Enzymatic Hydrolysis of Extruded-Expelled Soy Flour and Resulting Functional Properties

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ABSTRACT: Limited hydrolysis (4% degree of hydrolysis) of extruded-expelled soy flour protein (protein dispersibility index = 21) that was poor in solubility and other functional properties was evaluated at pilot-plant scale (5 kg of flour) with two endopeptidases and one exopeptidase. Some hydrolysates were merely spray-dried whereas others were jet-cooked at 104°C for 19 s before spray-drying. Solubility, emulsification capacity and stability, foaming capacity and stability, apparent viscosity, and sensory attributes were then characterized. The type of protease used and hydrothermal cooking affected functional and sensory properties. Protein solubility modestly increased with hydrolysis and jet cooking, but emulsification capacity decreased on hydrolysis and was not restored with hydrothermal cooking. Emulsion stability improved in the endopeptidase hydrolysates, but not in the exopeptidase hydrolysates. The foaming capacities of the hydrolysates for both types of enzymes were better than for the unhydrolyzed control. Highly stable foams were obtained after hydrolyzing with exopeptidase and hydrothermal cooking. Ten percent protein hydrolysate dispersions showed large losses in consistency coefficient and apparent viscosity, which increased significantly with hydrothermal cooking only for the unhydrolyzed control. Difference-from-control sensory evaluation indicated that both jet-cooked and non-jet-cooked enzyme hydrolysates were different from unhydrolyzed controls.

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KEY WORDS: Enzyme hydrolysis, extruded-expelled flour, functional properties, hydrothermal treatment, jet cooking, protein, sensory evaluation, soybean flour, soybeans, soy protein.

Food proteins are critical components of foods owing to their important functional or physicochemical and nutritional contributions. Food protein ingredients are increasingly expected to perform functional roles that are critical to processing efficiency and consumer acceptance of foods. Functional properties of proteins govern their performance and behavior in food systems during processing, storage, and final food preparation as well as relate to sensory qualities. Factors affecting functional properties of food proteins can be classified as intrinsic, extrinsic, and process parameters, and those functional properties can be modified and improved (1). The tools available to modify the structure of proteins and hence alter their functional properties are chemical modifications, heat treatments, and enzymatic modifications. Particular attention has been devoted to enzymatic hydrolysis of proteins (2) because the resulting hydrolysates preserve and frequently enhance biological values, as well as achieve physicochemical properties superior to those of the original protein. Numerous reports on enzymatic modification of soy proteins to improve functional properties are available (3–7) and generally agree that soy protein can be modified to obtain improved functional properties that can be used in highly nutritional products such as beverages and infant formulas.

Extruding-expelling (EE) is a relatively new process that combines extrusion and screw-pressing to recover oil from oil-bearing seeds (8). A cooked semifluid extrudate is obtained in which the cell walls are highly distorted or disrupted by using appropriate extrusion conditions, and the oil is immediately pressed while the extrudate is still in the hot semifluid state. Concerns about the environmental impact of hexane emissions and the safety of handling highly flammable hexane have rekindled interest in alternative oil recovery methods. Although hexane extraction of oil is still the dominant method, EE has several advantages including no use of organic or flammable solvent, low capital cost, use of simple machinery, and small production scale, all of which make EE ideally suited for identity-preserved processing of specialty soybeans. Despite these advantages, the protein in EE press cake has poor functional properties owing to exposure to high heat causing denaturation and thereby limiting its use in food.

Interest in using EE meals for human consumption has been increasing. Crowe *et al*. (9) and Wang and Johnson (10) characterized EE soybean flours for factors important in food and feed applications. They determined the ranges of protein and residual oil contents and protein dispersibility indices (PDI) that are possible with EE processing. Heywood *et al*. (11,12) showed that low-fat EE soy flour was less functional than other soy flours. Wang *et al*. (13) isolated protein (80% protein) from EE soy meal that had functional properties (emulsification, foaming, and protein dispersibility) similar to or better than those from white flakes. These isolated proteins had higher concentrations of glycinin relative to β-conglycinin compared with those from white flakes. Wang *et al*. (14) refunctionalized EE soy flour by using hydrothermal cooking (jet cooking), a treatment in which direct steam heating (150°C) and high shear were applied to the flour slurry. They reported significant improvement in solids dispersibility, protein dispersibility, and emulsification capacity of low (35 PDI) and moderately dispersible (60 PDI) EE meals by

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using hydrothermal cooking (154°C) with steam flashing (very short cooking period).

