 5% significance level ($p \leq 0.05$). Statistical analysis was performed using Microsoft Excel and SPSS 16 for windows.

Result and Discussion

Chemical Composition

The proximate composition of full-fat flour, defatted flour, and protein concentrate from winged bean are presented in Table 1. There are significant (α=0.05) differences among winged bean flours (full-fat and defatted) and winged bean protein concentrate on ash, protein, fat, and carbohydrates content except moisture content.

Table 1 : Chemical composition of full-fat flour, defatted flour and winged bean protein concentrate

Parameter	Full-fat winged bean flour	Defatted winged bean flour	Winged bean protein concentrate
Moisture content (%)	10.43±0.2[a]	10.16±0.2[a]	11.51±1.4[a]
Ash content (%)	3.24±0.4[a]	4.95±0.1[b]	7.15±0.3[c]
Crude protein content (%)[A]	34.88±0.2[a]	44.31±1.3[b]	68.20±1.3[c]
Crude fat content (%)	21.47±0.2[c]	1.41±0.2[b]	0.06±0.001[a]
Carbohydrate content (%)[B]	29.98±0.3[b]	39.17±0.5[c]	13.08±1.4[a]

Means in the same row with different letters are significantly different (α=0.05)

[A] Crude protein = N(%) x 6.25
[B] Carbohydrate content obtained by subtracting the sum of moisture, ash, crude protein, and crude fat from 100%

Data in Table 1 shows that protein content of defatted winged bean flour and winged bean protein concentrate are higher (44.31% and 68.20%, respectively) compared to full-fat winged bean flour (34.88%). The removal of fat increased ash and protein content. The similar finding was Onsaard [15] that lipid extraction in full-fat sesame flour can increase chemical composition of defatted sesame flour.

Carbohydrate content of defatted winged bean flour and full-fat winged bean flour is higher (29.98% and 39.17%, respectively) compared with winged bean protein concentrate (13.08%). The method of winged bean protein concentrate preparation was isoelectric precipitation that removed carbohydrate because carbohydrate was soluble in the solution during precipitation.

Water Holding Capacity (WHC)

Winged bean flours and protein concentrate had different water holding capacity significantly (α=0.05) (Figure 1). Water holding capacity of defatted winged bean flour increased compared to full-fat winged bean flour. A similar result was also found in the full-fat flour and defatted flour of beni [16], mucuna bean [17], drumstick seed [18], lentils [19], and chickpea [20]. Separation/removal of fat from the seeds will increase the water holding capacity on defatted flour [17].

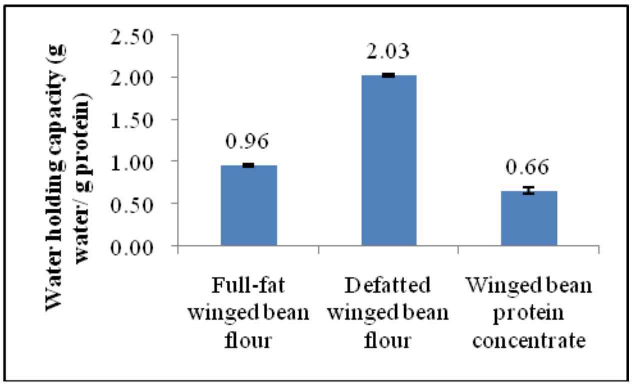

Figure 1: Water holding capacity of winged bean flours and protein concentrate

The low value of water absorption of winged bean protein concentrate may be caused by protein denaturation due to heat treatment during drying process. Denaturation exposes burried hydrophobic group of globular protein into protein surface that lowers the capability of protein to bind water.

In addition, protein concentrate that precipitated at the isoelectric pH have a low water holding capacity. The pH can affect the ability of the protein to bind water due to changes in the charge of the protein surface [21]. Therefore, the water holding capacity is very low at isoelectric point of protein. Other factors that affected water holding/water binding of protein are enviromental factors, pH, solvent, as well as the presence of fat and carbohydrates [22].

Oil Binding Capacity (OBC)

Winged bean flour and protein concentrate showed significantly different (α=0.05) in oil binding capacity (Figure 2). Oil binding capacity of defatted winged bean flour is higher than full-fat winged bean flour. A similar result was shown on the full-fat flour and defatted winged bean flour of beni [16], mucuna bean [17], drumstick seed [18], and lentils [19].

