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# Differential Centrifugation of Peanut and Soybean Protein Concentrates as Influenced by Preparation Technique and Heat Treatment[1]

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## ABSTRACT

Protein extracts prepared from defatted Florunner peanuts and Cobb soybeans were dried by spray-, freeze- and drum-drying techniques. Differential centrifugation was performed on 1.0% protein dispersions at 2,000 and 40,000 x g for 20 min at 24°C and at 200,000 x g for 1 hr at 15°C. Lowest supernatant protein content under all centrifugation conditions studied was observed in drum-dried protein preparations. Increased centrifugal force from 2,000 to 40,000 x g only slightly affected supernatant protein in freeze-dried and spray-dried oilseed preparations and did not affect drum-dried preparations. Heat treatment (70°C for 30 min) of the protein dispersions increased supernatant protein (2,000 and 40,000 x g) for spray- and drum-dried peanut, and for all soy protein preparations. Heat treatment only slightly affected ultracentrifuge supernatant protein. Sepharose 6B gel filtration indicated four major fractions in spray- and freeze-dried peanut preparations and three major fractions in spray- and freeze-dried soy protein preparations.


Key Words: peanut, soybean, centrifugation, protein solubility, gel filtration.

The complex nature of the major protein of peanuts has been well documented (Dawson, 1971; Evans et al., 1962; Neucere, 1969 and Tombs, 1965). In addition to varietal and genetic differences (Cherry, et al., 1973; Savoy, 1976) these proteins can vary in structure with respect to environmental conditions and method of preparation (Basha and Cherry, 1976; Shetty and Rao, 1974) because of environmental effects on dissociative-associative reactions. Heat treatment effects on peanut protein solubility and structure have been extensively investigated but are still not fully understood. Various forms of dissociated and aggregated peanut proteins have been observed electrophoretically following dry (Neucere et al., 1969; Neucere, 1972) or moist heat treatment of peanuts (Srikanto and Rao, 1974; Cherry and McWatters, 1975). In an extensive study on the effect of moist heat treatment on protein structure and solubility, Cherry et al. (1975) suggested that heat treatment alters peanut protein resulting in subunit or fragment formation which aggregate to larger molecular size components with increased heating. A similar mechanism has been suggested for heating effects on soybean proteins (Wolf, 1970).

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Because of the complexity of the protein, vast differences in protein structure and functionality in isolates prepared under different conditions would be expected. We have previously reported (Ahmed and Schmidt, 1979) functionality differences for peanut and soybean protein concentrates extracted under similar conditions but dehydrated by different processes. Spray-, freeze- and drum-drying techniques were compared. In the present study, we attempted to fractionate and characterize the proteins present in these concentrates. The effect of mild heat treatment of protein dispersions on structure and solubility was also evaluated.

## Materials and Methods

### Protein Extraction and Dehydration

The protein extraction and dehydration procedures have been previously discussed (Ahmed and Schmidt, 1979). Raw shelled peanuts (Florunner) which were blanched, ground and partially defatted under hydraulic pressure and ground soybeans (Cobb) were used. Protein concentrates were prepared according to the alkaline aqueous extraction procedure as described by Rhee et al. (1972) with modifications (Ahmed and Schmidt, 1979).

Protein dehydration techniques compared in the present study were: freeze-drying (Virtis model 25-5PC-4, Gardner, N.Y.) at 60°C; spray drying (Anhydro, Inc., N. Attleboro, MA) with gravity feed and atomization at 15 psi, inlet — 250°C, outlet-120°C and drum-drying (Blaw Knox, Model ALC-4, Buffalo, N. Y.) at 120°C with drum speed 0.5 rpm.

### Differential Centrifugation

A 1.0% protein dispersion of ground (20 mesh) protein preparation in 0.02M sodium phosphate (pH 7.0) was prepared. The dispersions were allowed to equilibrate at 24°C for 1 hr with continuous stirring. The pH was monitored during equilibration and the pH was readjusted to 7.0 when appropriate. Heat treatment of appropriate samples was done at 70°C for 30 min. For soluble protein determinations, aliquots were centrifuged at 24°C (to avoid cryoprecipitation) in a Sorvall RC-5 super-speed centrifuge (rotor SM 24) for 20 min at forces of 2,000 and 40,000 x g. A separate aliquot was centrifuged in a Sorvall OTD-2 preparative ultracentrifuge (rotor T-865) at 200,000 x g for 1 hr at 15°C. Protein content of the supernatants was determined by the Biuret procedure and results were calculated as percent of total protein determined by Kjeldahl. All tabulated data represent means of duplicate trials.

