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Some Functional and Nutritional Properties of Oat Flours as Affected by Proteolysis

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Oat flour was hydrolyzed with Alcalase (A) and Neutrase (N) to the following degree of hydrolysis (DH): 3.8 (A1), 7.6 (A2), 6.0 (N1), 10.4% (N2). The functional and nutritional properties of the resulting products were compared to those of control oat flours and commercial whole wheat and soy flours. Water hydration capacity and isoelectric solubility increased with increase in DH. Proteolysis reduced emulsifying stability, fat-binding capacity, and heat coagulability when compared to controls. These properties were comparable or higher when compared to soy flour, except for A2. Water-soluble proteins had fragments of 925, 645, 395, and 230 molecular weights in Alcalase-treated and 835, 565, 340, and 195 molecular weights in Neutrase-treated oat flours. No significant differences were observed in the protein efficiency ratio (PER) among controls and enzyme-modified oat flours. There was a significant ($p < 0.05$) but small decrease in the nitrogen apparent digestibility coefficient in Alcalase-treated oat flours.

Oat is an inexpensive source of good quality protein with high protein content (Youngs et al., 1982). Although oat is nutritionally superior to other cereals, only 2.3% of the total oat crop harvested is used for human consumption in Canada (Statistics Canada, 1985), mainly as rolled oat groats (breakfast cereals) or oat flour (bakery products and infant foods). High-protein oats have been proposed for the enrichment of conventional foods (D'Appolonia and Youngs, 1978) and for the development of unconventional products such as high-protein beverages (Cluskey et al., 1976). In spite of the good nutritional qualities of oats, lack of information on the functional properties appears to be the main constraint to increased oat utilization.

The functionality of processed domestic and wild oat groat products was determined by Chang and Sosulski (1985). The nitrogen solubilities of domestic and wild groat products were very low in the pH region around the isoelectric point (Chang and Sosulski, 1985), but this was due to the steam treatments used to inactivate lipase. Although oat protein concentrates and isolates have relatively high fat-binding capacities, the nitrogen solubility is poor at neutral and slightly acidic pH (Ma, 1983a,b). One approach to improve the nitrogen solubility and expand the range of functional properties offered by oat flours, concentrates, and isolates is enzyme modification.

Enzyme modification has been used extensively to improve the functional properties of proteins and to tailor the functionality of certain proteins to meet specific needs (Richardson, 1977). Proteolytic enzymes have been employed to solubilize plant proteins from various sources such as soy (Puski, 1975; Mohri and Matsushita, 1984), peanut (Beuchat et al., 1975), rapeseed (Hermansson et al., 1974), and oat (Ma, 1985).

Some of the most important protein functional properties are solubility, emulsification, gelation, water hydration and fat-binding capacities, viscosity, heat coagulability, and foaming. Proteolysis alters these properties by changing the molecular size, conformation, solubility, and strength of the inter- and intramolecular bonds of the protein molecules (Kinsella, 1976; Ryan, 1977).

Uncontrolled or prolonged proteolysis, however, most often results in the formation of bitter peptides and products with undesirable functional properties. The proteolysis reaction must, therefore, be carefully monitored and controlled in order to manufacture ingredients with the desired "degree of hydrolysis" (DH), as this parameter is the key to product quality (Adler-Nissen, 1982). The parameter DH is defined as the percentage of peptide bonds cleaved (Adler-Nissen, 1976). When the hydrolysis is carried out at pH 7 or above, DH can be conveniently monitored by the pH-stat technique: the amount of base, which is added to the reaction mixture to keep the pH

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constant, is proportional to DH (Adler-Nissen, 1982).

The objective of the present investigation was to determine the effect of partial proteolysis on some functional and nutritional properties of high-protein oat flour using Alcalase and Neutrase. Functional properties of enzyme-modified oat flours were compared with control oat flours and commercial whole wheat and defatted soy flours.

MATERIALS AND METHODS

Materials. Oat groats (*Avena sativa* L., variety Hinoat) were provided by Dr. V. D. Burrows, Ottawa Research Station, Agriculture Canada, Ottawa, Ontario. The groats were ground in a hammer mill (Smalley Manufacturing Co.) to pass through a screen with 3-mm holes. The enzymes used for hydrolyses were Alcalase [0.6 AU/g (Au = Anson unit)], a liquid food-grade preparation of subtilisin Carlsberg (*Bacillus licheniformis*), and Neutrase (0.5 AU/g), a liquid food-grade preparation of *Bacillus subtillis* neutral protease, both from Novo Industri A/S Bagsvaerd, Denmark. In addition to protease activity, Neutrase also contains β-glucanase activity. The crystalline enzyme reference standards of Alcalase (1.48 AU/g) and Neutrase (1.53 AU/g) were provided by Novo Laboratories Inc., Wilton, CT. All other chemicals were reagent-grade laboratory chemicals. Whole wheat flour was purchased from a local grocery store and ground in a cyclone mill (Cyclotec 1093 sample mill, Höganas, Sweden) to pass through a screen with 1-mm holes. Soy flour (SoyFluff) was provided by Central Soya Chemurgy Division, Fort Wayne, IN; it is used as a functional ingredient in bakery mixes and milk replacers.

