

## Protein Composition and Functionality of High-Protein Oat Flour Derived from Integrated Starch-Ethanol Process

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### ABSTRACT

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High-protein oat flour containing ~50% protein was derived as a by-product from an oat starch process integrated with ethanol production. The protein composition of the by-product was characterized in comparison to that of oat groats, the raw material of the process, to evaluate alterations occurring during processing. The proteins were fractionated according to their solubility. Amino acids of the fractions were analyzed as 9-fluorenylmethyl chloroformate derivatives by high-performance liquid chromatography, and molecular weight distribution was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. In addition, functionality (in terms of protein solubility, water absorption, and emulsifying capacity) of the high-protein oat flour was compared with that of a commercial soy concentrate at a pH range of 3.0-7.0.

Processing caused changes in the proportion of salt-soluble and alkali-soluble fractions, but not in the proportion of water-soluble or alcohol-soluble fractions. In oat groats, the alkali-soluble fraction accounted for 52% of the total nitrogen, and the salt-soluble fraction accounted for 28%. In high-protein oat flour, almost 80% of nitrogen was collected in the alkali-soluble fraction; the salt-soluble fraction accounted for <3% of the total nitrogen. The amino acid composition and molecular weight distribution of the protein fractions were not severely altered during processing. Solubility and emulsifying properties of the high-protein oat flour were competitive with those of the soy concentrate. Commercial soy concentrate, however, absorbed three to four times more water than did the oat flour over the pH range studied.

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Oat production accounts for ~20% of the total cultivated area in Finland (NBA 1992). During 1981-1990, the annual oat yield in Finland was approximately  $12 \times 10^8$  kg, which accounted for

2.5% of world oat production (USDA 1984, 1987, 1990). For the most part, oats are used as livestock feed. Recently, however, the nutritionally favorable attributes of oats, such as high protein, fat, and fiber content, have aroused considerable interest in increasing utilization of oats for human consumption (McMullen 1991).

Protein concentration in oat groats is high, typically ranging

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between 15–20% (McMullen 1991). Moreover, the nutritional quality of oat protein is fairly good. Lysine, methionine, and threonine are the limiting amino acids, although the lysine content in oats is somewhat higher than that in other cereals. The amino acid pattern of oats remains quite constant over a broad range of protein levels (Peterson 1976). Thus, an increase in protein content does not necessarily cause the same distinctive decline in lysine content in oats as it does, for instance, in wheat (Peterson and Brinegar 1986). Oats have a higher protein efficiency ratio (1.8–1.9) than does wheat, rye, or barley (Howe et al 1965, Lockhart and Hurt 1986). Utilization of proteins in food systems depends largely on their physicochemical properties, often defined as their functionality. Some oat protein concentrates and isolates have been prepared (Wu et al 1973, 1977; Cluskey et al 1978; Ma 1983a,b; Ma and Harwalkar 1984). Their functionality, especially their binding, emulsifying, and foaming properties, were promising for food-processing purposes.

The major alcohol producer in Finland, Alko Ltd., uses oats in a starch process integrated with ethanol production. This process consists of grinding steamed oat groats into a meal, homogenizing the meal with water, and screening the homogenate to separate dietary fiber. The remaining oat slurry is fractionated into starch and protein fractions with separators. The starch fraction is purified by hydrocyclones. The protein fraction is dewatered by a decanter, washed with water to remove possible off-flavors, and spray-dried. The result is referred to as *high-protein oat flour*. This process distributes the total protein from the groat into: hulls (10%), dietary fiber (32%), starch (1%), and high-protein fraction (55%).

The dietary fiber fraction derived from this process has been used successfully to increase the nutritional value of cereal products (Anonymous 1992). The present study was conducted to evaluate the possibility of using the high-protein oat flour as a food ingredient. The effect of processing on oat proteins was studied by comparing the protein composition of the high-protein oat flour to that of oat groats, which were used as raw material in the pilot starch process. The chemical composition was characterized by fractionation of proteins according to their solubility and by determining the amino acid composition and molecular weight distribution of these protein fractions. Functionality of the high-protein oat flour was determined in terms of solubility, water absorption, and emulsifying properties.

## MATERIALS AND METHODS

The materials were derived from the integrated starch-ethanol pilot process of Alko Ltd. (Rajamäki Factories, Finland). A wet protein fraction from the starch process was decanted to obtain a slurry containing ~25% solids. To remove possible off-flavors, the proteinaceous slurry was washed with cold tap water in two steps. First, 0.2 m<sup>3</sup> of the slurry was mixed with an equal amount of water and the mixture was separated (West Falia separator, SB7 36-076, Germany). The washing procedure was continued using the same separator with a steady feed of cold water. The total volume of water used for washing procedures was twice the volume of the proteinaceous wet slurry. After being washed and separated, the slurry was spray-dried to obtain high-protein oat flour.

