Functional Properties of Homogeneous Protein Fractions from Peanut (*Arachis hypogaea* L.)†

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Protein fractions, namely arachin, conarachin I, and conarachin II, from peanut (*Arachis hypogaea* L.) were isolated to homogeneity and their functional properties studied. Bulk density and water absorption and fat absorption capacities were measured. Solubility of the fractions varied as a function of pH, and more than 95% of protein was soluble at pH below 2.5 and above 7.0. The presence of NaCl decreased the solubility at low pH. The total protein of peanut had a value of 1.11 for emulsifying activity (EA), while arachin, conarachin II, and conarachin I had values of 0.90, 1.05, and 1.11, respectively, and their emulsifying stability (ES) values ranged from 72 to 300 s. The effects of variables such as protein concentration, pH, and NaCl concentration on the emulsifying properties of peanut protein fractions were studied. The foaming capacities of the fractions before and after hydrolysis with α-chymotrypsin were in the order total protein > arachin ≥ conarachin I > conarachin II and total protein > conarachin I > arachin > conarachin II, respectively. The foam stability of enzyme-hydrolyzed fractions followed the order conarachin I > total protein > arachin > conarachin II.

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is one of the major oilseeds of the world. Dehulled peanut contains 50–60% oil and nearly 30% protein. The major proteins of groundnuts are arachin and conarachins I and II, and they make up nearly 75% of the total proteins. The oil-free meal contains about 55–60% protein of good nutritional quality (Prakash and Rao, 1986).

A better understanding of protein functionality can be gained when the protein is purified. Many studies on the functional properties deal with the peanut flour or total proteins. The objective of this research was to study the functional properties of pure and homogeneous protein fractions of peanut to provide a better understanding of the individual and total protein system.

Solubility or extractability of peanut proteins has been studied (Rhee *et al.*, 1972; Ayres *et al.*, 1974; Basha and Cherry, 1976; McWatters *et al.*, 1976; McWatters and Cherry, 1977; Rhee, 1985). Effects of pH, salt concentration, and flour concentration on emulsifying properties of defatted peanut flour were also reported (McWatters *et al.*, 1976). Ramanatham *et al.* (1978) reported that peanut protein isolate had a higher emulsion capacity than peanut flour. Foaming and foam stability of peanut meal dispersions were influenced by pH adjustment (Lawhon *et al.*, 1972; McWatters *et al.*, 1976; Cherry *et al.*, 1979). Sekul *et al.* (1978) reported that foaming properties of peanut proteins increased significantly by partial hydrolysis with papain.

MATERIALS AND METHODS

Authenticated seeds of peanut, variety TMV-2, were procured from the Karnataka State Seeds Corp. Ltd., Mysore, India.

All chemicals were analytical reagents of the highest obtainable purity.

Preparation of Defatted Peanut Meal. The defatted peanut meal was prepared according to the procedure described previously (Monteiro and Prakash, 1994).

Extraction and Fractionation of Peanut Proteins. Peanut total proteins were extracted from defatted flour by stirring with 0.01 M phosphate buffer, pH 7.9, containing 0.5 M NaCl. The total protein extract was fractionated into arachin, conarachin II, and conarachin I by using a series of simple steps involving differential solubility, ammonium sulfate precipitation, and dialysis (Monteiro and Prakash, 1994).

Homogeneity of Peanut Protein Fractions. Homogeneity was established by the techniques of sedimentation velocity, polyacrylamide gel electrophoresis, gel filtration, and DEAE-cellulose chromatography as described elsewhere (Monteiro and Prakash, 1994).

Protein Solubility. Solubility in water or 0.2 M NaCl was determined by the method of Coffmann and Garcia (1977), and the protein concentration in the supernatants was determined according to the Lowry *et al.* (1951) procedure and expressed as percentage of initial total protein concentration.