Owing to increasing interest in EE processing of soybeans and use of highly heat-denatured EE meals for human foods, developing ways to improve the functionalities of these proteins is important. Proteolytic enzyme treatment is one option. Our aims were to evaluate two endopeptidases and one exopeptidase in enzymatic modification of EE soy flour proteins by limited hydrolysis (4% degree of hydrolysis, DH). Low DH is preferred because extensive hydrolysis (higher DH) produces smaller peptides that do not have desirable functionality and that produce bitterness.

EXPERIMENTAL PROCEDURES

EE flour. EE soy flour was purchased from Nutriant Co. (Cedar Falls, IA). The residual oil, fiber, and sugar contents of the flour were 7, 4.2, and 7.3% (db: dry basis), respectively. The moisture content was 2.62 ± 0.01% and the protein content (db) was 50.9 ± 0.12%. The PDI of the flour was 21 as determined by Eurofins Scientific Inc. (Des Moines, IA).

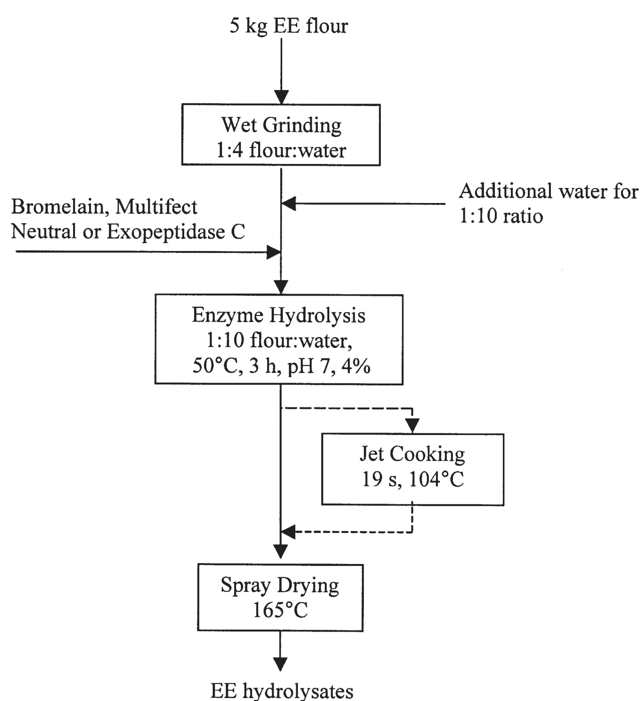
Enzymes. Two food-grade endopeptidases, Multifect Neutral (MN) and bromelain (BR), and one food-grade exopeptidase, Experimental Exopeptidase C (EEC), were used. MN and EEC were obtained from Genencor International Inc. (Rochester, NY) whereas BR was from Bio-Cat, Inc. (Troy, VA). BR is a proteolytic enzyme derived from pineapple stems and contained 2000 gelatin digestion units (GDU)/g activity. MN contained 1738 azo dye/g activity, and EEC contained 5.9 leucine-aminopeptidase units/mg protein activity. These enzymes were identified in lab screening tests to produce hydrolysates with better functional properties. The enzyme-to-substrate (E/S) ratio for each enzyme was varied to obtain approximately 4% DH in 3 h, and that ratio was used for the pilot-plant runs.

Optimization of E/S ratio. Our aim was to attain 4% DH at the end of 3 h of hydrolysis for each enzyme. DH was determined by using the pH-stat method (1,15), which determines %DH on the basis of the number of free titratable amino groups produced by hydrolysis of peptide bonds. The pH-stat method allowed us to monitor DH directly in real time. DH was calculated by using the equation: $DH = [(V_{NaOH} \times N_{NaOH})/(\alpha \times MP \times h_{tot})] \times 100\%$, where α is the degree of dissociation of α-amino groups, MP is the mass of protein (g), and h_{tot} is number of peptide bonds in the substrate (mequiv/g protein). The NaOH concentration was 2 N, and the α value was 0.44 for the hydrolysis temperature of 50°C and pH of 7.0. The h_{tot} value for soy proteins was 7.8 (15,16).

