Some Functional Properties of Peanut Proteins Partially Hydrolyzed with Papain

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Partial hydrolysis of peanut proteins by papain increased solubility in water and foaming capability and decreased viscosity. These results suggest that the incorporation of partially hydrolyzed peanut proteins in certain foods where these characteristics are desired has some advantages.

Oilseed proteins have been widely used as a source of proteins in animal feeds, but recent technological advances in protein research have shown them to be potential sources of protein for human comsumption (Gheyassuddin et al., 1970; Wilding, 1970; Smith, 1971; Ayres et al., 1974). In view of the increasing costs and limited supply of animal products (Kinsella, 1976), considerable research has been devoted to the development of nutritionally and functionally acceptable protein sources for fortifying food products (Sosulki and McCleary, 1972; Johnson, 1970; Lockmiller, 1972).

For their successful use in food, proteins should provide essential amino acids and possess critical functional properties, e.g., solubility, surface activity (foaming), emulsifying properties, etc., depending upon the desired use (Franzen and Kinsella, 1976; Adler-Nissen, 1976). These functional properties will govern the suitability of novel proteins as food supplements and as ingredients for fabricating new food products, even at the expense, sometimes, of the nutritional quality of the protein (Smith, 1971).

Partially hydrolyzed vegetable proteins have been used in food formulations for a number of years (Prendergast, 1973), but only recently has the versatility of these food ingredients been recognized. Chemical modification of protein is often required to produce more desirable properties that enhance the incorporation of the protein in some food formulations. Thus, hydrolysis by either acid or base, or occasionally by an enzymatic process, is employed to improve some functional properties of proteins. A serious deterrent to the wider use of protein hydrolyzates in food systems is the formation of bitter peptides caused by excessive (uncontrolled) hydrolysis of the protein. Proteins such as casein and those from soybean, when hydrolyzed by endopeptidases, are known to give rise to bitter products; some of these peptides have been characterized (Clegg and McMillan, 1974; Arai et al., 1970; Shiraishi et al., 1973). Many proteins have been examined for bitterness after hydrolysis, and studies have revealed that bitterness was related to the protein rather than to the enzyme used, although different enzymes gave rise to different intensities of bitterness (Clegg and McMillan, 1974).

Hydrolysis by either acid or base might require that the final product be neutralized, thereby increasing the concentration of salt (not desirable for certain end-uses). Use of the hydrolyzed product must be restricted where high concentrations of salts are undesirable (e.g., in low salt diets). Partial hydrolysis of peanut protein by papain (Sekul and Ory, 1977) offers an attractive, rapid, and economical method for increasing the versatility of this protein for use in foods, without increasing salt content of the final product. The partially hydrolyzed peanut protein is bland in taste, odorless, light in color, has increased solubility, and the microbial count after hydrolysis does not increase (Sekul and Ory, 1977). These characteristics should make it desirable for incorporation in certain types of food.

The physical properties of peanut flour hydrolyzed by bromelain, pepsin, and trypsin have been reported (Beuchat et al., 1975), but pepsin and trypsin are too expensive for commercial applications. Papain was found to be more desirable than bromelain in our earlier comparison (Sekul and Ory, 1977). Functional properties of cottonseed protein improved by treatment with various proteolytic enzymes have been reported (Arzu et al., 1972). The solubility of peanut protein from a defatted flour in water was significantly higher than that of cottonseed proteins. The better solubility is another desirable characteristic in possible commercial applications. Enzymatic hydrolysis and physical properties of soybean proteins (Puski, 1975) and of whey (Kuehler and Stine, 1974) have also been studied. This report describes the effects of partial hydrolysis of proteins in peanut flour by papain, compares the changes in some functional properties to those in unhydrolyzed (control) proteins, and suggests certain food applications for this product based on these properties.

EXPERIMENTAL PROCEDURES

Defatted flour from runner variety peanuts was obtained from the pilot plant of our Engineering and Development Laboratory (SRRC) and was used as received for unhydrolyzed controls. Papain-hydrolyzed peanut protein was obtained by a procedure modified slightly from that described earlier (Sekul and Ory, 1977). A deionized water dispersion of peanut flour (1:10, w/v) was treated with papain (0.5% total volume) at 45 °C for 15 min. The reaction mixture was then cooled, freeze-dried, and used for subsequent studies of the funtional properties. Results were compared with those of the unhydrolyzed flour.

