Physicochemical Properties and Yields of Sunflower Protein Enzymatic Hydrolysates As Affected by Enzyme and Defatted Sunflower Meal

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The effect of sunflower meals [industrial defatted sunflower meal (IDSM) and laboratory defatted sunflower meals (LDSM)] and enzymes (fungal protease, trypsin, and papain) on the yield and physicochemical properties of sunflower protein hydrolysates was investigated. The hydrolysate was prepared from the soluble portion of a sunflower meal−enzyme slurry after hydrolysis. Enzymatic hydrolysis solubilized a substantial portion of total protein content in all defatted sunflower meals, leading to a great increase in protein content of the hydrolysate. The type of enzyme significantly ($p < 0.05$) affected the product yield and protein recovery from IDSM. Papain gave the lowest product yield (47.4%) and protein recovery (83.8%) from IDSM substrate. More proteins were recovered from IDSM than LDSM with fungal protease. Higher protein recovery (96.4%) and protein content (80.4%) were obtained from 1% fat LDSM hydrolyzed with papain than from 18% fat LDSM or 1% fat IDSM. Fungal protease hydrolysates possessed significant antioxidant activity. All hydrolysates with papain had the highest emulsion capacity. Hydrolysate produced from a pilot-plant scale had similar characteristics to that from the laboratory scale. These results suggest that a proper enzyme and defatted sunflower meal should be considered for the commercial production of the protein hydrolysates because different enzymes and meals can result in different yields, functional properties, and compositions of the hydrolysates.

Keywords: *Sunflower protein; protease hydrolysates*

INTRODUCTION

Sunflower seed protein possesses many desirable properties including being free of any antinutritive factors (Sosulski and Fleming, 1977; Gassmann, 1983; Lusas, 1985). The quality of sunflower meal protein, however, relies on the oil extraction process. The common practice of processing conditions in the oil industry, such as incompletely dehulling and heat treatments under pressure during and after oil extraction, limits the uses of sunflower proteins in the food industry (Gassmann, 1983; Lusas, 1985). Proteins generated from these processes have poor solubility and functional properties (Sosulski, 1984). However, these proteins could be used in food and other industries if appropriate modification processes were developed (Parrado et al., 1991).

Enzymatic hydrolysis of food proteins is one of the most efficient methods for improving and expanding their functional properties. Partial hydrolysis of soy protein led to the development of a commercial whipping protein (Burnett and Gunther, 1947; Gunther, 1972). Limited hydrolysis of whey protein improved the emulsifying and forming properties (Kuehler and Stine, 1974). Hydrolysis of sunflower protein with trypsin and pepsin increased water solubility, water absorption, and foam expansion properties (Kabirullah and Wills, 1981). Parrado et al. (1991, 1993) hydrolyzed industrial defatted sunflower meal with a microbial neutral protease (Kerase) and reported that the resulting protein hydrolysate was highly soluble over a wide pH range of 2 to 10.

There has been increased interest in plant proteins as alternatives to animal protein used in cosmetic products because a current trend in the cosmetic industry is towards to the replacement of animal product or byproduct (Antonelli, 1993). If partially hydrolyzed sunflower protein with some specific physicochemical properties can replace animal-derived proteins, the utilization and value of sunflower proteins would be increased. Although there are some reports on sunflower protein hydrolysate, there is a lack of data describing protein yield, protein recovery, emulsion capacity, and antioxidant activity of hydrolysate with different enzymes. There is no single study that compares characteristics of hydrolysates produced from differently processed defatted sunflower meals. No study has been conducted to characterize large-scale production of sunflower protein hydrolysate. Therefore, the objectives of this study were (1) to investigate the effects of enzymes and sunflower meals (i.e., industrial defatted meal and laboratory defatted meals) on the yield, composition, color and functional properties of sunflower protein hydrolysates, and (2) to scale up the process of sunflower protein hydrolysate production and provide more information about production and characteristics of the hydrolysates for potential applications in industries.

MATERIALS AND METHODS

Materials. Dehulled industrial defatted sunflower meal (IDSM) and dehulled, nondefatted sunflower seed chips were obtained form the National Sun Industries (Enderlin, ND). The

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seeds were crushed and steamed at 100 °C for 30 min prior to oil extraction. Crude fungal (*Aspergillus*) protease (activity of 4 units/mg, catalogue no. P4032), trypsin (1000−2000 units/mg, catalogue no. T8128), and papain (1−2 units/mg, catalogue no. P3250) were purchased from Sigma Chemical Company (St. Louis, MO).

