Effect of Limited Proteolysis on the Functional Properties of Cottonseed Flour

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Cottonseed flour was treated with a protease at different ratios of the enzyme to the protein, and the effect of proteolysis on nitrogen solubility in water and 5% NaCl solution, water absorption, oil absorption and emulsifying capacity, foam capacity, and foam stability and bulk density was determined. Nitrogen solubility in water increased nearly 4-fold. However, nitrogen solubility in 5% NaCl solution was not altered. Water absorption capacity decreased by about 30% but oil absorption capacity was not affected. Emulsifying capacity increased by about 90% and foam capacity by about 40%. However, foam stability did not improve. Bulk density also increased due to proteolysis.

In the preceding paper (Rahma and Narasinga Rao, 1983), we have reported the results of a study of the effect of acetylation and succinylation of cottonseed protein on the gossypol content and functional properties of the flour. In this paper, we report the effect of limited proteolysis on the gossypol content and functional properties. To the best of our knowledge, no work on the effect of proteolysis on the gossypol content of cottonseed flour has been reported so far. The effect of proteolysis on the functional properties of soybean protein has been reported by Zakaria and McFeeters (1978) and Gunther and Staley (1979) and of sunflower protein has been reported by Kabirullah and Wills (1981).

MATERIALS AND METHODS

Cottonseed Flour. The source of cottonseed and the method of preparation of the flour are as described before (Rahma and Narasinga Rao, 1983).

Protease. Protease from *Aspergillus oryzae* was obtained from Amano Pharmaceuticals Co. Japan. According to the suppliers the enzyme has an activity of 10 000 units/g of enzyme. The unit of activity is defined as the quantity of the enzyme needed to produce an amino acid equivalent to 100 μg of tyrosine in 1 mL of filterate per reaction for 60 min at 37 °C.

Preparation of Enzyme-Modified Flour. The pH of a 25% aqueous dispersion of cottonseed flour was adjusted to 7.0 by the addition of 2 M NaOH. Enzyme in the solid form was added at different levels and the mixture left at 50 °C for 3 h with occasional shaking. At the end of the interval, the temperature was raised to 70 °C and left for 10 min to inactivate the enzyme. The material was then freeze-dried and powdered to pass through a 60-mesh size sieve. The protein dispersion which was kept at 50 °C for 3 h without added enzyme and subsequently freeze-dried served as the control.

Estimation of the Degree of Hydrolysis. The cottonseed flour was treated with varying amounts of the enzyme for 3 h at 50 °C as described above. An aliquot (3 mL) was mixed with an equal volume of 10% Cl_3AcOH solution and left for 15 min. The suspension was centrifuged for 20 min at 4000 rpm. The absorbance of the supernatant, after appropriate dilution, was read at 280 nm in a Shimadzu spectrophotometer. The blank was the cottonseed flour suspension kept at 50 °C for 3 h and mixed with 10% Cl_3AcOH solution.

Methods. The methods for determining total and free gossypol, nitrogen solubility in water and 5% NaCl, water and oil absorption capacity, emulsifying capacity, foam capacity and stability, and bulk density were the same as described in the preceding paper (Rahma and Narasinga Rao, 1983).

Table I. Total, Free, and Bound Gossypol Content of Enzyme-Hydrolyzed Cottonseed Flour[a]

sample	free gossypol, %	bound gossypol, %
cottonseed flour	1.52	0.20
cottonseed flour heated in solution at 50 °C for 3 h	0.31	1.41
cottonseed flour treated with enzyme at 30 mg/g of protein	0.69	1.03
cottonseed flour treated with enzyme at 70 mg/g of protein	0.89	0.84
cottonseed flour treated with enzyme at 100 mg/g of protein	0.91	0.82

[a] The total gossypol content of the flour = 1.72%.

RESULTS AND DISCUSSION

The extent of hydrolysis as a function of enzyme concentration is shown in Figure 1. The curve is a typical enzyme hydrolysis curve with an initial linear region followed by a plateau region. The plateau region was reached at an enzyme concentration of ~40 mg/g of protein.

The free and bound gossypol content of the flour is given in Table I. The flour had a total gossypol content of 1.72%, free gossypol of 1.52%, and bound of 0.20%. Heating the flour dispersion at 50 °C for 3 h decreased the free gossypol content and increased the bound gossypol content of the flour. This is consistent with the observation of Lyman et al. (1959) that at elevated temperatures gossypol binds to cottonseed proteins by interaction with the ϵ-amino groups of lysine residues. Proteolysis did not reduce the bound gossypol content to safe levels, although there was nearly a 40% reduction.

