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Study on the Effect of Control Variables on the Extraction of Peanut Protein Isolates from Peanut Meal (*Arachis hypogaea* L.)

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Abstract: The effect of control variables involved in the extraction of proteins and preparation of protein isolate from peanut meal flour have been investigated and optimized. These control variables include: temperature of the extraction medium, sample/water ratio, extraction time, effect of successive extraction steps and centrifugal speed. The pH-dependent protein solubility profile revealed that the region of minimum solubility (isoelectric point) of the proteins was at pH 4.5. The solubility reduced as the pH increased until it reached the isoelectric point which was followed by progressive increase in solubility with further increase in pH. The effect of temperature on the extraction of proteins indicated a slight decrease in the protein yield of about 17.6 and 15.0% as the temperature was increased from 40 to 60°C in both cold and heat pressed protein isolates, respectively. Protein yields in both samples were adversely affected at an increased temperature of 70°C. There was an increase in the yield of proteins with decreasing solid-water ratio while the yield of proteins increased as the centrifugal speed was increased.

Key words: Solubility, optimization of protein yield, control variables

INTRODUCTION

Food application of proteins such as the production of coating ingredients, emulsifiers, food additives, food blending formulations and different food products involves bringing proteineous materials into solution. Hence, the knowledge of protein yield and quality is an important factor in selecting particular vegetable proteins for possible food applications. Many factors affect the extractability of protein. These include quality of flour, solvent-to-flour ratio, pH, temperature and ionic strength of the extraction medium. However, maximum protein recovery during extraction process is of vital importance as it determines the protein content in the concentrate or isolate. Some of the important parameters that determine high protein quality and yield include; temperature of the extraction medium, extraction time, solid-liquid ratio, centrifugal speed and the effect of successive extraction steps. According to Aguilera and Garcia (1989) most extraction methods involve the use of flour-to-solvent ratios between 1:5 and 1:30, with 1 to 4 times repetitive extractions. Smaller particle size increases protein yield, while flaked or exploded material has higher diffusion coefficients, which gives lower yield. Sumner *et al*. (1981) studied the extraction yield of whole and dehulled yellow pea flour and reported that dehulled pea flour gave lower crude fiber and higher protein contents. A positive relationship between protein curd yields with protein recovery during solvent extraction in soybeans has been reported by Wang *et al*. (1983) and Rhee *et al*. (1972). Hettiarachchy *et al*. (1996) have reported that two repetitive extractions were generally adequate since the third and fourth did not significantly increase yield. The optimum flour-to-solvent ratio reported by Tzeng *et al*. (1988) for rapeseed and peanut was 1:20. This made it easier to handle the total amount of solvent and reduced

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water volume. In addition to these control parameters which determine the efficiency of the extraction processes, the technology involved in the preparation of protein isolates is also vital not only to the yield of the proteins but also in preserving the identity and structure of the proteins. Generally most technology involves the solubilisation of proteins in alkaline media (pH 10-12) and their subsequent precipitation at isoelectric point (PI). In this way, proteins are purified from non-protein substances such as sugars, fibres, lipids and other non-desirable substances in the final product. According to Lqari *et al*. (2001), there are varieties of modifications to protein isolation techniques based on the need to improve the yield of isolate, or to preserve the identity of the proteins in terms of the colour and stability of the amino acids. Several researchers have used various modification methods in their processes based on the chemical composition of the legumes. Okezie and Bello (1988) reported a method for the preparation of protein concentrates from winged bean flour and water with 0.1 M NaOH. Earlier scientists, for example Smith *et al*. (1959), prepared protein isolate from horse bean using extraction with water on dilute $Ca(OH)_2$ solution and rapid agitation. An alternative wet process called slurry centrifugation for precipitating protein isolate from cowpea was developed and reported by Horax *et al*. (2004). Coffman and Garcia (1977) prepared isolate from mung flour by mixing the flour with 0.001M NaOH at a ratio of 20:1 (water: flour) and allowed to stand for 1 h with moderate mixing at 15 min interval. Extraction of peanut proteins with water was reported to be influenced by pH, ionic strength, temperature, time and solid-to-water ratio (Rhee *et al*., 1972; Rustom *et al*., 1991). According to Abulude *et al*. (2006) processing methods have a greater effect on the physical and functional properties of peanut isolates. Two traditional methods are used for the extraction of peanut oil-cold pressed and heat treated methods. The meal/cake left after peanut oil extraction contains a substantial amount of protein (Kain and Chen, 2008). The purpose of this study therefore, is to optimize the extraction of peanut proteins with water from peanut flour obtained from cold pressed and heat treated processing methods and to study the effects of control variables on the extraction of proteins from flours obtained from the two oil extraction methods.