Functional Properties. A calibrated glass centrifuge tube was weighed, lyophilized protein fraction filled to 5 mL, and the tube weighed again. From the difference in weights the bulk density of the protein was calculated and expressed as grams per milliliter. Water absorption capacity was determined according to the method of Jones and Tung (1983). Fat absorption capacity was determined by the method of Sosulski *et al.* (1976). Emulsifying activity and stability were determined by the method of Pearce and Kinsella (1978). The absorbance at 500 nm at initial time zero was taken as emulsifying activity and the time required for the emulsifying activity to decrease by half as the emulsifying stability (Kato *et al.*, 1989). Foaming power and foam stability of peanut protein fractions in 0.01 M phosphate buffer, pH 7.9, were determined by the conductometric method of Kato *et al.* (1983) in a conductometer at 25 °C after the instrument was standardized with a 0.01 M KCl solution. Foaming power was determined by measuring the initial conductivity (C_i) of the foam and the foam stability corresponding to the time ($t_{1/2}$) at which the conductivity (C) reached half of its initial value ($C_i/2$) (Kato *et al.*, 1983).

Enzymatic Hydrolysis. The effect of enzymatic hydrolysis on the foaming properties of peanut protein fractions was studied in the presence of α-chymotrypsin. A 0.1% protein solution in 0.01 M phosphate buffer, pH 7.9, was adjusted to pH 8.1 with 0.1 N NaOH, and α-chymotrypsin was added in the ratio of 1:100. After incubation of the mixture for 2 h at 37 °C, the pH was immediately readjusted to pH 7.9 with 0.1 N HCl, and then the

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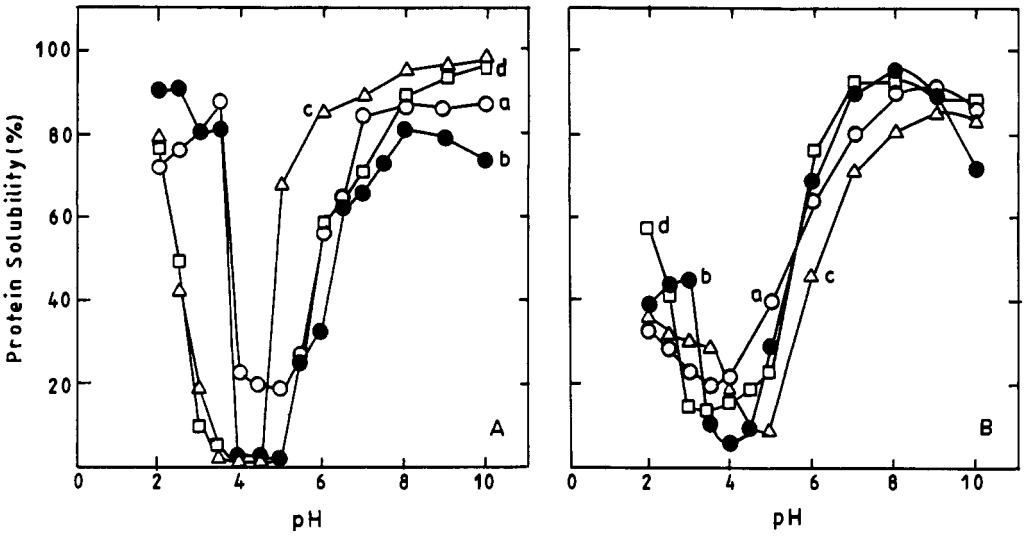

Figure 1. (A) Protein solubility curves of peanut protein fractions in water: (a) total protein; (b) arachin; (c) concarachin II; (d) conarachin I. (B) Protein solubility curves of peanut protein fractions in 0.2 M NaCl: (a) total protein; (b) arachin; (c) conarachin II; (d) conarachin I.

mixture was heated for 30 s at 90 °C to inactivate the α-chymotrypsin. Control proteins were obtained by similarly treating the protein fractions without α-chymotrypsin. The enzymatically hydrolyzed protein solution or the control was then subjected to conductivity measurements as described above (Kato *et al.*, 1983). The foaming properties of native (unhydrolyzed) peanut protein fractions were also included for comparison.

