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Extraction and Purification of Cowpea, Soybean, and Fishmeal Protein using Membrane Technology

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***Abstract* – Protein was extracted from cowpea, soybean, and fishmeal flours by ionic-strength and pH adjustment with different agitation speed at 250 and 900 rpm. After centrifugation, suspend solids in the extracts were removed by microfiltration, and the filtered extracts were purified by ultrafiltration. The protein extraction from cowpeas (51%) resembled that from soybeans (250 rpm) (55%) and exceeded that from fishmeal (20%); in the samples, extraction rate increased greatly as the agitation speed increased to 900 rpm. Ultrafiltration substantially purified the protein with minor loss of protein yields, 95% and 90% for soybean and cowpea flours, respectively. Low fat content in cowpea require less additional step to remove fat and purify the protein extracts. Therefore, cowpea flour can be an alternative protein source to soybean flour, and membrane technology is a viable option for extracting and purifying proteins such as those from cowpeas, and agitation substantially increased extraction rate.**

***Keywords* – Protein, Cowpea, Extraction, Purification, Ultra Filtration.**

I. Introduction

Interest in proteins has grown because of increased consumer awareness of quality nutritional foods. The International Food Policy Research Institute (IFPRI) has predicted that demand for meat will increase by 58 percent between 1995 and 2020 to 313 million tons because of income growth, urbanization, and changes in lifestyles and food preferences in addition to continuing population growth. It expressed concern about the availability and supply of protein and recommended more research to define the reasons for low consumption of alternative proteins and to identify ways to increase such consumption.

Cowpea is a valuable crop containing high nutritional quality (high in protein and minerals), drought and heat tolerance and relative short maturity [28]. Cowpea are low in fat, and high in dietary fiber and complex, slow-digesting carbohydrates and provides health benefits to prevent lifestyle-related chronic diseases like type II diabetes, cardiovascular disease, and cancer[28]. Like those of other legumes, cowpea proteins contain all the essential amino acids except the sulfur-containing amino acids [11]. However, absence of methionine in the cowpeas is not a serious limitation since a human diet including cereals, eggs, legumes and meats contains a significant amount of methionine [11].

Conventionally, the pH of slurry is adjusted to extract plant protein; solubilized protein is then precipitated by a decreasing the pH, and the protein is harvested for commercial use. However, Insoluble indigestible carbohydrate, toxic or potentially toxic factors, and protease inhibitors are extracted anddecrease nutritional quality of extracted protein during this process [5]. An alternative method, membrane filtration, can be used to avoid the use of chemicals during purification of protein extracts. Membrane filtration processes such as microfiltration and ultrafiltration have attractions which are low energy consumption, considerably shorter processing steps, greater separation efficiency and improved final product quality [4], [6], [7], [22]. The avoidance of potentially harmful chemicals may contribute to food safety without thermal treatment; and environmental protection.

To compare the relationship of extraction and purification rates of protein derived from cowpea, soybean, and fishmeal, we investigated contents of soluble protein and carbohydrates in extract after centrifuge separation, permeate after microfiltration, and retentate after ultrafiltration. To see the relationship of extraction rates in different agitation speeds, we agitated the slurry at different speeds (250 and 900 rpm).

II. Materials and Methods

A. Preparation of samples

Cowpea (TX08-74, College Station, TX) was planted in July 2013 on the field provided from AgriLife Research Center at Texas A&M University and harvested in September 2013. The grain was stored at 0-4°C until used. Soybean flour (Goodland Farms, Hearne, TX), and fishmeal flour (Producers Cooperative Association, Bryan, TX) were obtained and stored at 0-4°C until used. Cowpea was ground by a hammer mill using a screen (1531-0187, Fitzpatrick, Elmhurst, IL), and all flours were passed through a 20-mesh sieve (Seedburo Equipment Company, Chicago, IL) for use.