MATERIALS AND METHODS

Materials

Cold pressed peanut meal (CPM) and heat treated peanut meal (HPM) obtained as by-products from two peanut oil processing methods were from Qingdao Kerry Peanut Oil Co., Ltd. (Shandong Province, China). All chemicals and reagents used were of analytical grade and obtained from the chemistry department of Jiang Nan University, Wuxi, People's Republic of China.

Methods

CPM was oven dried at 40°C, ground into fine powder and stored in the refrigerator for protein extraction experiments. Some control parameters involved in the extraction of protein from peanut meal flour were optimized. These include temperature, pH, flour-to water ratio, extraction time, successive extraction steps and centrifugal speed. Proteins extracted from CPM and HPM are referred to as cold pressed peanut protein isolate (CPPI) and heat pressed peanut protein isolate (HPPI), respectively.

Effect of Temperature on the Extraction Yield of Protein Isolate

The effect of temperature on the yield of protein was evaluated by using a water bath with a thermostat to set and control temperature during the extraction process. Temperature levels considered for the evaluation of the effect of temperature on protein yield were: 20, 30, 40, 60 and 70°C.

Effect of Flour-to-Water Ratio on the Yield of Protein Isolate

The effect of flour-to-solvent ratio on the protein yield was studied. The flour-to-solvent ratios used were 1:3, 1:7, 1:10, 1:12 and 1: 15. Extraction was carried out at the optimum pH of 9.0 and a temperature of 40°C for 1 h. One molar NaOH and 1M HCl were used for all pH adjustments.

Effect of Extraction Time on the Yield of Protein Isolate

The effect of time of extraction on protein yield was evaluated by carrying out protein isolation using different extraction times between 30 and 150 min.

Effect of Successive Extraction Steps on the Yield of Protein Isolate

The effect of successive extraction steps on the extraction yield was evaluated by re-suspending the particulates obtained after centrifugation into additional distilled water and re-extracting the proteins under the same conditions for 1 h followed by centrifugation at 4000 rpm for 30 min. The supernatant was then precipitated at pH 4.5 using 1M HCl.

Effect of Centrifugal Speed on the Yield of Proteins

The effect of centrifugal speed on the rate of extraction of proteins during the separation of aqueous extract from the solid residue was studied. The different rotational speeds of centrifugation employed were: 1000, 2000, 3000, 4000 and 5000 rpm.

Colour Assessment of the Isolates

Instrumental colour readings were obtained from a Hunter lab colorimeter standardized against white tile with the following reflectance values: X = 98.84, Y = 99.99 and Z =105.37, used as reference. The colour codes were: Lightness (L), redness (+a), greenness (-a), yellowness (+b), blueness (-b). The L, a, b values of the standard white tile were 94.25,-0.83 and 0.79, respectively, where L = 100 (white), L = 0 (black).

Protein Solubility

Protein solubility was determined by the method of Sathe *et al*. (1982) with some modifications. The suspension (0.2%) of the flour in distilled water was adjusted to different pH values between 2 and 11 using either 1 M HCl or 1 M NaOH. Percent nitrogen in each supernatant was determined by micro Kjedahl method (AOAC, 1990). Percent soluble protein was calculated as percent nitrogen multiplied by 5.46 on wet basis.

RESULTS

Protein Solubility

Results show that solubility, to a greater extent, was pH dependent with the lowest solubility observed for both CPPI and HPPI at the isoelectric point of pH 4.5 (Fig. 1).

