Rapid Enzymatic Method for Partial Hydolysis of Oilseed Proteins for Food Uses

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ABSTRACT AND SUMMARY

Plant enzymes were used to obtain partially hydrolyzed oilseed proteins. Hydrolysis of oilseed proteins under different conditions of type of enzyme, enzyme concentration, temperature, and reaction time was investigated to develop optimum conditions. Pilot plant-produced defatted peanut flour treated with a 0.5% aqueous solution of papain at 45 C yielded a product that had 50% more soluble protein but with no increase in the microbial count of the original flour. Hydrolysis reached equilibrium in 10-15 min. Because of the mild reaction conditions of the method, enzymatic hydrolysis offers major advantages over nonenzymatic methods for modifying plant proteins for incorporation in specialty food products.

INTRODUCTION

The importance of high quality protein in human diets has long been recognized, but animal protein was generally considered the main source. Recently, there has been a growth of interest in the production of vegetable protein (1), and the use of partially hydrolyzed vegetable protein products in specialty foods is also increasing. Oilseed protein products are rapidly becoming important in food protein supplements that have unique functional properties. Modification of vegetable proteins to improve particular functional requirements in food systems is attracting considerable research attention, especially for incorporation into new, convenience-type products such as snacks, sauces, soups, and beverages. Soybeans are now the major source of protein for such purposes, but off-flavor and flatulence problems preclude more extensive usage. Defatted peanut flour, on the other hand, is bland, practically colorless, and has only trace amounts of flatus-causing sugars. Analyses of soluble carbohydrate profiles indicate that peanut flour is lower in raffinose and stachyose than either soybean or cottonseed flour (2-4). A higher level of peanut flour can be used in supplemented food items because it does not have a beany flavor.

Oilseed proteins contain globulins of large molecular weight such as arachin in peanuts and acalin-A (7-S protein) in cottonseed, which tend to precipitate from solution upon long standing and refrigeration. This can be partially prevented by the addition of high concentrations of buffers or salt, or by partial hydrolysis to polypeptides of smaller molecular weight. Hydrolysis can be achieved by acid or base, but this method requires prolonged heating times and the final products must be neutralized, thereby increasing the concentration of salt.

An endogenous protease in peanuts was first reported by Irving and Fontaine (5), who named the enzyme Arachain. Cameron and Mazelis (6) purified Arachain, examined the purified enzyme, and showed it to be a peptidase rather than a protease. Mainguy et al. (7) reported the activity of an endogenous protease in germinating peanut seeds, and van Huystee (8) studied the degradation of arachin by different plant proteases. Both groups studied enzymatic activity on arachin primarily to obtain basic information, not for applications in food processing. Yatsu and Jacks (9) reported acid proteinase activity in ungerminated cottonseed, and Arzu et al. (10) studied the improved functional properties imparted to cottonseed proteins by various proteolytic enzymes. Beuchat et al. (11) reported the physicochemical properties of peanut proteins after hydrolysis with plant and animal proteases but did not include all of those necessary for food applications (e.g. optimum temperature, best commercial enzyme, enzyme concentration, final microbial count in products).

In 1973, a method for partial hydrolysis of peanut proteins by activation of an endogenous protease in laboratory-prepared peanut flour was reported by Moseley and Ory (12,13). A reaction time of 9-10 hr was required to hydrolyze the proteins. In this long time, the bacterial count of the starting material could increase.

Because of the desirability of reducing energy consumption, an alternate method, using inexpensive, FDA-acceptable (Food and Drug Administration) plant enzymes, was sought that would yield a partially hydrolyzed protein product in less time and with no increase in bacterial count. This report describes optimum reaction conditions for partial hydrolysis of pilot plant-produced peanut flour (to simulate commercial peanut flours) by three commercial proteases, gives the bacterial contents of the product before and after hydrolysis, and suggests potential uses to supplement foods and beverages. For comparison, a limited study of these proteases on liquid cyclone process (LCP) cottonseed flour is included.

EXPERIMENTAL PROCEDURES

Materials

Pilot plant-prepared flour from 'Runner' variety peanuts was obtained from our Engineering and Development Laboratory, Southern Regional Research Center (SRRC). The peanuts were commercially blanched, flaked, and extracted continuously with n-hexane at 60 C, desolventized at 82 C under nitrogen, and subsequently ground to a flour. The flour was free of aflatoxin (as would be required for all protein products destined for food use). Cottonseed flour used in the experiments was also produced at SRRC. Papain (African papaya) was purchased from CalBiochem, San Diego, CA, and bromelain and ficin from Nutritional Biochemical Corp., Cleveland, OH. Reagents for gel electrophoresis of the proteins were obtained from Canalco Corp., Rockville, MD.