For evaluating the effect of washing procedures on the proteinaceous fraction, three additional samples were taken during washing: 1) unwashed slurry, 2) slurry washed with water in ratio of 1:1, and 3) washed slurry before spray-drying. These samples were freeze-dried and analyzed for chemical composition and solubility fractions. After they were dried, all samples were stored at room temperature.

The steamed oat groats were a mixture of different oat feed varieties cultivated in Finland. The groats used for analyses were ground with a Retch-mill (Type ZM 1, Germany), using a 1-mm screen. The protein, ash, and fat content of the samples were determined using AOAC (1984) methods. The starch content was determined using an enzymatic method of Boehringer Mannheim (catalog 207748). The conversion factor used in the protein calculations was 5.83.

## Protein Fractionation

Samples (10 g) were extracted with 100 ml of solvent in a 500-ml polypropylene centrifuge bottle equipped with a magnetic stirrer. The protein extracts were separated by centrifuging at 12,800 × *g* for 25 min at the temperature of the extraction. Each extraction was repeated three times, and the supernatants were combined. The extraction procedure was chosen according to the corresponding work for characterizing barley protein (Linko et al 1989), and according to the works of Wu et al (1972) and Robert et al (1983a,b) for characterizing oat proteins.

The extraction procedure (in three replicates) consisted of the following steps: 1) defatting the samples with 1-butanol; 2) extracting salt-soluble proteins with 1.0M NaCl; 3) dialyzing NaCl extracts and centrifuging to collect the supernatant (containing albumins and nonprotein nitrogen [NPN] compounds) and precipitated proteins, which represent globulins; 4) extraction of alcohol-soluble proteins, regarded as prolamins, with 55% (v/v) 2-propanol containing 1% 2-mercaptoethanol; 5) fractionation of 2-propanol extracts into water-soluble and water-insoluble prolamins by evaporating alcohol from the extract with a rotary evaporator, and by subsequent dialysis and centrifugation; 6) extraction of alkali-soluble proteins, regarded as glutelins, with 0.05M borate buffer (pH 10) containing 0.5% (w/v) sodium dodecyl sulfate (SDS). All the protein fractions were dialyzed and freeze-dried. The times and temperatures used in extractions and dialysis were as described by Linko et al (1989).

## Total Nitrogen, Protein, and NPN Determination

The nitrogen content of high-protein oat flour, oat groats, freeze-dried samples from three washing steps, protein extracts, and insoluble residues were determined by the Kjeldahl method. The protein content of freeze-dried extracts was analyzed using the method of Lowry et al (1951) for estimating the amount of sample required for protein hydrolysis and gel electrophoresis. The amount of NPN in NaCl extracts was determined after precipitating the proteins with trichloroacetic acid as described by Linko et al (1989).

## Amino Acid Analysis

Samples containing 5 mg of protein were hydrolyzed with 6M HCl under nitrogen in sealed tubes at 110°C for 24 hr. The amino acids were determined as 9-fluorenylmethyl chloroformate derivatives (Einarsson et al 1983). The high-performance liquid chromatography system (Shimadzu, Kyoto, Japan) that was used included two pumps (LC-6A), a solvent delivering system (SLC-6A), and an integrator (C-R3A). Amino acids were separated with a reversed-phase C<sub>8</sub> column (Superspher 60 RP-8, E. Merck, Darmstadt, Germany), which was placed in a gas-chromatograph oven (Shimadzu, GC-8A) to maintain the temperature at 45°C. Eluted derivatives were detected with a fluorescence detector (Shimadzu RF-530) at an emission wavelength of 310 nm and at an excitation wavelength of 260 nm. The solvents consisted of Na-acetate buffer (50 mM, pH 4.2), tetrahydrofuran, and acetonitrile at the ratio of 795:5:200 (solvent A), and acetonitrile and Na-acetate buffer at the ratio of 8:2 (solvent B). Amino acid derivatives were eluted at 1.2 ml/min with a linear gradient from 0 to 45% of solvent B over 40 min. At 42 min, the proportion of solvent B was increased to 100%, where it was held for 10 min and changed back to the initial 0% in 8 min. The injection volume of the samples was 5 µl. Taurine and L-norvaline were used as internal standards. A mixture of amino acid standards (Sigma AA-S-18, St. Louis, MO) was used for amino acid identification and quantitation.