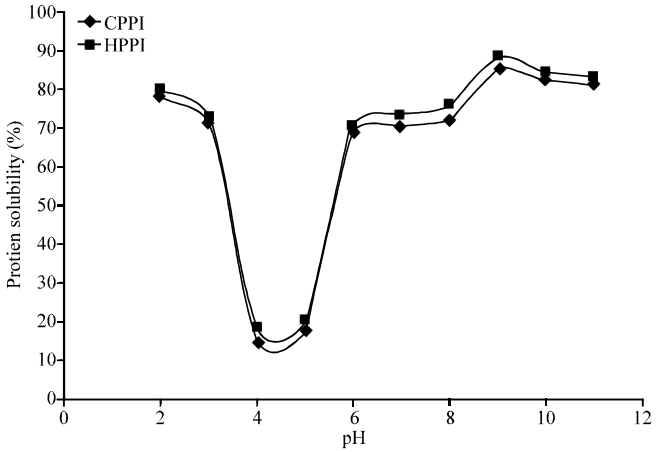

Fig. 1: Effect of pH on protein solubility

Effect of Temperature on Protein Extractability

The extraction yield for both samples was temperature dependent with optimum yields obtained at 40°C. Higher temperatures, above 40°C, recorded lower extraction yields (Fig. 2).

The Effect of pH of the Extraction Medium on the Extent of Protein Extractability

Results indicate that lowest yields were recorded at the isoelectric point of pH 4.5. A gradual increase in yield was observed between pH 5 and 10 (Fig. 3).

Effect of Extraction Time on Protein Yield

Optimum extraction time for maximum protein yield was 60 ±1 (min). Longer extraction times beyond 60 min recorded lower extraction yields for both CPPI and HPPI (Fig. 4).

Effect of Number of Extraction Time on Protein Yield

It could be seen from Fig. 5 that repetitive extraction times beyond the second extraction resulted in minimal increase in protein yield for both CPPI and HPPI (Fig. 5).

The Effect of Sample to Water Ration on Protein Yield

Results indicate that increase in sample to water ratio above 1:10 had minimal effect on the yield recorded for CPPI and HPPI. Lower sample to water ratios below 1:10 recorded lower protein extraction yields (Fig. 6).

