



ChemTech

## International Journal of ChemTech Research

CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555  
Vol.13 No.01, pp 30-37, 2020

# Characteristic of Cowpea (*Vigna unguiculata*) Protein Concentrates Protein Fraction on their Solubility

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**Abstract** : Cowpea (*Vigna unguiculata* L. Walp) has a protein content of 22,9% to obtain the protein in high concentrations, is made as a concentrates or isolates protein. The aim of study to determine of chemical and functional properties of flour and cowpea protein concentrates on their solubility. The study uses a completely randomized design with precipitation method based on solvent, there is distilled water, 5% salt solution, alkaline solution (NaOH 0,5 N) and ethanol 70%. The result showed that cowpea protein fractions of albumin, globulin, glutelin and prolamin had different minimum and maximum solubility. Flour and cowpea protein concentrates had different chemical composition and functional properties. Flour and cowpea protein concentrates can be develop in various food product.

**Keywords** : isoelectric point, functional properties, elektroforesis, albumin, globulin, glutelin, prolamin

## Introduction

Cowpea (*Vigna unguiculata* L. Walp) is a legume come from West Africa (1) and has been widely known in Indonesia. In terms of nutrition, cowpea ranged from 21.70 to 30.32% of protein, depending on the varieties and agronomic conditions (2, 3, 4, 5). The plant proteins are classified into albumin, globulin, glutelin and prolamin, based on their solubility in water, salt solution, alkali solution and alcohol solution (3). The method is often used for the extraction of concentrates/isolates proteins are alkaline extraction and protein precipitation by lowering pH at the isoelectric point or by heating (6).

The proportion of albumin 24.9%, globulin 66.6%, glutelin 4.7% and prolamin 0.7% (3). Study reported alkaline solution and isoelectric precipitation at pH 4.3 (7) and pH 4.5 (4). This study examines the chemical and functional properties of flour and cowpea protein concentrate by several methods, there is albumin isoelectric precipitation, globulin isoelectric precipitation, glutelin-isoelectric precipitation, and prolamin-acetone precipitation.

Ika Ratna Agustin *et al* /International Journal of ChemTech Research, 2020,13(1): 30-37.

DOI= <http://dx.doi.org/10.20902/IJCTR.2019.130104>

## Experimental

### Material

Cowpea bought from local market in Malang, 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. Then, there are ground and sieved in 80 mesh to obtain homogenous flour.

### Preparation of Protein Fraction Solubility

Determination of the maximum and minimum solubility of protein fractions are modified (8), with the solvent is distilled water (albumin), 5% salt solution (globulin) and alkaline solution/0.5 N NaOH (glutelin). Adjust supernatant protein at range pH 1-12 and determine the soluble protein with a spectrophotometer ( $\lambda=650\text{nm}$ ).

### Preparation of Protein Concentrate

Preparation of protein concentrate modified method (9), extraction of cowpea flour with each solvent (distilled water, 5% salt solution and alkaline solution/0.5 N NaOH). Separation of the the supernatant from the precipitate and adjust the supernatant at isoelectric point (solubility determine result), and then centrifugation at 4500 rpm for 15 min. Precipitate proteins (albumin, globulin, and glutelin) washed twice with distilled water, then neutralization to pH 6.0. Prolamin extraction (9), by adding acetone twice volumes of supernatant protein. Drying the precipitate proteins (albumin, globulin, glutelin and prolamin) with a vacuum dryer at 50 °C for 5 hours .

### Chemical Properties Analysis

Chemical analysis performed on flour and protein fractions, including moisture, fat, ash, protein and carbohydrate (by difference) (10).

### Functional Properties Analysis

#### Water Absorption Capacity (11)

One gram sample was added with 10 ml distilled water, the suspensions were allowed to stand at room temperature for 1 hr. The suspensions was centrifuge at 2000 rpm for 30 min. The volume of supernatant was measured and the water on the expressed as percent water absorption.