B. Extraction of protein

Extraction of protein was conducted following a modified method by different slurry concentrations, processing times, agitation speeds and periods with NaCl and NaOH [35]. The entire schematic diagram of the apparatus used for the protein extraction and purification using a microfiltration and ultrafiltration is showed at Figure 1.At room temperature, flours (500 g d.b.) were stirred with 4.5L of 0.15M NaCl solution for 1 hour, and then the pH was adjusted to 9.91 with 5M NaOH for 30 minutes to extract protein from the slurry. Some samples of slurry were agitated at 250 rpm and others at 900 rpm, using agitators (StirrerHotplate, Corning, MA) (A. Fig. 1). The slurries were centrifuged using a rotor (Sorvall RC-5C Plus centrifuge and SLA-1500 rotor) to separate the liquid from the cake at 9938 g-force for 30 min (C. Fig. 1). All cakes and liquids were stored at or below 4ºC until used.

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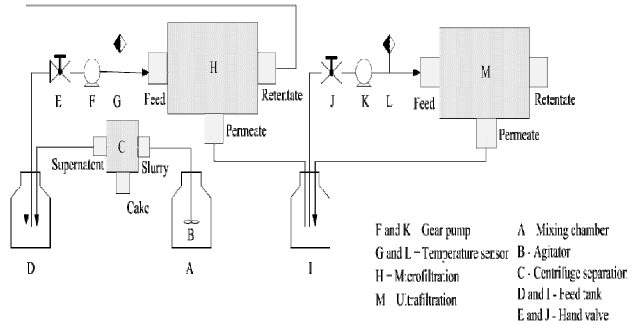

Fig.1. Schematic diagram of the apparatus used for the protein extraction and purification using a microfiltration and ultrafiltration

C. *Purification of protein extracts*

Protein purification was conducted by a simplified method [9]. Supernatants extracted at 250 rpm were filtered with stainless steel microfiltration (0.1 um, SCEPTER 4-750A-5P6, Graver Stainless Steel Membrane) to remove large insoluble substances (H, Fig. 1). The filtered liquid was re-filtered at room temperature using an ultrafiltration (polysulphone 8,000MWCO, PU 608, PCI membranes) to purify the protein (M, Fig. 1). Operating conditions for both filtration systems were 40 and 30 psi for inlet and outlet pressure, respectively. The unfiltered liquids in both steps were recirculating until they satisfied the mass balance which is an application of conservation of mass accounting for material entering and leaving system.

D. *Analysis of extracts*

Analyses of cowpea, soybean, and fishmeal flours, extracts and filtered liquids for protein, fat, ash and moisture contents were carried out according to the standard methods of the Association of Official Analytical Chemists [2]. Carbohydrates were determined by weight differences using the data or protein, fat, ash, and moisture content. The analytical values were achieved by calculating means of three determinations for each sample. Measurements were analyzed by an analysis of variance (ANOVA) using GLM (general linear model) procedure in SAS (2003). Results were shown as mean values with their standard error bars. The statistical significance of the differences between the averages in treatments was accessed by Duncan's multiple range tests. Differences were considered significant for $P<0.05$.

III. Results and Discussion

A. *Preparation of samples*

Table 1 shows the nutritional composition of cowpea, soybean, and fishmeal flours used in this research. Moisture contents of cowpea, soybean, and fishmeal flours were 6.51, 12.51, and 11.18%, respectively. The soybean and fishmeal flours contained significant amounts of fat, 20.55 and 11.87%, respectively; cowpea flour had only 1.34% fat. Therefore, soybean and fishmeal flours require an additional step to remove the fat and purify the protein extract using either mechanical or chemical methods. Fat is commonly removed using a solvent such as hexane,

Table 1: Composition of cowpea, soybean,and fishmeal.

Ingredient	Soybean	Cowpea	Fishmeal
Moisture	6.51	12.15	11.18
Fat	20.55	1.34	11.87
Protein	43.78	30.38	71.43
Ash	5.54	3.94	1.49
Crude fiber	5.46	3.64	0.56
Carbohydrate	24.68	60.69	14.66

