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Process Optimization of Tempeh Protein Isolate from Soybean (*Glycine max Merr*) and Cowpea (*Vigna unguiculata*) Mixture

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***Abstract*— Production of 'tempeh' (fermented soybean) protein isolates in Indonesia is still very low. It is necessary to increase the production with the alternative materials which is make by soybean and cowpea mixture. The utilization of this soybean cake (tempeh) as protein isolate raw material is expecting benefits both of soybean and cowpea component which can complement each other. Protein's content of protein isolates should be a minimum of 90% (db). Therefore, it is necessary to find the precipitation pH and the optimum level of purification in order to get tempeh protein isolate with high protein content. The analytical method used descriptive analysis. The optimal process of tempeh protein isolates from soybean and cowpea mixture conducted with pH 5 and pH 4 precipitation, the oil extraction was carried out at the beginning of the process (before extraction of protein) and before the drying stage. The result showed that the tempeh protein isolate of soybean and cowpea mixing have 75.12% (db) protein content. It was increasing in 20.67% to 50.16% from previous research.**

***Keywords*— Protein Isolate, Tempeh, Soybean and Cowpea**

I. INTRODUCTION

Tempeh is a traditional food which is made by fermentation. The fermentation process of tempeh is to form it with a higher digestibility, because its components have been broken down into simpler molecules ([12]). Soybeans is a main raw material in the manufacture of tempeh in Indonesia, meanwhile to fulfill the needs of it must be import. Therefore, it is possible to make it by soybean and cowpea mixture, in order to reduce the dependence of imported soybean and it able to improve of its local utilization in Indonesia.

Production of protein isolates is still very low in Indonesia. Needed is important to increase it which is use a mixture of soybean and cowpea as an alternative materials. Soybean has a limiting amino acids such as methionine and cysteine amino acids, while a limiting cowpea are tryptophan and tyrosine ([10]; [6]). By using tempeh is mixing by soybean and cowpea as a raw material for making the protein isolates, both of component soybean and cowpea are expected can be complement.

The process of plant materials protein isolation is begins with the extraction of proteins based on alkaline condition (above pH 10), at that pH is produced a maximal protein solubility. Therefore, when it obtained the next process is the protein precipitation with adjustment of solvent pH which is using hydrochloric acid (HCl) almost raise the isoelectric pH. According to [16], the using principle of acid is to decrease the pH of the protein solution, the pH of precipitation is generally set up for 4. The next step is washing with alcohol, with freeze drying, and finaly is sifting process with using a 70 mesh sieve produced a protein isolate powder ([14]). Based on [7], the protein content of tempeh protein isolate of soybean and cowpea mixture is still low at 50.16%. The protein content of protein isolate should be 90% (db) at least. To yield tempeh protein isolate of soybean and cowpea mixture is influenced by pH, precipitation and purification levels. This study was conducted to determine the pH of precipitation and purification optimum level and the proper way to make tempeh protein isolates of soybean and cowpea mixture, it produced with a high protein content.

II. Material and Methods

A. Materials and Equipment

The materials were used local soybean of production halls of East Java Province, local cowpea, Raprima yeast production of PT Aneka Fermentation Industries (AFI) Bandung Indonesia, paper and filter's cloth, distilled water, reagents Lowry, Follin, selenium, H_2SO_4, diethyl ether, nelson and arsenomolibdat reagent, cooking oil, borax acid, methyl red indicator and the blue, ethanol, HCl, NaOH, alcohol, benzene, phosphate buffer, and SDS. The equipment were used ovens, pH meters, spectrophotometers, bath, centrifuge, ashing furnace, vacuum oven, distillation apparatus, exicator, soxhlet, stirrer, blender and vortex as well as glassware.

B. Research Steps

The study was conducted at the Laboratory of Agricultural Product Chemistry and Biochemistry, Department of Agricultural Product Technology, Faculty of Agriculture Technology, University of Jember. Observation begun on October in the year 2010 until the year 2011 on January. Research held by four steps. That are making a soybean cake (tempeh) mixture of soybean and cowpea, determination of protein isoelectric pH soybean cake (tempeh) of soybean and cowpea mixture, making tempeh protein isolates of soybean and cowpea mixture, and the last, purification and drying of protein isolates.