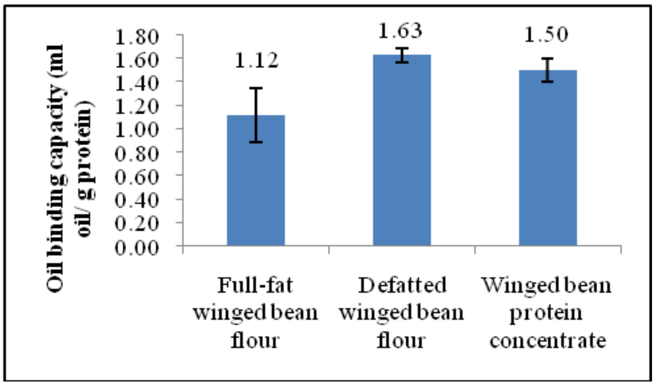

Figure 2: Oil binding capacity of winged bean flours and protein concentrate

The low oil binding capacity of winged bean protein concentrate was due to the exposure of group or cluster of hydrophobic proteins during the drying process. This reduces the ability of the protein to bind oil. In addition, the precipitation in isoelectric pH makes net charge of protein become neutral that protein molecules attract each other and minimilize interaction with water. Protein insolubility at the isoelectric point due to the neutral charge between protein molecules leads to coagulation due to hydrophobic interactions [23].

Emulsifiying Activity Index (EAI)

Winged bean flours and protein concentrate are different significantly (α=0.05) in emulsifying activity index (Figure 3).

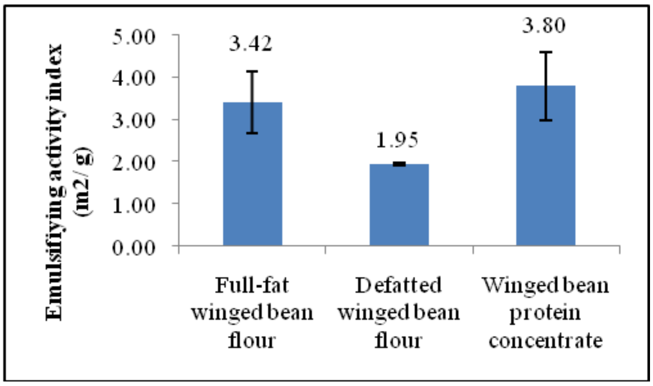

Figure 3: Emulsifying activity index of winged bean flours and protein concentrate

Full-fat winged bean flour had emulsifying activity index (EAI) that was higher (3.42 m^2/g) than that of defatted winged bean flour (1.95 m^2/g). Removal of fat by fat extraction reduced fat content of the flour and decreased the ability of flour to bind oil that make lower EAI of defatted flour compared to full fat flour. Meanwhile, cashew nut flour had higher EAI value (78.5 m^2/g) compared to its concentrate (65.6 m^2/g) and isolate (57.3 m^2/g) [7].

The higher value of EAI of winged bean protein concentrate than full-fat and defatted flour was due to the increase of hydrophobic amino acids in the protein surface so that the interaction of the protein with fat was higher. The high content of hydrophobic amino acids in a protein can increase hidrophobicity on the surface and expose hydrophobic groups which will promote interaction between protein and fat [24].

Soluble proteins on their fat surface can act as an emulsifier to form a film or membrane surrounding oil droplets that dispersed in a liquid medium thereby preventing structural changes such as coalescence, creaming, flocculation or sedimentation [6, 25]. In addition, other factors such as temperature, speed mixing, blender blades, the speed and addition of oil, pH, protein concentration, solubility, the presence of salt and water, can affect the activity of emulsion in proteins [26].

Emulsion Stability Index (ESI)

Winged bean flours and protein concentrate had significantly (α=0.05) different emulsion stability index (ESI). The results of emulsion stability index of winged bean flour and protein concentrate is presented in Figure 4.

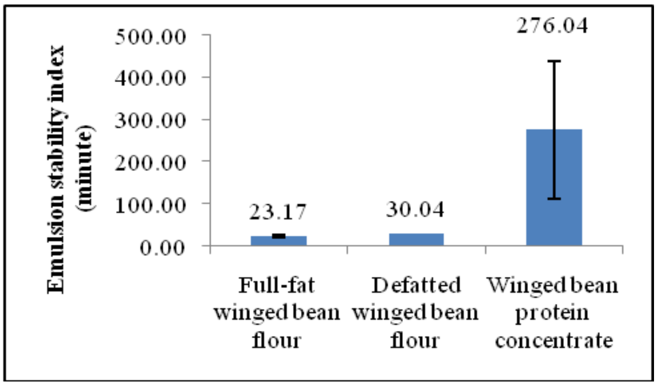

Figure 4: Emulsion stability index of winged bean flours and protein concentrate

It is known that the winged bean protein concentrate had the highest emulsion stability index and ESI is influenced by the high charge of the surface, high hydrophobicity of the surface, and high solubility. In addition, the balance of hydrophilic-lipophilic amino acid will determine the ability of the protein to form emulsion [27]. Proteins are composed of charged amino acids, uncharged polar amino acids and non-polar amino acids, which makes protein can function as an emulsifier, as it has both hydrophilic and hydrophobic groups that can interact with water and oil in the food system [28].

