

## **Functional Protein Isolation Technology Of The Cowpea (*Vigna Unguiculata*) As A Food Ingredient**

Choirul Anam<sup>1</sup>; Yuli Witono<sup>2</sup>

<sup>1</sup>Department of Agrotechnology, Faculty Of Agriculture, Darul Ulum Islamic University. The road. Airlangga number 3, Sukodadi, Lamongan, East Java. Zip code : 62253

<sup>2</sup>Department of Agricultural Technology, Faculty Of Agricultural Technology, University Of Jember. The road. Kalimantan II/24 Kampus Tegal Boto. Jember. East Java. Zip code : 68121

---

**Abstract:** Cowpea contains high enough proteins that amounted to 22.6% so potentially developed as a source of protein that can be applied as a functional food ingredient. This research studied the protein isolation techniques of Cowpea using fully functional modification of solvents and drying techniques. The first stage of the functional protein isolated from milk and flour of cowpea using solvents of ethanol and aquades. Results of the analysis of the yield and protein levels indicate that treatment of extraction using aquades from milk of cowpea is the best treatment to the value yield of 15,41% and protein levels of 76,41%. The second phase is conducted by using protein isolate extraction solvents ethanol and acetone with an ordinary oven dryer and vacuum oven. It is obtained results that purification with solvent acetone and drying using a vacuum oven is the best treatment with a yield of 16.64% and protein levels of 90,31%. In addition to the components of the protein, known to isolate proteins which is resulted from cowpea also containing components of water, fat, protein, carbohydrate and ash.

**Keywords:** cowpea, protein isolate, aquades, acetone, drying.

---

### **I. Introduction**

As one of the components of the protein constituent of food has a huge role in determining the quality of food products. The protein is able to interact with other compounds, either directly or indirectly, so that the effect on the application process, the quality and acceptance of the product. It is these properties that are referred to the nature of functional proteins, such as: water binding, solubility, viscosity, flower power, formation of the gel, surface activity and binding flavor. By exploiting the properties of these proteins, the protein can be an additional food ingredients that encourage the onset of flavor, texture and quality of other desired consumers (Kinsella et al., 1985). Some kinds of proteins from a variety of sources have been developed into products that have functional properties, either in the form of concentrates, isolates, dispersion, or hidrolisat. These products can be used as a food additive, such as: emulsifier, *flavor enhancers*, *texturizer*, *stabilizer*, *fat replacer* or suplemen nutritious foodstuffs.

Indonesia is currently still import food additive based on this protein. Soy protein concentrates and isolates, still have to be imported from the USA and China to meet the needs of the food industry of Indonesia. To that end, the need for development efforts of protein-based food additives from the materials are cheap and available in Indonesia, so as to reduce the dependence of food additive will be from other countries, increasing the value of the economy and the welfare of society.

Cowpea (*Vigna unguiculata* L.) contains a protein that is high enough, that amounted to 22.9 %, low fat content (1.4 %) and relatively high karbohidratnya content (61 %) (Rukmana and Oesman, 2000). The high protein content, making protein Cowpea has potential as an alternative replacement for animal protein. It is also supported by the Cowpea that is easily cultivated in Indonesia and the drying seed productivity high enough about 800 – 900 kg/ha on dry land and approximately 1700 kg/ha when land was given the watering (Robert, 1985).

Based on the subject, then it needs to be done about the research study of isolation technology of functional protein from Cowpea as a food ingredient through the test yield, protein and chemical properties of the resulting protein isolates. This research aims to know the technology of the production of functional protein isolates from Cowpea as an alternative source of protein functional food ingredient applications.

### **II. Research Methods**

#### **Materials and Research Tools**

Raw materials used in this research is the Cowpea (*Vigna unguiculata* L.) obtained from Malang, East Java. Chemicals used for isolation of functional protein include: aquades, ethanol 70%, acetone 70%, NaOH 0.1 N and HCl 1 N. Chemicals for chemical analysis include: reagent tube Lowry, kjeldahl, H<sub>2</sub>SO<sub>4</sub>, indicators pp

and shertoshiro, NaOH 45%, and petroleum ether. Chemicals used lacking specification Pro Analysis most branded Merck (Germany).