C. Making Tempeh of Soybean and Cowpea Mixture

Making tempeh of soybean and cowpea begun with preparing soybean and cowpea that have washed up clean. Because of the different characteristics so immersion process of soaking and boiling of soybean and cowpea are different.

For soybeans first, boiling for 15 minutes. Then stripping process bye squeezing the skin while washing up all regardless. Furthermore soybean soaked for 24 hours. The second, boiling for 30 minutes and followed by draining and cooling until really rough. After the second boiling, inoculated soybean as much as 1% tempeh yeast of soybean weight. The same stage is for a cowpea, but a rather different with soybean. The first difference is that it needs 10 minutes for the first boiling, a soaking time cowpea is 12 hours, while the second boiling was 20 minutes and inoculated with tempeh yeast as much as 2% of the weight of cowpea after the second boiling. After the two materials were inoculated with tempeh yeast then mix it. Mix ratio between soybean and cowpea is 60% soy and 40% cowpea. Then the packaging process use plastic which perforated. And done curing for 36 hours at room temperature.

D. Determination of the isoelectric pH Tempe Protein Mixture

Determination isoelectric pH begun with making tempeh mixture solution that is crushing 100 g tempeh and NaOH 0,1 N 500 ml solution with blender then filtered to get filtrate. Next done sedimentation for 4-5 hours and took supernata. Supernatan divide into 10 tubes and arrange pH are 3; 3,5; 4; 4,5; 5; 5,5; 6; 7; 8 and 10 with adding HCL 1 N. Determination protein consentrate in filtrate done with lowry method. Then made curve isoelectric pH between pH and consentrate. Isoelectric point of protein soybean mixture tempeh and cowpea are the lower protein consentrate who used as presipitation pH what is role in making tempeh protein isolate of soybean and cowpea mixture.

E. Making Mixture Tempeh Protein Isolate

Making proetin isolate begun with crushing 300 g soybean, cowpea tempeh and mixture deleting fat from tempeh (defating) with etanol 70%. Then done protein extraction with solution NaOH 0,1 N 1500 ml with blender. Then heating at 55°C for 30 minutes to increase the efficiency of protein extraction. Next done defating and parting non-protein residu with centrifugation, so will part between filtrate with sedimentation. Then sedimentation will throwed and done supernatan pH setting use HCL 0,1 N until reach isoelectric pH in order the protein will be sediment. In this step, determination pH isoelectric got two points differ isoeletric pH, so pH setting conducted steply. Because extraction is performed in alkaline condition in order to the first pH setting choised the higher pH (5), then setting for the lower pH (4), every after pH setting done sedimentation for 2 hours and separated sediment. Next step that sediment centrifugation with centrifuse at 4°C, 4000 rpm for 10 minutes. Sediment got is protein isolate.

F. Purification and Drying of Protein Isolate Tempeh Mixture

To lose acid residue and another compound like oil so done cleaning process wet protein protein isolates with the adding of 70% alcohol and centrifugation at a temperature of 4 ° C, 4000 rpm 10 minutes. Furthermore, by vacuum oven drying protein isolate to eliminate the smell of alcohol. Drying temperature of 40^{o}C for 12 hours. After drying, size reduction is done with blender, then sieving on the 80 mesh sieve and mixed soybean protein isolates produced soybeans and cowpea.

G. Research Design

The method used in this research is descriptive analysis of the average replications each parameter observations [15]. Each treatment was repeated three times. For ease of interpretation, the resulting data will then be diploting in graphical form.

H. Observations Parameter

The parameters observed were: protein content (Micro Kjedahl, [13], fat content (Soxhlet method), starch content (Nelson-Somogyi method), moisture content (oven method), ash content (Direct Method), protein solubility in various pH (Lowry method), Oil Holding Capacity (OHC) and Water Holding Capacity (WHC), the power and stability of the emulsion [9], power and foam stability [17].

III. Result and Discussion

A. Isoelectric Point

Isoelectric pH of soybean tempeh and cowpea tempeh and mixture ranges in pH 4 and 5. At that pH, the lowest protein concentration reached in soybean tempeh is about 1.9 mg/ml, in soybean and cowpea mixture tempeh are about 1.8 mg / ml, in cowpea tempeh is about 0.8 mg / ml. The existence some proteins with low concentration caused the isoelectric

pH conditions there are some proteins can not be deposited. Differences isoelectric point at various tempeh is caused by differences in the type and composition of amino acids. Isoelectric point is indicated with a minimum solubility at a pH. Solubility of tempeh protein at various pH is as shown in Fig. 1.