Solubility Determination. Solubility was determined over a pH range of 1–9 by a modification of Hagenmaier's procedure (Hagenmaier, 1972). Each peanut flour sample was added to deionized water to obtain a 5% suspension, and the pH was adjusted with 1.0 N HCl or 1.0 N NaOH while the mixture was stirred constantly for 30 min. The slurry was centrifuged at 15 000*g* for 15 min at 25 °C and filtered through Whatman No. 1 filter paper, and the filtrate was analyzed for nitrogen by the Kjeldahl procedure (AOAC, 1975). The percent soluble nitrogen in each sample was calculated and plotted against the corresponding pH values.

In separate experiments, the suspensions were adjusted to the desired pH (1–9), stirred for 30 min at 25 °C, and filtered through filter paper. The filtrate was discarded and the residue was air-dried to constant weight. A de-

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crease in residual weight (proteins plus a constant weight of nonprotein materials) indicated a gain in protein solubility; these values were also plotted against corresponding pH values.

Preparation of Peanut Flour Dispersion (PFD). For the determination of emulsion capacity, emulsion viscosity, and whipping properties, slurries were prepared as follows:

Flour (8 g) was added to 100 mL of deionized water (8% w/v) in an inverted pint-size Mason jar equipped with blender blades. The dispersion was mixed for 30-s periods at intervals of 30 s for a total of 2 min to attain maximum solubility. The pH of the medium was adjusted by the addition of acid or base, or by addition of acid followed by base in the two-step change of pH conditions described by McWatters et al. (1976). All procedures were carried out at 25 °C.

Emulsion Capacity. A modified procedure (Marshall et al., 1975) was employed to test emulsifying activity. Cottonseed oil containing 0.03% Oil Red-O, biological stain was added to 25-mL aliquots of PFD. Emulsions were formed in an inverted pint-size Mason jar with a 1-cm opening in the bottom center. Oil was delivered from a 50-mL buret with a 6-mm rubber tubing attached to the delivery tip. Addition of oil was controlled manually by a small hemostat. Addition of oil and blending action were interrupted every 10 min to prevent heat generation. Changes in viscosity and visual observation of the changes taking place identified the formation and collapse of emulsions. Emulsifying capacity is expressed as milliliters of oil emulsified by 25 mL of PFD at 25 °C.

Emulsion Viscosity. Emulsions were prepared as described above, with the exception that these prepared for viscosity studies contained 10 mL less oil than the amounts required to reach the breaking points. We added 80 mL of this material to a 100-mL beaker. Viscosity was determined with a Brookfield RVT viscometer and Model L Helipath stand, operated at 5 rpm with a B T-bar spindle and a 1 $^{7}/_{16}$ in. crosslength piece. Viscosity is reported in centipoises (CPS); each value is the average of 14 readings per sample.

Whippability. The preliminary 2-min mixing for preparing PFD was omitted in preparing emulsions for measuring whippability. We added 8 g of flour to 100 mL of deionized water and brought the mixture to the desired pH by adding either acid and/or base while stirring. The suspension was transferred to a small Pyrex mixing bowl and beaten at high speed for 3-min periods at intervals of 3 min for a total of 6 min in a kitchen-type mixer. The foaming material was then transferred to a graduated cylinder and readings were taken at 1, 30, and 60 min. Whippability is expressed as foam capacity, foam stability, percentage change, and percentage volume increase.

Polyacrylamide Gel Electrophoresis. Samples containing 300–400 μg of soluble proteins were analyzed by electrophoresis according to procedures described by Davis (1964). Protein concentration in solution was measured by the method of Lowry et el. (1951).

RESULTS AND DISCUSSION

In studying effects of enzyme treatment on functional properties of proteins, the enzyme must be inactivated at the end of the reaction to prevent the continuation of hydrolysis to smaller peptides and/or free amino acids that can induce bitter flavors. From an economic viewpoint, any treatment to remove or inactivate the enzyme increases the cost of the final product. In this procedure, papain is the protease for modifying the peanut proteins, and inactivation of the enzyme is apparently unnecessary because proteolytic activity reaches equilibrium in 10–15 min (Figure 1). This observation is confirmed by the activity curve and by observation of the gels in which no increase in protein degradation was noticed between 10 and 60 min.