#### Oil Absorption Capacity (11)

One gram sample was added with 10 ml soybean oil, the suspensions were allowed to stand at room temperature for 1 hr. The suspensions was centrifuge at 2000 rpm for 30 min. The volume of supernatant was measured and the oil on the expressed as percent oil absorption based on the original sample weight. (Density 0,89 g/ml).

#### Emulsifying Activity Index (EAI) and Emulsifying Stability Index (ESI) (12)

This method is used to measure the surface area of the droplet emulsion based on turbidity. Emulsion was taken 1 min after homogenization (2500 psi, 15 min) from the bottom of the tube and dissolved in 0.3% SDS solution. SDS solution absorbance was measured at a wavelength of 500 nm. Absorbance at time-0 is used to calculate the EAI.

$$\text{EAI (m}^2/\text{g)} = \{ (2.303)(2)(A_{500} \times \text{dilution}) \} / \{ (C)(\emptyset)(10.000) \}$$

$$\text{ESI (min)} = A_0 (\Delta t / \Delta A)$$

where: A : absorbance of the emulsion;  $\emptyset$  : oil volume fraction (0.25), C : concentration of protein (g / ml) (0.01 g / ml);  $\Delta A$  : A0-A10; A0 : absorbance at  $\lambda$  : 500 nm, t : 0 min; A10 : absorbance at  $\lambda$  = 500nm, t : 10 min;  $\Delta t$  : 10 minutes

### Foam Capacity and Stability (13)

1% sample were prepared in distilled water and adjusted to pH 7,4 with 1.0 N NaOH and 1.0 HCl. Volumes of 100 ml ( $V_1$ ) of suspension was blended for 3 min using high-speed blender, poured into 250 ml graduated cylinders and the volume of foam ( $V_t$ ) were immediately recorded at 0 min, 30 min and 60 min. Foaming capacity was calculated using the following equation:  $FC = V_t - V_1 / 100 \times 100\%$ . Foaming stability (FS) =  $V_t/V_1 \times 100\%$

### Swelling Power (14)

One gram sample was mixed with 10 ml distilled water in centrifuge tube and heated at 80°C for 30 min. The mixture was continually shaken during the heating period. After heating, the suspension was centrifuged at 3000 rpm for 15 min. The supernatant was decanted and the weight of paste taken. The swelling power was calculated as: swelling power = weight of the paste/weight of dry sample.

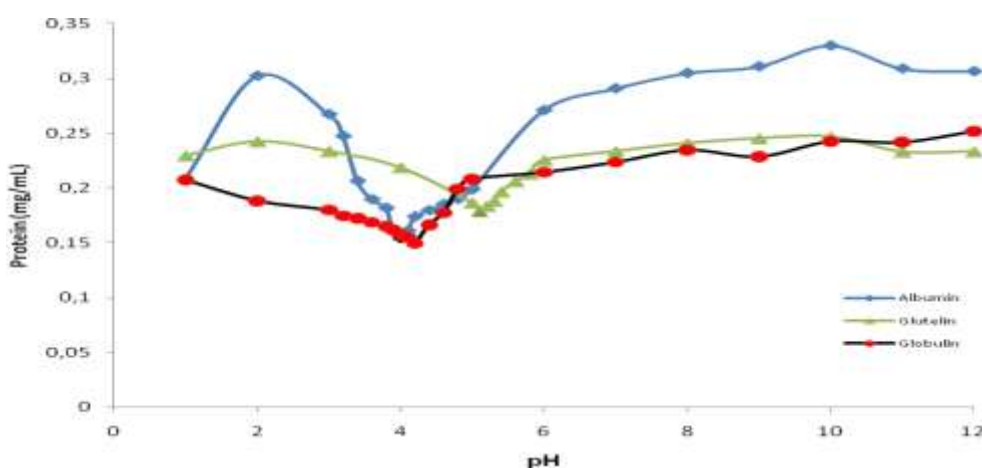
### SDS-PAGE (15)

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was modified method by using a 4% stacking gel and 12% separating gel. The samples were prepared included in the gel. Electrophoresis was performed at a constant voltage of 100 volts for 1.5 hours. Proteins were stained with Coomassie brilliant blue R-250 in waterbath-shaker for 1 hour at room temperature and destained with methanol and acetic acid overnight or until the color of the clear gel. Protein bands can be calculated using standard molecular weight (Sigma Chemical Co., St. Louis, MO) with a range of 10 to 260 kDa.