Emulsion stability is also influenced by the nature and condition of emulsification in protein, protein source and concentration, pH, ionic strength (type of salt and concentration) and viscosity in food system [27].

Foaming Capacity

Winged bean flours and protein concentrate had significantly (α=0.05) different foaming capacity. The results of foaming capacity of the winged bean flour and protein concentrate is presented in Figure 5.

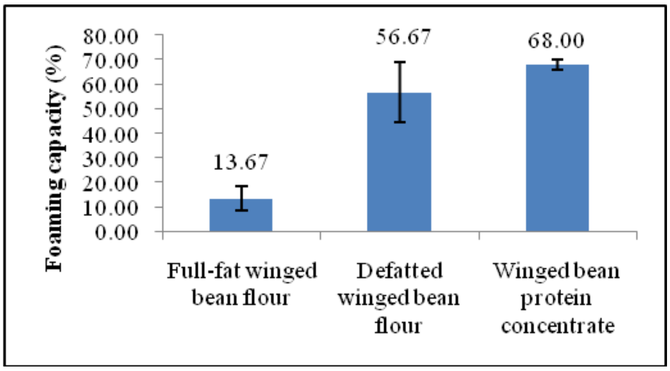

Figure 5: Foaming capacity of the winged bean flours and protein concentrate

Foaming capacity of defatted winged bean flour is higher than full-fat winged bean flour. This is due to extraction of fat of defatted winged bean flour. In chickpea protein, the removal of fat can improve the surface properties of chickpea protein [6]. Process of removal of fat can increase foaming capacity in the flour [17].

Foaming capacity of winged bean protein concentrate is higher than that of the full-fat and defatted winged bean flour. Winged bean protein concentrate had low fat content (0.06%) resulting of good foaming. Foam inhibitor, such as fat, can destroy the surface of air-water and water become insoluble [27]. Additionally, winged bean protein concentrate had the highest protein content that can increase the foaming capacity. The foaming capacity and stability is increased due to the high of protein concentration, which high concentrations of protein can increase viscosity and form a lot of cohesive protein layers at interface [23].

Foaming stability

Winged bean flours and protein concentrate had significantly (α=0.05) different foaming stability. Foaming stability of winged bean flours and protein concentrate are presented in Figure 6.

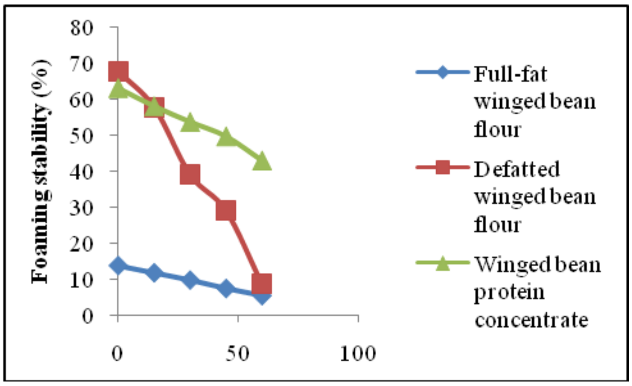

Figure 6. Curve of foaming stability of winged bean flours and protein concentrate

Foaming stability of full-fat winged bean flour at 0 min and 60 min were 13.67% and 5.33%, respectively; defatted winged bean flour at 0 min and 60 min were 67.67% and 8.67%, respectively; and winged bean protein concentrate at 0 min and 60 min were 63% and 43%, respectively.

Winged bean protein concentrate has a high foaming stability and very stable compared to the winged bean flours (full-fat and defatted). The high foaming stability of winged bean protein concentrate was related to the high concentration of protein. The foaming capacity and stability is increased due to the high protein concentration, which high concentration of protein can increase the viscosity and form a lot of cohesive protein layers at the interface [23]. Foam with a high concentration of protein will be more stable due to increase of film thickness [27].

Conclusions

Winged bean protein concentrate prepared by isoelectric precipitation had protein content of 68%. Protein concentrate had lower carbohydrate and fat content compared to full fat and defatted flour, meanwhile ash and moisture content were higher. Winged bean protein concentrate had high water holding capacity, oil binding capacity, and emulsifiying activity index. Winged bean protein concentrate was potential to be used in foods that require high emulsion stability index, foaming capacity, and foaming stability.

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