Hydrolysis Curves. The proteolytic activity of the enzyme preparations was compared with crystalline enzyme reference standards of Alcalase and Neutrase with hemoglobin as substrate (Novo Industri, 1978). The hydrolysis curves (degree of hydrolysis/time relationship) for oat flour hydrolyzed with Alcalase (0.61 AU/g) and Neutrase (0.50 AU/g), respectively, were obtained by the pH-stat technique, using the following hydrolysis parameters: substrate (S) = 12.5% oat flour [3% protein (N × 6.25)]; enzyme to substrate ratio (E/S) = 1.0 and 2.0% (enzyme to protein ratio = 3.5 and 8.8%); pH 8.0 (Alcalase) and pH 7.0 (Neutrase); temperature (T) = 50 °C; mass of protein (MP) = 22.8 g. The hydrolyses were carried out for 3 h in a 1-L reaction vessel, equipped with a stirrer, a thermometer, a pH electrode, and a delivery system for the admission of base. The hydrolysis curves were obtained by plotting DH as a function of time. DH was calculated from the base consumption during hydrolysis, following the equation as described previously (Adler-Nissen, 1982).

The DH was calculated with an average pK value of 7.1 at 50 °C (Steinhardt and Beychok, 1964). For further details of the pH-stat technique refer to Jacobsen et al. (1957). Corrected h_{tot} (total number of peptide bonds in the protein substrate, mequiv/g of protein) was calculated by taking into consideration the nonprotein nitrogen compounds present in the raw material. In the case of oat flour, h_{tot}(cor) was 7.15 mequiv/g.

Samples were drawn from the Neutrase/oat flour reaction mixture at regular intervals to determine the exact DH and TCA-soluble nitrogen (trichloroacetic acid). Exact DH was determined by the trinitrobenzenesulfonic acid (TNBS) method (Adler-Nissen, 1979). The percent TCA-soluble nitrogen was determined by the method described by Adler-Nissen (1982), with a slight modification in the concentration of TCA (20%). In the calculations above, corrections were made for the gradual, slight decrease in the amount of hydrolysis mixture caused by the drawing of samples.

Production of Enzyme-Modified Oat Flours. Four enzyme-modified oat flours were produced on a pilot-plant scale with use of Alcalase and Neutrase to cover a range of DH values, and production was monitored by the pH-stat technique. In addition, two controls were also prepared. The oat slurry was passed through a Comitrol Grinder equipped with a microcut head (Urshel Laboratories Inc., Valparaiso, IN) before the pH was adjusted for enzyme hydrolysis. All processing conditions were identical with those mentioned previously, except that when the calculated amount of base had been consumed, the enzyme inactivation was performed by heating at 75 °C for 5 min (after adjusting the pH to 7.0 in the case of Alcalase), followed by freeze-drying. The following samples were prepared: (1) untreated control (UC), no heat treatment, adjusted to pH 7.0 (no enzyme); (2) treated control (TC), adjusted to pH 7.0 and heated at 75 °C for 5 min (no enzyme); (3) Alcalase treated (A1), 1.0% E/S and to reach a preset DH value of 3; (4) Alcalase treated (A2), 2.0% E/S and to reach a preset DH value of 6; (5) Neutrase treated (N1), 1.0% E/S and to reach a preset DH value of 4; (6) Neutrase treated (N2), 2.0% E/S and to reach a preset DH value of 8.

The controls and enzyme-treated oat flours were analyzed for content of free amino groups by the TNBS method with L-leucine as the standard, to determine the exact DH values (Adler-Nissen, 1979).

Size Exclusion by HPLC. The molecular weight distribution profiles of the water-soluble proteins from enzyme-modified as well as unmodified oat flours were determined by HPLC on a TSK-2000SW column according to the method described by Vijayalakshmi et al. (1986) using a methanol-modified mobile phase. TSK-2000SW is useful primarily for peptides and low molecular weight proteins.

Chemical Analyses. Samples were analyzed for protein content (N × 6.25) and TCA-soluble nitrogen by the Kjeldahl method with a Kjel-Foss automatic 16210 apparatus (A/S N. Foss Electric, Hillrød, Denmark), lipid content with a Soxtec system HT6 (Tecator AB, Höganas, Sweden) using diethyl ether as extracting solvent, moisture and ash contents by AACC (1971), and neutral detergent fiber (NDF) by the method of Wainman et al. (1981) after the samples were pretreated with α-amylase. Chromic oxide in the feces was measured according to the method of Christian and Coup (1954).