### Gel Filtration

A 2.5 x 29.8 cm Sepharose 6B (Pharmacia Inc., Piscataway, N. J.) column was equilibrated with 0.02M sodium phosphate buffer (pH 7.0) to which 0.02% sodium azide had been added as a preservative. The column was calibrated and standardized according to manufacture's recommendations using Blue Dextran 2,000 for void volume ($V_O$) determinations and with standard protein mixtures: Aldolase (158,000 mol. wt.) and chymotrypsinogen A (25,000 mol. wt.); ovalbumin (45,000 mol. wt.) and ribonuclease A (13,700 mol. wt.).

To charactize the soluble proteins in the oilseed preparations, supernatants from 2,000 x g centrifugation were filtered through

celite and 1 ml portions were placed on the Sepharose 6B column. Elution was with 0.02M sodium phosphate buffer (pH 7.0) plus sodium azide. Fractions collected were monitored for protein content by measuring absorbance at 280 nm (Beckman Model 25 Spectrophotometer). While gel filtration was performed in duplicate, elution profiles from single trials are presented.

## Results and Discussion

Considerable lower soluble protein, as evidenced from supernatant protein at all centrifugation conditions (Table 1), was apparent in the drum-dried peanut concentrate (DDPC) than was observed for the spray-dried peanut concentrate (SDPC) or freeze-dried peanut concentrate (FDPC). This is probably due to protein insolubilization during the heat treatment involved in drum-drying.

**Table 1. Heating and Centrifugation effect on Supernatant Protein of Peanut Protein Concentrate.**

| Process Treatment | Centrifugal Force[1] | Supernatant Protein[2] | |
|---|---|---|---|
| | | Unheated[3] | Heated[4] |
| Freeze-dried | 2,000 | 65.7 | 63.3 |
| | 40,000 | 65.4 | 65.3 |
| | 200,000 | 41.4 | 28.5 |
| Spray-dried | 2,000 | 67.9 | 83.1 |
| | 40,000 | 62.1 | 83.3 |
| | 200,000 | 57.9 | 51.2 |
| Drum-dried | 2,000 | 18.6 | 31.1 |
| | 40,000 | 19.0 | 33.4 |
| | 200,000 | 22.1 | 25.3 |

[1]Relative centrifugal force ($X_g$); 2,000 and 40,000 for 20 min at 24°C; 200,000 for 1 hr at 15°C.

[2]Percent of total protein.

[3]A 1.0% protein dispersion in 0.02M phosphate buffer (pH 7.0); equilibrated at 24°C/1 hr.

[4]As above; heated at 70°C/30 min.

Increased centrifugation conditions from low speed (LS) at 2,000 x g to mid-speed (MS) at 40,000 x g for 20 min had no apparent effect on supernatant protein content. This suggests the absence of proteins of appropriate size to sediment at 40,000 x g in the preparations and that LS and MS centrifugation are effective in sedimenting similar protein components.

Ultraspeed (US) centrifugation for 1 hr lowered supernatant protein in the freeze- and spray-dried concentrates indicating the presence of intermediate-sized proteins in these preparations. The reduction in supernatant protein was more dramatic for the freeze-dried preparation. No effect was observed for US centrifugation of the DDPC suggesting that only lower molecular weight proteins are soluble in this concentrate. This may result from prior insolubilization of protein through aggregation or may result from dissociation to small molecular weight fragments during drum-drying. Low apparent solubility would suggest that the former has occurred.

Moderate heating (70°C for 30 min) of the protein dispersions resulted in a slight increase in LS and MS supernatant protein for the SDPC and DDPC while that of the FDPC was not affected by heating. Reasons for this difference are not readily apparent. Similar trends with heat treatment effects on protein solubility have been observed with a similarly prepared freeze-dried peanut preparation (Schmidt and Mendelsohn, 1977).

A decrease in the US supernatant protein was observed following heating of FDPC while that of SDPC and DDPC preparations was not affected by US centrifugation. Differences between the heating effect on the differential centrifugation profile may reflect differences in prior heating. It could be speculated that subsequent heating resolubilized previously aggregated proteins in the SDPC and DDPC prepared under drying conditions involving higher heat treatment. A slight aggregation may be taking place as a result of subsequent heating of the FDPC which was originally processed at a lower temperature.