## SDS-Polyacrylamide Gel Electrophoresis

Molecular weights of extracted proteins were determined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using a PhastGel system (Pharmacia, Sweden) with a 13-mm stacking gel zone (4.5% T, 3% C) and a 32-mm, continuous, 8–25% polyacrylamide gradient separating gel zone with 2% cross-linking. The buffer system was 0.112M acetate, 0.112M Tris (pH 6.4) in the gels and 0.20M tricine, 0.20M Tris, 0.55% SDS (pH 7.5) in the buffer strips. The samples of extracted protein fractions

were dissolved in sample buffer (10 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid, pH 8.0, 2.5% SDS, 5.0% 2-mercaptoethanol, 0.01% bromphenol blue) and heated at 100°C for 5 min. After centrifugation, 1 µl of sample containing 250–2,000 ng of protein was applied to the gels. The samples were run and stained using PhastGel separation and development units. The running conditions for one gel were programmed to 250 V, 10.0 mA, 3.0 W, 15°C, 65 Vh. Gels were stained at 50°C for 8 min with a solution of 0.1% Coomassie Blue R350 in 30% methanol and 10% acetic acid. The gels were destained with a mixture of methanol, acetic acid, and distilled water (3:1:6) for 23 min, after which they were treated with a preserving solution (5% glycerol, 10% acetic acid) for 5 min. The molecular weights of the extracted protein components were estimated by comparison with mobilities of molecular weight standards (catalog 17-0446-01, Pharmacia, Sweden).

### Functional Properties

The functionality (in terms of protein solubility, water absorption, and emulsifying capacity) of high-protein oat flour was compared to that of a commercial soy concentrate (Danpro S, Aarhus Oliefabrik A/S, Denmark). The methods of Morr et al (1985) and Sathé and Salunkhe (1981) were used for determining the protein solubility and water absorption at pH 3.0–7.0, respectively, as described by Lapveteläinen et al (1991). For solubility determinations, dispersions were mixed for 2 hr using a Gallenkamp shaker (200 rpm) before centrifugation. The emulsifying capacity was determined at pH 3.0, 5.0, and 7.0 according to the method of Vuilleumard et al (1990). A 0.075-g sample was dissolved in 50 ml of distilled water. The pH was adjusted during mixing using a magnetic stirrer for 30 min. Mixing of the solution continued using an Ultra-Turrax T25 homogenizer with an S25N 18G generator (Janke & Kunkel, GmbH & Co, KG, Stauffer, Germany) as a soy oil (Öljynpuristamo Oy, Finland) was added continuously at a rate of 25 ml/min from a funnel into the stirred mixture. The emulsifying capacity was determined by the amount of oil added at the inversion point using electrical resistance as detected by two electrodes in the bottom of the glass vessel that were connected to a multimeter (Normameter GWO 20). Mixing speed was set at 15,000 rpm at the beginning of homogenization, and then increased to 20,400 rpm after ~60 ml of oil had been added. A blank determination (the inversion point of oil added to water without a sample) was also made at each pH value. The emulsifying capacity was expressed as the difference per gram of sample.

## RESULTS AND DISCUSSION

The oat groats contained 16.8% protein, 7.1% fat, 2.1% ash, and 48.4% starch, dmb. Protein and fat contents were somewhat higher than those reported by Shukla (1975) and Lásztity (1983), but they are in accordance with the concentration ranges found for oats as reviewed by McMullen (1991). The protein content (51.6%) in high-protein oat flour was three times higher than

that in oat groats. The fat also concentrated considerably into this fraction during processing; fat content of oat flour was 28.0%, dmb. In high-protein oat flour, the ash content (1.3%) was one-half that in oat groats, and the starch content (11.2%) was one-fourth that in oat groats.

The composition of three freeze-dried slurry samples was studied to evaluate the impact of the washing procedures on the proteinaceous fraction. The starch content of this fraction (11.2%, dmb) did not change during washing. Protein and fat of the slurry concentrated slightly during washing procedures. The protein contents of the samples were: 48.7%, unwashed sample; 48.9%, sample washed (1:1) in water; and 50.9%, sample washed with a steady feed of water. The fat contents of the corresponding samples were 26.7, 28.3, and 29.2%, respectively. The washing procedures removed half of the minerals from the proteinaceous slurry. The ash content in the unwashed sample was 2.2%; it was 1.3% in the washed sample before spray-drying.

### Fractionation of Proteins According to Solubility

The nitrogen content of oat groats, high-protein oat flour, and the extracted fractions is shown in Table I. The amount of nitrogen in each fraction is also expressed as a percentage of the total nitrogen in the original sample. Processing mainly influenced the proportions of the salt-soluble and alkali-soluble fractions of oats. The percentage of salt-soluble nitrogen in oat groats was 27.5%; in the high-protein oat flour, it had decreased to 2.9%. In the groats, the majority (74%) of salt-soluble fraction consisted of globulins. The proportion of albumins and globulins for high-protein oat flour is given in Table I as a calculated difference (salt-soluble nitrogen – NPN), which accounted for ~35% of its salt-soluble fraction. Alkali-soluble proteins were 51.8% of total nitrogen in oat groats and 78.8% of total nitrogen in high-protein oat flour. The amount of alcohol-soluble nitrogen was similar in both samples: 11.0% in high-protein oat flour and 11.6% in oat groats.