The amino acid composition of the samples was determined with an amino acid analyzer (LKB Biochrom Ltd, Cambridge, England) equipped with an HP 3392A integrator, following hydrolysis with 6 N HCl (powder:acid = 1:1000) under nitrogen atmosphere at 110 °C for 24 h.

Functional Properties. Functional properties were determined on samples of product on an as is basis, except for emulsifying properties (adjusted to an equal protein basis). Nitrogen solubility was determined in a 1% (w/w) dispersion in distilled water. The pH of the sample dispersion was adjusted with 0.1 N HCl to values between 3.0 and 7.0, and the sample was magnetically stirred at room temperature for 45 min. The pH was readjusted if necessary, and after centrifugation at 10000g for 30 min, the supernatant was analyzed for nitrogen by the Kjeldahl method.

Water hydration capacity (WHC) was determined according to the method of Quinn and Paton (1979). The method for determining fat-binding capacity (FBC) was that described by Lin et al. (1974).

Emulsification activity and stability were measured according to the method of Yasumatsu et al. (1972). Heat

Table I. Proximate and Chemical Composition of Enzyme-Modified Flours in Comparison to Control Oat Flours and Commercial Whole Wheat and Soy Flours (Percent Dry-Weight Basis)

products	protein[a]	fat	ash	NDF[b]
untreated control (UC)	24.9	3.1	2.3	4.6
treated control (TC)	25.1	2.8	2.7	4.4
Alcalase treated (A1)	24.8	2.9	3.3	4.0
Alcalase treated (A2)	24.3	3.0	3.7	4.2
Neutrase treated (N1)	24.8	2.6	2.9	3.9
Neutrase treated (N2)	24.6	3.1	3.0	4.2
whole wheat	18.4	1.6	1.5	7.7
soy (defatted)	57.3	0.7	6.9	6.7

[a] N × 6.25. [b] Neutral detergent fiber.

coagulability (HC) was determined according to the method of Balmaceda et al. (1976) as described in Voutsinas et al. (1983).

Nutritional Evaluation. Diets were formulated to contain 1.6% nitrogen, 10% corn oil, 4% mineral mix (USP XVII, Teklad Test Diets, Madison, WI), and 1% vitamin fortification mix (Teklad). All the diets were made isocaloric on a digestible energy (DE) basis; corn starch was used to adjust DE to 3.92 kcal/g, and Celufil, a nonnutritive fiber (United States Biochemical Corp. Cleveland, OH), was used as a filler and given a DE value of zero.

The feeding study was done with Sprague–Dawley male rats, which were allotted at random in groups of 10 rats with approximately equal mean weights (58.6 ± 0.4 g) and assigned at random to the oat diets or casein diet for a 4-week test period. Throughout the study the rats were housed in individual stainless-steel wire screen cages equipped to collect feces in a room with constant humidity and temperature (49 ± 1% RH, 23 ± 1 °C) and a 12-h light and dark cycle. Food and water were offered ad libitum.

During a 4-week period, feed intake and weight gain were registered. The protein efficiency ratio (PER) was calculated as the weight gained per unit weight of protein consumed.

Feces were collected from days 14 to 21 to determine the apparent digestibility coefficient (ADC) of nitrogen. Chromic oxide (0.1%) was added to the diets to serve as an external indicator of digestibility.

Statistical Analysis. The results were subjected to analysis of variance (general linear models procedure), and treatment means were separated by Duncan's multiple-range test (Little and Hills, 1978).

Replications. All data on functional properties represent a mean value of at least duplicate analyses on each of the six oat flours and commercial whole wheat and soy flours.

RESULTS AND DISCUSSION

In this study, heat treatment was used to inactivate the enzymes. To differentiate between the effects of enzyme and heat treatment, two controls were used. The untreated control (UC) was the oat flour prepared without any heat treatment but adjusted to pH 7.0. The treated control (TC) was prepared exactly the same way as the enzyme-treated products, but without the addition of enzyme. The difference between the two controls indicates the effect of the heat treatment on the functional and nutritional properties.

Chemical Composition. Table I presents the proximate and chemical composition of the controls, enzyme-modified oat flours, and commercial whole wheat and soy flours. The protein, fat, and NDF were not markedly changed by enzyme modification, while the ash content was increased in enzyme-modified flours due to a higher content of salt as a result of pH adjustment to achieve the desired DH values. Whole wheat flour had lower protein, fat, and ash contents and higher NDF content than oat flours. Soy flour had the highest protein and ash contents when compared to oat and whole wheat flours.