Tools used include: blender (GMI), sentrifus (Yenaco model OUR YC-1180), spektronik 21 D (Melton Roy), pH meter (Jen Way type 3320, Germany), the magnetic stirrer (Stuart Scientific), vortex (Thermolyne type 16700), refrigerator, water bath (GFL 1083), analytical balance (Ohaus), electric heating (Gerhardt), spatula, vacuum ovens, vortex (Maxi MaxType 16700), regular oven, 80 mesh sieve and tools glasses.

This research is divided into three main parts, namely the preliminary research, research and research in phase 1, phase 2. Preliminary research done to determine functional protein isoelectric point of pH from Cowpea. Research phase 1 compares how the isolation of proteins directly from milk of Cowpea and indirectly from Cowpea Flour using a solvent of ethanol and aquades against the parameters yield and protein levels. Research phase 2 study of influence of washing using organic solvents (acetone and ethanol) against the nature of kimiadari isolates generated as well as the influence of different ways of drying (oven and vacuum oven) against yield and chemical properties of the resulting isolates.

Research of designed randomized factorial with two factors, namely A and B. On the research of tahap1 factor A (raw materials) consists of two levels namely milk cowpea, Cowpea and flour as well as factor B (solvent type) consist of 2 levels, namely aquades and ethanol. On the research of phase 2 factor A (organic solvent type) consist of 2 levels i.e. acetone and ethanol, as well as factor B (type of dryer) consists of two levels, namely the regular oven and vacuum oven. Each treatment combination is repeated 3 times. The data analyzed are descriptive for knowing the difference of each treatment group.

### **Implementation Of The Research**

- a. Research introduction: Cowpea soaked for 5 minutes, then peeled the skin arinya. Next the Cowpea blended with aquades use aquades comparison with material (6:2) and incubated at room temperature for 20 minutes. Next is added with NaOH 0, 1N NaOH by comparison with the material is equal to 5:1 and incubated at a temperature of 55°C for 30 minutes. Disentrifuse materials with a speed of 2000 rpm for 10 minutes. Supernatan the resulting pH is set each at pH 3, 4, 5, 6, 7, 8, 9, and 10 with the addition of HCl 1N. Each material is disentrifuse with a speed of 2000 rpm for 10 minutes. The resulting Supernatan is then analyzed using the method of terlarutnya protein Lowry (1951 *in* Walker, 2002) with modifications.
- b. Research phase 1: Cowpea soaked for 5 minutes, then peeled the skin arinya. Next the Cowpea blended each with aquades solvents and ethanol solvent and comparison with 6:2. Then added with NaOH 0, 1N NaOH: comparison with ingredient 5:1 and incubated at a temperature of 55°C for 30 minutes. Disentrifuse materials with a speed of 2000 rpm for 10 minutes. Supernatan in isoelectric point at pH kondisikan with the addition of HCl 1N. then dintrifuse returns with a speed of 2000 rpm for 10 minutes. The resulting sediment is protein isolates Cowpea wet then dried using an oven temperature of 60°C for approximately 18 hours. The resulting dried protein isolates analyzed parameters yield and protein levels. The same steps are performed for Cowpea Flour.
- c. Research phase 2: protein Isolates were wet from the best treatment research phase 1 then laundered using organic solvents namely acetone and ethanol with comparative material with organic solvents is equal to 1:3. Then in stirrer for 10 minutes and then at a speed of 2000 rpm sentrifuse for 10 minutes. The resulting sediment is protein isolates wetland have been purified. The sediment is then dried using 2 types of dryers, namely vacuum oven at temperatures over 50°C ± 8 hours and regular oven at a temperature of 60°C for approximately 18 hours. Dried protein isolates produced then analyzed parameters yield, protein (Sudarmadji, 1997), moisture content (AOAC, 2005), fat levels (Sudarmadji, 1997), the levels of ash (AOAC, 2005) and karbohidratnya levels (by difference).