In experiments to optimize the E/S ratio, 30 g of EE flour was mixed with water at a 1:10 ratio and titrated with 2 N NaOH in a 350-mL jacketed glass reactor connected to a circulating water bath at 50°C. The pH of the slurry was adjusted to 7.0 with 2 N NaOH and, after stabilizing the pH for about 10 min, enzyme was added and the slurry was stirred for up to 3 h. The pH of the slurry was maintained at 7.0 by using a pH-stat (718 STAT Titrino; Metrohm Ltd., Herisau, Switzerland), which added required base as hydrolysis proceeded. The volume of base consumed was converted into %DH by using the relation described above. The appropriate E/S ratio for each enzyme was used in pilot-plant trials.

Preparation of pilot-plant hydrolysates. Scheme 1 shows the procedure used for preparing 4% DH enzyme hydrolysates. EE flour (5 kg) was mixed with about 20 kg water to prepare a protein slurry for grinding with a wet grinder (Stephan U Sohne GmbH & Co., Hameln, Germany). The ground slurry was then transferred to a 400-L jacketed mixing tank (Walker Stainless Equipment Co., New Lisbon, WI), and more water was added to obtain a 10% solids dispersion. The slurry temperature was maintained at 50°C by circulating hot water through the tank jacket. The slurry pH was 6.35–6.40, which was then adjusted to 7.0 by adding 2 N NaOH and stabilized for 10 min before adding the enzyme. The slurry was then stirred at 13 rpm for 3 h. Enzyme hydrolysis caused the pH to drop quickly, and 2 N NaOH was added every 2 min for the first 30 min, every 6 min for next 30 min, and every 10 min for the next 2 h. Thereby, the pH was maintained at 7.0 ± 0.05 throughout the reaction period.

The hydrolysates either were cooked hydrothermally (jet cooked) in equipment previously described (13) or were spray dried without jet cooking. The U-shaped holding tube of the jet cooker provided 19 s of cooking time at 104°C. Jet cooking provides high shear and has been shown to refunctionalize soy flour protein (13), but the purpose of jet cooking in these experiments was to inactivate the enzyme and microorganisms. The slurry was then spray dried in an Anhydro compact spray dryer (APV Crepaco, Inc., Attleboro Falls, MA) using a spinning disk atomizer and 165°C air inlet temperature. The spray-



SCHEME 1

dried hydrolysate powders were placed in double polyethylene bags and stored at 4°C until tested for functionality. All hydrolysates were prepared in duplicate.

Protein and moisture determinations. The protein contents of the hydrolysates were determined by using a combustion-type nitrogen analyzer (Elementar Americas, Mt. Laurel, NJ). Moisture content (or % total solids) was determined by heating in a forced-draft oven at 130°C for 3 h.

Functional properties. Functional properties were characterized by using methods previously described (16,17). Protein solubility over the pH range of 3.0 to 9.0, emulsification properties (emulsification capacity and stability), and foaming properties (foaming capacity, specific rate constant of drainage, and rate of liquid incorporation into foam) were determined and compared among treatments.

For apparent viscosity of the hydrolysates, 10% protein dispersions at pH 7 were prepared in water with at least 20 min stirring. A cone-and-plate probe (60 mm × 2°) with a gap of 0.105 mm was used with an RS 150 rheometer (Haake, Karlsruhe, Germany) to apply shear over the range of 10 to 500 s^{-1}. Shear stress and apparent viscosity were monitored, and the flow curves were modeled using the Power Law model: $\tau = K\gamma^n$, where τ is shear stress, K is the consistency coefficient, γ is shear rate (s^{-1}), and n is the flow behavior index.

Peptide profiles. SDS-PAGE was performed to determine the effects of hydrolysis on soy protein polypeptide profiles. SDS-PAGE was carried out by using a SDS-Tris-glycine buffer system with 4% stacking gel and 13% resolving gel (Mini PROTEAN II Gel; Bio-Rad, Hercules, CA). A low-range MW marker from 66 to 6.5 kDa (M3913; Sigma, St. Louis, MO) and laboratory-purified β-conglycinin and glycinin were used as standards.