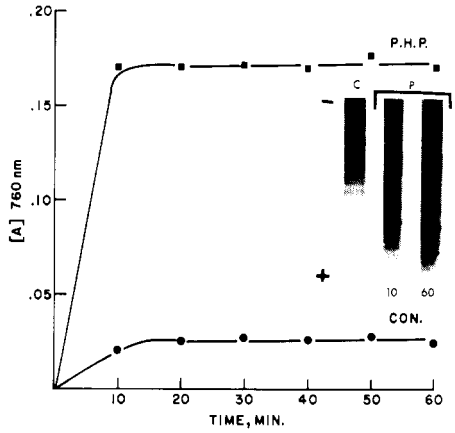

Figure 1. Proteolysis of peanut proteins measured by the Lowry method and by gel electrophoresis. Curves: P.H.P. = partial hydrolysis by 0.5% papain; CON = control (no papain). Temperature, 45 °C. Disc gel electrophoretic patterns, C = control (no papain); P = 10 and 60 min proteolysis with 0.5% papain, respectively.

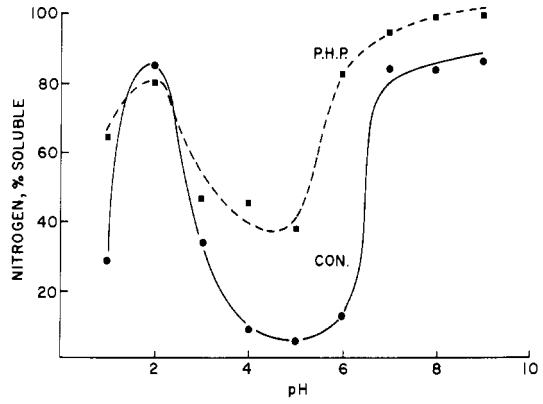

Figure 2. Nitrogen solubility (Kjeldahl) of peanut proteins. P.H.P. = partial hydrolysis by 0.5% papain; CON = control (no papain).

Table I. Emulsifying Properties of Peanut Proteins[a]

Protein	Volume Acid	Volume Base	pH[b]	Emulsion capacity, mL of oil/8 g of flour	Emulsion viscosity, CPS[c]
Control			6.7	118.5	16 000
Hydrolyzed			6.3	79.1	400
Control		1.2	8.2	110.1	25 000
Hydrolyzed		2.2	8.2	76.4	800
Control	4.9	6.1	4-8.2	103.2	32 000
Hydrolyzed	5.8	8.2	4-8.2	93.0	800

[a] Each sample contained 8% peanut flour, w/v. [b] pH adjusted with 1.0 N HCl and/or 1.0 N NaOH. [c] Average of 14 readings/sample.

Protein Solubility. Solubility of peanut proteins (based on nitrogen solubility) over a range of pH 1–9 is presented in Figure 2, and solubility of peanut protein based on actual weight of the flour is shown in Figure 3. In general, enzyme treatment improved solubility at all levels examined except at pH 2 and 8. Proteins of the control flour had minimum solubility between pH 4–5, which coincides with curves reported previously (Ayres et

Table II. Foaming Capacity and Foam Stability of Peanut Protein[a]

Protein	Volume Acid	Volume Base	pH[b]	Foam capacity, 1 min, mL	Foam stability, 60 min, mL	Change, %	Volume increase, %
Control			6.7	99.5	87	12.6	76.3
Hydrolyzed			6.3	139.0	*c*	*c*	125.5
Control		1.2	8.2	97.0	87	10.3	73.3
Hydrolyzed		2.2	8.2	182.0	98	46.2	148.0
Control	4.9	6.1	4-8.2	127.3	108	15.1	83.2
Hydrolyzed	5.8	8.2	4-8.2	367.5	305	17.0	238.4

[a] Each sample contained 8% peanut flour, w/v. [b] pH adjusted with 1.0 N HCl and/or 1.0 N NaOH. [c] Due to breakdown of foam before the 30 min reading interval, neither the milliliters of foam nor the percentage could be determined at the end of 60 min.