## Results and Discussion

### Protein Solubility

Generally, the protein solubility curve U-shaped and minimum solubility at pH 4-5 (isoelectric point), the same as in the legume proteins and other seed grains (16).



**Figure 1. Solubility of cowpea protein concentrate**

The minimum solubility of cowpea protein fractions at pH 4.0 (albumin), pH 4.2 (globulin), pH 5.0 (glutelin), and the maximum solubility at pH 10 (Figure 1). Cowpea protein solubility minimum at pH 4, 5, and 6, and a maximum solubility at pH 10 (17).

## Chemical Characteristics of Flour and Cowpea Protein Concentrates

Albumin has the highest moisture content while the lowest was found at globulin (**Table 1**). The high water content of albumin related to the water-soluble, certainly influenced by amino acid compositions. Albumin is rich in sulfur-containing amino acids, such as cysteine and methionin, as well as other essential amino acids, such as lysine compared with globulin (18). Globulin is a protein that unsoluble in water but soluble in calcium-magnesium salt solution that has a strong ionic bonds (19).

Albumin and glutelin had a protein content was lower than the other research of cowpea protein isolate (20, 21, 7). The lower protein content of the flour, globulin and prolamin because of non-protein components such as ash and carbohydrates (**Table 1**). Cowpea flour protein content was greater than Olalekan & Bosede (22). Cowpea protein content was depending on variety and geographical conditions (2, 3, 4, 5).

### Water Absorption Capacity (WAC)

Protein fraction that showed the highest WAC was glutelin (**Table 2**). WAC of cowpea flour are similiar to that reported by Khalid et al. (20). WAC of cowpea protein isolates ranged from 2.10-2.33 ml/g (20) and 1.9-2.21 ml/g (23). Water absorption of peanut protein depends on the type and concentration of protein, and the presence of non-protein components, such as saccharides, which increased water absorption (5).

### Oil Absorption Capacity (OAC)

The highest Oil absorption capacity of prolamin (**Table 2**). Oil absorption capacity of cowpea flour is lower than Khalid et al. (2012). Oil absorption capacity of albumin, globulin and glutelin (**Table 2**) is lower than commercial soy concentrate (ISOPRO) and soy protein isolate (SUPRO) (24, 25).

**Table 1. Chemical Composition of Flour and Cowpea Protein Concentrate**

Parameter	Cowpea Flour	Protein Concentrate			
		Albumin	Globulin	Glutelin	Prolamin
Moisture (%)	12.39±0.84 <sup>b</sup>	17.23±0.46 <sup>c</sup>	8.31±2.23 <sup>a</sup>	12.99±1.05 <sup>b</sup>	13.59±0.21 <sup>b</sup>
Protein (%)	25.79±0.12 <sup>b</sup>	73.06±1.72 <sup>d</sup>	46.16±0.15 <sup>c</sup>	72.26±1.31 <sup>d</sup>	17.23±1.68 <sup>a</sup>
Fat (%)	1.19±0.005 <sup>d</sup>	0.32±0.004 <sup>ab</sup>	0.62±0.16 <sup>c</sup>	0.40±0.17 <sup>bc</sup>	0.10±0.01 <sup>a</sup>
Ash (%)	3.14±0.03 <sup>a</sup>	5.85±0.63 <sup>a</sup>	7.97±1.45 <sup>a</sup>	4.89±0.17 <sup>a</sup>	6.88±1.7 <sup>a</sup>
Carbohydrate (%) (by difference)	57.48±0.76 <sup>d</sup>	3.54±1.89 <sup>a</sup>	36.95±0.46 <sup>c</sup>	9.44±0.59 <sup>b</sup>	62.26±0.09 <sup>c</sup>

Description: The average rate followed the same letter in the same row are not significantly different, LSD( $\alpha=0.05$ )

### Emulsifying Activity Index (EAI) and Emulsion Stability Index (ESI)

Emulsifying Activity Index (EAI) of flour and cowpea protein concentrates was shown at **Table 2**. Emulsifying activity index of soy protein isolate is 10.86 m<sup>2</sup>/g (26), 18.6 m<sup>2</sup>/g (27), 11 m<sup>2</sup>/g (28) and 45 m<sup>2</sup>/g (29).