As shown in Table 2, highest LS and MS supernatant protein was observed for the freeze-dried soybean concentrate (FSDC). An extremely low soluble protein level was observed in the drum-dried soy concentrate (DDSC). US centrifugation lowered supernatant protein content for the spray-dried soy concentrate (SDSC) and FDSC but did not affect that of the DDSC. These data follow a trend similar to that observed for peanut protein concentrates indicating the presence of only low molecular weight protein species in drum-dried preparations.

**Table 2. Heating and Centrifugation effect on Supernatant Protein of Soybean Protein Concentrates.**

| Process Treatment | Centrifugal Force[1] | Supernatant Protein[2] | |
|---|---|---|---|
| | | Unheated[3] | Heated[4] |
| Freeze-dried | 2,000 | 80.1 | 92.9 |
| | 40,000 | 75.4 | 97.1 |
| | 200,000 | 51.3 | 47.8 |
| Spray-dried | 2,000 | 62.1 | 72.1 |
| | 40,000 | 58.4 | 68.6 |
| | 200,000 | 50.6 | 44.5 |
| Drum-dried | 2,000 | 13.2 | 38.4 |
| | 40,000 | 12.4 | 36.0 |
| | 200,000 | 15.6 | 26.6 |

[1]Relative centrifugal force (Xg); 2,000 and 40,000 for 20 min at 24°C; 200,000 for 1 hr at 15°C.

[2]Percent of total protein.

[3]A 1.0% protein dispersion in 0.02M phosphate buffer (pH 7.0); equilibrated at 24°C/1 hr.

[4]As above; heated at 70°C for 30 min.

Heat treatment increased supernatant protein (LS and MS) for all soy protein dispersions. The US supernatant protein of FDSC and SDSC was not affected by heating suggesting that increases observed in LS and MS supernatants were due to solubilization of lower molecular weight components rather than dissociation of larger molecular weight aggregates.

The observed effects of centrifugation force on supernatant protein for these oilseed preparations follow trends similar to those observed by Shen (1976) for soy protein isolates. This suggests that a mid-speed centrifugation (40,000 x g) may be used to adequately determine soluble protein in oilseed preparations. However, other investigators (Hermansson, 1973) have shown differences in supernatant protein for other proteins analyzed by low and mid-speed centrifugation. Since oilseed proteins are extremely complex, analysis for supernatant protein using more than one centrifugal force may be advantageous to partially characterize the proteins.