Digestion Procedure

In a typical experiment, a distilled water dispersion of flour (1:10 w/v) was treated with the required amount of enzyme and rapidly transferred to a magnetically-stirred reaction vessel tempered by running water from a large, constant temperature bath. Samples were withdrawn at zero time and at 10 min intervals throughout the experiment for 1 hr. Enzyme concentrations ranged from 0.01% to 0.5% and the temperatures compared were 45, 55, and 65 C. The pH of the reaction was not controlled but was allowed to follow its own course to more closely approximate commercial conditions. The change varied from pH 6.4 before incubation to 6.1 at the end of 1 hr.

Enzyme Assays

Activity was measured by a modified Anson method

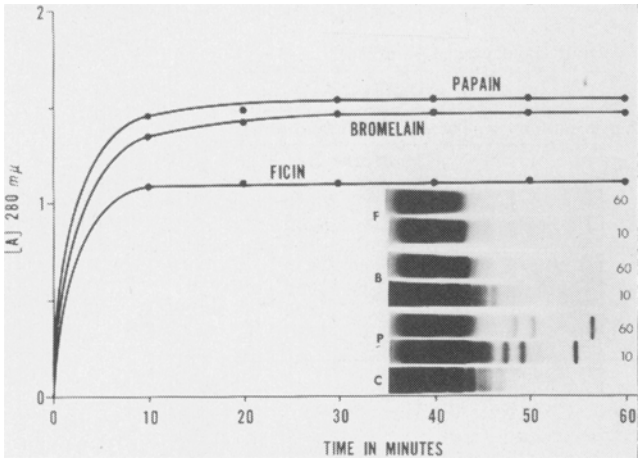

FIG. 1. Rates of activities of 0.5% each papain, bromelain, and ficin on peanut proteins at 65 C. Migration of gels from left to right. C = untreated control; p = papain; b = bromelain; f = ficin. Figures to right of gels are incubation time in minutes.

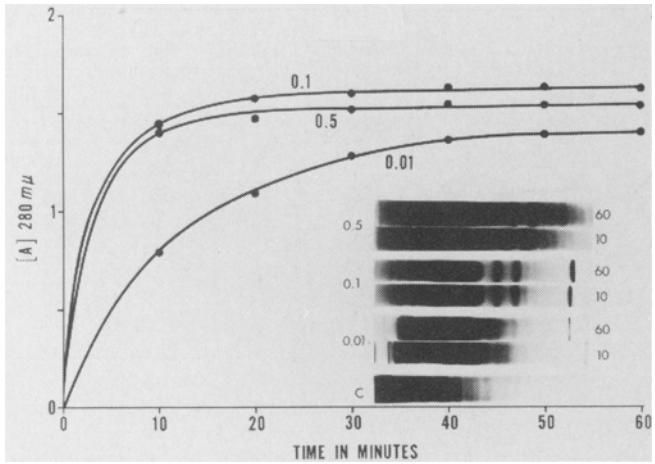

FIG. 3. Effect of different concentrations of papain on hydrolysis of peanut proteins at 45 C. Migration of gels from left to right. C = untreated control; figures to the left are percentages of papain; figures to right are incubation time in minutes.

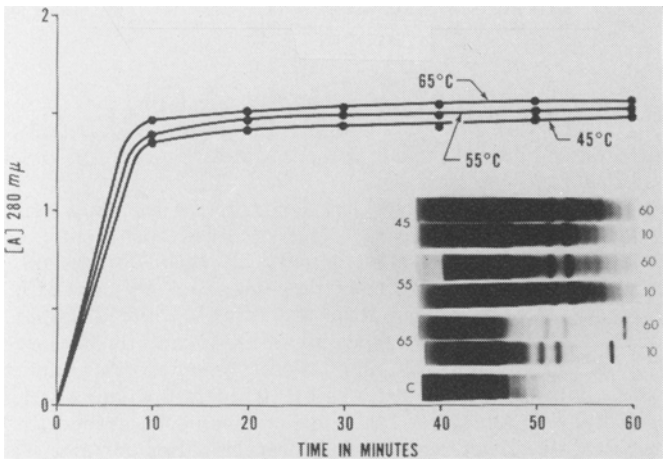

FIG. 2. Effect of different temperatures on hydrolysis of penaut protein by 0.5% papain. Migration of gels from left to right. C = untreated control. Figures to left are temperatures; to the right incubation time in minutes.

