Enzymatic Modification of Soy Protein Concentrates by Fungal and Bacterial Proteases

L.S. Bernardi Don, A.M.R. Pilosof[1,*] and G.B. Bartholomai[1]

Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, 1428-Buenos Aires, Argentina

Solubility, foaming capacity and foam stability of denatured soy protein concentrate obtained from toasted flour were improved by proteolysis with fungal or bacterial proteases. Emulsifying capacity was unchanged, but emulsion stability decreased; bacterial protease highly improved oil absorption. Also, the bacterial protease was able to solubilize more protein and gave products which foamed more than those obtained with the fungal enzyme. However, the stabilizing properties of the bacterial modified soy protein concentrate at the air/water or oil/water interface were inferior. By limited hydrolysis up to degree of hydrolysis 10% most functional properties were improved without greatly reducing emulsion stability and water absorption.

KEY WORDS: Hydrolysis, proteases, soy protein.

The expanded use of enzymes to modify protein functional properties has great promise for the food industry. Major advantages of using proteases compared to other agents include their specificity, their effectiveness at low concentrations and, under mild conditions, their general safety, thus eliminating the necessity for removing them from finished products (1). In addition, enzymatic hydrolysis of proteins does not reduce their nutritional value (2).

Proteolytic enzymes commonly used are pepsin, papain, ficin, trypsin, bacterial and fungal proteases. Differences in the modification behavior of different proteases would depend on their specific hydrolytic action on the substrate.

Many studies have demonstrated that limited hydrolysis of soya protein isolates improve some functional properties (3-6). The use of soya substrates other than acid-precipitated isolate has been reported in few cases. Bobalik and Taranto (7) improved whipping expansion of defatted soya flour by limited proteolysis. Adler-Nissen (8) found that by hydrolyzing soya concentrate to 3-5% degree of hydrolysis with alcalase, a protein with high foam expansion and stability could be obtained.

Soya protein concentrates are often denatured by extracting and/or processing conditions; therefore they have limited functional properties. The objective of the present work was to investigate the enzymatic modification of functional properties of denatured soy protein concentrates as related to the type of protease and the degree of hydrolysis.

EXPERIMENTAL PROCEDURES

Materials. Soy protein concentrate (SPC) was prepared by water washing a commercial defatted and toasted soy flour obtained from Genaro Garcia S.A. (Argentina). Approximate analysis of soy flour was: moisture, 9.0%; protein, (N × 6.25), 47%; and lipid, 1.0%. The washed soy flour was neutralized and freeze-dried at room temperature. The protein content (N × 6.25) was 62.7% (d.b.).

Two kinds of proteases were obtained from Biocon Cono Sur (Sao Paulo, Brazil)—a neutral fungal protease from *Aspergillus oryzae* and a *Bacillus subtilis* protease. Their activities were 400,000 Hu/g and 440,000 Du/g, respectively. One Hu is the amount of enzyme which will produce 1 g of tyrosine in 1 min at pH 5 and 50°C while acting on hemoglobin substrate. One Du is the amount of enzyme which will produce 1 g of tyrosine in 1 min at pH 9 and 50°C while acting on casein substrate. The pH optima for the proteases on these substrates were 5-7 for fungal protease and 9 for the bacterial protease.

Preparation of enzyme modified soya concentrates. The hydrolysis of SPC was carried out in a batch laboratory reactor at 50°C. The protein concentration was 6% and the pH was 6.75. In order to obtain enzyme treated samples of different degrees of hydrolysis, SPC was hydrolyzed for 1 hr with different enzyme to substrate ratios (E/S). E/S ranged between 0.1/100 and 1/100 for the fungal protease treatments and between 0.07/100 and 0.85/100 for the *Bacillus* protease treatments. Any residual enzyme activity was destroyed by heating the samples at 75°C for 10 min. The original pH was restored by adding dilute NaOH. Enzyme modified samples were freeze-dried at room temperature.

Degree of hydrolysis (DH) Trichloroacetic acid (TCA) soluble protein was measured according to Bernardi Don *et al.* (Bernardi: Don, L., A.M.R. Pilosof, and G.B. Bartholomai, unpublished data.). DH was calculated as the ratio of TCA soluble protein to total protein × 100. Relative percent error in DH was 1.5%.

Water absorption capacity (WAC) and oil absorption capacity (OAC). Water and oil absorption values were determined by the procedures of Kanterewicz *et al.* (9). The results were expressed as mL of water absorbed or mL of oil absorbed per g protein concentrate. Relative percent errors in WAC and OAC were 1.5% and 2% respectively.

Nitrogen solubility index (NSI). NSI was determined according to AOCS standard methods (10). Soluble protein was determined by using a Kjeltec Auto 1030 Analyzer (Tecator, Sweden). Relative percent error in NSI was 1%.