Table II presents the amino acid composition of control and enzyme-modified flours. Enzyme modification or heat treatment did not alter the amino acid composition of oat flours.

Hydrolysis Curves. The four hydrolysis curves obtained by hydrolysis of oat flour with Alcalase and Neutrase (E/S, 1.0 and 2.0%) are shown in Figure 1. The difference in kinetics between the two enzymes is apparent from the difference in the slope of the curves at a high E/S ratio. At a high E/S ratio, Alcalase is seen to be the more aggressive enzyme toward oat protein, even when its 20% higher activity is taken into account. The hydrolysis curves also serve to indicate the hydrolysis time needed to reach a desired DH value.

DH measured by the pH-stat technique slightly overestimates the DH value when compared to the TNBS method (Figure 2). This could be due to inhomogeneities in the hydrolysis mixture, delays in the response from the

Table II. Amino Acid Composition of Control Oat Flours, Enzyme-Modified Oat Flours, and Casein (Gram/16 g of N)

amino acid	UC	TC	A1	A2	N1	N2	casein
essential							
arginine	6.5	5.9	6.1	6.4	6.2	6.3	3.5
histidine	2.1	2.3	2.1	2.1	2.1	2.1	2.6
isoleucine	3.5	3.9	3.8	3.6	3.6	3.6	4.5
leucine	6.4	6.9	6.9	6.6	6.7	6.6	7.7
lysine	3.5	3.8	3.5	3.5	3.6	3.6	6.5
methionine[a]	1.2	1.0	1.0	1.0	1.0	1.0	2.6
phenylalanine[b]	4.6	5.3	5.0	4.8	4.9	4.8	4.6
threonine	2.9	3.2	3.1	2.9	3.0	3.1	3.7
valine	5.6	6.0	5.7	5.7	5.9	5.6	5.4
nonessential							
alanine	3.9	4.2	4.2	4.0	4.1	4.0	2.5
aspartic acid	7.2	8.2	7.9	7.2	7.9	7.8	6.0
half-cystine	3.4	3.1	2.9	2.8	3.3	3.1	0.8
glutamic acid	19.0	20.5	20.2	19.4	19.6	19.6	16.4
glycine	4.0	4.4	4.3	4.1	4.2	4.1	1.6
proline	3.3	3.6	3.3	3.3	3.1	3.3	9.2
serine	4.2	4.6	4.5	4.2	4.4	4.4	4.8
tyrosine	3.5	3.9	3.6	3.5	3.6	3.4	5.2
total	84.8	90.8	88.1	85.1	87.2	86.4	87.6
ammonia	14.1	15.9	14.6	14.2	14.7	14.5	9.0

[a] One-third to half can be supplied by cystine. [b] One-third to half can be supplied by tyrosine.

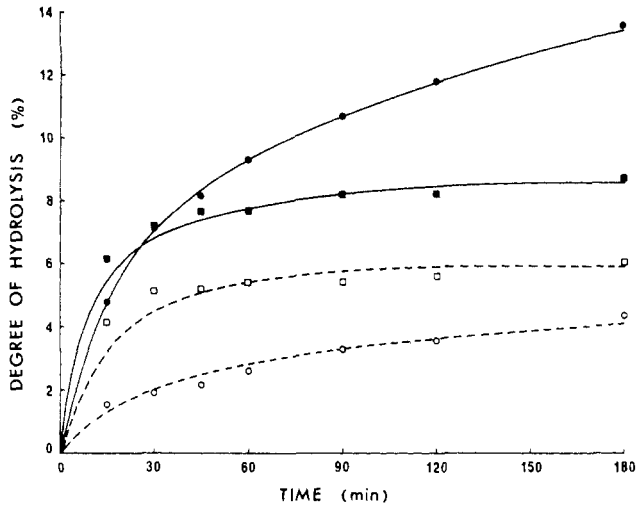

Figure 1. Hydrolysis curves obtained by hydrolysis of oat flour with Alcalase and Neutrase (E/S, 1.0% and 2.0%): (○) Alcalase 1.0%; (●) Alcalase 2.0%; (□) Neutrase 1.0%; (■) Neutrase 2.0%.

pH electrode, and increasing buffering capacity of the reaction mixture; but nevertheless, the technique yielded an approximate indication of the DH (Adler-Nissen, 1982).

A number of researchers have used percent TCA-soluble nitrogen formed during hydrolysis as a measure of proteolysis (Nakai et al., 1980; Miller and Groninger, 1976). In the present investigation, percent TCA-soluble nitrogen formed during proteolysis was compared to the exact DH (%) measured by the TNBS method (Figure 2). Although percent TCA-soluble nitrogen is not a precise measure of the peptide bonds cleaved, the data correlated well with the DH (%) for the given enzyme/substrate system. The correlation coefficients obtained with these two parameters for the two enzymes Alcalase and Neutrase were 0.96 and 0.98 (E/S 1.0 and 2.0%) and 0.94 and 0.96 (E/S 1.0 and 2.0%), respectively.