Laboratory Defatted Sunflower Meal (LDSM). LDSM was prepared by Soxhlet extraction of ground sunflower seed chips with hexane until the fat content was ~18 or ~1%. For the pilot-plant study, the sunflower seed chips were ground and defatted with hexane at 50 °C in an Armfield solvent extractor (model FT29, Hampshire, England). LDSM was different from IDSM in that mild heat was used during oil extraction, and therefore, it was assumed that more native proteins remained in LDSM.

Sunflower Protein Hydrolysate Production. The preparation of sunflower protein hydrolysate was described by Parrado et al. (1991) with some modifications as follows. IDSM or LDSM was washed/mixed with ninefold water at pH 4.5, and settled/floated into lignocellulosic, soluble, and proteinaceous fractions (LCF, SF, and PF, respectively). The LCF and upper, clear portion of SF were removed by reduced pressure. The PF and lower, turbid portion of SF were centrifuged at 15 100g for 15 min. These processes were repeated twice. After centrifugation, the PF was hydrolyzed for 80 min with 2% fungal protease (25 °C, pH 8.0), 0.75% trypsin (37 °C, pH 8.1), or 2% papain (25 °C, pH 7.0, with 0.0082% EDTA and 0.019% cysteine-HCl) on a dry weight basis in a vessel equipped with a stirrer, thermometer, pH electrode, and buret for delivering 0.5 N NaOH. The different enzyme concentrations and hydrolysis conditions used were based on the protease activity, cost, and optimal pH and temperature recommended by the manufacturer.

For the laboratory scale study, the hydrolysis was accomplished in a 2-L beaker with 5% total solids of PF. The suspension after hydrolysis was heated to 90 °C for 10 min, cooled, and then centrifuged at 15 100g for 15 min. The sludge was resuspended in water with an equal amount of the supernatant and recentrifuged. The first and second supernatants (soluble portions) were combined, evaporated, and freeze-dried. For the pilot-plant scale study, the hydrolysis was conducted in two 20-L buckets with papain under the conditions just described. The suspension after hydrolysis was heated in steam to 90 °C in a still retort, cooled, and centrifuged at 3330*g* in a continuous Centrico Westfaliar separator (model CA150, Elgin, IL). The sludge was suspended and recentrifuged twice to recover protein hydrolysate (soluble fraction). The supernatants were combined, concentrated to ~23% solid in a climbing-film evaporator, and freeze-dried.

Degree of Hydrolysis. The degree of hydrolysis, defined as the percentage of number of peptide bonds cleaved divided by the total number of peptide bonds in a protein, was calculated from the consumption of base (NaOH) by the pH-stat method of Adler-Nissen (1977, 1986). By taking the average of p*K* values of α-amino groups in polypeptides reported by Adler-Nissen (1977), we used the p*K* values of 7.60 at 25 °C and 7.35 at 37 °C for the calculation.

Chemical Analysis. Moisture and ash were determined according to AOAC Methods 925.10 and 923.03 (1990), respectively. Total nitrogen was determined by the Kjeldahl method, and protein content was calculated by multiplying Kjeldahl nitrogen by a conversion factor of 5.5 (Sosulski and Sarwar, 1973; Gassmann, 1983). Fat was determined by the procedures of Osborne and Voogt (1978).

Protein Molecular Mass Determination. Defatted sunflower meal, PF, and hydrolysate were examined for polypeptide molecular mass distribution by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with a gradient gel of 10−20% based on the procedure of Laemmli (1970). Samples were extracted on a magnetic stirrer with 0.05 M sodium phosphate buffer (1 g:10 mL) at pH 7.5 for 90 min. The slurry was centrifuged at 1960*g* for 10 min to remove residues. The protein concentration of supernatant was determined by the Biuret method and adjusted to 5 mg/mL with distilled water. An equal volume of SDS-sample buffer containing 10% 2-mercaptoethanol was added to the protein solution. After boiling for 2 min, 40 μL of the cooled solution containing 100 μg of protein was loaded into the gel. Electrophoresis was performed in a BioRad Protean II chamber at 100 V for 8 h. The gel was stained with Coomassie Brilliant Blue R-250.

Emulsion Capacity. Emulsion capacity was determined according to Ethen (1987) with some modifications. The device consisted of a Tekmar tissumizer (SDT-1810), a variable autotransformer, a glass spice jar, a volt-ohm-milliammeter, an oil reservoir, and a circulating water bath with the thermostat at 20 °C (Ethen, 1987).