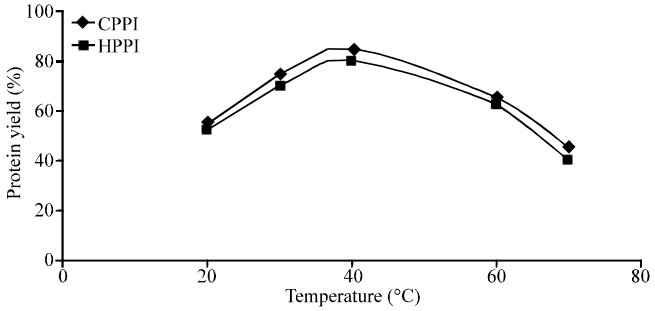

Fig. 2: Temperature level (°C) of extraction medium vs. protein extractability

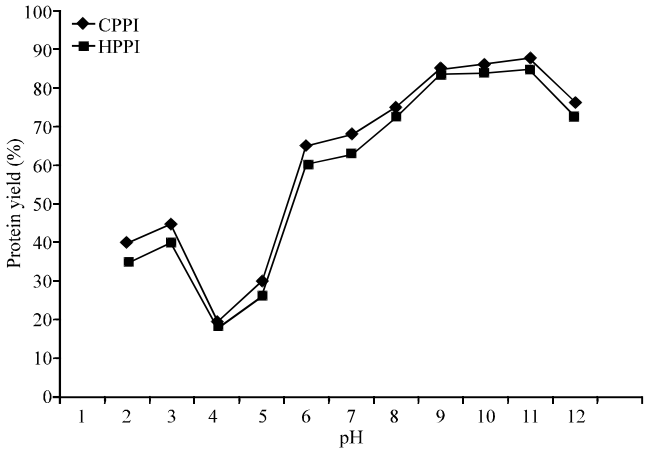

Fig. 3: Effect of pH on degree of protein extractability

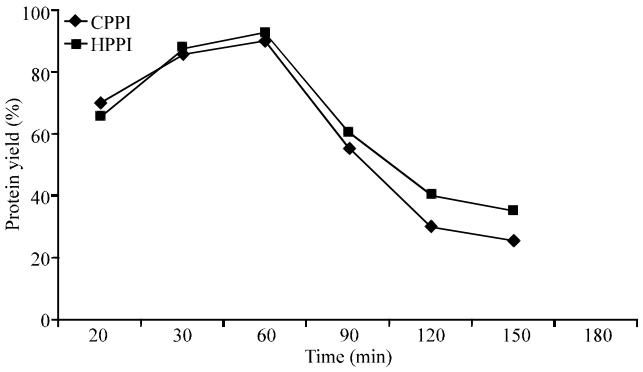

Fig. 4: Effect of duration of extraction process on protein yield

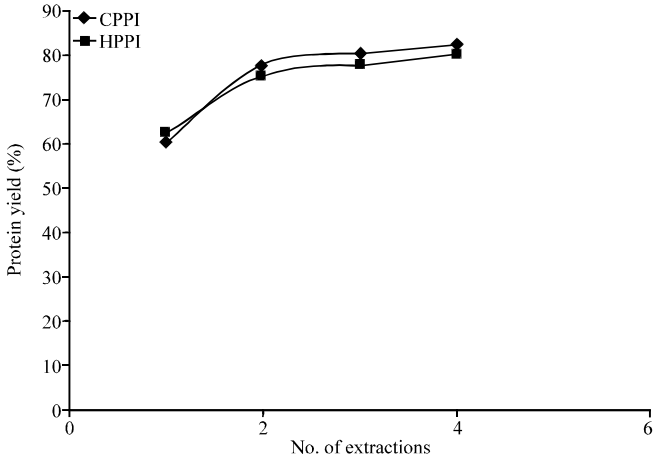

Fig. 5: The effect of the number of extraction times on protein yield

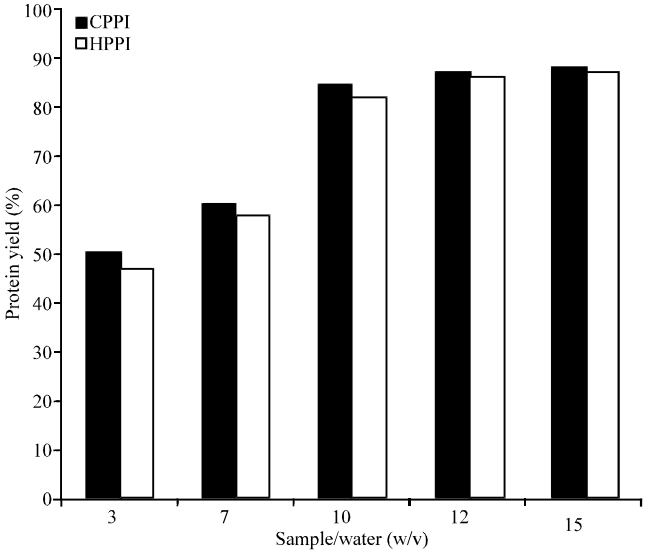

Fig. 6: Effect of sample-to-water ratio on protein yield

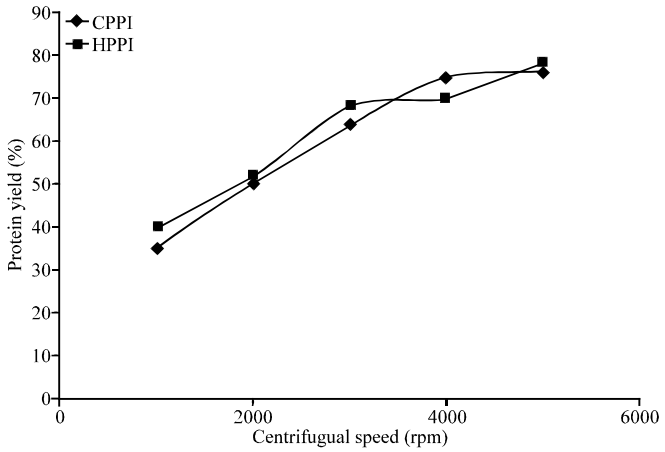

Fig. 7: Effect of centrifugal speed on protein recovery

Effect of Centrifugal Speed on Protein Yield

Results show that recovery rate of CPPI and HPPI increased with an increase in centrifugal speed (Fig. 7).