Extent of Proteolysis. Table III gives the DH values preset from the base consumption for the pilot-plant-scale production of enzyme-modified flours. However, the exact DH values obtained by the TNBS method on freeze-dried products varied significantly from the preset DH values as given in Table III. This increase could be partly due to the enzyme inactivation procedure. Since the reaction mixture was quite viscous, the time taken to increase the temperature to 75 °C was long enough for further hydrolysis. This is supported by the DH value obtained for the TC when compared to the UC (Table III).

Table III. Comparison of Methods To Measure the Extent of Proteolysis

products	DH, % pH-stat	DH, % TNBS[a]	TCA-sol N,[a] %
UC	0.0[b]	0.0[b]	10.5
TC	0.0[b]	1.2	10.4
A1	3.0	3.8	25.9
A2	6.0	7.6	39.1
N1	4.0	5.9	27.2
N2	8.0	10.4	34.7

[a] Freeze-dried samples analyzed. [b] DH given a zero value.

TCA-soluble nitrogen was also determined on the freeze-dried products to see whether it changes with DH. Since the DH value obtained for TC is greater than that for UC, it would be expected to have a higher TCA-soluble nitrogen. But heat treatment did not alter the TCA-soluble nitrogen when compared to the untreated control. This indicates that the intermediate-size peptides formed during the heat treatment without the presence of enzyme are precipitable with TCA. This will be further elaborated in the following section. From the results presented in Table III, it is obvious that TCA-soluble nitrogen is not the best measure of proteolysis, even though it is widely used by many researchers, because the extent of proteolysis by different enzymes on the same substrate cannot be compared due to differences in enzyme specificities and chemical reactions taking place during the heat inactivation procedure. This statement can be further elaborated by comparing A2 and N2 for DH and TCA-soluble nitrogen. According to the TCA-soluble nitrogen data, A2 is more hydrolyzed than N2. In contrast, the TNBS method indicates that N2 is more hydrolyzed than A2. This illustrates that Neutrase has a tendency to hydrolyze oat proteins to more intermediate peptides that are precipitable by TCA than does Alcalase. Alcalase probably hydrolyzes proteins to small peptides and even to free amino acids, which are soluble in TCA, and thus gives a higher TCA-soluble nitrogen value.

Size Exclusion by HPLC. The molecular weight distribution profiles of the water-soluble proteins (pH 7.0) from enzyme-modified as well as control oat flours are shown in Figure 3. Heat treatment of the control flour

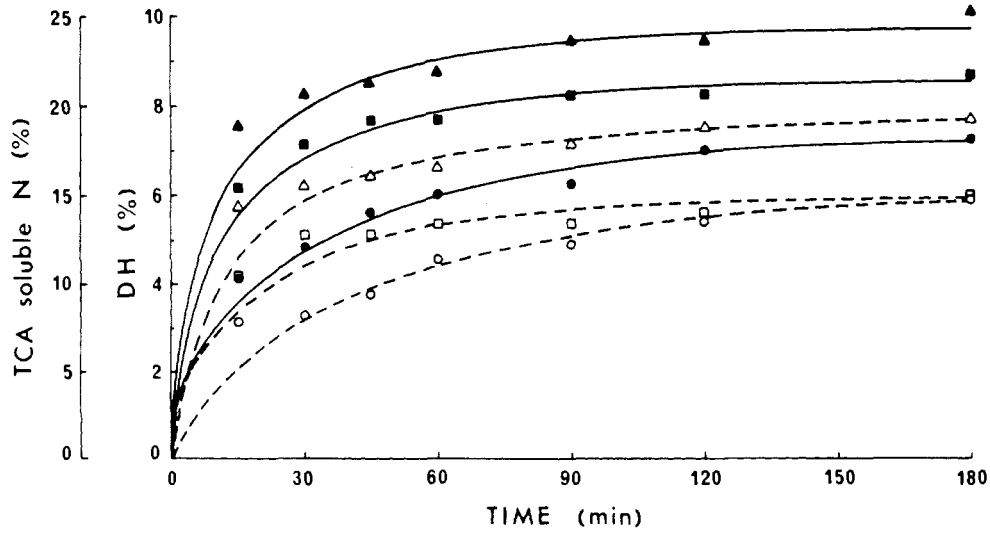

Figure 2. Comparison of DH by the pH-stat technique and the TNBS method and the extent of proteolysis as measured by TCA-soluble nitrogen in the Neutrase/oat flour system with an E/S of 1.0% (- -) and 2.0% (—): pH-stat (□ and ■); TNBS (○ and ●); TCA-soluble nitrogen (△ and ▲). Open symbols are for 1.0% Neutrase, and closed symbols are for 2.0% Neutrase.