All samples were made up to protein solutions of 3 mg/mL by placing a sample containing 100 mg of protein in 33.3 mL of 0.01 M sodium phosphate buffer (pH 7.0). The jar with 33.3 mL of the solution was weighed and then placed into the water bath apparatus. Corn oil was delivered at 9 mL/min. The emulsion capacity testing process was timed from the time the tissumizer was turned on. At 10 s, the transformer setting was 20%; at 20 s, it was set at 40%; and by the first 50 s, it was set at 100% (generating 48 000 rpm of the spindle). The gradual increase in the spinning reduced entrapping air. The oil tube was clamped when a sudden jump in the electrical resistance was observed. The final sample weight was measured. The emulsion capacity was calculated as the grams of oil per 100 mg of protein required to reach an infinite electrical resistance minus a blank containing 33.3 mL of the phosphate buffer.

Antioxidant Activity. Antioxidant activity was determined with a Metrohm Rancimat (model 679, Herisau, Switzerland). Sunflower meal or hydrolysate (0.10 g) was dispersed in fresh crude sunflower oil (49.90 g) by a sonicator, and 5.0 g of each suspension was used to determine antioxidant activity based on the induction time of oil oxidation. The air speed and block temperature of the instrument were set at 20 L/min and 98 °C, respectively (AOCS, 1987). Butylated hydroxyanisole (BHA) and oil blank were used as positive and negative controls, respectively.

Color. Hunter color was measured on a Gardner colorimeter (model XL-23, Gardner Lab Inc., Bethesda, MD). The instrument was standardized with a standard white tile [lightness (L) = 91.94, redness (a_L) = −1.03, and yellowness (b_L) = 1.14].

Statistical Analysis. Experimental data were analyzed by analysis of variance with the general linear model by Duncan's multiple comparison (SAS Institute, 1988). The significant level was defined as 0.05 probability or less. All treatments were run in two replicates.

RESULTS AND DISCUSSION

Proximate Composition. The analysis of proximate composition showed that freeze-dried sunflower meal protein hydrolysates contained 1−3% moisture, 63−80% protein, 1−17% fat, and 6−10% ash, depending on meals and enzymes used (Table 1). LDSM hydrolysates with papain had higer protein concentrations than those with fungal protease (Table 1). The opposite was true for IDSM. Therefore, papain was more suitable to solubilize LDSM proteins and fungal protease was more appropriate for IDSM proteins. High-fat meal resulted in high-fat hydrolysates (Table 1). All LDSM hydrolysates had lower ash content than the raw meal.

Enzyme hydrolysis of defatted sunflower meals enriched the protein content of the soluble portion (hydrolysate) and depleted the protein in the remaining insoluble material. Papain hydrolysates had a protein content that was ~1.7 times their PF values and two times their meal values (Table 1). Laboratory low fat meal (LLFM) hydrolysates had the highest protein content among three meals based on the same enzyme used. Papain- and trypsin-LLFM hydrolysates contained 79−80% protein which is ~18% higher than other treatments. Protein contents of IDSM hydrolysates

Table 1. Proximate Composition of Sunflower Meal and Enzymatic Hydrolysates[a]

meal[b]	substrate	enzyme	moisture (%)	protein (%)	fat (%)	ash (%)
LHFM	raw meal		6.6b	36.0d	18.2a	7.5b
	PF[c]		63.7a	42.4c	15.0ab	
	PF	fungal protease	2.4c	63.3b	16.9a	5.5a
	PF	trypsin	1.8c	65.9b	10.4b	5.6a
	PF	papain	1.8c	70.8a	11.4b	5.7a
LLFM	raw meal		8.3b	42.9d	1.1a	10.0a
	PF		68.9a	49.5c	1.2a	
	PF	fungal protease	3.4c	74.3b	1.7a	6.2bc
	PF	trypsin	3.2cd	78.9a	1.0a	6.6b
	PF	papain	2.2d	80.4a	0.9a	5.7c
IDSM	raw meal		5.2b	29.5d	1.5a	9.4b
	PF		78.1a	35.1c	0.5b	
	PF	fungal protease	2.0c	65.9a	0.9b	6.3c
	PF	trypsin	1.8c	64.7a	0.6b	9.9a
	PF	papin	1.3d	61.7b	0.7b	9.6ab

[a] Data are means of two treatment replicates on a dry weight basis except that moisture is on a wet weight basis; means within the same meal in the same column followed by different letters are significantly different ($p < 0.05$). [b] LHFM = laboratory high-fat meal; LLFM = laboratory low-fat meal; IDSM = industrial defatted sunflower meal. [c] Proteinaceous fraction.