Colour Assessment of CPPI, HPPI and SPI (Soy Protein Isolate)

The Hunter L-values for CPPI, HPPI and SPI (used as control) are as follows: CPPI, 90.1; HPPI, 48.5 and SPI, 86.0.

DISCUSSION

Generally, solubility reduced as pH increased until it reached the isoelectric point. At pH levels above isoelectric point an increase in protein solubility was observed. Similar observation was reported for soy bean and winged bean according to Okezie and Bello (1988) and Vani and Zayas (1995). However, HPPI appeared to be slightly more soluble than CPPI. The solubility profile of a protein provides some insight into the extent of denaturation or irreversible aggregation and precipitation which might have occurred during the isolation process. It also gives an indication of the types of foods or beverages into which the protein could be incorporated. Factors such as concentration, pH, ionic strength and the presence of other substances influence the solubility of protein. The characteristics described above can be understood on the basis of the overall ionic charge of the protein with the pH. At low pH values, most of the carboxyl and amino groups from the lateral amino acid chains are protonated in the -COOH and $-NH_3^+$ forms, respectively and the overall charge of most protein molecules is positive. As the pH increases some of the carboxyl groups are dissociated into $-COO^-$ and H^+, according to their dissociation constants and the positive charges associated with the proteins diminish up to the isoelectric point, where these are neutralized (Cherry and McWatters, 1975). At this point, the protein cannot be hydrated by water molecules, due to the modification of its tertiary and quaternary structures and its solubility reaches a minimum value. As the pH increases even more, the amino groups dissociate into $-NH_2$ and H^+ and the overall protein charge becomes negative due to the presence of $-COO^-$ groups and can consequently be hydrated and dissolved in water. The mechanism can also be explained in terms of the prevalent charge on the constituent amino acids of proteins at various pH values. According to Kinsella (1979), amino acid exists in three different forms depending on the pH value. At isoelectric pH, minimum solubility takes place because of minimum repulsion among the constituent amino acids. The balance in positive and negative charges

minimizes the electrostatic repulsion and this reduces solubility of proteins at isoelectric pH. At both extremes of the pH scale, electrostatic repulsion improves and this enhances solubility; as observed in the current study at pH levels of 2 and 9 (Fig. 1). The high solubility of these isolates in the acidic pH range and alkaline pH range indicate that these isolates may be useful in the formulation of acidic food like protein rich carbonated beverages and bakery products. Since, protein solubility affects other functionalities like emulsification, foaming and gelation (Kinsella, 1976), the high solubility of the proteins indicates that they could have promising food applications.

A decrease in protein yield of about 17.6 and 15.0% was observed in CPPI and HPPI, respectively as the temperature was increased from 40 to 60°C (Fig. 2). The decrease was more profound as the temperature was increased to 70°C. The decrease in protein yield may be attributed to thermal degradation of the proteins. Sathe *et al.* (1982) reported the formation of insoluble aggregates with sulphur-rich proteins in soybean flour when heated to 70°C and above. This aggregation depends on several factors which among them is the heating medium. Since, the extraction was carried out in aqueous medium, the decrease in protein yield may have been due to reduced solubility of the protein with increase in temperature. Voutsina *et al.* (1983) also reported similar observations. The increased temperature affected HPPI slightly more than CPPI.