The nitrogen solubility in water increased with an increase in enzyme concentration. Hydrolysis at a 100 mg of enzyme/g of protein level increased the nitrogen solubility in water almost 4-fold (Figure 2). However, such a marked effect was not observed in 5% NaCl solution. This could be due to the fact that the solubility of the control itself was fairly high. It was of interest that heating the cottonseed flour dispersion in water for 3 h at 50 °C reduced the nitrogen solubility from 31 to 18%. A similar effect of heat on the solubility of soybean proteins has been reported (Puski, 1975). Kabirullah and Wills (1981) have

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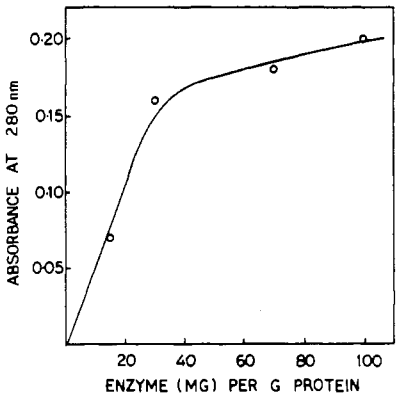

Figure 1. Extent of hydrolysis of cottonseed proteins as a function of enzyme concentration.

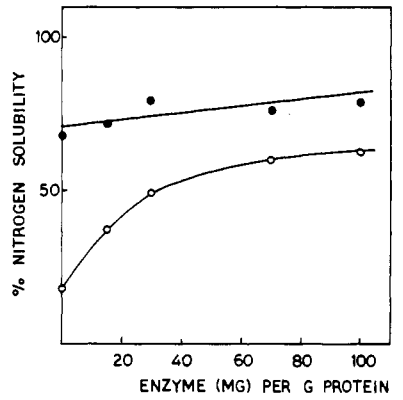

Figure 2. Nitrogen solubility of cottonseed flour as a function of enzyme concentration: (○) in water; (●) in 5% NaCl solution.

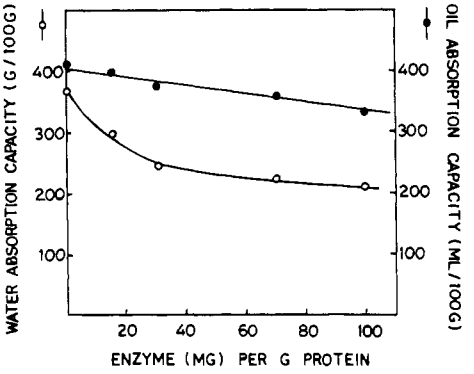

Figure 3. Water absorption capacity and oil absorption capacity of cottonseed flour as a function of enzyme concentration: (○) water absorption; (●) oil absorption.

reported that partial hydrolysis of sunflower proteins with proteases increased the nitrogen solubility.

The water absorption capacity of cottonseed flour decreased after treatment with protease. After an initial decrease of about 30% there was no further decrease at higher levels of enzyme (Figure 3). Oil absorption capacity showed only a slight decrease (Figure 3). It is not easy to understand the decrease in water absorption capacity. Hydrolysis may be expected to increase the number of hydrophilic groups. However, in spite of an increase in number, conformational features may be responsible for the decrease in water absorption capacity. Kabirullah and Wills (1981) have reported that the partially hydrolyzed sunflower protein isolate had a higher absorption of moisture.

The emulsifying capacity (EC) of cottonseed flour increased after hydrolysis (Figure 4). There was a 2-fold increase in EC when the flour was treated with the enzyme

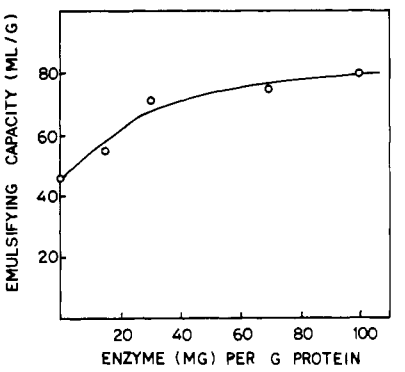

Figure 4. Emulsifying capacity of cottonseed flour as a function of enzyme concentration.

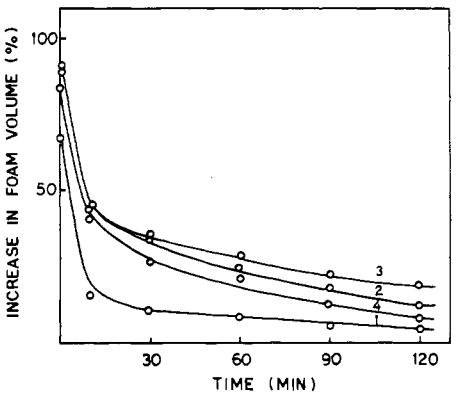

Figure 5. Foam capacity and stability of cottonseed flour as a function of enzyme concentration: (1) untreated cottonseed flour; (2) 30 mg of enzyme; (3) 70 mg of enzyme; (4) 100 mg of enzyme.

Table II. Bulk Density of Protease-Treated Cottonseed Flour

sample	bulk density, g/mL
cottonseed flour	0.290
cottonseed flour heated in solution at 50 °C for 3 h	0.333
cottonseed flour treated with enzyme at 30 mg/g of protein	0.410
cottonseed flour treated with enzyme at 70 mg/g of protein	0.440
cottonseed flour treated with enzyme at 100 mg/g of protein	0.444

at a 100 mg of enzyme/g of protein level. Zakaria and McFeeters (1978) have reported that limited hydrolysis of soy protein with pepsin increased the EC. However, Kabirullah and Wills (1981) have reported that partial hydrolysis of sunflower proteins with proteases decreased the EC.