with fungal protease, trypsin, and papain were 65.9, 64.7, and 61.7%, respectively (Table 1). Parrado et al. (1991) reported that protein hydrolysate produced from IDSM contained 77.6% protein. The difference in protein content was mainly attributed to different sources of industrial meal, enzymes, hydrolysis conditions, and product yields. Parrado et al. (1991) used IDSM containing 36% protein, Kerase enzymes with an activity of 5000 AU/mg, and protein concentrate of 58.3% as the substrate. For the production of sunflower protein hydrolysate to be more practical for scaling-up, we used crude enzymes and PF containing 35.4% protein as the substrate for producing IDSM hydrolysate. We eliminated the tedious, stepwise aqueous ethanol washing procedures that can add production cost, denature proteins, and decrease the functional properties (Gassmann, 1983).

Product Yield and Protein Recovery. When hydrolyzed with papain, LHFM and LLFM had significantly higher product solid yields than IDSM based on PF substrate (54.3 and 60.6% versus 47.4%). However, the recovery of solids in hydrolyzate calculated from raw LDSM and IDSM was not significantly different (34.2−36.7%). There were no significant differences in product yield from LLFM among three enzymatic treatments (60.2−62.7%). However, when laboratory high fat meal (LHFM) was hydrolyzed, papain gave the lowest product yield (54.3%) and fungal protease gave the highest (62.4%).

Of the three meal PF substrates hydrolyzed by papain, LLFM gave the highest protein recovery (96.4%). More protein in LDSM-PF substrate than IDSM-PF substrate was hydrolyzed by papain to produce hydrolysate. Based on raw meals, however, IDSM had higher protein yields (77.0−90.8%) than LDSM (61.8−66.9%). This difference was due to the greater loss of protein (mostly soluble protein) in LDSM during PF preparation compared with IDSM. LDSM lost 32% protein on the average, whereas IDSM lost 8%. The great loss of protein in LDSM may require more effective methods to increase the recovery of proteins in the SF. No differences in protein recovery from LDSM occurred among three enzymes. However, LHFM had lower protein recoveries (88.6−90.5%) than LLFM from PF hydrolysis (91.5−97.1%). When IDSM was hydrolyzed, papain gave the lowest protein recovery from PF (83.8%) and raw meal (77.0%), whereas trypsin produced the highest recovery (98.7% from PF and 90.8% from raw meal). High protein recovery (96.4−98.7% of substrates) could be achieved with a proper combination of enzyme and meal. Papain or trypsin with LLFM, and trypsin or fungal protease with IDSM gave higher protein content and protein recoveries.

Color. The color of papain hydrolysate was significantly lighter than the other protease hydrolysates. The lightness (Hunter L value) of papain LDSM-hydrolysate was 34.8−35.1, whereas the L value of fungal protease and trypsin hydrolysates was 32.6−33.9. Papain hydrolysate was less yellow (b_L value, 9.7) compared with trypsin and fungal protease counterparts (b_L values of 13.2 and 11.4, respectively). The redness (a_L value) of IDSM hydrolysate was 4.5, smaller than that of LHFM hydrolysate (6.5) and LLFM hydrolysate (5.3). Differences in meal composition and hydrolysis conditions (pH and temperature) may account for the difference in color of hydrolysates.

Emulsion Capacity. The types of enzymes and meal significantly influenced the emulsion capacity of hydrolysates (Table 2). Emulsion capacity of protein and hydrolysate increased after the hydrolysis of sunflower meals with papain. All papain hydrolysates had higher emulsion capacity than other enzyme hydrolysates (Table 2). The hydrolysate produced from LHFM (18% fat) with papain had the highest emulsion capacity (107.3 g of oil/100 mg of protein), whereas the trypsin hydrolysate had the lowest (35.1 g/100 mg of protein). Fungal protease and trypsin did not increase emulsion capacity of protein in LDSM hydrolysates. When hydrolyzed by papain, laboratory defatted meals resulted in higher emulsion capacity of the product compared with the industrial meal. The trypsin-catalyzed hydrolysis of IDSM increased emulsion capacity, but the enzymatic hydrolysis of LHFM decreased emulsion capacity based on the unit weight of protein. Therefore, the emulsion capacity of sunflower protein depended on the source of protien (nature of protein) and enzyme. The enzyme specificity and defatted sunflower meal composition/properties may have interactions with respect to emulsion capacity.