RESULTS AND DISCUSSION

Protein Solubility. The protein solubility profiles of peanut protein fractions are shown in Figure 1. The profiles in water (Figure 1A) showed a U-shaped pattern and are typical of and similar to many such profiles reported in the literature for peanut proteins (Rhee, 1985; McWatters *et al.*, 1976). The peanut protein fractions showed decreasing solubility with decreasing pH, minimum solubility at the isoelectric point, and resolubilization at pH values acidic to the isoelectric point. It is well-known that the solubility of globular proteins varies considerably as a function of pH. The protein solubility curves for peanut protein fractions showed a broad range of solubility between pH 2.0 and 10.0, and more than 95% of the protein was solubilized at pH below 2.5 and above 7.0.

These results show that solubility properties of isolated peanut protein fractions vary from those of total proteins. The data obtained in the present study show that in water the soluble fraction was highest (87%) at pH 3.5 for total protein, 91% for arachin at pH 2.5, and 98% for conarachin II and conarachin I at pH 10.0. The isoelectric points ranged between pH 4.0 and 5.0 for all of the peanut protein fractions. The peanut protein fractions showed relatively higher solubilities (67–89%) at pH 7.0 than those of peanut isolates (33–73%) (Berardi and Cherry, 1981; Madhavi *et al.*, 1989; Kim *et al.*, 1992). The peanut protein fractions were also found to be more soluble compared to protein isolates obtained from other oilseeds such as soybean isolates (40–63%) (Schwenke *et al.*, 1981) and safflower meal protein (27–65%) (Paredes-Lopez *et al.*, 1988). The protein solubility values of the peanut protein fractions obtained in this study were higher than the values reported for peanut isolates (15.1–55.6%) (Kim *et al.*, 1992).

Study of the solubility of peanut protein fractions in the presence of 0.2 M NaCl revealed that the solubility decreased at pH below 4.0 and then, after reaching a maximum, decreased again (Figure 1B). The pH affects charge and electrostatic balance within and between proteins. Below and above the isoelectric point, proteins have a positive or negative charge which enhances solubility. At the isoelectric point the charge is zero, attractive forces predominate, and molecules tend to associate (Kinsella, 1984). The first decrease in solubility in the presence of NaCl is because of salting-in, and the second decrease, after a maximum is reached, is due to salting-out effects (Damodaran and Kinsella, 1982; Prakash, 1986; Prakash and Rao, 1986). The presence of NaCl decreased the solubility of peanut protein fractions particularly at acidic pH. In the acidic region the number of charges is reduced and NaCl competes with protein for water. Thus, hydrophobic interactions could have increased in peanut protein fractions, probably followed by aggregation of proteins and loss of solubility.

Functional Properties. Bulk densities of peanut total protein, arachin, conarachin I, and conarachin II were found to be 0.253, 0.312, 0.080, and 0.084 g/mL, respectively. Water absorption capacity values of the peanut total protein, arachin, conarachin I, and conarachin II were 1.45, 1.30, 1.53, and 1.49 g of H_2O/g of protein, respectively. Fat absorption capacity values obtained for total protein, arachin, conarachin I, and conarachin II were 1.22, 1.28, 1.26, and 1.29 g of oil/g of protein respectively.

Emulsifying Properties. The emulsifying activities (EA) and the emulsifying stabilities (ES) of the peanut protein fractions are shown in Figure 2A. The total protein of peanut had a value of 1.11 for EA, whereas arachin, conarachin II, and conarachin I gave lesser values of 0.90, 1.05, and 1.10, respectively. These values were higher than the values reported earlier (Kim *et al.*, 1992) for peanut protein isolates. The ES values obtained from the graph (Figure 2A) were 84, 72, 216, and 300 s for total protein, arachin, conarachin II, and conarachin I, respectively.