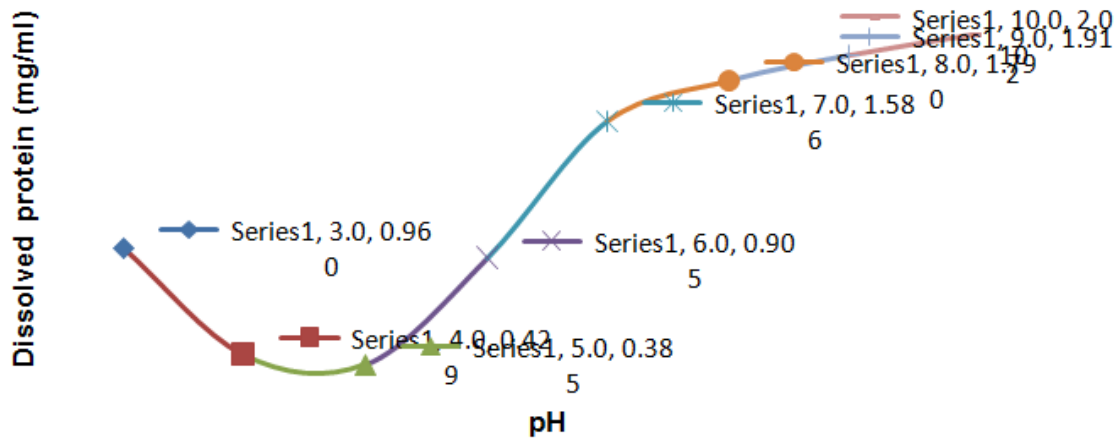
## **III. Results And Discussion**

### **Determination of Isoelectric Point of pH Functional Protein from Cowpea**

The process of isolation of protein from vegetable-based protein ingredients beginning with the extraction at pH alkaline (pH above 9), those obtained at pH maximum protein solubility. After having obtained a solution of proteins so the next process is the deposition by means of solvent pH settings using hydrochloric acid (HCl) approached the isoelectric pH. According to Winarno (1985) the principle of the use of acid is to lower the pH of the solution pH, protein precipitation are generally set up to 4.

Isoelectric point indicated by the solubility minimum at a certain pH. Cowpea protein solubility at pH range is shown in Figure 1. The concentration of dissolved proteins shows a decline with the decline in the value of the pH. the pH of the early material was 13.8 which was later reduced by the addition of 1 N HCl to reach points of the desired pH. The lower the concentration of dissolved proteins means the more protein isolates may be deposited on the point of the pH, so it can be inferred that the isoelectric point of pH 5 is a protein Cowpea because it has the lowest solubility value of 0.385 mg/ml. At pH below 5 indicates an increase in dissolved

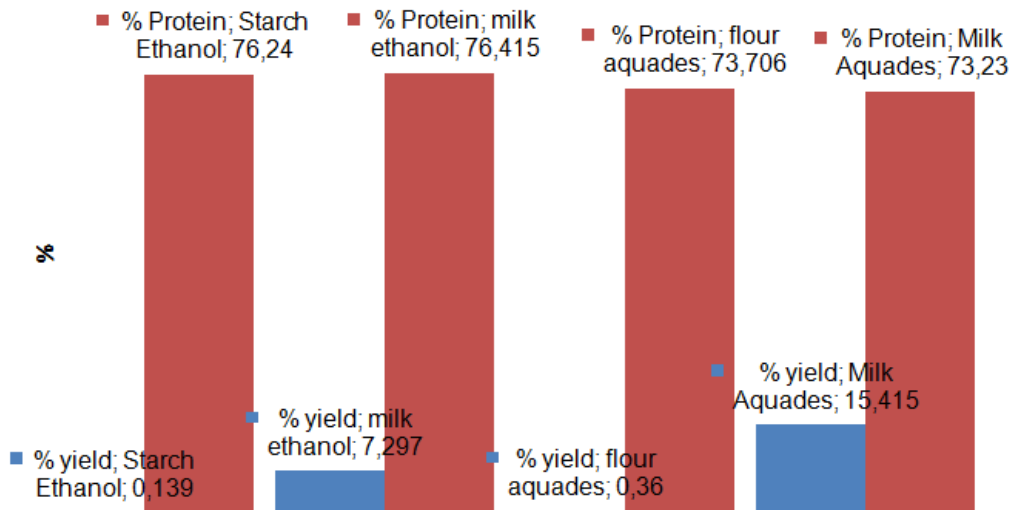
protein that can be caused by protein denaturation process of acid into amino acids and short peptides-peptides of other causes protein solubility rate goes up again.