The foam capacity of cottonseed flour increased on proteolysis. At enzyme levels of 30 and 70 mg the foam volume increased by nearly 80% (Figure 5). However, at a 100-mg level, it decreased slightly; but even then it was higher than that of the untreated sample. Foam stability of the untreated flour was not high; it collapsed within about 10 min. Treatment with the protease did not improve the stability.

Puski (1975) has reported that the foam capacity of soy protein slightly increased due to enzymatic treatment but the foam stability was very low. Kabirullah and Wills (1981) have also reported a similar observation with sunflower proteins.

The bulk density of cottonseed flour increased due to the action of the protease (Table II). It increased from

a value of 0.33 g/mL to a value of 0.44 g/mL at an enzyme level of 100 mg.

This study indicated that limited proteolysis did not reduce the free or bound gossypol content of cottonseed flour to safe levels. However, it improved some functional properties such as nitrogen solubility in water, emulsifying capacity, and foam capacity.

Registry No. Protease, 9001-92-7; gossypol, 303-45-7.

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Rapeseed Protein Isolates: Effect of Processing on Yield and Composition of Protein

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Countercurrent extractions of defatted rapeseed meal, alternately at pH 9.5 and pH 12.0 in the successive stages, ensured highest dissolution of meal protein (~92%) and phytic acid (~70%). The resulting extract provided, upon consecutive precipitations at pH 6.0 and 3.6, two protein isolates containing ~60% and ~12%, respectively, of meal protein and distinctly different levels of phytic acid (9.8% and 4.3%, respectively). As much as ~70% of phytic acid, but only ~14% of protein, contained in the meal could be removed by extraction at pH 4.0. The resulting meal residue provided, upon extraction at pH 11.0 and subsequent precipitation of the protein from the extract at pH 4.7, a protein isolate containing only 0.2% phytic acid. The color of the protein isolates could be considerably improved by the use of sodium bisulfite in the solvent for protein extraction. Rapeseed protein isolates containing about 10% phytic acid exhibited a portein efficiency ratio of 2.2, as compared to a value of 2.5 for casein.

Seeds of rape, *Brassica napus*, and related cruciferous oilseed crops, such as *Brassica campestris*, *Brassica juncea*, *Brassica carinata*, *Brassica nigra*, and *Sinapis alba*, are a rich source of edible protein (Ohlson and Anjou, 1979) having favorable nutritional properties (El Nockrashy et al., 1975; Mukherjee et al., 1979; Jones, 1979). Technological processes that have become known for the isolation of such seed proteins are based essentially on dissolution of the protein from the defatted seed meal and subsequent recovery of the protein isolate by precipitation (Sosulski and Bakal, 1969; Owen et al., 1971; Girault, 1973; Kodagoda et al., 1973; Thompson et al., 1976; Gillberg and Törnell, 1976a; El Nockrashy et al., 1977). Current interest in large-scale production of these proteins for use in food prompted us to investigate the effect of various processing parameters on the yield and composition of rapeseed protein isolates. The results reported here form the basis for layout, design, and operation of a pilot plant that has recently been installed for the production of protein isolates from cruciferous oilseeds.

EXPERIMENTAL SECTION

Materials. All Chemicals and reagents used were of analytical grade. Technical-grade hexane, purified by distillation, was used for extraction.

Seeds of *B. napus*, cultivar Erglu, harvested in 1979, were used throughout. The seeds were crushed, the lipids were extracted with hexane in Soxhlet apparatus, and hexane was removed from the defatted meal at 40–50 °C in an oven with forced air circulation. Finally, the meal was finely ground to pass a 0.2-mm screen. The rapeseed meal thus obtained contained 41.0% protein; nonprotein nitrogen constituted 10.0% of the total meal nitrogen.

Analytical Methods. Nitrogen was determined by digestion of the samples according to a semimicro Kjeldahl procedure (Association of Official Agricultural Chemists, 1970) followed by measurement of ammonium ions in the digest using an Orion Research Microprocessor Ion Analyzer/901. Protein content was calculated as percent nitrogen × 6.25. Nonprotein nitrogen in solid samples was determined by extraction of the samples with 10% trichloroacetic acid and measurement of nitrogen in the extract. The liquid samples were treated with trichloroacetic acid at a concentration of 10% in the final mixture, protein precipitated was separated by centrifugation, and nonprotein nitrogen in the extract was determined.

Phytic acid was extracted from the samples according to Wheeler and Ferrel (1971); however, 0.4 M perchloric acid was used as solvent. Phytic acid was recovered as sodium phytate, via ferric phytate, and phosphorus content of the sodium phytate solution was determined with molybdenum blue reagent (Bartlett, 1959). On the basis of phosphorus content, phytic acid was calculated as *myo*-inositol hexaphosphate.

The color of the protein isolates was measured as follows. The sample, 25 mg, was dissolved in a solution consisting

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