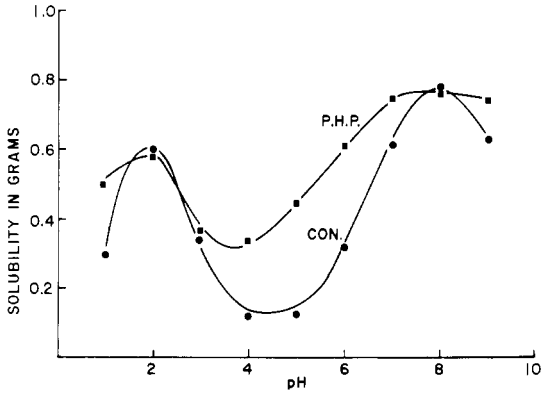

Figure 3. Peanut protein solubility (weight measure). P.H.P. = partial hydrolysis by 0.5% papain; CON = control (no papain).

al., 1974; Conkerton and Ory, 1976). The partial hydrolysis of peanut proteins by papain did not produce bitter peptides, and the final product was almost tasteless (Preston, 1976).

One interesting observation made in these studies of solubility after papain hydrolysis was the similarity of curves obtained by measuring actual solubility (weight of protein in solution) and those obtained by measuring the nitrogen solubility in solution by the Kjeldahl procedure. For beverage applications where high protein solubility is desired (milk-type drinks, acid pH fruit-flavored beverages, dry soup, sauce, or gravy mixes), partially hydrolyzed peanut proteins seem to have an advantage over unhydrolyzed proteins.

Hydrolysis of peanut proteins of aqueous slurries of defatted flour by papain should be of commercial interest. Papain is one of the least expensive FDA-approved vegetable enzymes; there are economic or FDA health-related restrictions on the addition of animal or microbial enzymes to food formulations; and our current and earlier studies have shown that papain is free of peptidase activity (Sekul and Ory, 1977). Industrial sources of microbial proteases, to be commercially attractive, would have to be crude or partially purified preparations that would have some peptidase activity that can yield bitter peptides. Protein solubility profiles over a wide range of pH values are frequently used as an index to select suitable proteins for a desired application, e.g., use in soup or gravy mixes, in beverages, in food or salad dressings where good solubility is desired, in thick or frozen desserts, or in other fluid products.

Viscosity and Emulsifying Capacity. The emulsifying capacity and the viscosity of emulsions of partially hydrolyzed peanut protein at various pHs were generally lower than for the unmodified flour (Table I). Products from papain hydrolysis of peanut protein apparently differ substantially from those described by Beuchat (Beuchat et al., 1975), who used bromelain, pepsin, and trypsin. Under their conditions, trypsin completely destroyed the emulsifying capacity of the modified peanut proteins. Hydrolysis by papain decreased viscosity of the flour dispersion by as much as 73%, but did not completely destroy all emulsifying capacity. Thus, peanut flour with reduced viscosity could be used to evaluate the thickening ability of peanut proteins, a property of practical interest in semithick foods, pudding-like desserts, or hard frozen confections. Partially hydrolyzed protein, however, may be less desirable than unhydrolyzed protein for these applications.

Foaming Capacity and Stability of Foam. The results of the foaming experiments are shown in Table II. Partial hydrolysis of peanut proteins significantly increased both foaming capacity and foam volume at all levels examined, especially where the pH was adjusted in two steps prior to formation of the foam. This two-step process increased foaming capacity threefold. Although stability of foam at acid pH (6.3/6.7) is low (less than 30 min), stability at higher pH (8.2) is longer lasting, suggesting more potential for partially hydrolyzed peanut proteins in nonacidic pH foods. Foaming capacity is an important protein functional property for several food formulations. These results suggest that papain-modified peanut protein flour should find use in products such as frozen desserts, soft mix ice creams, dessert and pie toppings, etc.

In summary, papain hydrolyzates of peanut proteins have increased foaming capacity and improved protein solubility, suggesting that partially hydrolyzed peanut protein have advantages in foods or beverages where these characteristics are desired.

