egg white was due to heat denaturation of the ovomucin–lysozyme complex. In general, foam persistence is the result of the interaction of several factors, including surface tension, viscosity, temperature, pH, ionic strength, and concentration of protein in solution (Briskey, 1968; Hansen and Black, 1972).

All yeast protein isolates except those prepared by heat precipitation at pH 6.0 from alkaline extracts showed good emulsifying activity and were slightly superior than soy isolate. The yeast protein isolates prepared from water extracts and precipitated with heat possessed lower emulsifying activity than the samples precipitated without heat. Lawhon and Cater (1971) also found that some functional properties of protein isolates from glandless cottonseed processed with heat were inferior to those of isolates from unheated meal.

The yeast proteins lowered the surface tension of aqueous solutions. However, they were not as effective as soy isolates in this respect. This property was reflected in the lower foam stability of the yeast protein isolates compared to soy isolate.

Yeast protein isolates, especially those obtained by water extraction (Vananuvat and Kinsella, 1975b, 1975c) which are low in nucleic acids and have a good amino acid balance, should have potential commercial application in meat emulsions, ground meats, and bakery goods. These isolates possess a light creamy color, little flavor, and good emulsifying properties.

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Received for review October 28, 1974. Accepted April 4, 1975.

# Physicochemical Properties of Peanut Flour as Affected by Proteolysis

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Defatted peanut flour was hydrolyzed with pepsin, bromelain, and trypsin. Nitrogen solubility was increased substantially in water at pH 4.0–5.0 and in 0.03 *M* $Ca^{2+}$ at pH 4.0–11.0. Water adsorption by the flour when exposed to various relative humidities was increased as a result of hydrolysis. Emulsion capacities in water and in 0.5 *M* NaCl were completely destroyed during digestion and water- and oil-retaining properties were reduced when compared to control samples. Gel electrophoretic patterns showed substantial qualitative changes in enzyme-treated peanut protein. Patterns were different for each of the hydrolysis treatments.

Physicochemical properties of defatted peanut flour depend upon naturally occurring characteristics associated with the peanut kernel as well as processing conditions to which the kernel is exposed during conversion to flour. Behavioral properties of peanut proteins in the presence of carbohydrates, fat, water, and other food ingredients are of greatest interest, since protein comprises approximately 60% of defatted flour. Peanut flours represent potentially valuable ingredients in the formulation of protein-fortified food products.

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Modification of vegetable and animal proteins to improve particular functional requirements in food systems has attracted considerable research attention. Moist and dry heat treatments (McWatters and Heaton, 1974; Neucere et al., 1969; Neucere, 1972), acid hydrolysis (Better and Davidsohn, 1958; Fontaine et al., 1946; Higgins et al., 1941), fungal fermentation (Beuchat et al., 1975; Quinn and Beuchat, 1975), and frozen storage in the presence and absence of reducing agents (Cherry and Ory, 1973) were found to have marked effects on the physicochemical properties of both peanuts and peanut flours. Proteolytic enzymes were reported to improve functional properties of proteins from cottonseed (Arzu et al., 1972), soybean (Roozen and Pilnik, 1973), rapeseed (Hermansson et al., 1974), whey (Kuehler and Stine, 1974), and egg (Grunden et al., 1974). This paper describes the effect of enzymatic hydrol-

ysis of protein with pepsin, bromelain, and trypsin on physicochemical properties of peanut flour.

## MATERIALS AND METHODS

**Substrate.** Peanut flour was obtained from J. L. Ayres, Gold Kist Research Center, Lithonia, Ga. Flour preparation consisted of slicing whole spin-blanched No. 1 runner peanuts, extracting with hexane, and desolventizing for 3 hr at 82° in a vacuum dryer. Meal was ground at 11,200 rpm with a Kolloplex (Alpine American) mill to yield, on a percentage basis: protein, 61.0; oil, 1.6; fiber, 4.0; ash 4.5; and moisture, 5.7.