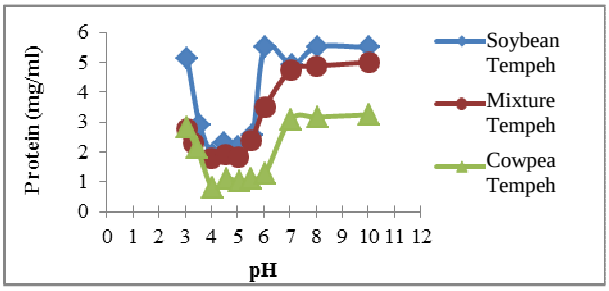

Fig. 1. Soybean Protein Solubility at Various pH

B. Yield

Highest yield is in soybean tempeh protein isolate, then mixture tempeh protein isolate and the lowest is the cowpea tempeh protein isolate. Yield of protein isolate from soybean, mixture and cowpea tempeh are respectively 6.8%, 4.2% and 2%. This is caused by the soybean protein content higher at around 35% [8] compared to cowpea which is about 21% [11]. The yield of various types of tempeh protein isolate is shown in Fig. 2.

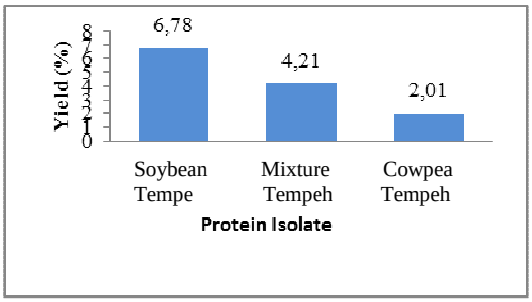

Fig. 2. The Yield of the Protein Isolates from Various Tempeh

C. Proximate Composition

Chemical composition of protein isolate from soybean, mixture, and cowpea tempeh are seen in Table 1.

TABLE I
PROTEIN ISOLATE COMPOSITION OF VARIOUS TEMPE IN PROXIMATE

Component	Amount (%)		
	Soy tempeh	Mixture tempeh	Cowpea tempeh
Protein	69.28	70.83	74.72
Fat	22.58	17.11	2.31
Starch	0.90	2.14	2.25
Water	5.22	5.71	9.53
Ash	1.25	1.05	1.56

D. Protein Content

Table 1 can be seen the highest protein content of tempeh protein isolate is cowpea tempeh (82.59% db), then mixture tempeh (75.12% db), and the lowest is soybean tempeh (73.10% db). Supposedly protein of protein isolate is 90% [5] and the highest levels should be at soybean tempeh protein isolate. However, since the fat content of soybean tempeh the protein isolate is most in order that the protein level is lowest.

E. Fat Content

Table 1 shows that the protein isolate of soybean tempeh has the highest fat content is 22.58%. Mixture tempeh protein isolate has fat content is about 17.11%, while the lowest fat content is protein isolate of soybean tempeh (18%) [1], it is higher than cowpea (1.4%) [4].

However, when compared with protein isolates according to reference [5], the fat content of the resulting protein isolate is still too high. Fat level of protein isolates according to reference [5] is about 0.5%. It is thought that fat strongly bound with fat solvent and hydrophobic group (extraction media) the extract is less powerful.

F. Starch Content

Based on the observation of the starch content of various tempeh protein isolate, starch content was highest is protein isolate of cowpea tempeh, then protein isolates of mixture tempeh and which had the lowest starch content is soybean tempeh protein isolate. This is according to reference [1] for cowpea has a high carbohydrate content (61%), while soybean has a lower carbohydrate content (34%).

G. Water Content

Table 1 shows the highest water content of protein isolate is in cowpea tempeh protein isolate is about 9.53%, while the water content of protein isolate of soybean tempeh and tempeh mixture do not so significant is about 5.5%. This can be caused by the protein and starch content of protein isolate from cowpea tempeh is bigger than mixture and soybean tempeh protein isolates. Protein and starch have characteristic binding water in order the water is more difficult evaporated.

H. Ash Content

Ash is inorganic substances residual a result of burning organic material. Ash content and composition depending on the material and how ashing. Table 1 shows that the ash content of third tempeh protein isolates are not much different, it is about 1.2 to 1.5%.