Sensory attributes. Three separate tests for difference-from-control sensory evaluation were conducted with 2.5% sample dispersions in water according to procedures of Meilgaard *et al*. (18). The samples tested were: no enzyme hydrolysis, no jet cooking control; no enzyme hydrolysis, jet-cooked control; enzyme-treated, jet-cooked experimental treatment; and enzyme-treated, no jet cooking experimental treatment. Untrained panelists compared each treatment to a reference, which was the no enzyme hydrolysis, no jet cooking control sample. Each enzyme treatment was compared with control samples in separate sessions. Sample size was 10 mL. Panelists were instructed to swallow each sample for complete evaluation of the aromas and flavors and re-tasting was allowed. To overcome problems with residual effects of bitter and astringent compounds, the test was designed so that each panelist tasted only two samples to compare with a reference sample R. The panelists were instructed to taste the reference sample and then taste each sample and rate the degree of difference from the reference in the scale of 0 = no difference to 10 = extreme difference. The samples were presented in 1-oz (30-mL) plastic cups labeled with randomly selected 3-digit numbers. Samples were presented in partitioned booths under fluorescent light. Panelists were provided crackers and water to rinse their mouths.

Statistical analysis. The SAS system (Version 6.0, SAS Institute, Inc., 1996) was used (general linear model, PROC GLM) to compare the means, where appropriate, by using least squares difference at P <0.05.

RESULTS AND DISCUSSION

Optimal E/S ratio. Figure 1 shows plots of mean (3–7 runs) DH over time for the three enzymes studied. All three enzymes exhibited the characteristic exponential hydrolysis curve, with MN being more active during the first 30 min. For BR and EEC, however, an apparent plateau region was not reached after 3 h. The solids contents of the hydrolysates ranged from 92 to 96%, whereas the protein contents ranged from 46.5 to 54% (Table 1). The average DH values reached by 3 h were 4.2 ± 0.2, 4.6 ± 0.4, and 4.0 ± 0.2 for BR, MN, and EEC, respectively. The corresponding E/S ratios were 0.026, 0.48, and 0.84 g enzyme/g protein (db), respectively. Bromelain activity was high and required less enzyme to reach a similar DH than did the other two enzymes. Both endopeptidases had smaller optimal E/S ratios compared with the exopeptidase.

SDS-PAGE profiles. Figure 2 shows the peptide profiles for all three enzyme hydrolysates, control, glycinin, β-conglycinin standards, and markers. All enzymes almost completely hydrolyzed the α′-, α-, and β-subunits of β-conglycinin. No differences among enzyme type (endo- or exopeptidase) were observed in hydrolyzing β-conglycinin, which seemed to be more susceptible to hydrolysis than were the glycinin subunits. We previously reported similar results for hydrolyzing different soybean substrates with MN (16). The acidic subunits of glycinin, ranging between 36 and 44 kDa in molecular size, were partially hydrolyzed by BR and EEC, but not by MN. The basic subunits of glycinin were not hydrolyzed by any of the

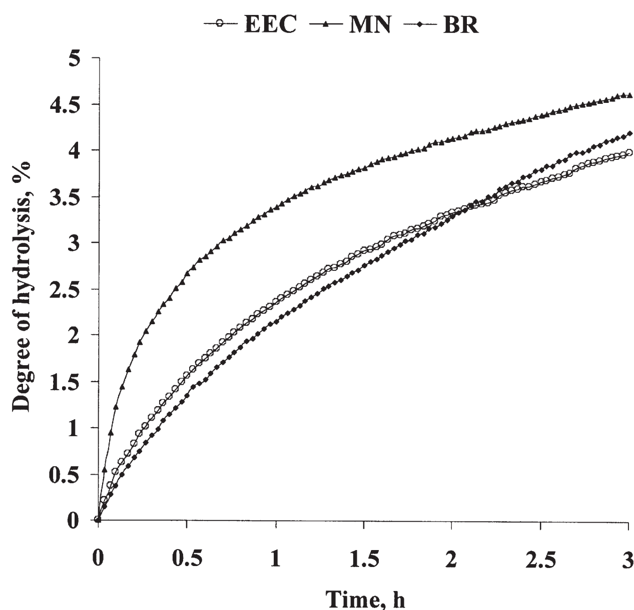


FIG. 1. Effects of reaction time on hydrolysis in laboratory-scale experiments. MN, Multifect Neutral (▲); BR, bromelain (◆); and EEC, experimental exopeptidase C ✣.