**Hydrolysis.** Fifteen grams of peanut flour was placed in 250-ml flasks, 75 ml of deionized water was added, and the slurry was adjusted to pH 2.0, 4.5, or 7.6 ± 0.1 with 1.0 *N* HCl or NaOH, and tempered for 10 min in a water bath at 50°. Pepsin (EC 3.4.4.1), bromelain (EC 3.4.4.24), and trypsin type II were obtained from Sigma Chemical Co., St. Louis, Mo. Solutions were prepared in deionized water at concentrations resulting in ratios of 1:75 (enzyme–protein, w/w) when 10 ml was added to the warmed peanut flour substrate. Hydrolysis was carried out under constant agitation for 10, 30, and 50 min. After hydrolysis, all test samples were heated at 90° for 10 min to inactivate enzymes. Hydrolysates were then cooled to room temperature and adjusted to pH 6.9 ± 0.1, the original pH of the unadjusted 15% peanut flour slurry.

Nonhydrolyzed samples included control 1, a 15% flour slurry receiving no pH–heat treatment, and Controls 2, 3, and 4, 15% slurries adjusted to pH 2.0, 4.5, and 7.6, respectively, heated 50 min at 50°, 10 min at 90°, cooled, and finally adjusted to pH 6.9. Samples from specific treatments were combined, freeze-dried, and pulverized.

**Nitrogen Solubility.** Nitrogen (proteins, peptides, free amino acids) solubility of test samples was measured in deionized water and 0.03 *M* $Ca^{2+}$ (as $CaCl_2$) over a pH range of 2.0–11.0. Each sample was added to the solvents to yield a 2% suspension and pH was adjusted and maintained by the addition of HCl or NaOH over a 45-min period with occasional agitation. Suspensions were centrifuged at 9750 × *g* for 10 min and 25 ml of supernate was analyzed for nitrogen by the Kjeldahl procedure (AOAC, 1970).

**Protein Solubilization.** Samples of soluble protein were prepared by grinding 0.1 g of control or enzymatically digested peanut flour in 1 ml of water with a mortar and pestle and centrifuging this mixture at 43,500*g* for 30 min. The soluble protein in the supernates was then measured by the technique of Lowry et al. (1951) using bovine serum albumin as a standard. These preparations were also used for electrophoretic analyses.

**Polyacrylamide Gel Electrophoresis.** Electrophoresis of samples containing 200–600 μg of soluble protein was performed on low bis 10% polyacrylamide disc gels according to the procedures outlined by Canalco (1973) and Cherry et al. (1970).

**Water Adsorption.** Equilibrium moisture contents (EMC) of hydrolyzed and control samples at various equilibrium relative humidities (ERH) were determined at 21° using a method similar to that reported by Kilara et al. (1972). ERH of 12, 33, 52, 75, and 97% were maintained in closed desiccators containing saturated solutions of LiCl, $MgCl_2$, $Mg(NO_3)_2$, NaCl, and $K_2SO_4$, respectively, as described by Rockland (1960). After test samples were allowed to equilibrate above the salt solutions for 12 days, duplicate 2-g samples were removed, dried under vacuum at 70° for 24 hr, and weighed, and the EMC were determined.

**Emulsion Capacities.** Emulsion capacities of samples were determined by a procedure adapted from Carpenter and Saffle (1964) and Inklaar and Fortuin (1969). Two grams of sample and 23 ml of either deionized water or 0.5 *M* NaCl were added to an improvised jar and blended 30 sec at low speed using an Osterizer blender. Peanut oil (Gold Kist Ravo) was added from a buret to the blending sample at a rate of 0.4 ml/sec until the emulsion breakpoint was reached. The breakpoint was defined subjectively as that point when emulsion coalescence broke to yield liquid separation and substantial loss in consistency.