I. Functional Characteristic

1) Protein Solubility at Various pH: Protein solubility at various pH is the identification of soluble protein contained in a protein isolates. Protein solubility in various tempeh protein isolate can be seen in Figure 3.

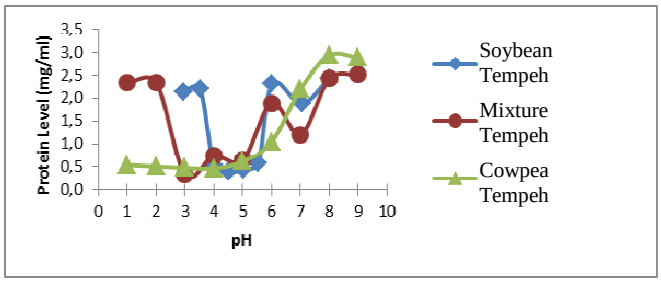

Fig. 3. Protein Solubility from Various Tempeh Protein Isolate

Fig. 3 shows that the protein solubility are varies at different pH. The third lowest protein solubility type of this tempeh ranges at pH 3 to 5. Protein solubility will increase pH above or below isoelectric pH, because the protein contains a positive or negative load. Protein solubility at pH above isoelectric pH dominated by amino groups which act as the alkaline groups. Whereas the protein solubility at pH below isoelectric pH dominated by carboxyl groups which act as acid groups.

2) Oil Holding Capacity (OHC): Analysis results of OHC value various types of tempeh protein isolates can be seen in Table 2.

TABLE II
OIL HOLDING CAPACITY (OHC) OF PROTEIN ISOLATES FROM VARIOUS TEMPEH

Sample	OHC (%)
Soy tempeh	131,18
Mixture tempeh	192,73
Cowpea tempeh	330,20

OHC of mixture tempeh protein isolate is 192.73%, this means protein isolate of mixture tempeh can bind oil 1.93 times weigth of protein isolates. Third the protein isolates produced has OHC higher than soybean protein isolate (107%) [3]. The order OHC, the highest was protein isolate of cowpea tempeh, mixture tempe protein isolate and the smallest was soybean tempeh protein isolate. This is becaused by the protein content of cowpea protein isolate was higher than the mixture and soybean tempeh protein isolate, so the capacity binding oil is also high. Proteins are able to bind water and fat. Besides allegedly fat content of soybean tempeh protein isolate high made low fat absorption because protein have bound fat.

3) Water Holding Capacity (WHC): Analysis results of WHC of various tempeh protein isolates can be seen in Table 3.

TABLE III
WATER HOLDING CAPACITY (WHC) OF PROTEIN ISOLATES FROM VARIOUS TEMPEH

Isolat Protein	WHC (%)
Soy tempeh	167,94
mixture tempeh	229,36
cowpea tempeh	292,27

Table 3 shows that the protein isolate from mixture tempeh has WHC of 229.36%, whereas for bakery products only need about 60-70% ([2]). Whereas the order of the highest WHC is cowpea tempeh protein isolates, followed by a mixture and soybean tempeh protein isolate. This is becaused by the cowpea tempeh protein isolate has highest protein and starch content.

4) Emulsion Activity and Stability: Emulsion activity and stability of protein isolates from various tempeh can be seen in Figure 4 and 5.