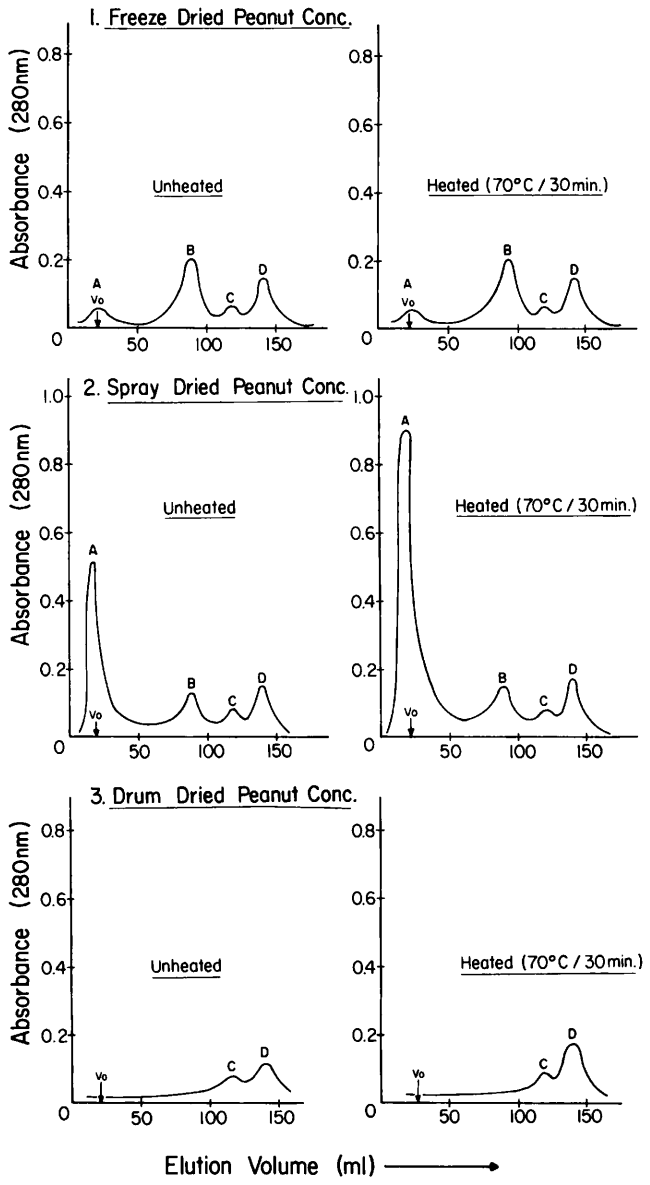


**Fig. 1. Elution profile of soluble protein (2,000 x g) for peanut protein preparations chromatographed on Sepharose 6B: 1. Freeze-dried peanut conc. (FDPC); 2. Spray-dried peanut conc. (SDPC); 3. Drum-dried peanut conc. (DDPC). Apparent molecular weight of fractions: A - large mol. wt. primarily excluded from gel; B - 1.6 x $10^5$; c - 3.0 x $10^4$ and D - 1.2 x $10^4$.**

## Gel Filtration of Protein Preparations

The elution profile for peanut protein preparations chromatographed on Sepharose 6B is presented in Figure 1. Four peaks were observed for SDPC and FDPC supernatants. The approximate molecular weights (calculated from standard curves) for these fractions were: A - large molecular weight primarily excluded from the gel; B - 1.6 x $10^5$; C - 3.0 x $10^4$ and D - 1.2 x $10^4$.

The physical chemical characteristics of peanut proteins and their associative-dissociative reactions are extremely complex. Therefore, comparison of molecular weight estimates of protein fractions with those previously reported for peanut proteins fractionated under different conditions is difficult. Arachin, the most investigated peanut protein fraction, has been suggested to exist as a 14.6 S (330,000 mol. wt.) dimer which can reversibly dissociate into 9.5 S (180,000 mol. wt.) subunit by lowering ionic strength (Tombs, 1965). Larger polymeric (17.7 S) and smaller dissociated fragment of arachin have also been reported (Neucere, 1969; Shetty and Rao, 1974; Tombs and Lowe, 1967). The other major peanut protein fraction, conarachin, apparently associates from 7.8 S (140,000 mol. wt.) to 12.6 S (295,000 mol. wt.) as ionic strength decreases (Dechary et al., 1961.) Under the ionic conditions of these experiments, therefore, fraction A (Fig. 1) probably consists of aggregated forms of both major peanut protein fractions while component B may correspond to partially dissociated forms of arachin. The lower molecular weight components contained in fraction C are probably further dissociated peanut protein components.

Considerably higher content of the high molecular component (A) was observed in the soluble portion of the SDPC than in the FDPC. This does not corraborate the previous observed effects of ultracentrifugation on these protein preparations (Table 1). The FDPC was more affected by ultracentrifugation than was SDPC, suggesting a larger content of higher molecular weight proteins in the freeze-dried preparation. Further analysis of components A and B would be necessary to clarify this. A loss of high molecular weight protein aggregates through the drum-drying process previously suggested is corraborated in Figure 1. Only the low molecular weight fractions were apparent in the DDPC.

Heat treatment (70°C/30 min) did not qualitatively alter the elution patterns of the peanut preparations. A slight increase in content of component A for SDPC and a slight increase in component D for DDPC were apparent from elution profiles. These data suggest that increases in protein solubility observed with heating (Table 1) of the SDPC and DDPC dispersions are a result of increased solubilization of the individual components rather than a dissociation phenomenon. Aggregation with heating of FDPC previously intimated from ultracentrifuge solubility data cannot be detected from gel filtration elution data. Perhaps the

differences are too subtle for detection by these techniques.

Gel filtration elution profiles for soy protein preparations chromatographed on Sepharose 6B are presented in Figure 2. Three major fractions were observed for soy protein with approximate molecular weights as follows: A - large molecular weight primarily excluded from gel, B - 1.6 x $10^5$ and C 1.4 x $10^4$.