**Figure 1.** Cowpea protein solubility at different pH

**Production of Cowpea Protein Isolate Dairy Cowpea Flour and Cowpea Using Solvents of ethanol and Aquades**

Production of protein isolates from two ingredients, milk directly from the Cowpea (MC) and indirectly from Cowpea Flour (CF) using aquades and ethanol solvents at each material. The results of the analysis of the yield shows treatment with SKT aquades is the best treatment with a yield of 15.415 followed MC CF aquades ethanol, and ethanol respectively CF of 7.297; 0.360; and 0.139. While the results of the analysis showed protein levels of ethanol as the best treatment MC with protein 76.415 followed CF ethanol, MC aquades and aquades each CF 76.240; 73.706; and 73.230. The best treatment is selected by using the parameters of the total protein is derived from a combination of yield and protein levels, so the selected treatment MC aquades as the best treatment.



Blue colour = % yield; Red colour = % Protein

**Figure 2.** The histogram value yield and protein levels at different stages of research treatment 1

Referring to the rules of the FAO (2007) which stated that the minimum protein levels of a protein isolates are 90 (db), then all the treatments in phase 1 studies have not found to be eligible. Hence continued research phase 2 to increase the purity of protein isolates by conducting protein isolates, wet washing using organic solvents.

**Purification of Cowpea Protein Isolates using a combination of different types of Solvent (ethanol and acetone) and Drying (Oven and Vacuum Oven)**

Purification of Cowpea protein isolates, aims to increase levels of a protein from the protein isolates produced. Purification of protein isolates was performed using solvents of ethanol and acetone is combined with the technique of drying oven temperature of 60oC and regular oven vacuum 50oC. The results of the analysis of the yield in a regular oven acetone treatment shows 60oC of 18.536 is best treatment followed by ethanol, acetone vacuum vacuum oven and ethanol respectively of 18.078; 16.638; and 16.521. While the results of the analysis showed protein treatment vacuum oven acetone as the best treatment with the highest protein levels of 90.305 (db) followed by acetone, ethanol and vacuum oven ethanol oven each of 86.649; 82668 and 82585 (db).

The main objective of the 2nd stage of the treatment is to increase levels of a protein from the protein isolates produced so that greater than 90 (db) in accordance with the provisions of the FAO (2007), therefore the chosen treatment vacuum oven as acetone is the best treatment in the research phase 2.