NPN compounds were 1.7 mg/g in high-protein oat flour and 1.3 mg/g in oat groats. In the work on protein-rich by-product derived from the corresponding barley starch process, Linko et al (1989) found that the amount of NPN compounds was considerably higher in high-protein barley flour than it was in barley grains. The increase in NPN compounds in barley was caused by endogenous proteases during processing conditions suitable for proteolytic activity. In the oat starch process, some degradation of the proteins may also occur, but not to the same extent as it did in the barley starch process. In fact, the proportion of NPN compounds decreased; they accounted for 4.6% of total nitrogen in oat groats and 1.9% of total nitrogen in high-protein oat flour. The oat grains had been steamed before processing to inactivate lipases. Most probably, the steaming also inactivates the other enzymes, such as the endogenous proteases; thus, the formation of NPN compounds during oat starch processing remains low.

The NPN compounds were also affected by the washing procedures before spray-drying the flour. The two washing steps

TABLE I  
Distribution of Nitrogen (dmb) in the Protein Fractions of High-Protein Oat Flour and Oat Groats\*

Fraction	High-Protein Oat Flour			Oat Groats		
	N, mg/g	SD <sup>b</sup>	% of Total N	N, mg/g	SD	% of Total N
Total nitrogen	88.5	0.3	100	29.2	0.2	100
Salt-soluble nitrogen compounds	2.6	0.2	2.9	8.0	0.4	27.5
Albumins	0.9 <sup>c</sup>	...	1.0 <sup>c</sup>	0.8	0.0	2.8
Globulins				5.9	...	20.1
Nonprotein nitrogen	1.7	0.2	1.9	1.3	0.2	4.6
Alcohol-soluble nitrogen, prolamins	9.7	0.7	11.0	3.4	0.3	11.6
Alkali-soluble nitrogen, glutelins	69.8	0.6	78.8	15.1	0.2	51.8
Residue	3.0	0.1	3.4	1.9	0.3	6.4
Total nitrogen recovered	85.1	...	96.1	28.4	...	97.3

<sup>a</sup> Mean of three replicates

<sup>b</sup> Standard deviation.

<sup>c</sup> Represents both albumins and globulins for high-protein oat flour. Obtained by subtracting nonprotein nitrogen from salt-soluble nitrogen.



decreased the amount of NPN compounds in proteinaceous slurry by 46%. Washing with tap water also removed approximately one third of the albumins and globulins, but it did not considerably change the proportion of alcohol-soluble and alkali-soluble proteins.

Relative to the other cereals (wheat, barley, and rye), oats have lower levels of alcohol-soluble and higher levels of salt-soluble proteins. These two fractions are regarded as major storage protein fractions in oats (Robert et al 1983b). Alcohol-soluble proteins account typically for 10–15% of the total seed protein, but there is a large variation in quantities reported for salt-soluble and alkali-soluble proteins. The range reported for either fraction spans from 10 to 80% of total seed protein (Peterson and Smith 1976; Wieser et al 1980; Robert et al 1983b, 1985), mostly due to the method used for fractionation. Optimum extractants for salt-soluble and alcohol-soluble proteins of oats are 1M NaCl in 0.05M Tris, pH 8.5, and 52% ethanol (v/v), respectively (Kim et al 1978, Peterson 1978). Using these conditions, Robert et al (1983b) reported that salt-soluble and ethanol-soluble proteins recover 51 and 12%, respectively, of the total protein extracted. The residual proteins, generally regarded as glutelins, become totally solubilized with a solution of 0.5% SDS and 0.6% 2-mercaptoethanol. They recovered 37% of the total seed protein. However, based on electrophoretic separation, oat glutelins consist mainly of the same type of molecules as those found in prolamins and, especially, in globulins. This has led to the suggestion that globulins account for 70–80% of the total proteins

in oats, and actual glutelins account for less than 10% (Robert et al 1983b, 1985).

In the present study, the proportion of salt-soluble proteins obtained for groats was lower than that reported by Robert et al (1983b). The solubilizing capacity of 1M NaCl depends on pH (Peterson 1978). Thus, unbuffered NaCl may leave unsolubilized globulin molecules that are bound to other constituents, such as phenolics, lipids, and carbohydrates. As the proportion of alcohol-soluble proteins in groats was in accordance with earlier reports (Peterson and Smith 1976, Kim et al 1978, Ma 1983a, Robert et al 1983b), the proteins that remained unsolubilized in NaCl-extraction were most probably collected in final extract and regarded as glutelins, which accounted for 52% of the total nitrogen of groats.