Table IV. Nitrogen Solubilities of Enzyme-Modified Oat Flours in Comparison to Control Oat Flours and Commercial Whole Wheat and Soy Flours

products	nitrogen solubility, %		
	pH 3.0	pH 5.0	pH 7.0
UC	19.9	16.6	16.6
TC	19.9	16.6	29.9
A1	36.8	33.5	38.5
A2	48.5	51.9	51.9
N1	39.6	38.0	38.0
N2	47.1	43.8	47.1
whole wheat	41.9	27.9	23.3
soy	48.7	8.7	56.0

produced an increase in the peak that corresponds to an 8200 molecular weight. This indicates that slight hydrolysis has taken place due to the heat treatment, as measured by DH (Table III). With both Alcalase and Neutrase, this peak disappeared almost completely. Since Alcalase and Neutrase hydrolyzed the polypeptides to simple peptides and amino acids, aggregation of the peptides was probably not possible with the heat treatment given to inactivate the enzymes. Both Alcalase- and Neutrase-modified flours has a negligible peak that corresponds to a 19 000–21 000 molecular weight. In contrast, Ma (1985) observed a large polydispersed component with an average molecular weight of about 18 000 by gel filtration. This difference can be attributed to the specificities of the different enzymes used in both studies.

The major peaks in Alcalase-hydrolyzed flours were those that, on the average, correspond to 925, 645, 395, and 230 molecular weights, and among those the most important peak is at 395 molecular weight, which may be a di- or tripeptide. It seems that this peptide is released from the insoluble portion of the proteins. Similarly, with Neutrase, on the average the major peaks were at 835, 565, 340, and 195 molecular weight. There are a number of peaks beyond the elution volume of 25 mL (Figure 3). These are nonproteinaceous substances coming out of the column beyond its inclusion limit.

Solubility. The solubilities of enzyme-modified oat flours in comparison to controls and commercial flours are given in Table IV. Minimum solubility for all the flours was near pH 5 except for wheat flour, which is near pH 6 as shown by Chang and Sosulski (1985). Heat treatment of the control flour did not affect the solubility around the isoelectric point (pH 5). But it is interesting to note that the nitrogen solubility has been almost doubled for TC at pH 7. This increase in solubility could be partly attributed to the 8200 molecular weight polypeptide, which is soluble in water, as discussed in the previous section (Figure 3). But Chang and Sosulski (1985) observed a very low solubility due to steam treatments used to inactivate lipase.

Enzyme-modified oat flours had 2–3 times greater solubilities at pH 5 than the controls. With increase in DH,

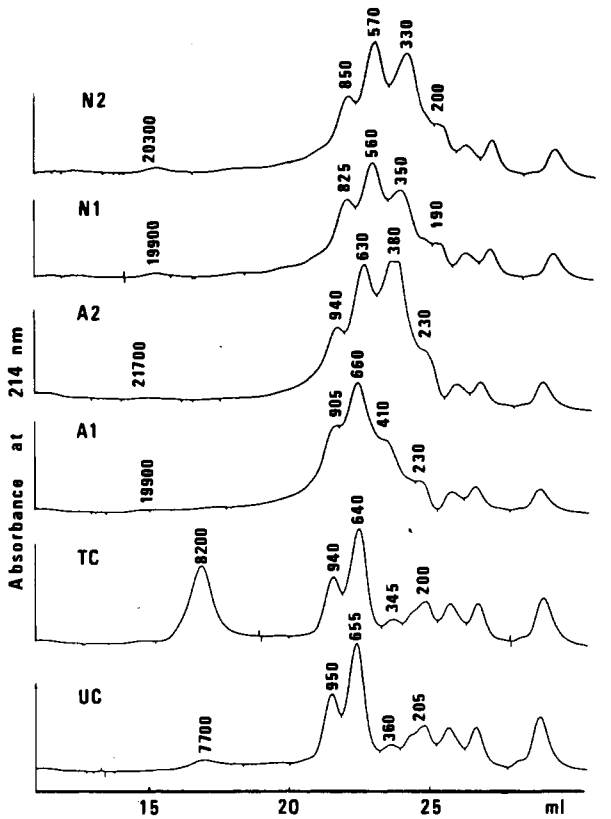

Figure 3. High-performance liquid chromatography of water-soluble proteins in controls as well as Alcalase- and Neutrase-treated oat flours. Molecular weights (daltons) are given in the figure. Key: UC, untreated control; TC, treated control, DH = 1.2%; Al, Alcalase treated, DH = 3.8%; A2, Alcalase treated, DH = 7.6%; N1, Neutrase treated, DH = 5.9%; N2, Neutrase treated, DH = 10.4%.