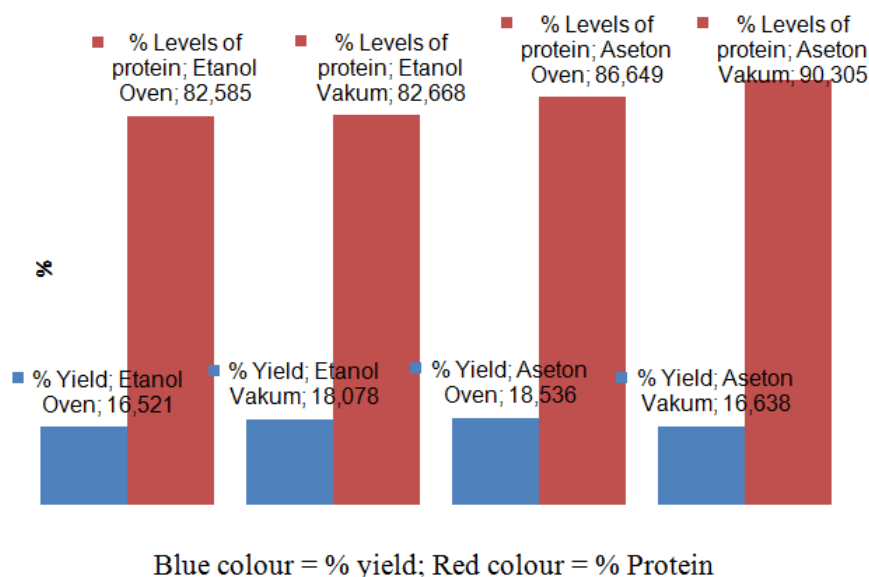


Figure 3. The histogram value yield and protein after purification and drying stages.

**Chemical Properties Of Cowpea Protein Isolates**

Chemical properties of Cowpea protein isolates, as indicated in table 1 below.

Table 1. The composition of the Cowpea protein isolates on different treatment

Component	The levels of / Treatment			
	Etanol oven	Aseton oven	Etanol vakum	Aseton vakum
Water (% wb)	9,991 ± 0,497	10,010 ± 1,112	7,756 ± 0,784	7,932 ± 0,188
Protein (% db)	82,585 ± 0,700	86,648 ± 2,524	82,688 ± 2,731	90,305 ± 0,170
Fat (% db)	1,183 ± 0,142	1,154 ± 0,413	1,227 ± 0,150	1,050 ± 0,095
Carbohydrates (% db)	13,340 ± 1,057	10,130 ± 2,517	13,438 ± 2,574	8,784 ± 4,064
The grey levels (% db)	2,972 ± 0,130	2,067 ± 0,009	2,667 ± 0,198	3,852 ± 0,149

\* Components are expressed as average value ± standard error of mean (SEM)

**Moisture Content**

Table 1 indicates that the moisture content of protein isolates the Cowpea (10.010%) in a regular oven, the acetone treatment followed by the result of the treatment using ethanol for ordinary oven vacuum, acetone and ethanol vacuum each of 9.991%; 7.932%; and 7.756%. Moisture content of protein isolates that are processed with vacuum dryer moisture content lower than isolates that use ordinary oven penengring. This can be caused because the vacuum oven drying, drying can be done on a fast time and a lower temperature so as not to damage the material.

**Fat Content**

Isolate the protein that has the highest fat content i.e. 1.227% (db) is found on the vacuum's treatment of ethanol, followed by the result of the process of using ethanol, acetone regular oven oven and vacuum the acetone respectively of 1.183%; 1.154%; and 1.050% (db) (table 1). This suggests that leaching using a solvent acetone is more effective at lowering the fat content in the isolates compared with leaching using a solvent of ethanol. However when compared with protein isolates according to FAO (2007) the fat content of protein

isolates produced Cowpea is still too high. Fat content of protein isolates according to FAO (2007) is approximately 0.5%. This allegedly fat bound with hydrophobic cluster and fat solvents (media ekstraksinya) less powerful power extracts.

### **Carbohydrate Levels**

Carbohydrate levels of Cowpea is of 61% (Aak, 1989) and experienced a decline during the production process of protein isolates. Based on the results of observations (table 1) shows that the highest levels of carbohydrates found in the regular oven ethanol treatment of 13.340% followed ethanol vacuum, acetone and acetone vacuum oven each of 13.438%; 10.130%; and 8.784% (db). Treatment of washing with acetone is more effective in lowering the content of carbohydrates of isolates than washing with ethanol.

### **The Grey Levels**

Ash is the result of the burning of the residual inorganic an organic material. Ash and composition depending on the ingredients and how to pengabuannya. Table 1 shows that the levels of ash four isolates protein which is about 2.067% – 3.852% (db).