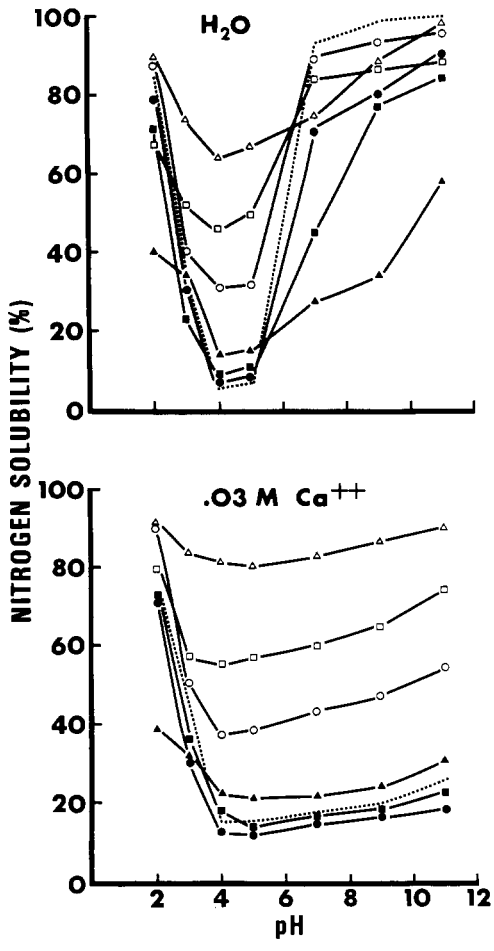


**Figure 1.** Nitrogen solubility profiles for enzyme-treated and nontreated peanut flour. Dashed line indicates no pH–heat treatment (control 1); solid lines indicate 50-min treatment at 50° followed by 10 min at 90°. Symbols: triangles, pH 2.0; squares, pH 4.5; and circles, pH 7.6 during heat treatment; open triangles, pepsin treatment; open squares, bromelain treatment; and open circles, trypsin treatment; closed triangles, squares, and circles indicate controls 2, 3, and 4 for respective enzyme treatments. All samples were adjusted to pH 6.9 prior to freeze-drying and analytical examination.

**Liquid Retention.** Four-gram samples of hydrolyzed and control peanut flours were combined with 20 ml of deionized water or peanut oil in 30-ml centrifuge tubes. Slurries were stirred occasionally over a 30-min period at 24° and then centrifuged at 15,000*g* for 15 min. The volume of decanted supernate was measured and milliliters of liquid retained per gram of sample was determined.

## RESULTS AND DISCUSSION

**Nitrogen Solubility.** Nitrogen solubility profiles of control and of enzymatically hydrolyzed peanut flour are shown in Figure 1. Solubilities of the nontreated peanut flour (control 1) in deionized water over the pH range tested are comparable to those previously reported (Lawhon et al., 1972; Rhee et al., 1972, 1973). Water-dispersible nitrogen (protein, peptides, free amino acids) was lowest at pH 4.0–5.0, a range bracketing the isoelectric points of most peanut proteins. Decreasing the pH to below 4.0 caused a sharp increase in the percentage of soluble nitrogen. The same was true when the pH was elevated from 5.0 to neutrality. Greater than 90% of the peanut flour nitrogen was