TABLE 1
Solids and Protein Contents of Spray-Dried Extruded-Expelled (EE) Soy Flour Hydrolysates

	Solids content, (% as is)		Protein content, (% dry basis)	
Hydrolysates	Uncooked	Jet cooked	Uncooked	Jet cooked
Control (no enzyme)	94.6	94.5	46.6	51.4
Multifect Neutral	95.1	95.7	53.3	53.2
Bromelain	94.7	92.5	52.5	53.9
Experimental exopeptidase C	95.5	96.2	53.1	53.6

enzymes, as they are located in the interior of protein molecules (19). The MN hydrolysates showed new peptide bands in the 24- to 36-kDa ranges that were not seen in the hydrolysates of the other enzymes. Some low-MW peptides were also observed at the bottom of the gels for all enzymes. These observations conform to our previous results (16). The resulting peptide profiles and the interaction of these peptides with themselves and other constituents determine the functional properties of the hydrolysates.

Solubility profiles. Nitrogen solubility profiles for control and enzyme hydrolysates are shown in Figure 3. These profiles are the typical V-shaped profiles for soy protein, having low solubility at the isoelectric point (pH ~ 4.5). The solubilities of all enzyme hydrolysates increased compared with the control (no enzyme, uncooked), except at pH 3. The EE soy flour, which experienced high temperatures during preparation due to EE, has much of its proteins in a denatured and aggregated state. Such proteins have low solubility. The type of protease (endo- or exopeptidase) did not seem to affect solubility profoundly at pH 3 and above, as all uncooked hydrolysates had significantly higher solubility than did the control. This conforms with our previous observations (16) on solubility of 2 and 4% DH hydrolysates from various soybean substrates.

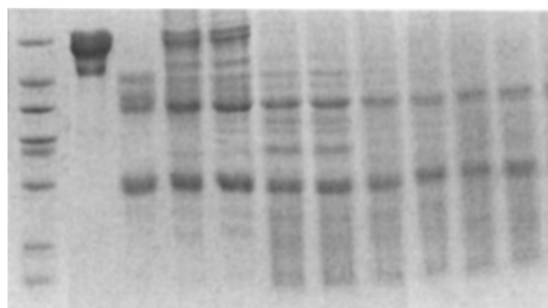
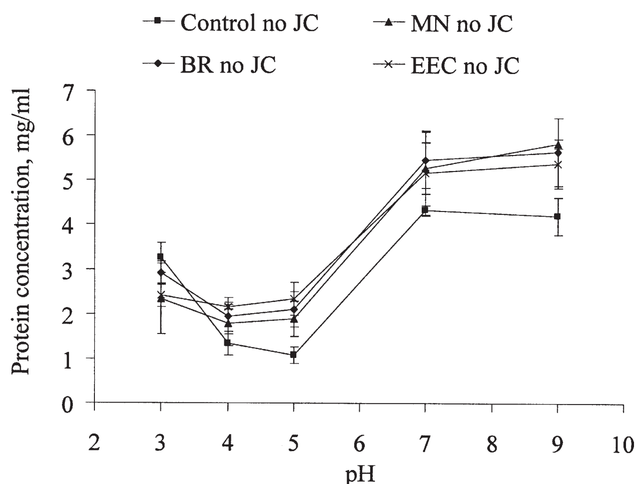


FIG. 2. SDS-PAGE profiles for hydrolysates (4% degree of hydrolysis) of extruded-expelled (EE) soy flours. Lane M, molecular weight markers 66, 44, 36, 29, 24, 20, 14.2, and 6.5 kDa from top down, respectively. Lanes 1, β-conglycinin; 2, glycinin; 3, jet-cooked control; 4, uncooked control; 5, MN, hydrolyzed and jet cooked; 6, MN, hydrolyzed and uncooked; 7, EEC, hydrolyzed and jet cooked; 8, EEC, hydrolyzed and uncooked; 9, BR, hydrolyzed and jet cooked; and 10, BR, hydrolyzed and uncooked; 1 mg protein/lane. For other abbreviations see Figure 1.

Figure 3 also shows the positive effect of jet cooking on solubility for the control sample in the range of pH 7–9. Jet cooking of enzyme hydrolysates before spray drying, however, did not considerably change the solubility. Wang and Johnson (20) also observed restoration of solubility after jet cooking, changing from a flat solubility profile for low-PDI soy flour to one that is more typical of native soy protein. Why jet cooking improves