Kabirullah and Wills (1981) also reported that the emulsion activity and emulsion stability were lost in all the hydrolysates produced by partial hydrolysis of sunflower protein isolate with pepsin and trypsin irrespective of the degree of hydrolysis. They speculated

Table 2. Effect of Enzymes and Sunflower Meal on Emulsion Capacity (EC) of Hydrolysates[a]

	EC (g of oil/100 mg of protein)			EC (g of oil/g of hydrolysate)[b]		
treatment	LHFM[c]	LLFM[d]	IDSM[e]	LHFM[c]	LLFM[d]	IDSM[e]
fungal protease	61.1b(a)	50.6b(a)	49.2c(a)	387.3b(a)	373.6b(a)	318.9b(a)
trypsin	35.1c(c)	51.8b(b)	67.4b(a)	232.2c(b)	405.3b(a)	441.8a(a)
papain	107.3a(a)	95.3a(b)	86.5a(c)	741.8a(a)	764.9a(a)	532.3a(b)
control (raw meal)	59.9b(a)	60.8b(a)	53.2c(a)	201.5c(ab)	237.8c(a)	156.9c(b)

[a] Data are means of two treatment replicates; means within a column followed by different letters are significantly different ($p < 0.05$); means within the same EC category in the same row followed by different letters are significantly different ($p < 0.05$). [b] Estimated from the emulsion capacity based on g oil/100 mg protein. [c] Laboratory high-fat meal. [d] Laboratory low-fat meal. [e] Industrial defatted sunflower meal.

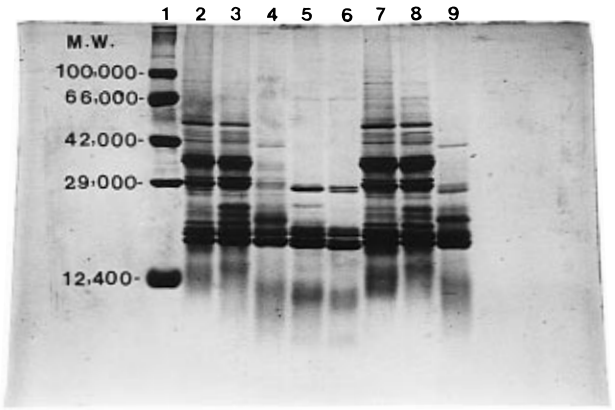

Figure 1. SDS-PAGE pattern of polypeptides in laboratory/pilot-plant defatted sunflower meals and their proteinaceous fraction (PF) and protein hydrolysates. Lane 1 is protein molecular mass marker (molecular mass is shown on the left). Lanes 2 and 3 are polypeptides in laboratory low-fat meal (LLFM) and PF, respectively. Lanes 4, 5, and 6 represent protein hydrolysates of PF with papain, fungal protease, and trypsin, respectively. Lanes 7, 8, and 9 are polypeptides in defatted sunflower meal, PF, and papain hydrolysate in the pilot-plant study, respectively.

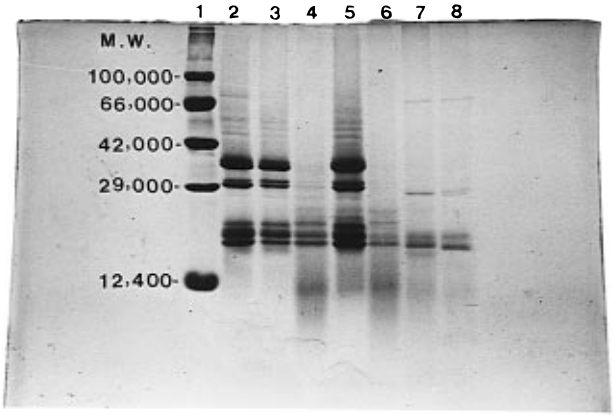

Figure 2. SDS-PAGE pattern of polypeptides in industrial defatted sunflower meal (IDSM), proteinaceous fraction (PF), and protein hydrolysates. Lane 1 is protein molecular mass marker (molecular mass is indicated on the left). Lanes 2, 3 and 4 are polypeptides in IDSM, PF, and papain-IDSM hydrolysate, respectively. Lanes 5, 6, 7, and 8 represent polypeptides in control hydrolysate (PF with no enzyme), papain-PF hydrolysate, fungal protease-PF hydrolysate, and trypsin-PF hydrolysate, respectively.

that the degree of hydrolysis (2.3−11.8%) resulted in the protein molecules being too small to entrap the fat globules to form and stabilize the emulsion. Turgeon et al. (1992) and Gauthier et al. (1993) studied the emulsifying property of whey peptide fractions hydrolyzed from whey proteins and found that the fraction with larger molecular weight and more hydrophobic areas of peptides gave better emulsifying properties. Thus, the enzyme specificity and molecular weight of polypeptides may account for the difference in the emulsion capacity because the content of larger molecular weight of polypeptides in papain hydrolysate was higher than that of fungal protease and trypsin hydrolysates (Figures 1 and 2).