#### Molecular Weight Distribution

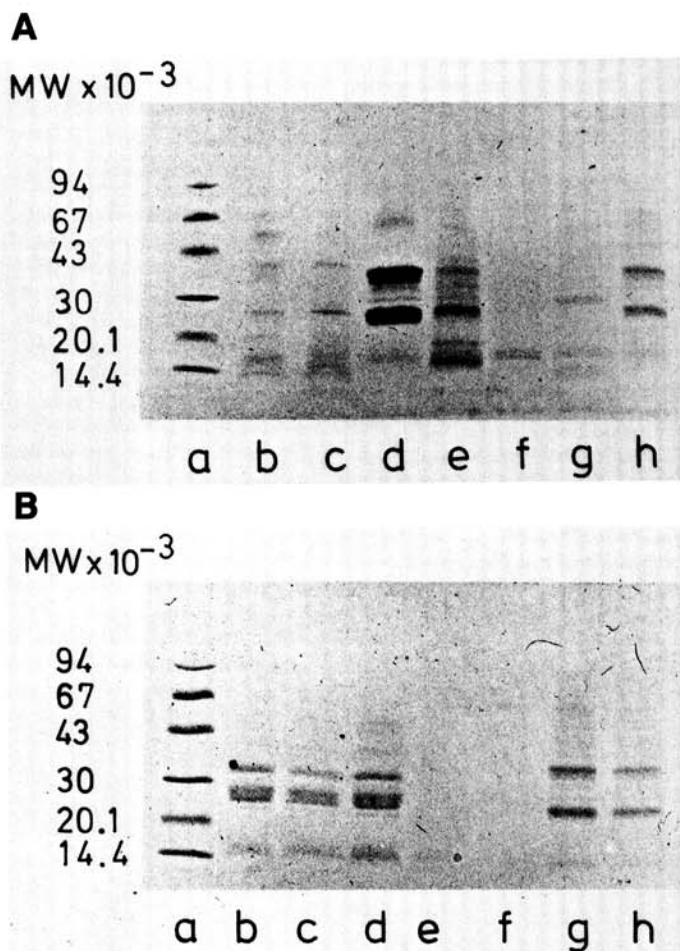
The molecular weight distribution determined by SDS-PAGE for the protein fractions did not differ considerably between groats and high-protein oat flour (Fig. 1A,B). Albumin fractions of both samples contained polypeptides with molecular weights (MW) of 14,000–17,000, 27,000, and 44,000 (b and c in Fig. 1A). The groats also showed additional bands at MW of 56,000 and 68,000 that were not detectable in the protein-rich flour. Galle et al (1988) proposed that these proteins with higher molecular weight were contaminating bands caused by globulins.

Globulins of oats consist of proteins with two distinct MW ranges: 32,00–43,000 ( $\alpha$  group) and 20,000–25,000 ( $\beta$  group). In the absence of reducing agents,  $\alpha$ - and  $\beta$ -polypeptides are linked together, resulting in major electrophoretic bands at MW of 52,000–70,000. Because globulins contribute considerably to the residual glutelin fraction of oats, the electrophoretic separations of these two fractions are very much alike (Brinegar and Peterson 1982; Robert et al 1983b, 1985; Galle et al 1988). In the present study, the electrophoretic separation was performed under reducing conditions. Major polypeptides of globulins and glutelins were detected at MW of 27,000 and 41,000 (d,e in Fig. 1A and g,h in Fig. 1B). High-protein oat flour showed bands also at MW of <20,000, which may be due to the degradation of some globulin proteins during processing.

Robert et al (1983a) studied molecular weight patterns of nine oat cultivars of different protein contents and reported the major prolamin polypeptides to have MW between 22,000 and 40,000. In addition, minor bands at MW of 15,000–16,000 were apparent. In our work, electrophoretic separation was determined separately for water-insoluble and water-soluble prolamins, the former displaying the typical electrophoretic pattern reported earlier (Robert et al 1983a, Galle 1988). Water-insoluble prolamins in both samples had major polypeptides with MW of 28,000 and 34,000. Furthermore, polypeptides with MW of 15,000 were also detected (b–d in Fig. 1B). Water-soluble prolamins refer to proteins that remained unprecipitated during the evaporation of 2-propanol from the alcohol extract before freeze-drying. In oat groats, this fraction showed only one slight band at the MW of 18,000, while high-protein oat flour had three bands at MW of 17,000, 19,500, and 32,000 (f,g in Fig. 1A). Electrophoretic patterns determined separately for water-soluble and water-insoluble oat prolamins have not been reported before.