isoelectric solubility also increased, when a particular enzyme/substrate system was compared (Table IV). Soy flour had the least solubility at pH 5, but at pH 3 and 7 the solubilities were greater than those of the enzyme-modified oat flours. Proteolytic enzymes have been frequently used to enhance the solubility of plant protein products (Hermansson et al., 1974; Puski, 1975; Beuchat et al., 1975; Mohri and Matsushita, 1984; Ma, 1985). The increase in solubility is due to the reduction in the molecular size of the proteins, exposing more charged and polar groups to the surrounding water (Jones and Tung, 1983). The increased solubility would be advantageous in the utilization of oat flours in food applications, such as in ground meat emulsions, where the functionality of oat proteins may be high.

Water Hydration and Fat-Binding Capacities. The water hydration capacity of TC was greater than that of UC (Table V). This may be due to the effect of heat treatment on the solubility of TC at pH 7. Also, starch

Table V. Water Hydration and Fat-Binding Capacities, Emulsifying Properties, and Heat Coagulabilities of Enzyme-Modified Flours in Comparison to Control Oat Flours and Commercial Whole Wheat and Soy Flours

products	DH, %	WHC[a]	FBC[b]	EA,[c] %	ES,[d] %	heat coag, %
UC	0.0	1.4–1.5	57.4	60.0	62.8	12.6
TC	1.2	2.6–2.7	62.2	62.9	65.7	5.4
A1	3.8	2.8–2.9	58.9	63.8	56.3	0.0
A2	7.6	2.9–3.1	57.6	20.7	4.9	0.0
N1	5.9	3.0–3.2	47.7	61.7	60.3	0.5
N2	10.4	3.2–3.4	46.5	60.6	59.3	0.0
whole wheat flour		0.9–1.0	35.1	41.8	62.4	40.8
soy flour		2.2–2.3	38.5	60.3	60.3	0.2

[a] Gram of H_2O/gram of flour (water hydration capacity). [b] Gram of oil/100-g sample on the basis of 14% moisture (fat-binding capacity). [c] Emulsion activity. [d] Emulsion stability.

Table VI. Weight Gain, Feed Intake, PER, and Nitrogen ADC of Rats Fed Diets Containing 1.6% N[a]

diet	wt gain, g	feed intake, g	PER	N ADC,[b] %
UC	70.6[b]	321.8[b]	2.25[b]	81.2[b]
TC	74.4[b]	326.6[b]	2.20[b]	81.0[bc]
A1	76.1[b]	332.8[b]	2.19[b]	79.9[c]
A2	72.7[b]	313.7[b]	2.20[b]	78.3[d]
N1	73.6[b]	319.5[b]	2.19[b]	82.0[b]
N2	77.2[b]	329.8[b]	2.24[b]	81.4[b]
casein	130.6[a]	371.7[a]	3.35[a]	89.0[a]
CV, %	10.1	13.9	6.7	1.6

[a] Mean values were calculated on 10 rats. [b] Apparent digestibility coefficient. Means within a column followed by the same superscript letter are not significantly different ($p < 0.05$).

gelatinization may play a role in increasing the WHC of TC. WHC increased with an increase in DH, when a particular enzyme/substrate system was compared. Similar to solubility improvement, limited proteolysis may increase WHC by increased exposure of polar and ionic groups to an aqueous environment. Ma (1985) also observed a significant increase in WHC after proteolytic treatment of oat protein concentrate with trypsin, but the effect of heat treatment on the native protein concentrate was not given. A slight increase in water absorption was reported in rapeseed protein concentrates treated with pepsin and papain (Hermansson et al., 1974); and an increase in water-adsorbing capacity was observed for pepsin-, bromelain-, and trypsin-treated peanut flour (Beuchat et al., 1975). WHC values of whole wheat flour and soy flour were quite low compared to all the oat flours that were heat treated.

The fat-binding capacity of TC was higher than UC. Enzyme modification decreased the FBC of oat flours compared to TC. But all the oat flour products had greater FBC when compared to whole wheat or soy flours. The enzyme-modified products with higher WHC had lower FBC.

Emulsifying Properties. Emulsion activity (EA) and emulsion stability (ES) of TC were greater than for UC at pH 7.0 (Table V). This again can be explained by the increase in solubility at pH 7.0. A1 and N1 gave fairly good EA and ES. But with an increase in DH, EA and ES were decreased in A2 and N2. The decrease in EA and ES was much more pronounced in A2 than N2, even though DH was higher in N2. In comparison to soy flour, all the oat flours had good emulsifying properties, except A2. This illustrates that limited enzyme modification may not be detrimental to the emulsification properties of oat flours.