which can be potentially harmful if the extracted protein is used as a food source. Because cowpea flour contains so little fat, it could serve as a safe source of protein-rich food. Fishmeal is used mainly for animal feed while soybean and cowpea flours have been used for animal feed and human food. In our study, fishmeal contained much more protein (71.43%) and less soluble carbohydrate (14.66%) than soybean and cowpea flours (43.78 and 30.38% protein and 24.68 and 60.69% soluble carbohydrates, respectively). Fishmeal contained less ash (1.49%) and crude fiber (0.56%) than soybean and cowpea flours (5.54 and 3.94% ash and 5.46 and 3.64% crude fiber, respectively). Our findings regarding the nutritional content of cowpea, soybean, and fishmeal flours were in agreement with data in the literatures. Soybean seed from raw soybeans consists of approximately 18.4% fat, 47.2% protein, 5.3% ash, and 29.1% carbohydrates on a dry weight basis [15]. Cowpeas contain approximately 1.9% fat, 24.8% protein, 6.3% ash, 3.6% crude fiber, and 63.6% carbohydrates on a dry weight basis [31]. Fishmeal, derived from fatty fish fishmeal herring contains and 9.8% fat, 77.2% protein, and 13% ash; fishmeal derived from white-fish contains approximately 5.4% fat, 71.7% protein, and 22.8% ash [36].

Soy and cowpea proteins are high-quality protein sources that provide all essential amino acids except that containing sulfur (methionine) [11]. Humans consume dietary proteins with nitrogen and amino acids, including the nine essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) [33]. FAO recommend methionine consumption for adults (16 mg/g protein) [12], [13], [20], however, cowpea only contains (5.4 mg/g protein) [18], and soybean (10.7 mg/g protein) [24]. However, absence of methionine in the legumes is not a serious limitation since a human diet including cereals, legumes and animal proteins contains a significant amount of methionine [11]. Fishmeal produced in North America varies widely in processing conditions and quality because there is no standard for fish meal quality. However, high-quality fishmeal contains high levels of available lysine and methionine, which plant protein supplements lack [1].

B. *Extraction and purification of protein with agitation at 250 rpm*

About 55% and 51% protein was extracted from soybean and cowpea flours, respectively, with 250 rpm agitation (Fig. 2). Useless particles such as impurities, suspended particles and microorganisms were removed by microfiltration. Protein was substantially purified by ultrafiltration (94.7% and 90.1% for soybean and cowpea

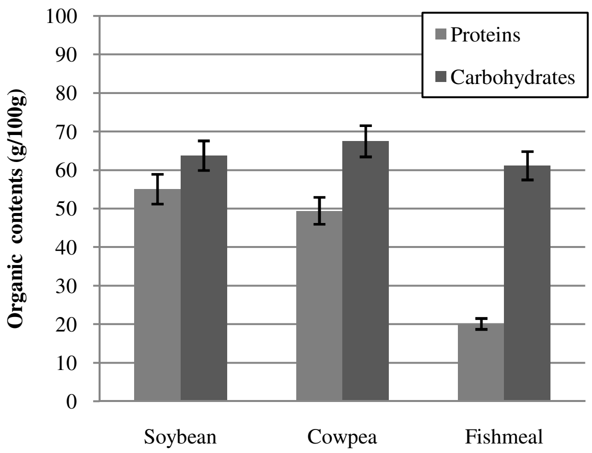

Fig.2. Solubility of protein and carbohydrate in supernatant after extraction with NaCl and NaOH compared with initial content in flour at 250 rpm.

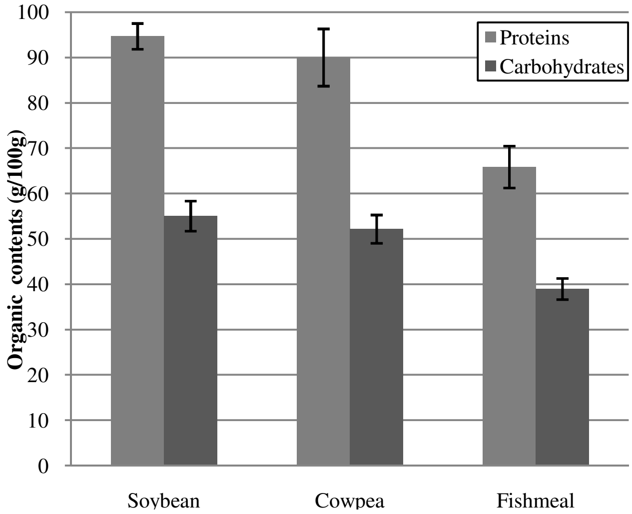

Fig.3. Purification of protein and carbohydrate in retentate with ultrafiltration compared with permeate after microfiltration at 250 rpm.