#### Amino Acid Composition

The amino acid composition of each protein fraction is illustrated in Table II. Proportions are given as mole percent of the sum of amino acids determined. Asparagine and glutamine are included with aspartic acid and glutamic acid, because the amides are hydrolyzed to the corresponding acids during the HCl hydrolysis. Robbins et al (1971) estimated that 69% of these two amino acids in oats are in the form of amides. Tryptophan was not determined because it decomposes during acid hydrolysis. Also cysteine was undetermined with the HPLC method applied in this study. The coefficient of variation (CV) of amino acid contents was generally below 10%. Histidine was an exception, with an average CV of 18%. In addition, in some cases, phenylalanine and isoleucine had a CV above 10%. The proportions given for



**Fig. 1.** SDS-PAGE of protein fractions isolated from high-protein oat flour and oat groats. **A:** a = standard, b = albumins of groats, c = albumins of flour, d = globulins of groats, e = globulins of flour, f = water-soluble prolamins of groats, g = water-soluble prolamins of flour, h = glutelins of flour. **B:** a = standard, b = water-insoluble prolamins of groats, c and d = water-insoluble prolamins of flour, e = water-soluble prolamins of groats, f = water-soluble prolamins of flour, g = glutelins of groats, h = glutelins of flour.

methionine are based on single determinations only.

A high proportion of glutamic acid is characteristic of the amino acid composition of oats. Also aspartic acid, leucine, and arginine are reported in relatively high concentrations. The proportion of cysteine, histidine, and methionine typically is low (Robbins et al 1971, Pomeranz et al 1973, Peterson 1976.) We also found high concentrations of glutamic acid. For groats, aspartic acid, serine, alanine, glycine (in albumins), and proline (in albumins and prolamin fractions) were detected in fairly high amounts. The lowest proportions obtained were for methionine and phenylalanine, and in some cases, for lysine (globulins, water-insoluble prolamins) and for histidine (water-insoluble prolamins, glutelins).

Processing did not considerably change the amino acid patterns of the protein fractions (Table II). High-protein oat flour showed practically the same amino acid composition in the glutelin fraction as did oat groats. Also, the differences between the two samples were slight in the albumin fraction, with the exception of methionine, the proportion of which was four times higher in oat flour than it was in groats. The globulin fraction also had a noticeable increase in the proportion of methionine during processing. Another distinctive change in the globulin fraction was detected in the aspartic acid content, which decreased by 40% during processing. In the water-insoluble prolamin fraction, all amino acids, except glutamic and aspartic acids, showed somewhat larger proportions in flour than they did in groats. In water-soluble prolamins, processing caused the largest relative changes in methionine, arginine, and histidine content, which had decreased

by 70, 50, and 40%, respectively. The amount of glutamic acid in water-soluble prolamins had increased by ~20% during processing.

The water-insoluble and water-soluble prolamin fractions differed in their amino acid patterns. This was obviously caused by the absence of the major polypeptides with MW of 28,000–34,000 in the water-soluble prolamin fraction. Characteristics of the amino acid composition of water-soluble prolamins included: a high amount of serine, glycine, asparagine, and alanine; and a distinctively lower amount of glutamic acid than was found in water-insoluble prolamins. The same type of differences in the amino acid composition was reported earlier for the corresponding prolamin fractions of barley by Linko et al (1989).

Processing increased the proportion of limiting amino acids (lysine, threonine, and methionine) in globulin and water-insoluble prolamin fractions. From a nutritional point of view, changes occurring for limiting amino acids are most critical in the globulin and glutelin fractions because they contribute ~80% of the total nitrogen in the samples. The amount of threonine in the glutelin fraction remained unchanged during processing, whereas the proportions of lysine and methionine decreased slightly by ~10–15%.

### Functional Properties

The functionality (protein solubility, water absorption, and emulsifying capacity) of protein-rich oat flour was compared with that of commercial soy concentrate. The results of functionality determinations are shown in Table III.

TABLE II  
Amino Acid Composition (mol %)<sup>a</sup> of the Protein Fractions of High-Protein Oat Flour and Oat Groats