The effect of protein concentration on the turbidity of peanut protein fractions is shown in Figure 2B. The protein concentration varied from 0.05% to 0.5%. The turbidity (absorbance at 500 nm) of the arachin-stabilized emulsion was higher than those of other emulsions of peanut protein fractions at all concentrations. As the protein concentration increased from 0.5 to 5.0 mg/mL the turbidity of the emulsions of peanut protein fractions increased gradually, attained a maximum at 3 mg/mL protein concentration, and decreased afterward.

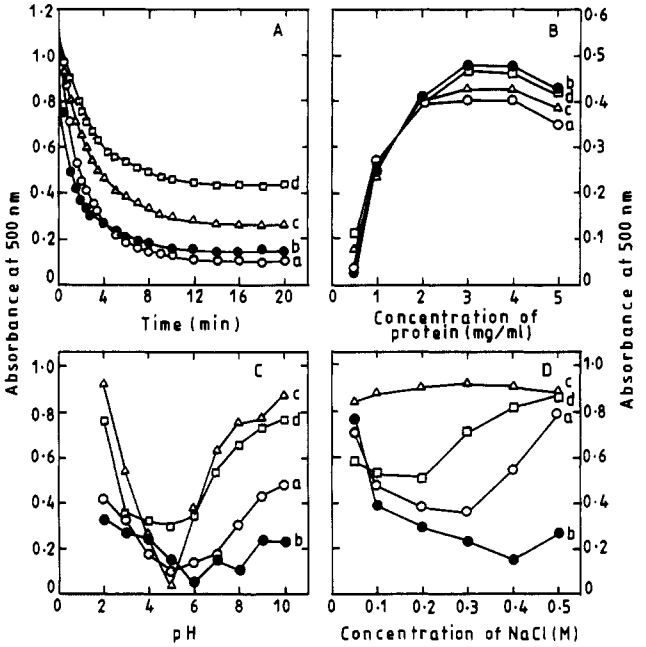

Figure 2. (A) Emulsifying properties of peanut protein fractions: (a) total protein; (b) arachin; (c) conarachin II; (d) conarachin I. (B) Effect of protein concentration on the emulsifying properties of peanut protein fractions: (a) total protein; (b) arachin; (c) conarachin II; (d) conarachin I. (C) Effect of pH on the emulsifying properties of peanut protein fractions: (a) total protein; (b) arachin; (c) conarachin II; (d) conarachin I. (D) Effect of NaCl concentration on the emulsifying properties of peanut protein fractions: (a) total protein; (b) arachin; (c) conarachin II; (d) conarachin I.

Effects of pH on emulsifying properties of peanut protein fractions are shown in Figure 2C. The turbidity (absorbance at 500 nm) for peanut protein fractions decreased toward pH 5.0 and 6.0 and then increased. The turbidity curve for conarachin II sharply decreased at the isoelectric point (pH 5.0) of the protein, whereas for conarachin I the curve decreased gradually. Arachin first showed a decrease, then a slight increase, and then a second decrease. After pH 8.0, an increase in the absorbance at 500 nm was seen.

To investigate the effect of NaCl concentration on emulsifying properties, proteins (0.1%) were dissolved in phosphate buffer, pH 7.9, containing various concentrations of NaCl ranging from 0.05 to 0.5 M. After emulsification, turbidity of the emulsions was measured at 500 nm. The turbidity of the peanut protein-stabilized emulsions is shown in Figure 2D. Although the turbidity of the arachin-stabilized emulsion was almost the same as that of the conarachin II-stabilized emulsion at 0.05 M NaCl, it decreased considerably at 0.1 M NaCl and further decreased up to 0.4 M NaCl. The same is the case with peanut total protein also up to 0.3 M NaCl concentration. However, after 0.3 M NaCl concentration, turbidity increased with increase in NaCl concentration. There was a gradual decrease up to 0.2 M NaCl in the case of conarachin I, and there was an increase up to 0.5 M NaCl concentration. With conarachin II there was a slight increase up to 0.3 M NaCl and after that there was a slight decrease.