flours, respectively) (Fig. 3). In contrast, fishmeal flour had a lower percent of protein extracted (20.1%) and lower purification (65.9%) by ultrafiltration. This result implies that as initial extraction rates increased, the purification rates by membrane filtration increased. In other words, ultrafiltration purified effectively the protein in permeate after microfiltration, and there were still subtle but important loss of protein (5.3, 10.0, and 34.1% respectively) and significant permeate of soluble carbohydrates (44.92, 47.80, and 61% from cowpea flour, soybean flour, and fishmeal, respectively). Retentate during microfiltration and permeate during ultrafiltration steps were being recirculate thus the protein and carbohydrates remaining in permeate would be present in the flow.

Microfiltration with a pore size of 0.01–2 μm can selectively separate particles with molecular weight (MW) of >200 kDa such as bacteria from smaller solutes [16], [29]. Ultrafiltration with MWCOs in the range of 1-300 kDa and with a pore size of ~0.01 μm can separate colloids like proteins from small molecules like sugars and salts [29]. Therefore, in this research the proteins after microfiltration in permeate were substantially purified with higher protein and lower carbohydrate content after ultrafiltration in retentate. During the membrane processes, we expected the purification rates of cowpea flour, soybean and fishmeal to be similar. In other words, we did not expect the fishmeal to lose a substantial amount of protein during ultrafiltration. However, the fishmeal lost approximately 34% of its protein and 60% of its carbohydrates. Previous studies have reported the protein sizes of cowpeas, soybeans, and fishmeal flours. The proteins in soybeans consist of approximately 90% globulins, with trypsin inhibitor, cytochrome, and polymers constituting the rest [21]. Approximately 53.2% globulin, 23.2% albumin, 13.5% basic glutelin, 8.7% acid glutelin, and 1.7% prolamin are presents in cowpea seed [34]. The main components of soy protein are 7S and 11S globulins and tertiary structures of the sub-units of these globulins [25]. Reference [30] shows 11S protein precipitates faster and forms larger aggregates, and 11S gels have a higher water holding capacity, higher tensile values, higher hardness and expand more on heating. Therefore, the 11S protein has a different functionality from 7S protein in that it makes much harder Tofu gels. The 7S globulin unit is a trimmer made up of α' (~72 kDa), α (~68 kDa), and β (~52 kDa) subunits [32]. The 11S globulin unit is a hexamer made up of polypeptide (~20 kDa) and acidic polypeptide (~35 kDa) linked together via a disulfide cross-link [27]. Those units are larger than ultrafiltration (8 kDa MWCO) and smaller than microfiltration pores (0.05 μm). Reference [14] investigated globulin and albumin fractions from 81 wild forms and 110 cultivated forms of cowpea seed. They found that major subunit fractions of proteins ranged from 49 to 62 kDa and that ranged from 24 to 94 kDa globulins and albumins, respectively. Cowpeas have considerable amounts of salt-soluble proteins, mainly vicilin 7S globulin, and lesser amounts of legumin-like 11S globulins such as in other legume seeds [8]. In the current research, those major protein components of soybeans and cowpeas remained in the retentate passed through microfiltration and filtered by ultrafiltration. However, fishmeal had a smaller protein particle size and so the proteins in fishmeal continuously passed through both microfiltration and ultrafiltration membranes. In previous research [23], the electrophoretic patterns of herring protein hydrolysate, in which is a major band of MW in herring protein hydrolysate was less than 6.5 kDa, while the soluble fraction of the un-hydrolyzed herring protein was in various bands in the range of 14.2 to 45 kDa. In this research, those major hydrolysate protein components of fishmeal passed through ultrafiltration and remained in permeate. Fishmeal lost more protein during ultrafiltration than did cowpea and soybean flours because the average MW of fishmeal protein is lower than that of proteins from cowpeas and soybeans.

The major sugars in soybeans are glucose, fructose, sucrose, raffinose, and stachyose [10], [37]. Soybeans contain approximately 5.3% soluble sugars [17].