**Table I. Physicochemical Properties of Enzyme-Treated and Nontreated Peanut Flour**

| Treatment[a] | Heating conditions at 50°[b] pH | Heating conditions at 50°[b] Time, min | Soluble protein,[c] mg/ml of $H_2O$ | Emulsion capacity, ml of oil/g of sample $H_2O$ | Emulsion capacity, ml of oil/g of sample 0.5 *M* NaCl | Liquid retention, ml/g of sample $H_2O$ | Liquid retention, ml/g of sample Oil |
|---|---|---|---|---|---|---|---|
| Control 1 | 6.9 | *d* | 24.3 | 36 | 26 | 0.63 | 1.79 |
| Control 2 | 2.0 | 50 | 14.6 | 39 | 32 | 1.45 | 1.79 |
| Pepsin 1 | 2.0 | 10 | 42.8 | 31 | 32 | 1.14 | 1.74 |
| Pepsin 2 | 2.0 | 30 | 44.3 | 0 | 0 | 0.86 | 1.77 |
| Pepsin 3 | 2.0 | 50 | 45.0 | 0 | 0 | 0.83 | 1.76 |
| Control 3 | 4.5 | 50 | 12.0 | 36 | 23 | 1.03 | 2.05 |
| Bromelain 1 | 4.5 | 10 | 44.2 | 29 | 17 | 1.03 | 2.23 |
| Bromelain 2 | 4.5 | 30 | 40.9 | 0 | 0 | 0.96 | 2.20 |
| Bromelain 3 | 4.5 | 50 | 45.6 | 0 | 0 | 0.73 | 2.11 |
| Control 4 | 7.6 | 50 | 24.0 | 34 | 25 | 0.95 | 2.01 |
| Trypsin 1 | 7.6 | 10 | 42.8 | 35 | 35 | 0.70 | 1.95 |
| Trypsin 2 | 7.6 | 30 | 42.0 | 35 | 35 | 0.58 | 2.09 |
| Trypsin 3 | 7.6 | 50 | 42.9 | 0 | 0 | 0.58 | 1.91 |

[a] No enzymes added to controls. [b] Following these treatments, all samples except control 1 were heated 10 min at 90° and readjusted to pH 6.9 prior to freeze-drying and analyses. [c] Soluble protein extracts used for electrophoretic studies (see Figure 2). [d] No heat treatment.

soluble in the alkaline pH range. The nitrogen solubility of control 2 (pepsin control) at pH 2.0 was substantially lower than those of controls 1, 3 (bromelain control), and 4 (trypsin control). Otherwise, pH adjustment and heat treatment had little effect on nitrogen solubilities below 5.0. Above pH 5.0, however, a marked suppression in soluble nitrogen of controls 2, 3, and 4 was noted. The effect was greatest on control 2 which had been adjusted to pH 2.0 and back to 6.9, intermediate on control 3 which had been adjusted to pH 4.5 and back to 6.9, and least on control 4 which had been adjusted to pH 7.6 and back to 6.9. These data suggest that changes in peanut proteins exposed to highly acidic conditions are less reversible than are those taking place in proteins exposed to alkaline conditions or to pH manipulations which do not take the proteins through their entire isoelectric range.

Nitrogen solubility profiles of enzymatically hydrolyzed peanut flour in water were markedly different from their respective controls and control 1 (no pH–heat treatment). Lowest solubilities for 50-min pepsin-, bromelain-, and trypsin-treated samples were 64, 46, and 30%, respectively, at pH 4.0. Although hydrolyzed samples had higher nitrogen solubilities than their respective controls in the alkaline pH range, they were less soluble than the nontreated control 1. There is probably an ionic strength effect due to the addition of HCl and NaOH during the pH adjustment procedures which is influencing the nitrogen solubilities of the test samples. It is unlikely, however, that ionic strength differences would be sufficient to alter the relative order of change in solubilities among hydrolyzed and control samples.

Nitrogen solubility profiles for enzymatically hydrolyzed and control peanut flour samples in 0.03 *M* $Ca^{2+}$ (as $CaCl_2$) are also shown in Figure 1. Solubilities of all controls between pH 2.0 and 5.0 in 0.03 *M* $Ca^{2+}$ were similar to those noted for water; however, very little increase in nitrogen solubility of controls was noted in the pH 5.0–11.0 range. Similar data were reported by Rhee et al., (1972). They noted that at pH 2.0–3.0, peanut protein extractability was enhanced in 0.01–0.10 *M* $CaCl_2$ but suppressed in solutions containing 0.25 *M* or higher $CaCl_2$. Likewise, they showed little increase in protein extractability at pH above 4.0 in 0.01–1.00 *M* $CaCl_2$. As shown in Figure 1, enzyme treatment of peanut flour greatly increased the nitrogen solubility at pH 2.0–11.0 in 0.03 *M* $Ca^{2+}$. Again, pepsin treatment resulted in the greatest increase. Lowest solubilities in the profiles were at pH 4.0–5.0, where values of 81, 57, and 38% were measured for pepsin-, bromelain-, and trypsin-treated flours, respectively.