Emulsifying properties. For determining emulsifying properties, all the samples were dispersed at 3% (w/w) in distilled water. Emulsifying capacity (EC) was determined according to Kanterewicz *et al.* (9) and was defined as mL oil/100 mL emulsion before phase inversion occurred. Emulsion stability (ES) was determined after 24 hr according to Elizalde *et al.* (11), and a stability rating (SR) was calculated as:

[1]Members of Consejo Nacional de Investigaciones Cientificas y Tecnicas de la Republica Argentina.

*To whom correspondence should be addressed.

$$SR = \frac{100 - M(24)}{100 - M(0)} \times 100$$

where M(24) refers to the percent moisture of the bottom of emulsions after 24 hr at 45°C and M(0) to the percent moisture of freshly prepared emulsions. The relative percent error was 2%.

Foaming properties. Foaming capacity (FC) was determined in duplicate by placing 30 mL of 3% (w/w) dispersions in a graduated cylinder and stirring it for 3 min at 6,000 rpm in a Griffin and George stirrer. The percent increase in volume was calculated as:

$$\% \text{ Volume increase} = \frac{\text{Final volume} - \text{Initial volume}}{\text{Initial volume}} \times 100$$

Relative percentual error was 2%.

Foam stability (FS) was determined in duplicate by measuring the liquid drained from 10 mL foam contained in a graduated cylinder as a function of time. FS was expressed as the drainage half time defined as the time (min) required to drain 50% of the maximum amount of drained liquid. Relative percent error was 1.7%.

RESULTS AND DISCUSSION

Protein solubility. The data in Figure 1 show that the solubility at neutral pH of SPC was greately increased by proteolysis. There were similar effects by both fungal and bacterial proteases; the greatest changes in solubility occurred up to DH 10%; however, the bacterial protease was able to solubilize more protein than the fungal protease at the same DH values. This effect was more remarkable at the highest DH values. Increased protein solubility after enzymatic hydrolysis is common; the solubilities or extractabilities of soybean protein (3,4), heat-treated coconut meal (12), rapeseed concentrate (13), and fish protein concentrate (14,15) are substantially increased by proteolysis.

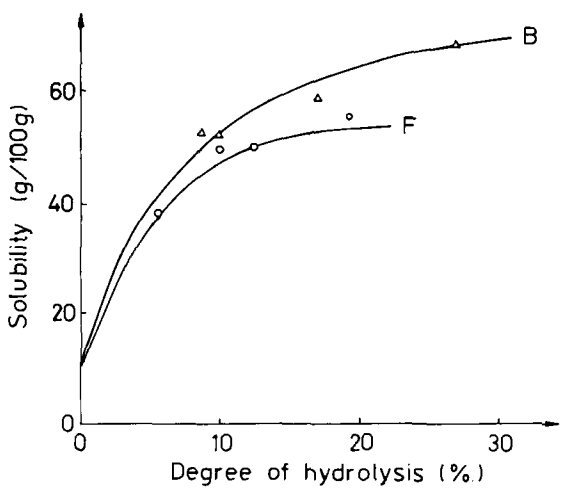

FIG. 1. Solubilities at neutral pH of soy protein concentrates hydrolyzed to different degrees with fungal (F) or bacterial (B) protease.

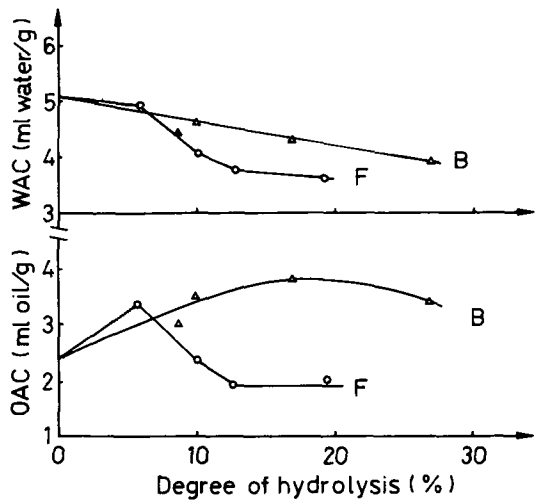

FIG. 2. Water (WAC) and oil (OAC) absorption capacities of soy protein concentrates hydrolyzed to different degrees with fungal (F) or bacterial (B) protease.

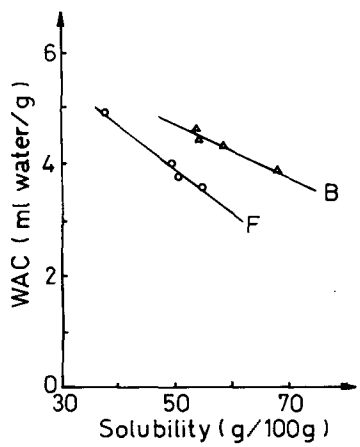

FIG. 3. Correlations between water absorption capacity (WAC) and solubility of soy protein concentrates hydrolyzed to different degrees with fungal (F) or bacterial (B) protease.