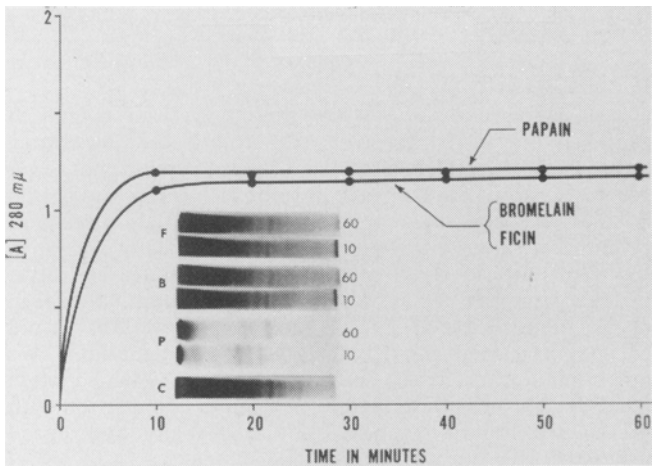

FIG. 4. Rates of activities of 0.5% each papain, bromelain, and ficin on cottonseed proteins at 45 C. Migration of gels from left to right. C = untreated control; P = papain; B = bromelain; F = ficin. Figures to right of gels are incubation time in minutes.

(14). The hydrolyzed flour (1 ml) was treated with 5.0 ml of 0.5M trichloro acetic acid (TCA) and allowed to stand at room temperature for 30 min, then filtered through filter paper. Differences in optical density measured at 280 nm were indicative of enzyme activity. Samples withdrawn for disc electrophoretic analyses were cooled to lower the reaction temperature and centrifuged at 27,000 x g for 15 min at 4 C to obtain the soluble proteins. Samples of 0.01 ml, containing ca. 200 μg protein, were applied to the gels.

Disc Electrophoresis

Analyses were performed according to Davis (15). Gels at pH 9.5 contained 7% acrylamide and 0.18% crosslinking agent. Tris-glycine buffer at pH 8.3 was used in the reservoir. Runs were carried out in a Buchler apparatus equipped for 12 columns (7.5 mm long, 5.0 mm ID) for ca. 1 hr at a constant current of 3 mA/column. The running dye, bromophenol blue, was allowed to migrate to within 6 mm of the end of the column. The gels were stained overnight in a solution of 0.5% amido black in 7% acetic acid, then destained for 24-36 hr in 7% acetic acid in a circulating flow, destaining reservoir.

Solubility Determinations

To ascertain the differences in solubility before and after hydrolysis, a 10% flour sample was dispersed in water, stirred at room temperature for 1 hr, and centrifuged. The clear filtrate was discarded and the dried residue weighed and compared to a sample treated with 0.5% papain at 45 C for 60 min. A decrease in residual weight indicated gain in solubility of protein.

Microbial Plate Count Determinations

Hydrolyzed flours were collected in both liquid and freeze-dried forms. These, plus the unhydrolyzed flour, were sent to a commercial analytical laboratory for microbial analysis.

RESULTS AND DISCUSSION

The method that utilizes endogenous proteases is not economically desirable for commercial applications because of the long reaction times and the large amount of energy needed to effect partial hydrolysis. In developing a new method, experiments were first designed to study relative rates of activity on peanut flour of three commercially available plant proteases (papain, bromelain, and ficin). The rates of activities of these enzymes shown in Figure 1 indicate that maximum hydrolysis (shown by the curves on the left) was obtained with papain. The electrophoretic gels illustrate the actual extent of hydrolysis by each enzyme.

The importance of including gel electrophoresis to show

TABLE I

Microbial Counts of Peanut Flour and Partially Hydrolyzed Samples

Treatment		Liquid samples		Freeze dried samples	
Time min	Temp (C)	Total	Thermophiles	Total	Thermophiles
		(Counts per gram)		(Counts per gram)	
Control	–	600	100	670	180
30	45	830	120	760	240
30	55	880	460	570	140
30	65	980	250	1100	430
60	45	780	250	770	320
60	55	2900	330	660	240
60	65	690	130	740	240