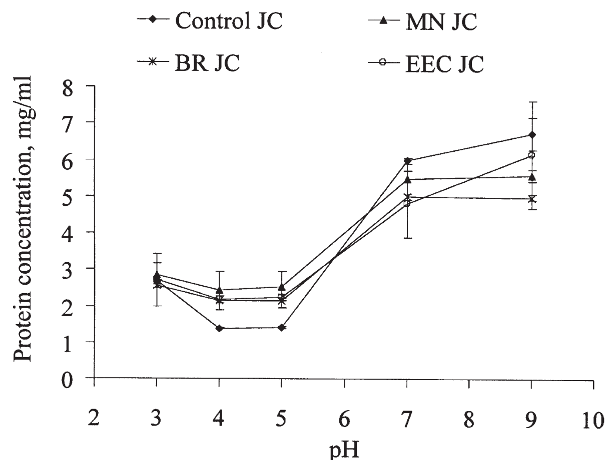


FIG. 3. Water solubility profiles for uncooked (no JC) (top), and jet-cooked (JC) (bottom) spray-dried controls and enzyme hydrolysates of EE soy flour.

TABLE 2
Foaming Properties[a] of 0.5% w/w Dispersions of EE Soy Flour Hydrolysates

Enzymes	Foaming capacity, (mL/mL)		Liquid incorporation rate, V_i (mL/min)		Specific liquid drainage rate constant, D [1/(mL-min)]	
	Uncooked	Jet cooked	Uncooked	Jet cooked	Uncooked	Jet cooked
Control (no enzyme)	1.14[c]	1.83[a]	11.4[d]	23.0[c]	0.11[b]	0.06[b]
Multifect Neutral	1.79[a]	1.83[a]	34.9[a]	34.2[a]	0.02[b]	0.02[b]
Bromelain	1.83[a]	1.83[a]	27.4[b]	23.2[c]	0.03[b]	0.04[b]
Experimental exopeptidase C	1.37[b]	1.70[a]	3.8[e]	14.4[d]	0.35[a]	0.05[b]

[a]Means of four measurements of duplicate preparations. Values sharing common superscripts are not significantly different ($P < 0.05$). D, calculated as $1/(V_{max} \times t_{1/2})$, where V_{max} is the volume of liquid incorporated into the foam (mL), and $t_{1/2}$ is the time to drain one-half of the liquid incorporated into foam (min). For abbreviation see Table 1.

soy protein solubility is not readily apparent, but these authors reported that jet cooking disrupts large protein aggregates caused by exposure to ethanol during processing. High-shear mixing during cooking could prevent further formation of large aggregates. They also speculated on a role for deamidation in improving solubility.

Foaming properties. Foaming capacity (FC), liquid incorporation rate into foam (V_i), and the foam drainage rate constant (D) for uncooked and jet-cooked controls and 4% DH enzyme hydrolysates are shown in Table 2. The FC for the uncooked control was 1.14, which was slightly lower than that reported by Jung *et al*. (16) for a similar substrate (1.4), but in the range reported by Rickert *et al*. (17) for soy protein isolates (1.13) and glycinin (1.19). Hydrolysis by the endo- and exopeptidases significantly increased FC. BR and MN hydrolysates exhibited higher increases in FC (FC = 1.8) compared with EEC hydrolysates (FC = 1.37). The SDS-PAGE profiles indicated the enzymes were effective in hydrolyzing the subunits of β-conglycinin, which possess lower foaming capacity (17). The largely intact glycinin molecules, which contain subunits consisting of disulfide-linked acidic and basic polypeptides, may have helped increase foaming capacities of the hydrolysates. Because foaming is a surface-active property of proteins, increased solubility and smaller polypeptides following hydrolysis would enhance foaming properties.

Jet cooking, however, had mixed effects on FC. The control and EEC hydrolysates were significantly ($P < 0.05$) improved whereas BR and MN hydrolysates showed no improvement. Jet cooking seemed to have affected foaming behavior of hydrolysates differently, mainly owing to their different peptide profiles and improved solubilities following hydrolysis. FC values for the uncooked control and hydrolysates closely followed the trends in the liquid incorporation rate into foam (V_i) values, which could also be interpreted as liquid-to-foam conversion rate. Hydrolysis increased V_i values for uncooked hydrolysates, except for EEC. Jet cooking increased these values for the control and EEC hyrolysates, but not for the BR and MN hydrolysates. A similar effect was also observed for FC.