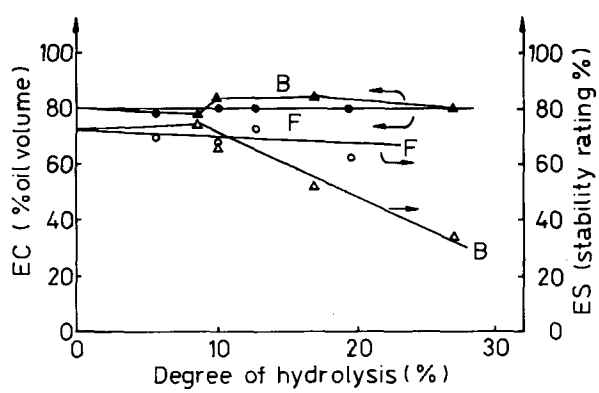

FIG. 4. Emulsifying capacities (EC) and emulsion stabilities (ES) of soy protein concentrates hydrolyzed to different degrees with fungal (F) or bacterial (B) protease.

Water and oil absorption. Figure 2 shows that WAC was decreased by proteolysis; the decrease in WAC was greater when the fungal protease was used. As shown in Figure 3, NSI and WAC of SPC hydrolyzed samples were negatively correlated. Correlation equations were:

$$WAC = 6.66 - 0.0403\ NSI \qquad (R = -0.983;\ P < 0.02)$$

for fungal protease and

$$WAC = 7.95 - 0.0809\ NSI \qquad (R = -0.960;\ P < 0.05)$$

for bacterial protease, respectively.

The hydrolytic action of proteases causes disruption of the protein network, which is responsible for the imbibition and holding of water and, hence, would decrease it. Concomitantly, the elimination of the protein crosslinkages which prevent solubilization and the reduction of the molecular weight would allow the protein to solubilize more easily.

The oil absorption capacity (OAC) of SPC was greatly increased by proteolysis with the bacterial protease (Fig. 2). However, when fungal protease was used, OAC increased up to DH 5% and then decreased.

Emulsifying properties. Figure 4 shows that the emulsifying capacity of SPC was not changed by proteolysis with fungal protease. Bacterial protease increased EC from 80 to 82% oil volume for DH, 10 and 17%. The stabilities of emulsions formulated with the fungal-SPC hydrolyzed samples slightly decreased as DH increased. When the hydrolysis was performed by the bacterial protease, the stabilities of emulsions were modified little up to DH 10%; increasing DH greatly decreased the stability of emulsions.

In addition, different mechanisms of destabilization were observed. During 24 hr aging of emulsions formulated with the fungal-SPC hydrolyzed samples, practically no oil layer was apparent. However, oil separation was observed for all emulsions formulated with the bacterial-SPC modified samples and it increased as DH increased. Thus, creaming was the prevailing mechanism of destabilization for the fungal-SPC modified samples while coalescence occurred for the bacterial-SPC ones.

Foaming properties. Figure 5 shows the foaming capacities and foam stabilities of the fungal and bacterial modified SPC. The FC curves show a similar shape maximum FC was achieved at DH 10-12%. However, the bacterial-SPC hydrolyzed samples showed better FC.

Increasing DH of fungal-SPC hydrolyzed samples increased foam stability (FS). Maximum FS increase occurred at DH 10% for the bacterial ones. Higher DH values decreased FS.

Different mechanisms of foam destabilization were observed. For the fungal-SPC hydrolyzed samples, drainage of liquid from the foam occurred without visible modification of bubble size. The foams from the bacterial-SPC samples showed a rapid collapse of small bubbles and accumulation of very large bubbles as the foam drained. This behavior suggests that while bacterial-SPC modified samples are more appropiate than the fungal ones for foam formation, they do not successfully associate at the surface for matrix formation, resulting in the rapid collapse of the lamellae.