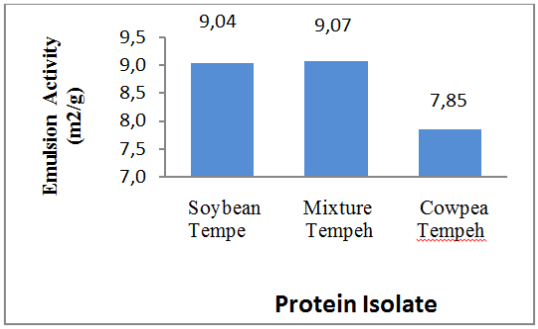

Fig. 4. Emulsion Activity of Protein Isolates from Various Tempeh

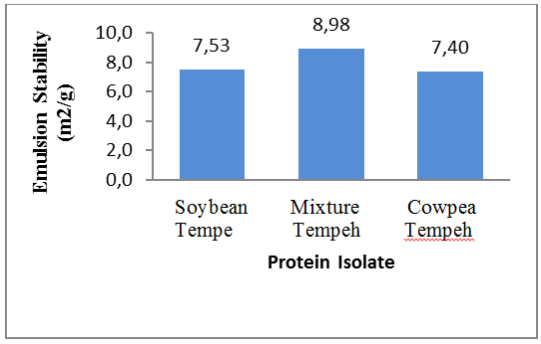

Fig. 5. Emulsion Stability of Protein Isolates from Various Tempeh

The highest capacity and emulsion stability of protein isolate was mixture tempeh, then soybean tempeh and the lower was cowpea tempeh. Emulsion capacity and stability be affected by protein content and conformational. Protein isolate of mixture soybean tempeh and cowpea guessed produce protein isolate with protein content and conformational synergize to form and stabilize emulsions. When compared with emulsion capacity of soybeen isolate 10 m^2/g ([18]), so emulsion capacity of protein isolate produced was good. Means protein isolate of tempeh produced can be used as emulsifier for food such as mayonnaise, margarine and other emulsion-based products.

5) Foaming Capacity and Stability: Analysis results of foaming capacity and stability can be seen in Figure 6 and 7.

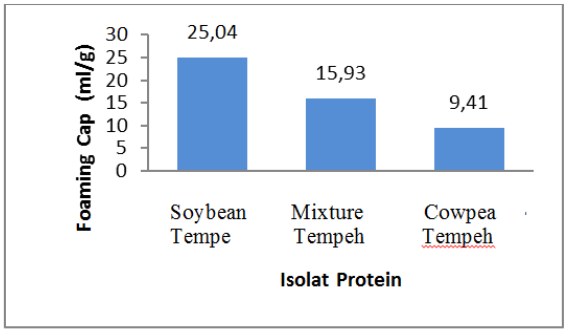

Fig 6. Foaming Capacity of Protein Isolates from Various Tempe

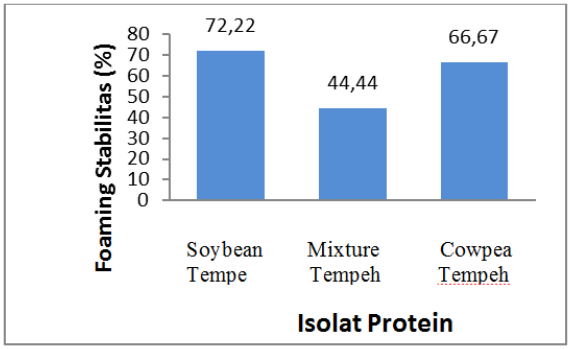

Fig. 7. Foaming Stability of Protein Isolates from Various Tempe

Fig. 6 can be seen that the highest foaming capacity is soybean tempeh protein isolate, followed mixture and cowpea tempeh. This caused by soybean tempeh protein isolate has more hydrophobic cluster. Because of the hydrophobic groups on the interface will facilitate polypeptide that will produce foam with high stability.

Fig. 7 can be seen that the highest stability is soybean tempeh protein isolate, then cowpea tempeh protein isolate and the lower is mixture tempeh protein isolate. This caused protein content of soybean tempeh protein isolate tempeh and strach content of cowpea tempeh protein isolate make high viscosity. Solution with higher viscosity has a higher foam stability than the solution with lower viscosity. However, when compared with the foaming capacity of soybean protein isolate is 40.88 ml/g ([3]), then the foaming capacity of soybean protein isolate produced is not good, so this protein isolate is less suitable for food what need foaming capacity such as ice cream and whipped cream.

IV. Conclusions

Optimal making the protein isolates from soybean and cowpea tempeh mixture was conducted with multilevel precipitation at pH 5 and 4. The oil extraction was done early in the process (before protein extraction) and drying. Protein isolate produced has protein content of 75.12% (db) increased by 20.67% from the previous study that was 50.16%, fat 17.11% and starch 2.14%. The isolate has functional characteristic such as: OHC 192.73%, WHC 229.36%, emulsion power 9.07 m^2 /g, emulsion stability 8.98 m^2 /g, foaming capacity 15.93ml/g, and foaming stability 44.44%.

Needs to be further research relevance to use protein isolate from soybean and cowpea tempeh mixture for food. Hoped there are more observation to know how to make protein isolate from soybean and cowpea tempeh mixture with standard content of protein, fat, and starch.

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