TABLE II

Relative Solubility of Peanut Flour before and after Hydrolysis with Papain

Treatment	Original weight	Weight of residue	Weight in solution	Relative solubility[a]
Control (no hydrolysis)	5.00	2.87	2.13	1.00
Papain, 0.5%, 45 C, 1 hr	5.00	1.87	3.13	1.47
Papain, 0.5%, 65 C, 1 hr	5.00	1.75	3.25	1.52

[a]Compared to the unhydrolyzed control, taken as unity.

actual degradation of larger proteins was discovered in earlier experiments, when it was found that measuring hydrolysis of oilseed proteins solely by colorimetric or spectrophotometric methods did not give a true indication of the extent of hydrolysis. Certain materials present in peanut flour apparently contributed to the color and/or to the electrometric titration value without actually hydrolyzing the globulins. Some titration curves showed increases in optical density (suggesting proteolysis) in solutions devoid of enzymes. Measuring hydrolysis by these methods was not completely reliable; better results were obtained when reaction mixtures were read at 280 nm in conjunction with gel electrophoretic analyses of the partially hydrolyzed products.

The optical density measurements shown in Figure 2 indicate that 65 C produced the best results. This observation, however, does not correlate with results obtained by gel electrophoresis. Degradation of larger proteins to smaller polypeptides shows that a more extensive breakdown product results at 45 C. This temperature would be more desirable for a commercial operation because the lower heat needed to effect maximum hydrolysis would lower production costs.

Figure 3 compares three concentrations of papain on the hydrolysis of peanut protein. Comparison of gel electrophoresis protein profiles with optical density measurements favors the 0.5% concentration of the three compared to obtain sufficient partial hydrolysis in 10 min, for potential application in a commercial process.

Conditions of temperature, enzyme concentration, and reaction time necessary to produce a desirable partially hydrolyzed product are also illustrated in Figure 3. Observation of the gel profiles for the control flour (c) and the 0.5% treated flour (top 2 gels) shows that essentially the same patterns are obtained at the end of 10 min as were obtained after 1 hr, indicating that partial hydrolysis is achieved at relatively low temperatures and short reaction times. Although the two curves for 0.1% and 0.5% papain suggest that both concentrations yield the same hydrolysis, as measured by optical density of the solutions, the electrophoretic gels for these reaction products gives a more accurate indication of the true degradation of larger globulins into smaller polypeptides. Based upon this comparison, it is obvious that 0.5% enzyme produces better hydrolysis than does 0.1%. Hydrolysis reaches equilibrium in 10-15 min at 45 C and 0.5% papain, suggesting that these conditions should be adaptable for producing partially hydrolyzed peanut protein in a continuous process for commercial food applications.

Other oilseed proteins being considered for food uses were tested under the same set of conditons: Figure 4 shows the relative rates of activity of papain, bromelain, and ficin on a cottonseed flour prepared by the LCP process. Hydrolysis of cottonseed flour is quite different from that obtained with peanut flour, apparently because of the difference in solubility of cottonseed proteins under the conditions of the test, i.e., pH, temperature, and water. A totally different set of conditions would be needed for greater hydrolysis of cottonseed proteins. For commercial use, however, the greater solubility of peanut proteins in water, without added buffers or salts, would be an advantage.

Table I lists the microbial counts of the peanut flours before and after partial hydrolysis at different temperatures. At 45 C, under the conditions employed, there was very little increase in the microbial content of the partially hydrolyzed material. A partially hydrolyzed peanut protein product formed under the described conditions, therefore, would probably be acceptable by the FDA in most food products.

Table II shows the increased solubility of peanut flour proteins after hydrolysis for 1 hr by 0.5% papain at 45 C and 65 C. Solubility at both temperatures increased by ca. 50%, again confirming 45 C as the most economically feasible temperature.

The data obtained in these studies indicates that treatment of proteins in peanut flour with proteolytic enzymes, especially papain, produces changes in the electrophoretic patterns and improves solubility of the proteins. Several microbial protesases approved for food applications have been advertised in recent issues of food industry periodicals, but none of them were tried in these tests. Although microbial proteases might catalyze proteolysis faster than the plant enzymes we studied, they are single cell organisms and would also contain peptidases that could rapidly hydrolyze the polypeptides to free amino acids. This increase in free amino acids could affect the flavor of the final product and limit its applications. Papain, because of its properties and the mild reaction conditions it requires, offers major advantages over nonenzymatic methods in

modifying plant proteins for food uses.

ACKNOWLEDGMENTS

Technical assistance was provided by E.D. Blanchet; drawing of figures was by J.H. Conkerton; photography was done by J.J. Bergquist.

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[Received July 28, 1976]