The fraction of SPC made soluble by treatment with both proteases exhibited improved foam expansion and

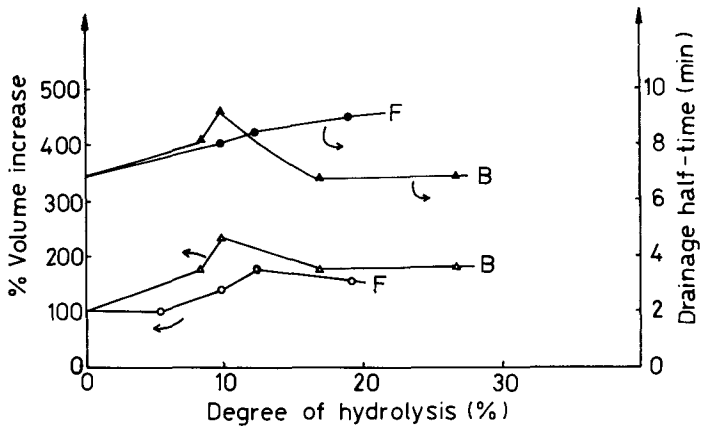

FIG. 5. Foaming capacities and foam stabilities of soy protein concentrates hydrolyzed to different degrees with fungal (F) or bacterial (B) protease.

foam stability (Fig. 6). FCs of SPC soluble fractions were higher than that corresponding to total SPC and it was similar for all DHs irrespective of the protease. FS of SPC soluble fractions was also superior to that of the unmodified or modified total SPC. However, FS showed a maximum improvement for DH 10%. Again, different behaviors during foam destabilization were observed. Drainage from foams formulated with the fraction solubilized by the bacterial protease were accompanied by a total collapse of the foam.

This experiment indicates that the soluble fraction of hydrolyzed SPC is responsible for the increased FC and FS exhibited by the total hydrolysate reaction product, namely solubilized and insoluble fractions in admixture.

From the results of this study we concluded that solubility, foaming capacity and foam stability of denatured soy protein concentrates could be improved by treatment with both fungal and bacterial protease. Emulsifying capacity was practically unchanged and emulsion stability decreased as DH increased. Water absorption decreased, while bacterial protease treatment highly improved oil absorption, the treatment with the fungal enzyme improved it up to DH 5%. Increasing DH above 5% decreased hydrophobicity (OAC).

Differences in the hydrolytic action of the enzymes on the soy substrate would be the principal basis for the observed differences in the functional behavior of modified SPC. The *Bacillus subtilis* protease is a mixture of metalloprotease and serine protease with broad specificity, which releases mainly terminal hydrophobic-COOH amino acids (16). *Aspergillus oryzae* protease is a mixture of aspartic, metallo, serine protease and carboxypeptidase with very broad specificity. This could explain the increased hydrophobicity (OAC) of SPC hydrolyzed by the bacterial protease.

Differences in the action of the two enzymes were apparent from the results. Generally, the *Bacillus subtilis* protease solubilized more protein and gave products which foamed more than those obtained by the fungal enzyme treatment. The better foaming properties of SPC hydrolyzed by the bacterial protease could be related to the higher solubility and hydrophobicity that showed these products when compared to the SPC hydrolyzed by the fungal protease at the same DH values.

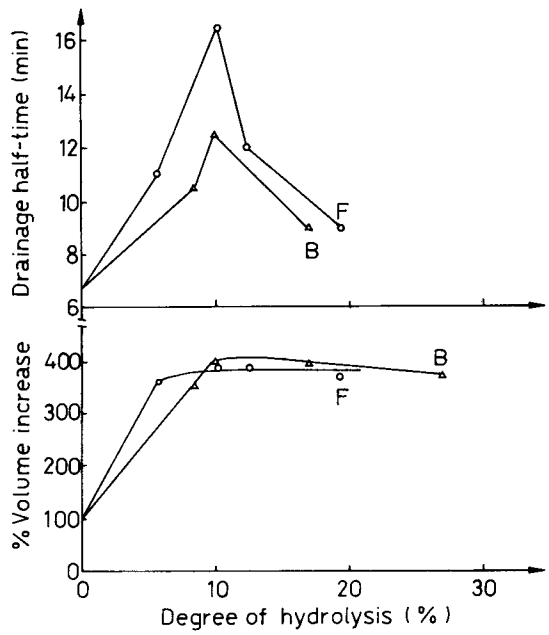

FIG. 6. Foaming capacities and foam stabilities of the soluble fraction of soy protein concentrates hydrolyzed to different degrees with fungal (F) or bacterial (B) protease.

However, the stabilizing properties of the bacterial modified SPC at the air/water or oil/water interface were inferior as was demonstrated by the coalescence of the oil or air bubbles in the emulsions or foams made with the bacterial modified samples.

Structural factors other than hydrophobicity should be also considered. The facility of protein-protein interaction and surface denaturation at the oil/water or air/water interface may be concerned in emulsion and foam stability (17).

Differences in the dependence upon hydrophobicity between emulsifying and foaming capacities suggest that more extensive hydrophobicity and unfolding protein molecules are required at the air/water interface than at the oil/water interface.

The results of this study also agree with the concept of limited enzyme hydrolysis (16). Limited hydrolysis up to DH 10% give the maximum improvment in most functional properties of SPC while avoiding loss of emulsion stability or water absorption.

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