The solubility of protein isolates is of great importance in liquid protein supplements, in which the appearance of insoluble sediment is undesirable. Hence, an important characteristic of protein isolates used for some food applications is solubility at an appropriate pH to the food or beverage. The solubility profiles shown in Fig. 3 are pH dependent with minimum solubility in both samples observed between pH 4.5 and 5.5 which is expected as it falls within the precipitation pH range. Similar observations have previously been reported by McWatters *et al.* (1976). Both CPPI and HPPI recorded high solubility at pH levels of 9 and 10.

The effect of time of extraction on protein yield from both samples is shown in Fig. 4. The results indicate that the yield of proteins was fairly stable between 30 and 60 min of extraction. This implies that most of the extractable proteins were solubilized during the first 30 min of extraction. This will include most of the albumins present in the protein. However, for longer extraction times, the percentage yield of proteins increased with increasing extraction time until an optimum yield was obtained after 60 min of extraction time. Further increase in extraction time proved to be counter-productive as longer extraction times (i.e., >60 min) resulted in lower protein yields. The percentage yield increased from 85 to 90% and from 87 to 92% for HPPI and CPPI, respectively with an increase of extraction time from 30 to 60 min.

The effect of number of extraction times on protein yield was evaluated by re-suspending the residues obtained after centrifugation into additional distilled water and re-extracting the proteins under the same conditions. Protein yield increased from about 60% in the first extraction to about 78% in the second extraction. Further extraction at the third and forth times did not improve protein yield but bulk which is indicative of the fact that soluble sugars and other particulates were extracted which increased the total yield but not protein yield. The results indicate that the protein yield increases initially with the number of extraction times before reaching an optimum value (Fig. 5). The protein extraction obeys the well mechanism for solid-liquid mass transfer which assumes that solid extraction from a solid/liquid ratio increases until a position of equilibrium is attained, after which the extraction of solute remains relatively constant (Prabhudesal, 1988).

There was an increase in the yield of proteins with decreasing solid-to-water ratio. The mechanism governing the extraction of proteins must follow the dissolution and/or diffusion kinetics (Fig. 1). This kinetics is governed by a driving force related to the gradient of the component concentration between the solid and liquid phases. The concentration of the material in the liquid at equilibrium can be related to the solid phase by a partition coefficient (Davis *et al.*, 2007). There is no significant difference between sample ratios of 1:10, 1:12 and 1:15, therefore 1:10 was chosen for optimum extraction.

Minimal differences in protein yield were observed between CPPI and HPPI indicating that oil extraction method had very little or no effect on the sample-to-water ration used as a variable in protein extraction.

The centrifugal speed was varied over the range of 1000 to 5000 rpm during the extraction of protein from the aqueous solution of the flour in order to separate the aqueous extract from the solid residue (meal) (Fig. 7). There was progressive increase in the yield of proteins as the centrifugal speed was increased. This might be due to high rate of compaction of solid phase as a result of the higher centrifugal speed (Lawhon *et al*., 1981). Nonetheless 4000 rmp was chosen as the optimum since 5000 rpm resulted in tight compact and makes it difficult to remove the samples from the centrifuge cup.

The colour of CPPI and HPPI was determined with Hunter colorimeter using soy protein isolate (SPI) as control. CPPI recorded the highest L-value (90.1) compared to HPPI (48.5) and SPI (86.0), non the less HPPI exhibited a much lighter colour as compared to the original meal flour.

CONCLUSIONS

The high solubility in the alkaline and acidic pH range indicates that the isolate may be useful in the formulation of food like protein rich beverages and bakery products. Since, protein solubility affects other functionalities like emulsification, foaming and gelation, the high solubility of the proteins indicates that they could have promising food applications. Optimal conditions have been obtained in the preparation of protein isolates from peanut meal flour. These optimum conditions include pH 9, temperature 40°C, pH level of 4.5 for protein extractability, extraction time of 60 min, extraction times of two, sample-to-water ratio of 1:10 and a centrifugal speed of 4000 rpm. With regards to the effect of oil extraction method on colour, CPPI had a better colour than HPPI. On the whole this study can be considered to be environmentally friendly as a potential waste product has been used to extract proteins which could be functionally utilized in third world countries where protein-energy-malnutrition is prevalent.

ACKNOWLEDGMENT

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