The selection of 0.03 *M* $Ca^{2+}$ was chosen for investigation since this level has been prescribed as a minimum in the formulation of imitation milk. Evidence from the present study suggests that enzymatic hydrolysis of peanut flour modifies protein to the extent that it is highly soluble in 0.03 *M* $Ca^{2+}$ at a pH range normally associated with liquid milk. Further studies are required to assess the effect of proteolysis on organoleptic properties of hydrolyzed peanut protein solutions.

**Protein Solubility and Gel Electrophoresis.** Table I shows protein solubility data for control and test flours. Extracts from nontreated flour and from control 4 contained similar quantities of protein, whereas controls 2 and 3 contained substantially less. Lowering the pH of the pepsin control to 2.0 and that of the bromelain control to 4.5 followed by heating evidently altered the peanut proteins irreversibly to less soluble forms. Adjusting the trypsin control to pH 7.6 during the heating procedure did not require passing the proteins through their isoelectric points. This procedure did not significantly change the solubility of peanut protein. Enzymatic hydrolysis increased the soluble protein as compared to respective controls. Approximate threefold increases were noted after pepsin digestion, fourfold after bromelain, and twofold after trypsin treatment. These levels of protein solubility were evident after only a 10-min enzyme treatment. Further digestion times did not significantly increase the amount of soluble protein. These data are generally consistent with those obtained for nitrogen solubility as shown in Figure 1. Any inconsistences are probably due to nonprotein nitrogen measured by the Kjeldahl technique.

Electrophoretic patterns of proteins in water-soluble fractions of control and enzymatically hydrolyzed peanut meal are shown in Figure 2. Patterns showed that the proteins of pepsin and bromelain controls (controls 2 and 3) were markedly different from those of nontreated flour. Heating of controls 2 and 3 under acidic conditions not only altered protein solubility as shown by decreased nitrogen solubility, but also changed the proteins qualitatively. No major (dark-staining) components were detected in 0.5–1.5-cm region of gels of the pepsin and bromelain controls. Two minor (light staining) bands were detected in region 0.5–1.5 cm and a number of major components were

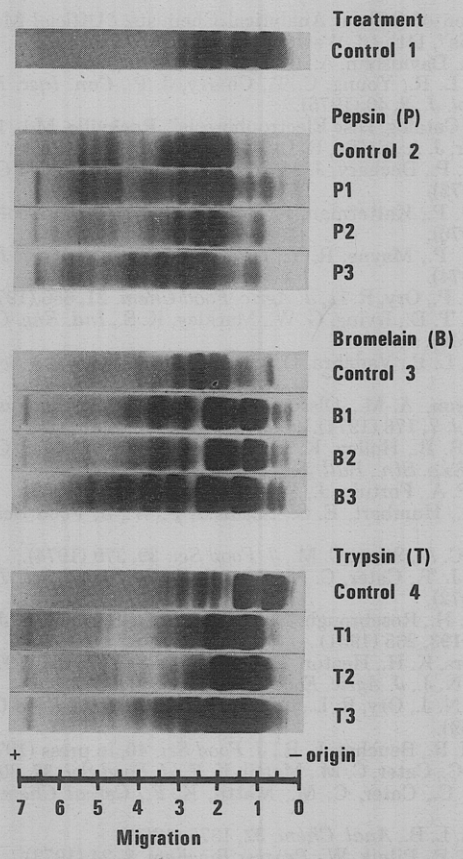


**Figure 2.** Polyacrylamide gel electrophoretic patterns of enzyme-treated and nontreated peanut flour. See Table I for description of treatments.

clearly shown in regions 2.0–4.0 and 4.5–5.5 cm of gels of the pepsin control. The gel pattern of the bromelain control showed three minor components in region 0.5–1.5 cm and a number of dark-staining bands in region 2.0–4.0 cm.