Protein emulsion capacity decrease if the protein concentration was greater (30, 24). If the low protein concentration, protein adsorption on the surface of the water-oil through a process of diffusion can be controlled, as it will spread over the surface before it can be adsorbed. If a higher protein concentration, activation energy occurs that does was greater that not allow the on going inhibitory protein diffusion migration (31), this may explain the cause of the emulsifying activity index decreased with increasing protein concentration.

Emulsion stability index of flour and cowpea protein concentrates was showed at **Table 2**. Prolamin had a high ESI than the others, emulsion stability index Kabuli chickpea-ultrafiltration (19.7 minutes) Boye et al. (32), green lentils-IEP (17.8 minutes), soy protein isolate, 0.80 minutes (26). The factors that cause differences in emulsifying stability index is pH, droplet size, the total charge, interpartial action, viscosity, and conformation of proteins (33).

**Table 2 . Functioal Properties of Flour and Cowpea Protein Concentrates**

Parameter	Cowpea Flour	Protein Concentrates			
		Albumin	Globulin	Glutelin	Prolamin
WAC (mL/g)	1.33±0.06 <sup>b</sup>	1.53±0.12 <sup>c</sup>	1.37±0.06 <sup>bc</sup>	2.43±0.15 <sup>d</sup>	0* <sup>a</sup>
OAC (mL/g)	0.77±0.15 <sup>c</sup>	0.38±0.06 <sup>ab</sup>	0.27±0.06 <sup>a</sup>	0.49±0.09 <sup>b</sup>	2.86±0.42 <sup>d</sup>
EAI (m <sup>2</sup> /g)	5.38±0.45 <sup>a</sup>	4.20±0.86 <sup>a</sup>	9.56±1.87 <sup>b</sup>	5.65±0.64 <sup>a</sup>	17.60±3.29 <sup>c</sup>
ESI (minutes)	26.75	27.35	39.13	41.22	46,12
Foam Capacity (%)	40.00±8.72 <sup>b</sup>	45.33±9.87 <sup>b</sup>	29.33±3.06 <sup>a</sup>	26.00±7.21 <sup>a</sup>	18.00±2.0 <sup>a</sup>
Foam Stability (%)	7.33	6.67	0.67	4.67	5.67**
Swelling power (g)	4.66±0.46 <sup>c</sup>	5.01±0.23 <sup>c</sup>	3.51±0.51 <sup>b</sup>	6.34±0.40 <sup>d</sup>	1.76±0.48 <sup>a</sup>

Description: Same letters in the same row means are not significantly different, LSD ( $\alpha = 0.05$ )

\* soluble in water (not able to absorb water)

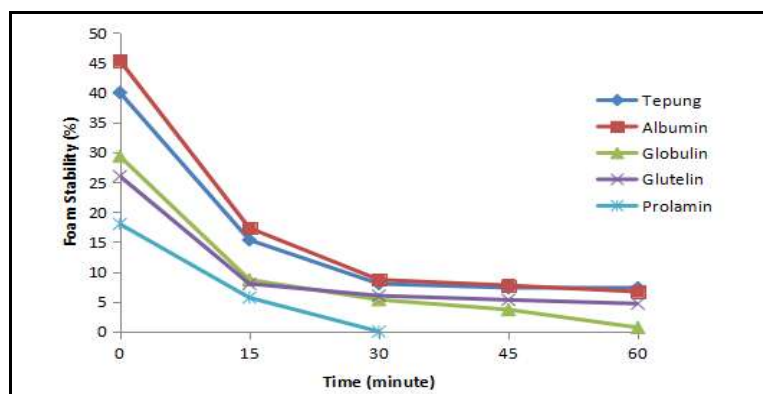
\*\* foam stable only 15 minutes early then decrease slowly until 30 minutes