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Received for review August 15, 1977. Accepted March 7, 1978. Names of companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned.

Factors Affecting the Retention and Extraction of Yeast Chromium

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Retention of radioactive chromium chloride by Brewer's yeast, *Saccharomyces carlsbergensis*, was stimulated by glucose and phosphate. Glucose levels to 25%, increased the amount of chromium retained per gram of cells but high glucose also inhibited growth. Addition of phosphate enhanced Cr retention more than 2.5-fold over the unsupplemented controls. The rate and maximal incorporation of radioactively labeled inorganic chromium salts, which yield little biological activity, was similar to that of organic chromium complexes that display in vitro insulin potentiating activity. The release of the labeled Cr from the yeast cells was pH dependent and more than 85% of the labeled Cr could be extracted with dilute ammonium hydroxide. Disruption of cells with teichozyme-Y released a similar amount of labeled chromium but much lower amounts were extracted with ethanol. Insulin potentiating activity was greatest in the ammonia extract and was lower in ethanol and teichozyme extracts. Essentially all of the radioactive chromium incorporated into the yeast was present in the soluble portion of the cell. These data define conditions for the growth of Brewer's yeast for optimal incorporation of labeled Cr and the conditions for extraction of a biologically active γ-labeled product.

Brewer's yeast, the richest known source of an organic form of chromium, was used in the treatment of diabetes more than a century ago (Herepath, 1854). Several decades later, Glaser and Halpern (1929) described an insulin potentiating effect of yeast by demonstrating that incubation of insulin with a yeast extract potentiated the hypoglycemic action of insulin. McCay (1952) recommended a daily dietary supplement of Brewer's yeast for older people and suggested that yeast would lessen the daily insulin requirement. However, it was not until 1959 that the primary active component of yeast, effective in the treatment of diabetes, was postulated to be an organic form of chromium (Mertz and Schwarz, 1959).

Doisy et al. (1976) studied the effect of Brewer's yeast extract, high in insulin potentiating activity, on 14 subjects over the age of 65 with impaired glucose tolerance. After 1–2 months, glucose tolerance tests were normal for approximately half of the subjects. Liu et al. (1977) also reported that supplementation of the diet of 15 hyperglycemic women with a yeast extract resulted in an improved tolerance to glucose. In both studies release of endogenous insulin and fasting levels of serum cholesterol and triglycerides were reduced.

Little is known of the form or forms of Cr utilized by yeast or higher animals and man. We investigated the optimal conditions for the synthesis of a biologically active form of Cr by yeast and methods to extract this active product. Retention of inorganic Cr salts and biologically active synthetic organic Cr complexes by yeast was also studied.

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MATERIALS AND METHODS

Brewer's yeast, *Saccharomyces carlsbergensis* (ATC No. 9080) was grown in 4-L Erlenmeyer flasks containing 2 L of purified synthetic medium (Toepfer and Polansky, 1970) or in medium containing 1% peptone, 0.03% phosphate, 10% glucose, pH 4.5. Chromium chloride, 500 μCi/L, was added prior to sterilization in an autoclave at 15 psi for 15 min. Cells were grown routinely at 26 °C, shaking at 125 rpm, in a New Brunswick Controlled Environment Incubator Shaker. Ten days after innoculation, cells were harvested by centrifuging at 10000g for 15 min and washed with 10 volumes of metal-free deionized water until the counts in the cells were constant.

Cells were extracted with ammonium hydroxide by adding 20 mL of 0.1 N NH_4OH to 14 g of wet cells, followed by shaking for 60 min at 175 rpm at 30 °C. Cells were then centrifuged at 15000g for 15 min and washed with water until the counts remaining in the cells were constant. The extract was then concentrated in vacuo and an aliquot was removed and assayed for insulin potentiating activity as described (Mertz and Roginski, 1971). Cells extracted with ethanol were treated similarly except 50% ethanol was added instead of dilute base and the cells were steamed in an autoclave for 5 min. Yeast cells were also treated with teichozyme-Y which was a generous gift from Dr. Norm Lin, Worthington Biochemical Corpora-