Digestion of peanut flour with pepsin and bromelain resulted in considerable changes in the lower half of the gels (region 4.5–7.0 cm). The changes in the protein patterns of peanut flour treated with these enzymes, especially pepsin, became most prevalent by 50 min. The gel patterns of the bromelain-digested flour did not resemble its control or the nontreated flour. Six major bands were distinctly shown in region 1.0–5.0 cm and four minor components were present in region 0.5–6.8 cm which were not clearly distinguished in the control. The dark-staining components in region 1.0–2.5 cm had mobilities similar to the major diffuse bands detected in the trypsin-treated samples and may be altered forms of arachin, the major storage globulin in peanuts (Cherry et al., 1973).

The gel pattern of the trypsin control was very similar to that of the nontreated peanut flour. This observation supports an earlier suggestion that adjusting the pH of the trypsin control to 7.6 followed by heating did not alter the proteins enough to substantially affect their solubility. However, qualitative changes were noted in the soluble protein fraction of trypsin-treated flour. Arachin appears in the 0.5–1.5-cm region of the electrophoretic pattern of nontreated flour but is not clearly shown in trypsin-treated preparations. Instead, two diffuse major bands were detected in region 1.0–2.5 cm and a number of smaller components were present in the lower halves of the gels. These major bands may result from arachin which was partially digested by trypsin to yield forms having increased electrophoretic mobilities.

Changes in soluble proteins of peanut flour caused by proteolytic enzyme digestion as detected by gel electrophoresis resemble those resulting from fermentation of peanut

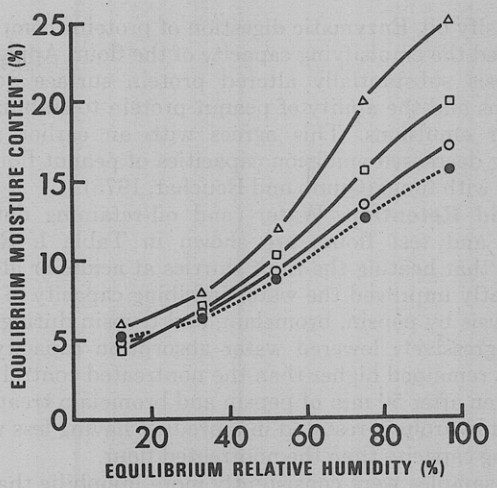


**Figure 3.** Moisture adsorption isotherms of enzyme-treated and nontreated peanut flour. Symbols are the same as those described for Figure 1.

meal with some fungi (Beuchat et al., 1975) and growth of fungi on viable peanut kernels (Cherry et al., 1974). These studies showed that "standard" peanut protein electrophoretic patterns were distinctly modified as a result of fungal growth. Biochemical transformations in proteins included decomposition of large molecular weight globulins such as arachin to smaller components, followed by rapid qualitative and quantitative decreases in these latter constituents as fungal growth progressed.

**Water Adsorption.** Moisture adsorption isotherms for 50-min enzyme-treated samples and the nontreated sample are shown in Figure 3. All three test enzymes caused the peanut flour to adsorb more water than the nontreated control at specific ERH values. The 10- and 30-min enzyme hydrolysis samples (data not presented) showed adsorption isotherms intermediate between 50-min enzyme-treated samples and their respective controls. Increased water adsorbing capacities of enzyme-treated peanut proteins are probably related to increased numbers of polar sites, such as carboxyl and amino groups, which appear on the proteins as a result of hydrolysis. The increased EMC in enzyme-treated flours cannot be attributed entirely to proteolysis, however, since controls 2, 3, and 4 (not shown in Figure 3) also exhibited abilities to adsorb slightly more water than control 1. Peptization and permanent configurational changes occurred during heating processes under acidic and alkaline pH conditions. Better and Davidsohn (1958) also noted that heat and pH may alter the effectiveness of using pepsin to solubilize proteins in peanut meal.