Amino Acids <sup>b</sup>	Alcohol-Soluble <sup>c</sup>									
	Water-Soluble <sup>c</sup>		Salt-Soluble <sup>d</sup>		Insoluble in Water		Soluble in Water		Alkali-Soluble <sup>f</sup>	
	Flour	Groats	Flour	Groats	Flour	Groats	Flour	Groats	Flour	Groats
Ala	9.6 (0.1)	11.3 (0.2)	9.0 (0.1)	7.7 (0.1)	6.6 (0.1)	6.0 (<0.1)	9.0 (0.1)	9.1 (0.1)	8.1 (0.2)	8.9 (0.1)
Arg	2.0 (<0.1)	1.6 (0.1)	7.9 (...)	6.6 (...)	3.2 (0.1)	2.3 (0.1)	1.1 (<0.1)	2.0 (0.2)	6.1 (0.4)	6.4 (...)
Asp	10.3 (1.0)	10.8 (0.3)	8.9 (0.3)	15.0 (1.4)	3.6 (0.2)	4.4 (0.2)	13.3 (1.0)	11.7 (0.9)	12.8 (1.0)	12.9 (0.5)
Glu	23.9 (1.1)	22.6 (0.7)	27.8 (0.7)	27.8 (2.3)	47.7 (1.3)	60.0 (0.6)	20.8 (0.6)	16.5 (0.6)	26.4 (0.7)	26.6 (0.9)
Gly	9.5 (0.4)	9.4 (0.3)	6.1 (0.1)	5.1 (0.5)	2.4 (0.1)	1.3 (0.1)	13.2 (0.8)	14.5 (0.6)	5.4 (0.2)	5.3 (0.2)
His	3.2 (0.6)	2.6 (0.6)	2.9 (0.4)	2.5 (0.3)	0.8 (0.1)	0.7 (<0.1)	1.1 (0.2)	1.8 (0.4)	1.9 (0.6)	2.5 (0.5)
Ile	1.8 (0.3)	1.6 (0.1)	2.3 (0.2)	3.4 (0.8)	1.9 (0.1)	1.2 (<0.1)	0.5 (0.1)	0.7 (0.1)	3.1 (0.1)	3.0 (0.4)
Leu	4.1 (0.7)	4.1 (0.5)	4.6 (0.4)	5.2 (0.4)	6.8 (0.4)	4.5 (0.1)	2.5 (0.1)	2.8 (0.1)	5.5 (0.2)	5.5 (0.4)
Lys	3.4 (0.2)	3.8 (0.2)	2.3 (0.2)	2.0 (0.2)	0.6 (<0.1)	0.3 (<0.1)	1.7 (0.2)	1.7 (0.3)	2.2 (0.2)	2.6 (0.1)
Met	1.6 (...)	0.4 (...)	2.2 (0.1)	0.8 (...)	2.4 (...)	2.0 (...)	0.4 (...)	1.1 (...)	1.4 (...)	1.6 (...)
Phe	1.3 (0.2)	1.3 (0.1)	1.7 (<0.1)	2.9 (0.4)	3.0 (0.3)	1.8 (0.1)	0.8 (0.3)	0.7 (<0.1)	2.9 (0.3)	2.4 (0.2)
Pro	6.2 (0.4)	5.3 (0.2)	5.8 (0.4)	4.3 (0.3)	8.7 (0.4)	5.8 (<0.1)	7.3 (0.5)	7.3 (0.3)	4.8 (0.2)	4.7 (0.2)
Ser	9.7 (0.3)	9.5 (0.2)	7.0 (0.1)	7.1 (0.3)	4.1 (0.1)	3.5 (0.1)	18.1 (0.3)	18.6 (0.3)	7.3 (0.2)	7.3 (0.1)
Thr	5.4 (0.1)	5.9 (0.2)	5.4 (0.1)	4.4 (0.2)	2.5 (0.1)	2.1 (<0.1)	4.2 (<0.1)	4.4 (<0.1)	4.9 (0.2)	4.9 (0.1)
Tyr	6.5 (0.1)	7.1 (0.1)	3.3 (...)	3.0 (...)	2.0 (0.1)	1.4 (0.1)	5.1 (0.3)	5.6 (0.1)	3.7 (0.5)	3.3 (...)
Val	2.6 (0.3)	2.7 (0.1)	3.1 (<0.1)	3.4 (0.2)	4.0 (0.3)	2.7 (<0.1)	1.0 (0.1)	1.2 (0.1)	3.6 (0.2)	3.5 (<0.1)

<sup>a</sup> Standard deviations appear in parenthesis.

<sup>b</sup> Ala = alanine; Arg = arginine; Asp = asparagine + aspartic acid; Glu = glutamine + glutamic acid; Gly = glycine; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; Phe = phenylalanine; Pro = proline; Ser = serine; Thr = threonine; Tyr = tyrosine; Val = valine.

<sup>c</sup> Albumins.

<sup>d</sup> Globulins.

<sup>e</sup> Prolamins.

<sup>f</sup> Glutelins.