Antioxidant Activity. The antioxidant activity of sunflower meal hydrolysate varied with the enzymes. However, only hydrolysates produced from fungal protease exhibited significant antioxidant activity compared with the negative control (Table 3). LLFM hydrolysate had the highest antioxidant activity (i.e., longer induction time of oil oxidation) among three fungal protease hydrolysates (Table 3). The antioxidant activity of LLFM hydrolysates of 2000 ppm in crude oil was comparable to butylated hydroxyanisole at 200 ppm.

The nature of the antioxidant constituents in the enzymatic hydrolyzates was not very clear. Although phenolic compounds are well-known antioxidants (Bishov and Henick, 1972; Pratt et al., 1981) and they are present in defatted sunflower meal and protein

Table 3. Antioxidant Activity of Sunflower Meal and Hydrolysate[a]

		induction time (h)		
sample[b]	concentration (ppm)	fungal protease	trypsin	papain
BHA (+ control)	200	10.28a	10.36a	10.29a
LLFM hydrolysate	2000	9.88a	8.46b	8.96b
LHFM hydrolysate	2000	9.43b	8.25b	8.91b
IDSM hydrolysate	2000	9.24b	8.21b	8.60b
oil bank (− control)	0	8.44c	8.40b	8.55b

[a] Data are means of two treatment replicates; means within a column followed by different letters are significantly different ($p < 0.05$). [b] BHA = butylated hydroxyanisole; LLFM = laboratory high-fat meal; LHFM = laboratory low-fat meal; IDSM = industrial defatted sunflower meal.

hydrolysate (Gassmann, 1983; Parrado et al., 1991), the antioxidant activity of our hydrolysates may not be simply related to phenolic compounds because only fungal protease produced the antioxidant activity of hydrolysates based on the same defatted sunflower meal. The antioxidant activity may be attributed to amino acids and peptides and their association with other constituents in the fungal protease hydrolysate. Because fungal protease is a mixture of aspartic protease, metalloprotease, serine protease, and carboxypeptidase (Adler-Nissen, 1986), the broad specificity could play an important role in antioxidant activity. The hydrolysis catalyzed by fungal protease could produce amino acids, sulfhydryl compounds, and peptides due to its exopeptidase and endopeptidase activity. Papain and trypsin did not have exopeptidase activity and

Table 4. Effect of Enzymes and Sunflower Meals on the Degree of Hydrolysis[a]

enzyme	substrate (PF)	degree of hydrolysis (%) at time 20 min	40 min	60 min	80 min
fungal protease	LHFM[b]	8.6c(d)	10.7d(c)	12.1de(b)	12.9ef(a)
	LLFM[c]	8.6c(d)	10.7d(c)	12.1de(b)	13.5de(a)
	IDSM[d]	11.5a(d)	14.9a(c)	17.4a(b)	19.3a(a)
trypsin	LHFM	7.1d(d)	9.2e(c)	10.5e(b)	11.6f(a)
	LLFM	7.2d(d)	9.3e(c)	11.1e(b)	12.0f(a)
	IDSM	9.4bc(d)	13.3b(c)	15.9b(b)	17.4b(a)
papain	LHFM	11.8a(c)	13.6b(b)	14.7bc(a)	15.7c(a)
	LLFM	9.4bc(d)	11.7cd(c)	13.6cd(b)	14.9cd(a)
	IDSM	10.0b(d)	12.0c(c)	13.3cd(b)	14.6cd(a)

[a] Data are means of two treatment replicates; means within a column followed by different letters are significantly different ($p < 0.05$); means within a row followed by different letters in parenthesis are significantly different ($p < 0.05$). [b] LHFM = laboratory high-fat meal. [c] LLFM = laboratory low-fat meal. [d] IDSM = industrial defatted sunflower meal.

therefore no production of amino acids from sunflower protein was expected. Amino acids and peptides have been shown to act as primary antioxidants whose activity varies with their concentration, environmental pH, and other compounds present (Marcuse, 1960; Bishov and Henick, 1972, 1975; Hayes et al., 1977). Marcuse (1960) and Bishov and Henick (1972, 1975) also reported that amino acids and low molecular weight peptides had a synergistic effect with phenolic antioxidants in herring oil emulsion and in model systems with linoleic acid. The synergistic effect of amino acids was explained on the basis of chelation of prooxidative trace metals or by regeneration of oxidized primary antioxidants (Marcuse, 1960). Thus, the antioxidant activity of our fungal protease hydrolysates may be related to the concentration and synergistic effect of amino acids and peptides with phenolic compounds.