The results obtained in this study are in close agreement with the emulsifying properties of defatted peanut flour (McWatters and Cherry, 1975) and with the effect of pH, salt concentration, and flour concentration on emulsifying properties of peanut flour (McWatters *et al.*, 1976) and soybean flour (McWatters and Holmes, 1979) reported earlier.

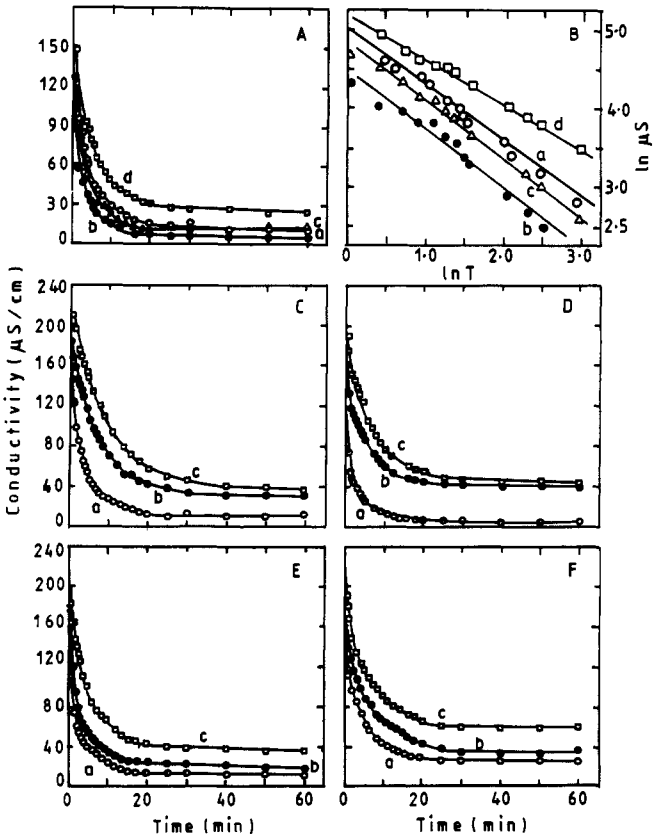

Figure 3. (A) Foaming properties of peanut protein fractions: (a) total protein; (b) arachin; (c) conarachin II; (d) conarachin I. (B) log plot of the foaming properties of peanut protein fractions: (a) total protein; (b) arachin; (c) conarachin II; (d) conarachin I. (C) Foaming properties of enzymatically hydrolyzed peanut total protein: (a) native; (b) control; (c) α-chymotrypsin-hydrolyzed. (D) Foaming properties of enzymatically hydrolyzed arachin: (a) native; (b) control; (c) α-chymotrypsin-hydrolyzed. (E) Foaming properties of enzymatically hydrolyzed conarachin I: (a) native; (b) control; (c) α-chymotrypsin-hydrolyzed. (F) Foaming properties of enzymatically hydrolyzed conarachin II: (a) native; (b) control; (c) α-chymotrypsin-hydrolyzed.

Foaming Properties. Figure 3A shows the foaming properties of peanut protein fractions. Total protein gave a value of 148 μs/cm, whereas arachin and conarachin I each gave a value of 128 μs/cm and conarachin II gave a value of 112 μs/cm as the foaming capacity. The foam stability values obtained from the graph were 180, 90, 180, and 360 s, respectively, for total protein, arachin, conarachin II, and conarachin I. The foam capacity of peanut protein fractions was in the order

total protein > conarachin I > arachin > conarachin II

and the foam stability followed the order

conarachin I > conarachin II ≥ total protein > arachin

A log log plot of conductivity of peanut protein fractions vs time is shown in Figure 3B. Analyses were done with protein fractions to arrive at a hierarchy of the foaming ability of different protein fractions.