## **IV. Conclusion**

The manufacture of protein isolates, Cowpea is best done directly from milk Cowpea using solvent aquades and precipitation at pH 5. Followed by washing using acetone and drying using a vacuum oven temperature of 50°C for approximately 8 hours. The resulting protein isolates have a yield of 16.638%; moisture content 7.932% (wb); protein 90.305% (db); 1.050% fat levels (db); carbohydrate levels of 8.784% (db); and the grey levels 3.852% (db). Furthermore it needs to be examined regarding the constituent components of the protein in Cowpea protein isolates and the nature of its current status. In addition it needs to be done further research on methods of decreasing the levels of fat in the Cowpea protein isolates to fit the standard. Further development of protein isolates Cowpea in its application as a food ingredient is expected to meet the needs of a still-functional protein source revisited until today.

## **Bibliography**

- [1]. Aak. 1989. *Soy*. Kanisius, Yogyakarta.
- [2]. AOAC. 2005. *Official Methods of Analysis of The Association of Official Analytical Chemist*. Virginia USA: AOAC Inc. Arlington, Virginia.
- [3]. Amin, A. M., Ahmad, A. S., Yin, Y. Y., Yahya, N., & N. Ibrahim. 2007. Extraction, Purification and Characterization of Durian (*Durio zibethinus*) Seed Gum. *JurnalFood Hydrocollods*, 21:273–279.
- [4]. CODEX STAN 175–1989. *Codex General Standard For Soy Protein Product*. www.codexalimentarius.org/[diakses 14 April 2014].
- [5]. Davis, D. W., Oelke, E. A., Oplinger, E. S., Dolll, J. D., Hanson, C. V., and Putnam, D. H. 2003. Alternative Field Crops Manual Cowpea. Di dalam Kabas, O., Yilmaz, E., Ozmerzi, A., dan Akinci, I. Some Physical and Nutritional Properties of Cowpea Seed (*Vigna sinensis L.*). *J. Food Eng.*, 79 : 1405 – 1409.
- [6]. FAO. 2007. *Isolated Soybean Protein (ISP)*. <http://www.fao.org/docrep/t0532E/t0532e07.htm> [6 Maret 2010]
- [7]. *Geankoplis, C.J. 1993. Transport Processes and Unit Operation th 3 edition*. India: Prentice Hall.
- [8]. Kinsella, J.E., Damodaran, S. and German, B., 1985. Physicochemical dan Funtional Properties of Oilseed Proteins with Emphasis on Soy Proteins, *Dalam : Altschul, A.M. dan Wilcke, H.L. eds. New Protein Foods*. Academic Press, Inc., New York, pp: 107-179.
- [9]. Kinsella and Shetty.1985. *ACS Symp.* Dalam : Damodaran,S. 1997. *Food Proteins and Their Application*. New York: Marcel Dekker, Inc.
- [10]. Maryani, S. 2003. Komak Bean Potential As Raw Protein Isolates. Thesis: IPB Faculty Of Agricultural Technology. Bogor.
- [11]. Maulana, L. 1999. Isolation of proteins of winged bean, mung bean, and Pea Tolo With NaOH. Thesis.: Food Technology Courses Unpad. Bandung
- [12]. Rhee, K. C. 1994. *Functionaly of Soy Protein*. Dalam : N.S. Hettiarachy and G.R. Zielger (eds). *Protein Functionaly in Food System*. New York: Marcell Dekker Inc.
- [13]. Robert, E. A., (1985): *Grain Legumes Crops*. Collin, London.
- [14]. Rukmana, R. dan Oesman, Y.Y., 2000. *Cowpea Cultivation and the prospect of a Farmer*. Karnisius.Yogyakarta.
- [15]. Sudarmadji, S., B. Haryono and Suhardi.1997. *The procedure of Analysis for Food and agriculture*. Liberty, Yogyakarta.
- [16]. Winarno, F.G. 1985. Soy Food Future. *In : Utomo and Antarlina. 1998. Potential Nuts Komak (Dolichos Lablab L.) as raw Protein Isolates. Proceedings Of The National Seminar - Food Technology Experts Association Of Indonesia, Yogyakarta.*