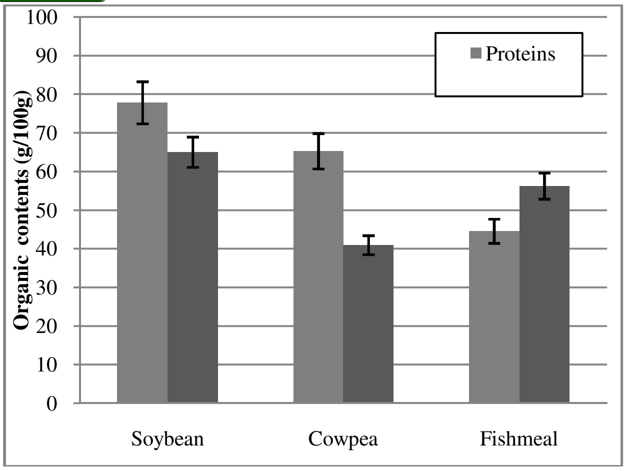

Fig.4. Solubility of protein and carbohydrate in supernatant after extraction with NaCl and NaOH compared with initial content in flour at 900 rpm.

Reference [4] shows that cowpeas contain 13.75-19.75% total soluble sugars. Cowpea varieties contained approximately 40.6% starch glucose, 3.6% verbascose,2.7% starchyose, and 1.6% sucrose on a dry basis [26]. Therefore, cowpeas had higher soluble sugar content than did soybeans and thus the simple sugar contents would be released through retentate with water during ultrafiltration. Researcher used monosaccharides such as fructose and glucose (150 Da), sucrose (340 Da), raffinose (504 Da), and stachyose (666 Da) for nanofiltration, which is used to selectively separate particles smaller than those filtered in microfiltration. They mentioned that nanofiltration is an effective way to separate small organic solutes such as oligosaccharides, low MW peptides, inorganic salts, amino acids, and other low MW materials [38]. Nanofiltration retains the small organic solutes as retentate and passes smaller particles through as permeate. Therefore, during ultrafiltration, low MW particles such as sugars that might be retained as retentate after nanofiltration and water would pass through as permeates. In this research, a combination of microfiltration to remove large insoluble substances and ultrafiltration to remove low MW sugars achieved a high yield of purified protein. During ultrafiltration of fishmeal, there was a substantial loss of protein content (34.1%) with carbohydrates. Use of a smaller pore size than 6.5 kDa could help prevent this loss. In other words, less fishmeal protein would penetrate to permeate, and more fishmeal protein would remain in retentate.Thus, ultrafiltration with appropriate pore size is essential to high protein yield.

C. Effect of agitation speed

The yield of protein obtained with an agitation speed of 250 rpm ranged from 20% (fishmeal) to 55% (soybean). Fig.4 shows that in all samples, the protein extraction rate was higher at 900 rpm than at 250 rpm. At this speed, high proportions of protein were extracted (approximately 78% and 65% of soybean and cowpea protein, respectively). Again, fishmeal had a lower extraction rate(45%), but the rate was higher than that at 250 rpm. Agitation enhances the bond strength for water-based primers but decreases it for acetone-based primers [19]. Therefore, the rate of physically-separated starch granules and protein bodies' increases as agitation speed of slurries does. This confirms that increasing extraction speed provides more mechanical force from the agitator to break bonds between components by enhancing hydration. Ultimately, it results in increased protein extraction rate

IV. Conclusion

With 250 rpm agitation, the protein extraction rate from cowpeas resembled that from soybeans and was higher than that from fishmeal. The protein extraction rate increased substantially when the agitation speed was increased to 900 rpm. Ultrafiltration greatly purified the protein with minor loss of protein yields for soybean and cowpea flours and substantial loss of soluble carbohydrates except from fishmeal protein. Therefore, cowpea flour can be an alternative protein source to soybean flour, and membrane technology is an alternative option for purifying proteins.

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Dr. Yongjae Lee

is a Head of Separation Science at Food Protein R&D Center at Texas A&M University, USA. In this occupation he directly leads the Separation group in the lab and in the pilot plant research on the separation technologies of food, dairy, feed, beverage, specialty ingredients, biotechnology and biofuel, and oil & gas. He also hosts short courses in the area of membrane, and other traditional and advanced separations techniques. He has more than 12 years of industrial experience in the field of food chemistry, food flavor, microbiology, chemical engineering, and agricultural engineering. He has published several papers in journals and has presented numerous invited lectures in various national and international meeting.

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