Degree of Hydrolysis (DH). DH is an important index during hydrolysis because it influences product yield, protein recovery, functional properties, and organoleptic quality (e.g., bitter taste) of hydrolysates (Adler-Nissen, 1986). Enzyme and sunflower protein substrate affected the degree of hydrolysis (Table 4), which was probably due to different enzyme specificity. Crude enzymes (fungal protease and papain) have a broad spectrum of specificity, whereas trypsin has a high specificity. When hydrolyzed with fungal protease and trypsin, the DH of IDSM was higher than that of LDSM, thus indicating that these enzymes hydrolyzed IDSM proteins (denatured proteins) more rapidly. At 20 min, IDSM-fungal protease and LHFM-papain had the highest DH (11.5−11.8%), and the DH of laboratory meals with trypsin had the lowest (7.1−7.2%). The lowest of DH with trypsin could be attributed to its high specificity for lysine and arginine. After 80 min, DH ranged from 11.6 to 19.3%, with the lowest value given to LHFM-trypsin hydrolysate and the highest to IDSM-fungal protease (Table 4). Of the three enzymes, papain produced the highest DH value from the laboratory meals but yielded the lowest DH from the industrial meal. This result suggests that papain hydrolyzed the denatured protein more slowly than the native protein. The lower DH resulted in lower solubility of proteins, leading to the lower protein recovery (83.8%) and product yield (47.4%) of papain-IDSM hydrolysate. The DH increased with hydrolysis time, which was in agreement with Parrado et al. (1991). They found that hydrolysis of industrial sunflower meal protein concentrate with neutral protease Kerase to 18.8% DH was efficient in solubilizing sunflower protein and recovered 81.9% of the protein present in the substrate. We recovered 83.8, 98.7, and 96.9% protein from PF with papain, trypsin, and fungal protease at DH of 14.6, 17.4, and 19.3%, respectively (Table 4).

Table 5. Chemical Composition of Defatted Sunflower Meal and Protein Hydrolysate (Pilot-Plant Scale)[a]

sample	moisture (%)[b]	protein (%)[c]	fat (%)[c]	ash (%)[c]
meal	11.4a	40.3a	1.2a	9.1a
PF	77.4b	47.3b	—[d]	—[d]
hydrolysate	5.6c	68.6c	1.2a	7.0b

[a] Data are means of two treatment replicates; means within a column followed by different letters are significantly different ($p < 0.05$). [b] On a wet weight basis. [c] On a dry weight basis. [d] Not determined.

Protein Molecular Mass Distribution. Gel electrophoretic patterns showed that LDSM and PF had six major bands of polypeptides representing molecular masses of 54 000, 39 000, 31 000, 22 000, 20 000, and 18 000 Da (Figure 1). After enzymatic hydrolysis, the concentration of polypeptides ≥31 000 Da was much lower, as indicated by the lighter bands or by the band disappearance (Figure 1). Papain-LDSM hydrolysate (lane 4) had wider polypeptide distribution although the major polypeptides had molecular masses of 18 000, 20 000, 23 000, 29 000, 36 0000, and 42 000 Da. The larger polypeptides (≥36 000 Da) may be one of the reasons for contributing to emulsion capacity (Table 2). Fungal protease and trypsin hydrolysates were primarily composed of polypeptides of 18 000, 20 000, and 29 000 Da (Figure 1) and peptides. The defatted sunflower meal, PF, and hydrolysate produced from the pilot plant had the similar protein distribution to the laboratory products (Figure 1).

The protein pattern of IDSM and PF was similar to laboratory counterparts except there was a lower polypeptide concentration at 54 000 Da (Figure 2). The control (without adding enzyme) hydrolysate did not change the molecular mass distribution when compared with PF (lanes 2 and 5). Polypeptide patterns of hydrolysates produced from papain with industrial meal and PF were essentially the same (lanes 4 and 6). The polypeptides with molecular mass of 31 000 and 39 000 Da in IDSM were reduced to an insignificant level (Figure 2). Smaller polypeptides and peptides were major components of enzymatic hydrolysates. Kabirullah and Wills (1981) and Parrado et al. (1993) also reported that sunflower protein hydrolysates with a degree of hydrolysis of ≥12.0% were mainly composed of low molecular mass proteins and peptides.