### Foam Capacity and Stability

Foam capacity is the ability of the protein to stabilize the foam surface area/unit weight of protein to stabilize the film or coating the surface of the internal and external forces (34). **Table 2** show the foam capacity of flour and cowpea protein concentrates. Foaming capacity of cowpea flour on other research is 57% (35), 16.33% (22), whereas the protein concentrates and isolates cashews have foam power by 40% and 45% (36).

Foaming stability of globulin and albumin protein concentrates did not reach 60 mins (**Figure 2**). Foam stability of prolamin is lower related to the low concentration of prolamin protein (17.23%).

The ability to form foam affected by protein source, temperature, pH, protein concentration, and the foaming time. Foam stable which occurs when low surface tension and high viscosity so that the surface of the colloidal solution, solid shape, amorphous (not formed), the surface of solid film. The high stability of the foam on the whole legume flour due to the complex carbohydrates in seed epidermis (37).



**Figure 2. Foam Stability of Flour and Cowpea Protein Concentrate**

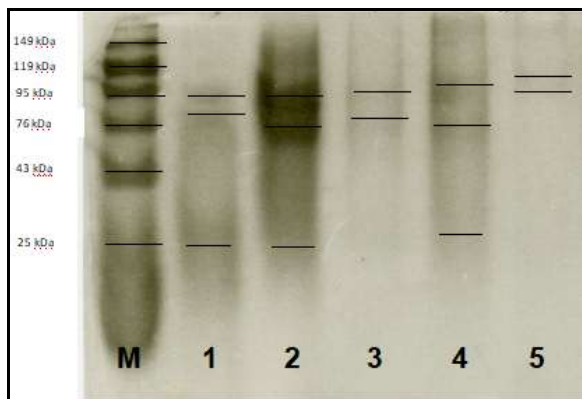
### Swelling Power

Swelling power of flour and cowpea concentrate protein showed at **Table 2**. Glutelin has the highest swelling power, but was lower than cowpea flour swelling power (35). Swelling power depend on water absorption, glutelin have high water absorption so it have a high swelling power compared with prolamin which dissolve in water.

### Protein Electrophoresis Profile

The identification of the protein profile in the flour and cowpea protein concentrates by SDS-PAGE, albumin obtained 3 bands (18.01, 34.67, 51.35 kDa). Other studies on albumin has 4 major sub-units (27 to 30 kDa) and 4 minor subunits (81 to 93 kDa) (38). Globulin obtained 2 bands (39.46 and 58.45 kDa), in cowpea reported 4 major sub-units (65, 60, 56, and 50 kDa) (3).

SDS-PAGE results showed glutelin has 3 bands (56.45, 37.60 and 16.68 kDa).



**Caption: M (Marker); 1 (Cowpea Flour); 2 (Albumin); 3(Globulin); 4 (Glutelin); 5 (Prolamin)**  
**Figure 3. SDS-PAGE Analysis of Flour and Cowpea Protein Concentrate**

Glutelin separates into several bands with molecular weight of 101, 68, 31, and 29 kDa (3). Prolamin obtained 2 bands (60.25 and 45.11 kDa). Cowpea prolamin containing 4 bands (105, 62, 59 and 54 kDa) , where is prolamin have more hydrophobic amino acids than flour protein fractions (3).

### Conclusion

Preparation of cowpea protein concentrate with various solvents obtained only albumin and glutelin protein concentrate that has concentration more than 70%. Cowpea protein concentrate have characteristic functional properties different. Albumin and glutelin has foam capacity is quite good suitable for meat products, whipped toppings, ice cream. Globulin has good emulsifying ability, suitable for product sauce, cake. Proteins were preparation with alcohol which prolamin fraction has good functional properties of beverage products, whipped toppings, salad dressings.

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