TABLE III  
Protein Solubility, Water Absorption, and Emulsifying Capacity of High-Protein Oat Flour and Soy Concentrate<sup>a,b</sup>

pH	Protein Solubility, %		Water Absorption, g/g of Sample		Emulsifying Capacity, g of oil/g of sample	
	Flour	Soy	Flour	Soy	Flour	Soy
3.0	61.2 ± 0.3	15.1 ± 0.4	1.1 ± 0.01	4.5 ± 0.28	358 ± 54	363 ± 50
3.5	8.1 ± 0.6	5.8 ± 0.1	1.0 ± 0.10	4.2 ± 0.23	...	...
4.0	2.9 ± 0.2	2.4 ± 0.5	1.2 ± 0.03	4.0 ± 0.15	...	...
4.5	2.1 ± 0.2	4.2 ± 0.2	1.3 ± 0.09	3.5 ± 0.18	...	...
5.0	3.0 ± 0.1	4.3 ± 0.3	1.3 ± 0.13	3.6 ± 0.14	436 ± 36	311 ± 21
5.5	4.5 ± 0.5	7.1 ± 0.2	1.4 ± 0.02	4.3 ± 0.08	...	...
6.0	7.2 ± 0.5	16.0 ± 0.3	1.5 ± 0.06	4.7 ± 0.23	...	...
6.5	10.6 ± 0.9	18.8 ± 0.3	1.2 ± 0.02	5.2 ± 0.21	...	...
7.0	18.0 ± 2.0	20.1 ± 0.4	1.3 ± 0.01	5.4 ± 0.29	410 ± 12	311 ± 31

<sup>a</sup> Danpro S.

<sup>b</sup> Values are given as a mean of three replicates ± standard deviation.

Both samples showed low protein solubility (<21%) in the range of pH 3.5–7.0. At pH 3.0, however, the solubility of oat protein was distinctively higher, ~61%. Soy concentrate absorbed three to four times more water than did high-protein oat flour. The water-absorption capacity of oat flour remained fairly constant (1.0–1.5 g per gram of sample) over the pH range studied. The water-absorption capacity of soy concentrate was 3.5–3.6 g per gram of sample at pH 4.5–5.0, and it increased to 5.4 g per gram of sample at pH 7. The emulsifying capacity was similar for both samples at pH 3.0; at pH 5 and 7, the oat flour emulsified a greater amount of oil than did the soy concentrate.

The solubility profile obtained for high-protein oat flour is in accordance with the results of Wu et al (1973, 1977) and Ma (1983a,b), who found the solubility of oat proteins to be near or below 20% at pH 4–6, but over 60% at pH values below 3 or above 8. Solubility data reported for soy protein mostly concern soy isolates; their solubility is typically very low at pH 4–5 and higher at the acidic and alkaline sides of the isoelectric pH range (Hermansson 1979, Visser and Thomas 1987). However, several factors, such as protein source and processing history, affect the solubility and related functional properties of proteins. Soy protein concentrates are prepared from defatted flour by eluting soluble components with dilute aqueous acid, aqueous alcohol, or water combined with heat treatment. The functionality of the product depends on the processing conditions used (Kinsella 1979, Visser and Thomas 1987). Our results for the solubility of soy concentrate agree well with those of Hutton and Campbell (1977), who reported the nitrogen solubility of soy concentrate to remain below 25% at pH 5, 6, and 7. Soy concentrate studied in this work is intended for use in meat products, where good water- and fat-binding properties are required. According to Visser and Thomas (1987), soy products with low solubility properties do not necessarily form real emulsions; most probably, their stabilizing effect is based on good binding properties.

Wu et al (1977) and Ma (1983a) reported that the emulsifying and water-holding properties of oat protein concentrates are comparable to those of soy isolate. Protein concentrates in those studies were prepared by alkali extraction followed by filtering and freeze-drying. Ma (1983b) used two methods for preparing oat protein isolates: 1) extraction with salt solution, and 2) extraction with alkali solution followed by isoelectric precipitation. Both the emulsifying properties and water-holding capacity were lower in oat isolates than they were in soy isolate (Ma 1983b).

## CONCLUSIONS

Processing caused changes in proportions of isolated protein fractions, but the alterations in their amino acid and polypeptide patterns were fairly small. Alkali-soluble glutelins were the most abundant protein fraction, both in high-protein oat flour and in oat groats, although the proportion of glutelins was higher in the former than in the latter. The amino acid composition and molecular weight pattern of glutelins, however, seemed to remain unchanged during processing. The proportion of salt-soluble proteins decreased most distinctively. Part of the globulins decompose, and some of them may also dissolve in the processing water. On the other hand, because the amino acid and the polypeptide profiles of the globulins and glutelins were much alike, there is also the possibility that globulins undergo some conformational changes that make them insoluble to 1M NaCl, and thus they are extracted in the alkali fraction. Washing the high-protein oat flour with cold water was an efficient way of decreasing the amount of NPN compounds. Washing with water also decreased the minerals of the flour, but major changes in protein or fat contents were not detected. The functional properties of a protein product dictate its use in various food applications. High-protein oat flour showed a lower water-absorption capacity than did soy concentrate, but they had comparable solubility and emulsifying properties. The potential of high-protein oat flour for utilization in food systems needs to be assessed in further studies.

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