Pilot-Plant Sunflower Meal Protein Hydrolysate. Papain gave LDSM hydrolysate a higher protein content, a lighter color, and higher emulsion capacity as discussed earlier, so it was used for the pilot-plant study. The protein concentration of pilot-plant-produced hydrolysate on a dry weight basis was 68.6%, ~21% higher than substrate PF and 28% higher than raw meal (Table 5). Crude protein recovery was 85.0% from PF and 57.5% from raw meal. Protein content and protein recovery in the pilot-plant study were lower than those in the laboratory study as described previously. This difference was probably due to soluble solid loss in the pilot plant where a smaller centrifugal force (3300*g*) was applied to the sunflower meal soluble fraction and hydrolysate slurry. The SDS-PAGE polypeptide patterns of sunflower meal, PF (substrate), and papain hydrolysate were similar to those in the laboratory study (Figure 1).

Color decreased in lightness (*L* value) after enzymatic

Table 6. Color of Defatted Sunflower Meal and Protein Hydrolysate (Pilot-Plant Scale)[a]

sample	Hunter color		
	L	a_L	b_L
raw meal (as-is form)	82.84a	1.17a	8.72a
raw meal (freeze-dried)	57.53b	3.48b	13.53b
proteinaceous fraction (freeze-dried)	60.16c	2.50c	12.21c
hydrolysate (freeze-dried)	45.55d	6.15d	14.41d

[a] Data are means of two treatment replicates; means within a column followed by different letters are significantly different ($p < 0.05$).

Table 7. Antioxidant Activity and Emulsion Capacity of Defatted Sunflower Meal and Papain Hydrolysate (Pilot-Plant Scale)[a]

antioxidant activity			emulsion capacity	
sample	concentration (ppm)	induction time (h)	sample	oil/sample (g/100 mg)
meal	2000	8.80a	meal	58.8a
hydrolysate	2000	8.98a	hydrolysate	129.0b
BHA[b] (+ control)	200	10.43b	ISP 620[c]	32.8c
oil blank (− control)	0	8.43a	ISP 710[c]	48.3d

[a] Data are means of two treatment replicates; means within a column with different letters are significantly different. [b] Butylated hydroxyanisole. [c] Isolated soy protein (Protein Technologie International, St. Louis, MO).

hydrolysis (Table 6). Raw meal after freeze-drying also decreased in L value. The color of enzymatic hydrolysate was redder (greater a_L value) and more yellow (greater b_L value) than raw meal. The brown color of the hydrolysate was due to the oxidation of polyphenolic compounds (Gassmann, 1983) and the Maillard reaction (Dworschak, 1980) during processing. Polyphenolic compounds could be reduced before hydrolysis by stepwise aqueous/ethanol washing procedure (Parrado et al., 1991).

Pilot-plant papain hydrolysate at ≤2000 ppm did not have antioxidant activity (Table 7), which is in accordance with the laboratory study discussed earlier. The antioxidant activity of the defatted sunflower meal was not significant compared with the control. The emulsion capacity of the hydrolysate was higher than raw meal on a protein weight basis (Table 7). Hence, the enzymatic hydrolysis significantly increased the emulsion capacity of sunflower protein. It was also found that sunflower meal and protein hydrolysate possessed higher emulsion capacity than isolated soy proteins (Table 7). A similar result was reported by Sosulski and Fleming (1977) who found that sunflower flour and protein concentrates had higher oil emulsification than soybean counterparts. They suggested that sunflower products might have specific applications in emulsion-type meats. We used the sunflower protein hydrolysate produced on the pilot-plant scale as a protein base for a cosmetic product (skin lotion) and conducted human subject tests. The results showed no significant difference in product preference when compared with the control (an animal protein-base lotion formulated by a local cosmetic company; unpublished data).

Conclusions. Different sunflower meals and enzymes resulted in different characteristics of hydrolysates. Papain hydrolysate gave the highest emulsion capacity. Fungal protease hydrolysate had significant antioxidant activity. Enzymatic hydrolysis solubilized a substantial portion of protein content of sunflower meals and enriched the protein content of hydrolysates. Trypsin and fungal protease gave higher protein recoveries than papain from IDSM. LLFM hydrolysates with papain and trypsin had a high protein content (79−80%) and high protein recovery (96−97%) from the substrate. The papain and fungal protease hydrolysates could be used as an emulsifier, antioxidant, and/or plant protein base for cosmetic products.

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