The results of this investigation also indicate that for good emulsifying properties the product should not contain a high quantity of small peptides and amino acids. According to Adler-Nissen and Olsen (1979), the optimal conditions for emulsification seem to be at a DH value where the hydrolyzate consists of approximately equal amounts of soluble and insoluble material. They also indicated that the molecules should not be too small, and a certain solubility seems to be necessary for achieving the optimal emulsifying properties.

Heat Coagulation. Heat coagulation was determined as the percentage loss in solubility of a protein after heating (98–100 °C, 30 min). As seen in Table V, A1, A2, and N2 do not show any coagulation on heating, whereas N1 and soy flour showed slight coagulation on heating. Among the controls and whole wheat flour, whole wheat flour exhibited the greatest coagulability. From the results obtained from oat flours, it can be stated that heat coagulation decreases as the extent of proteolysis increases.

Even though soy flour had a high protein content, the functional properties of oat flours were better than for soy flour under the conditions tested.

Nutritional Evaluation of Controls and Enzyme-Modified Oat Flours. Weight gain, feed intake, PER, and nitrogen ADC for the experimental diets containing oat flours are summarized in Table VI. Significant differences were not observed among the oat flour diets for weight gain, feed intake, and PER. Casein had the highest weight gain, feed intake, PER, and nitrogen ADC.

Although significant differences ($p < 0.05$) were observed in nitrogen ADC values among oat flours, the differences were small. Alcalase-treated oat flours had lower nitrogen ADC values compared to Neutrase-treated oat flours. Heat treatment did not alter the nitrogen ADC in TC when compared to UC. But Sosulski et al. (1985) observed that when oat flakes were cooked, the protein ADC was slightly decreased. In general, the 10% lower digestibility of the oat nitrogen should account, in part, for the lower PER values relative to casein, the differences in lysine content being the major factor (Table II).

Although Alcalase and Neutrase did not alter significantly the nutritional properties of oat flours, the alteration of the physicochemical properties of oat protein by enzyme treatments can significantly improve solubility and water hydration capacity with good emulsification properties. In order to use the enzyme-modified oat flours in baked products, including intermediate-moisture foods, these flours have to be tested in food systems to achieve the desired end result. Beuchat et al. (1975) reported that defatted peanut flour hydrolyzed with pepsin, bromelain, and trypsin exhibited increased nitrogen solubility and water adsorption when exposed to various relative humidities, in comparison to the untreated control. It was suggested that the increased water-adsorbing capacity of enzyme-treated peanut flour may have important implications in the formulation of intermediate-moisture foods. This was further illustrated by incorporating the enzyme-treated defatted peanut flour in the preparation of cookies (Beuchat, 1977). Incorporation of enzyme-treated defatted peanut flour improved dough handling, top grain, appearance, increased the diameter, and reduced the height of the cookies. Improvement of oat protein functionality by physical, chemical, or enzymatic modifications could help to promote the utilization of this nutritionally valuable crop in a wide range of food products.

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Effects of Deamidation with Chymotrypsin at pH 10 on the Functional Properties of Proteins

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The effect of deamidation on the functional properties of proteins was investigated by proteolytic deamidation in an controlled condition. The emulsifying and foaming properties of egg white proteins (ovalbumin, lysozyme) and soy proteins (7S globulin, 11S globulin) were improved by the enzymatic deamidation with chymotrypsin at pH 10. About 20% of the asparaginyl or glutaminyl residues in these proteins was deamidated by the treatment with chymotrypsin without proteolysis or with a slight proteolysis. The surface hydrophobicity and the flexibility detected by digestion velocity of deamidated proteins increased, resulting in the improvement of the foaming and emulsifying properties. Thus, proteolytic deamidation was proposed to be a useful method for the improvement of functional properties of food proteins.

Various enzymatic and chemical modifications of food proteins have been attempted to improve the functional properties. These will offer the possibility for food application of unutilized protein sources, if the safety for food use is solved. We have noted and studied the deamidation of proteins as the most promising method to improve the solubility and surface properties (Matsudomi et al., 1982, 1985 a,b). The deamidation of proteins is expected to cause increases in the solubility and flexibility due to an increase in the negative charge of proteins.

The mild acid treatment to deamidate proteins was very effective to improve their functional properties. However, the mild acid treatment causes deamidation of asparaginyl and glutaminyl residues in proteins together with not only denaturation but also the cleavate of peptide bond. Therefore, a mild deamidation is desirable to investigate the effect of deamidation on the functional properties of food proteins.

In a previous paper (Kato et al., 1987), we reported that proteases have the deamidation activity of proteins in an alkaline pH region where proteolytic activity was at the minimum. Especially, ovalbumin and lysozyme were

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