Foaming Properties after Enzymatic Hydrolysis. The effect of enzymatic hydrolysis on the foaming properties of peanut protein fractions is shown in Figure 3C–F. The collapse of the foam of the native and α-chymotrypsin-hydrolyzed total protein of peanut is depicted in Figure 3C. Taking into account the initial conductivity, C_i, as a measure of the foam capacity, one can see that the conductivity improved after hydrolysis with α-chymotrypsin. C_i of enzyme-hydrolyzed total protein reached a

value of 213 μs/cm, whereas it was only 148 μs/cm for the native total protein. Similarly, arachin (Figure 3D), conarachin II (Figure 3E), and conarachin I (Figure 3F) in their native form had values of 128, 112, and 128 μs/cm, respectively, for foaming capacity. After enzymatic hydrolysis, the foaming capacities of arachin, conarachin II, and conarachin I increased to 190, 184, and 192 μs/cm, respectively, and the order was

total protein > conarachin I > arachin > conarachin II

The foaming capacities of the control protein fractions were slightly lower than that of the protein fractions hydrolyzed by α-chymotrypsin (total protein, 184 μs/cm; arachin, 165 μs/cm; conarachin II, 178 μs/cm; conarachin I, 158 μs/cm), although values were slightly increased when compared with that of native protein fractions. In all cases, the foam capacity increased initially and then decreased with time. The measure of foam stability ($t_{1/2}$) represents stages of foam destabilization. $t_{1/2}$ reflects an early stage of destabilization, and it describes the capability of the protein to diffuse at the interface and to be adsorbed very quickly, stabilizing the air bubbles just after formation.

The enzymatically hydrolyzed proteins showed higher foam stabilities (total protein, 9 min; arachin, 7 min; conarachin II, 5 min; conarachin I, 10 min) compared to the native ones (total protein, 3 min; arachin, 1.5 min; conarachin II, 3 min; conarachin I, 6 min) and the order was

conarachin I > total protein > arachin > conarachin II

The foam stability of the control peanut protein fractions was less than that of the chymotrypsin-hydrolyzed protein fractions (total protein, 6.5 min; arachin, 5.5 min; conarachin II, 2 min; conarachin I, 6.5 min).

Enzymatic hydrolysis by α-chymotrypsin can be used to improve the foaming properties of peanut protein fractions. Since enzymatic hydrolysis of peanut protein fractions causes an increase in foaming capacity, this hydrolysis may contribute to a larger extent for proteins poor in foaming capacity. Alkaline treatment of proteins is generally used for food application to improve functional properties such as solubility, emulsifying, and foaming properties. However, excessive alkaline treatment results in undesirable effects on food proteins such as lysinoalanine formation and racemization of amino acids (Kato *et al.*, 1987). On the other hand, proteolytic hydrolysis in a mild alkaline condition is a favorable method for application in food systems. Insolubility of the peanut protein fractions in the isoelectric pH region can be minimized by hydrolyzing the protein fractions with proteolytic enzymes especially to solubilize lower molecular weight fractions as compared to that of the native protein.

The above results present a comparative study of the hierarchy of the foaming, emulsifying, and nitrogen solubility properties of the pure protein fractions of peanut along with the effect of enzymatic hydrolysis on such functional properties. Apparently the total protein of peanut has a higher foaming capacity compared with those of arachin, conarachin II, and conarachin I, whereas upon hydrolysis the foaming stability of conarachin I increases as compared to that of total protein. On the other hand, the functional properties of arachin are unaltered as a result of enzymatic hydrolysis. These results give, for the first time, an indication that the functional properties of peanut proteins can be quite different when they are looked into as a total protein and pure protein fractions. The observed differences in functional properties of the peanut proteins might, as well, arise from polymorphism, subunit structure, hydrophobicity and hydrophilicity profiles of the isolated protein fractions, and also the protein–protein interactions in the solvent system. A detailed investigation on the physicochemical and molecular changes in the peanut protein fractions would throw more light on the physical, chemical, and enzymatic properties of the different protein fractions in solutions.

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