Stability is another important foaming characteristic, which is described by the specific drainage rate constant (D); smaller D values indicate more stable foams. We observed highly unstable foams for uncooked exopeptidase hydrolysates (D = 0.35), followed by the uncooked control (D = 0.11). Both endopeptidase hydrolysates produced highly stable foams. This conforms to observations of Jung *et al*. (16) in which they reported improved foam stability for 4% MN hydrolysates. Foam stabilities after jet cooking the hydrolysates followed similar trends to FC and V_i. Foams were stabilized for control and EEC hydrolysates, and BR and MN hydrolysate foams were not affected. Highly stable foams were obtained after jet cooking, with no difference between control and endo- and exopeptidases. Stabilization of foams of jet-cooked EE soy flour was reported by Wang *et al*. (14).

Emulsification capacity and stability. Emulsification capacity (EC) is the amount of oil that can be emulsified under specified conditions per unit weight of protein, and emulsification stability (ES) describes the ability of protein to form an emulsion that remains unchanged over time at a given temperature and gravitational field (1). Mean EC and ES values for control and enzyme hydrolysates, jet cooked or uncooked, are shown in Table 3. Limited hydrolysis (4% DH) by both endo- and exopeptidase treatments significantly reduced the EC for enzyme hydrolysates compared with the control, with the exopeptidase hydrolysates showing the lowest EC values. This was in contrast to the generally recognized trend that EC increases with limited protein hydrolysis up to 5% DH (2,4,5,21). Extensive hydrolysis does have a detrimental effect on EC, but in our case, we observed decreased emulsification even at 4% DH. Jung *et al.* (16) also observed decreased EC for their MN hydrolysate of EE meal at as low as 2% DH. They attributed the decreased EC to the substrate's content of native-state protein and decreased hydrophobicity following hydrolysis. Their EE flour had a PDI of 68, but the PDI of our EE soy flour was only 21, indicating our protein was more denaturated. Jung *et al*. (16) observed increased EC values for low-PDI soy substrates following hydrolysis that were mainly due to increased solubility. Our results for EC were different, as we did not observe improved EC although solubility slightly increased on hydrolysis. The mode of enzyme attack (endo- or exopeptidase) reduced the EC values of the hydrolysates to varying degrees.

Jet cooking did not improve the EC values of either the control or the enzyme hydrolysates. Although the effects of jet

TABLE 3
Emulsification Properties of 2% w/w Dispersions of EE Soy Flour Hydrolysates[a]

Hydrolysates	Emulsification capacity (g oil/g protein)		Emulsification stability (min)	
	Uncooked	Jet cooked	Uncooked	Jet cooked
Control (no enzyme)	1935[a]	1785[a,b,c]	67[e]	267[a]
Multifect Neutral	1702[b,c]	1801[a,b]	153[b,c]	124[c,d]
Bromelain	1456[d,e]	1568[c,d]	190[b]	87[d,e]
Experimental exopeptidase C	1288[e,f]	1230[f]	80[e]	103[d,e]

[a]Means of four determinations on duplicate preparations. Values sharing common superscripts are not significantly different ($P < 0.05$). For abbreviation see Table 1

cooking on EE flour hydrolysates have not been reported before, Wang *et al*. (14) observed increased EC values for unhydrolyzed 35- and 65-PDI EE flours for a 24-s residence time. Our jet cooking procedure had a 19-s residence time and may be the reason for less increase in EC values with jet cooking.

The ES values of uncooked hydrolysates significantly improved two- to threefold over the control with endopeptidases (BR and MN) but did not improve with exopeptidase. Larger peptides are believed to provide emulsion stability, whereas smaller peptides resulting from extensive hydrolysis have a detrimental effect. The SDS-PAGE profiles indicated that the hydrolysates were different, with EEC showing extensive hydrolysis of β-conglycinin subunits. No new bands were observed for EEC, indicating the resulting peptides were short chain. This may partly explain the lack of a significant increase in emulsion stability for this enzyme hydrolysate. Short peptide chains would lead to decreased viscosity of the continuous phase and, although capable of adsorbing to the emulsion interfaces, they cannot form sufficiently strong barriers to prevent coalescence (2). Jet cooking the hydrolysates did not result in consistent trends in emulsion stability with regard to enzyme type. MN and EEC hydrolysates showed no significant change, but BR hydrolysates showed a significant reduction. The unhydrolyzed (no enzyme) control showed fourfold improvement in emulsion stability with jet cooking. Because jet cooking disrupts large protein aggregates owing to high-shear mixing (10) to improve solubility, emulsion stabilities of unmodified and longer peptide chains also may have increased compared with those of the 4% DH hydrolysates.