Aqueous soybean protein extracts have been suggested to contain four major components (2 S, 7 S, 11 S and 15 S) (Wolf and Briggs, 1956; Obara and Kimura, 1967). It can be speculated from molecular weight approximations that peak B (Fig. 2) corresponds to the 7 S globulin or dissociated forms of the larger fractions while the proteins contained in peak C may approximate those of the 2 S fraction and/or other dissociated forms. Larger aggregated proteins would be expected in peak A.

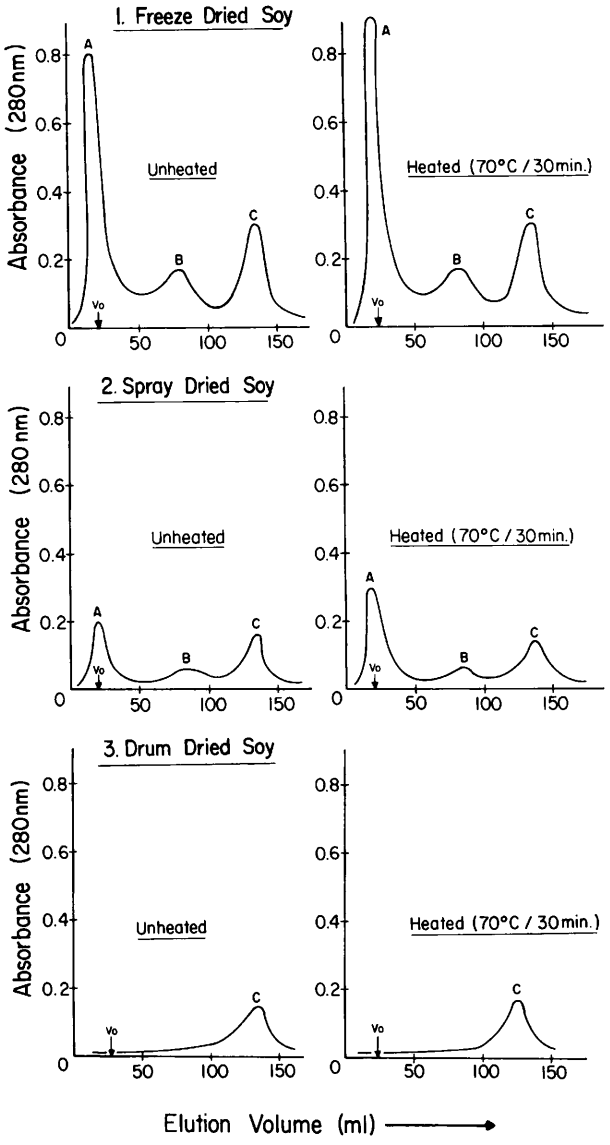


**Fig. 2. Elution profile of soluble protein (2,000 x g) for soy protein preparations chromatographed on Sepharose 6B: 1. Freeze-dried soy conc. (FDSC); 2. Spray-dried soy conc. (SDSC); 3. Drum-dried soy conc. (DDSC). Apparent mol. wt. of fractions: A - large mol. wt. primarily excluded from gel; B - 1.6 x $10^5$ and C - 1.4 x $10^4$.**

Peak A was considerably larger in FDSC than in the SDSC. The FDSC also had higher soluble protein than did SDSC (Table 2). The elution profile indicates that soluble protein differences noted between FDSC and SDSC may result from differing concentration of the high molecular weight component. As observed for DDPC (Fig. 1), only low molecular weight protein components were detectable in the gel filtration elution profile for DDSC.

Heating at 70°C for 30 min had no qualitative effect on gel filtration profiles for soy protein. Slight increases in content of component A for SDSC and FDSC and in component C for DDSC were apparent with heating.

Differing trends with respect to drying method effects on supernatant protein and gel filtration data were observed for soybean and peanut protein concentrates. Lower LS and MS supernatant protein and a larger peak A on gel filtration was observed in SDSC compared to FDSC. A reverse trend was observed with the peanut protein system where similar supernatant protein was observed in FDPC and SDPC. However, a much smaller peak A was apparent in FDPC than in SDPC. Qualitative differences may exist in oilseed protein preparations with respect to processing effects which may not be fully accounted for by supernatant protein and gel filtration data. More definitive techniques may be necessary to characterize these differences and the complex protein interaction which may be occurring.

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