From a practical viewpoint, the increased water-adsorbing capacity of enzyme-treated peanut flour at specific ERH may have important implications in the formulation of intermediate-moisture foods. At an ERH of 60%, a level generally regarded as minimum for the growth of microbes in foodstuffs, the EMC of nontreated flour was 10% compared to 14% for the 50-min pepsin-treated flour. Conceivably, a food product could be developed using pepsin-digested flour which would be safe from the standpoint of not supporting microbial growth and yet contain significantly more water than a product formulated using nontreated flour. Such a product might not exhibit the characteristic mouth-drying sensation often associated with vegetable proteins.

**Emulsion Capacity.** Emulsion capacities of controls and enzyme-hydrolyzed peanut flour are listed in Table I. Values were determined for both water and 0.5 *M* NaCl systems. Capacities were higher in water than in the NaCl system for most samples and pH adjustment and heating did not result in substantial changes in the capacity of flour

to emulsify oil. Enzymatic digestion of proteins completely destroyed the emulsifying capacity of the flour. Apparently hydrolysis substantially altered protein surface activity strengths and the ability of peanut protein to stabilize oil-in-water emulsions. This agrees with an earlier report showing decreased emulsion capacities of peanut flour fermented with fungi (Quinn and Beuchat, 1975).

**Liquid Retention.** Water- and oil-retaining data for control and test flours are shown in Table I. Results showed that heating the flour slurries at acidic or alkaline pH greatly improved the water-imbibing capacity of flour. Hydrolysis by pepsin, bromelain, and trypsin during heating progressively lowered water absorption capacity. Capacities remained higher than the nontreated control, however, even after 50 min of pepsin and bromelain treatment. Trypsin hydrolysis resulted in a product having less water-retaining capacity than the nontreated flour.

Test samples were consistently more lipophilic than hydrophilic. Although heating at pH 2.0 did not affect oil-retaining characteristics of the flour, heating at pH 4.5 and 7.6 tended to enhance these characteristics. Oil retention by peanut flour was not significantly changed from respective controls as a result of hydrolysis with pepsin, bromelain, or trypsin.

Liquid retention properties of peanut protein may affect food processing conditions where water or oil is incorporated as ingredients along with the peanut flour. Overall qualities of food products, such as shrinkage during processing, mouthfeel, and storage stability, are affected by liquid retention properties of their constituent ingredients.

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Received for review December 19, 1974. Accepted March 6, 1975.

# Pilot Plant Production of an Edible White Fraction Leaf Protein Concentrate from Alfalfa

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This paper describes the development of a pilot plant scale wet fractionation process to obtain an edible white protein fraction from fresh alfalfa (*Medicago sativa L.*). Expressed alfalfa juice is given a flash heat treatment to agglomerate preferentially the green pigmented proteins which can then be separated by continuous high-speed centrifugation. The chlorophyll-free soluble protein remaining in the supernatant is precipitated by heating to 80° and separated by centrifugation. An off-white to light-tan, bland, protein concentrate containing approximately 90% protein is obtained. The product and its processing behavior can be improved by the addition of sodium metabisulfite to the fresh alfalfa prior to processing. The major product from the process, called the Pro-Xan II process, is a dehydrated alfalfa meal. The remaining products include the alfalfa solubles fraction and a feed-grade protein–xanthophyll concentrate. The latter is prepared by heat coagulating, pressing, and drying the agglomerated green protein fraction. Yields, compositions, and other processing data from the pilot plant operation are discussed.

Leaf protein concentrates (LPC's) were first described over 200 years ago (Rouelle, 1773) and have been studied extensively during the past 30 years (Bickoff et al., 1947; Kohler et al., 1968; Pirie, 1971a). However, the use of LPC in the human diet has remained almost nonexistent while other newly developed protein concentrates and isolates from soy, cottonseed, peanut, whey, and other sources are already being incorporated into human foods at a rapidly expanding rate (Hammonds and Call, 1972; Holsinger et al., 1973; Rooney et al., 1972). In spite of its high protein content and good nutritive value (Singh, 1967; Woodham, 1971; de Fremery, 1972; Olatunbosun et al., 1972), LPC has been rejected by most human consumers and food product

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