Apparent viscosity. The shear stresses developed at the applied shear rates fitted the Power Law very well ($R^2 > 0.99$). Table 4 lists the consistency coefficients (K) and flow behavior indices (n) for 10% w/w controls and hydrolysate dispersions. The K value, a measure of dispersion thickness, decreased significantly, four- to eightfold, for uncooked hydrolysates compared with the control and did not improve with jet cooking. Jet cooking the control, however, had a very large and significant impact on K value with a fivefold increase. The K values were not affected by the type of protease used for hydrolysis. The changes in K values due to hydrolysis as well as jet cooking also were reflected in apparent viscosity at both 10 and 500 s^{-1} shear rates. At 500 s^{-1}, all hydrolysate dispersions had much reduced viscosities compared with 10 s^{-1}, indicating shear-thinning. Cleavage of a few peptide bonds produces protein hydrolysates with drastically reduced viscosity compared with that of intact parent proteins (21). The decreased viscosity is also attributable, in part, to increased protein solubility following hydrolysis (16).

The n values significantly increased for BR and MN (endopeptidase) hydrolysates compared with the control but did not increase for the exopeptidase hydrolysate. Jet cooking significantly reduced n values for the controls but not the enzyme hydrolysates. Viscosity is one of the important functional properties of soy proteins, especially for incorporating into meat and dairy products and beverages. Jet cooking is one means of enhancing this important property.

Sensory properties. Difference-from-control sensory evaluations for 4% DH EE hydrolysates are presented in Table 5. All MN-treated hydrolysates were different from control treatments. The jet-cooked MN hydrolysate was different from all

TABLE 4
Power Law Parameters for 10% w/w Dispersions of Spray-Dried EE Soy Flour Hydrolysates[a]

Enzymes	Consistency coefficient, K (Pa-s^n)		Flow behavior index, n		Apparent viscosity (Pa-s) At 10 s^{-1}		Apparent viscosity (Pa-s) At 500 s^{-1}	
	Uncooked	Jet cooked	Uncooked	Jet cooked	Uncooked	Jet cooked	Uncooked	Jet cooked
Control (no enzyme)	1.7[b]	8.5[a]	0.66[b]	0.42[c]	0.75	2.24	0.20	0.23
Multifect Neutral	0.20[b]	0.43[b]	0.74[a]	0.70[a,b]	0.11	0.22	0.04	0.07
Bromelain	0.29[b]	0.89[b]	0.74[a]	0.64[b]	0.16	0.38	0.06	0.09
Experimental exopeptidase C	0.41[b]	0.72[b]	0.67[b]	0.66[b]	0.2	0.35	0.06	0.09

[a]Means of four measurements on duplicate preparations. K or n values sharing common superscripts are not significantly different ($P < 0.05$). For abbreviation see Table 1.

TABLE 5
Sensory Evaluation of Difference Between EE Soy Flour and Hydrolysates[a]

Treatments	Mean	Panelists
Control without jet cooking	2.69[c]	39
Control with jet cooking	2.89[c]	35
Multifect Neutral with jet cooking	6.41[a]	41
Multifect Neutral without jet cooking	4.73[b]	38
Control without jet cooking	2.26[b]	26
Control with jet cooking	3.57[a,b]	27
Bromelain with jet cooking	5.00[a]	26
Bromelain without jet cooking	3.92[a]	26
Control without jet cooking	2.06[b]	34
Control with jet cooking	5.50[a]	34
Exopeptidase with jet cooking	6.48[a]	33
Exopeptidase without jet cooking	6.09[a]	34

[a]Main effect means are responses of 45 panelists, 0 = no difference to 10 = extreme difference. Means sharing common superscripts are not statistically different ($P < 0.05$).

of the other treatments. The BR hydrolysates were different from the uncooked control, but the same as the jet-cooked control. The exopeptidase-treated samples were different from the uncooked control, but the same as the jet-cooked control. A sensory test session was conducted separately for each enzyme hydrolysate and all controls were the same for each session. For MN and BR hydrolysates, the panelists scored all controls the same, whereas panelists found them to be different during the EEC session.

These tests indicated only difference-from-the-control, and panelists did not identify specific flavors, but comments indicated that most of the hydrolysates were more bitter, astringent, or beany than the control samples. Descriptive analysis will need to be conducted to determine the types of flavors that developed during hydrolysis.

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