

Protein Composition of a High-Protein Barley Flour and Barley Grain

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ABSTRACT

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The hot air-dried, proteinaceous by-product from a barley starch pilot plant was compared with the raw material of the process, 10% dehulled barley grain. The by-product, high-protein barley flour, has been used for feeding pigs, cows, and sheep. The proportions of four protein fractions, isolated according to their solubility, and the nonprotein nitrogen content were evaluated. The polypeptide distribution of the protein fractions was observed by vertical sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by densitometric scanning. The amino acids of the fractions were determined by gas chromatography of their pentafluoropropionyl-2-propyl esters. The protein content was approximately three times greater in the barley protein flour than in barley grain. The most abundant protein fraction was that of alkali-soluble

"glutelins" (45%) in the protein flour and that of alcohol-soluble "prolamins" (35%) in barley grain. During processing, the proportion of prolamins and water- and salt-soluble "albumins" + "globulins" decreased, as the proportion of glutelins increased. In the barley protein flour, 80% of the salt-soluble nitrogen compounds was nonprotein nitrogen. The most remarkable differences between the amino acid compositions of the protein flour and its raw material were detected in glutelin fractions. The sum of the essential amino acids determined was 6-21% lower in all protein fractions of protein flour compared with that of the barley grain. The polypeptide distribution differed most in the albumin and globulin fractions.

Barley has an important role as a feed grain in most Western countries. In Finland barley constitutes 50% of all cultivated cereals. Approximately 81% of annual barley production is used for feed, 9% for seed, 8% for malt and alcohol production, and only 2% for human consumption (The Agricultural Economics Research Institute 1986). Alko Ltd., the major producer of alcohol in Finland, has set up a barley starch process integrated with ethanol production. The process consists of grinding of barley seeds and screening of bran, mixing the meal with water at 50°C, homogenizing and screening the fiber fraction, concentrating the remaining barley slurry, and separating it into starch and protein fractions. The protein fraction was isolated by decanting, then dried by a direct hot-air stream and called "high-protein barley flour." From the seed protein, 6% was passed into bran, 22% into the fiber fraction, less than 0.5% into A-grade starch, 30% into B-grade starch, and 42% into the protein fraction. So far, the high-protein flour has been used only as a feedstuff for cattle and pigs. However, its high protein content justifies its evaluation as a foodstuff ingredient.

The proportions of different protein fractions, the molecular weights, and the amino acid composition of barley protein have been widely studied, as reviewed by Shewry and Mifflin (1983) and Lásztity (1984). The main emphasis of the present work was to compare the high-protein barley flour and the raw material of the process, partially hulled barley grain, to evaluate the changes that occur in the composition of the protein during processing. The intention was to separate the different protein fractions according to their solubility using as mild a procedure as possible. After fractionation, the molecular weights of the protein components and the amino acid compositions were studied. The usefulness of the high-protein flour as a foodstuff ingredient is discussed according to the results.

MATERIALS AND METHODS

Materials were obtained from the integrated ethanol-starch pilot process (Alko Ltd., Rajamäki Factories, Finland). The 10%-dehulled barley grain was a mixture of different feed-barley varieties cultivated in Finland. The grain was ground through a 1-mm screen (Tecator Cyclotec 1093). High-protein barley flour was produced from the proteinaceous by-product by decanting and direct hot air-drying. Both samples were stored at 4°C. The

average composition of the samples for protein, ash, and fat content was determined by using AOAC (1984) methods. The coefficient 5.83 was used in protein calculations.

Protein Fractionation

Samples (10-g) were extracted with 100 ml of solvent in a 500-ml polypropylene centrifuge bottle by stirring with a magnetic stirrer. Separation of the protein extracts was made by centrifuging at $12,800 \times g$ for 20 min at the temperature of the extraction. Each extraction was repeated three times and the supernatants were combined. Three replicates of the whole extraction procedure were made as follows. The barley samples were defatted with 1-butanol (2×10 ml g^{-1} for 30 min, then 1×10 ml g^{-1} for 60 min at 4°C) (Shewry et al 1977). After centrifugation the residues were extracted with 0.5M NaCl (3×10 ml g^{-1} for 60 min at 4°C) to dissolve "albumins," "globulins," and nonprotein nitrogen (NPN) (Shewry et al 1978b, Byers et al 1983). The NaCl extracts were dialyzed for 48 hr against distilled water at 4°C, and the precipitated globulins were separated by centrifuging at $16,000 \times g$ for 25 min at 4°C. Both fractions were frozen and freeze-dried.

A mixture of 55% (v/v) 2-propanol and 1% (v/v) 2-mercaptoethanol (3×10 ml g^{-1} for 60 min at 20°C) was used for extracting the "prolamins" (Shewry et al 1978a,b; Byers et al 1983). When the prolamins extract was freed from alcohol using a rotary evaporator (El-Negoumy et al 1977), some of the proteins precipitated. This mixture of the water-soluble and precipitated prolamins was dialyzed against distilled water for 48 hr at 4°C. After centrifugation, both prolamins fractions were freeze-dried separately.

"Glutelins" were extracted with 0.05M borate buffer (pH 10) containing 0.5% (w/v) sodium dodecyl sulfate (SDS) (Ingversen and Kjøie 1973, El-Negoumy et al 1977, Shewry et al 1978b). After dialyzing for 6 hr at room temperature and 18 hr at 4°C, the extracts were freeze-dried.

The nomenclature of albumins, globulins, prolamins, and glutelins is valid only for the original barley grain proteins. Because of the changes occurring in high-protein barley flour during processing, the terms water-soluble, salt-soluble, alcohol-soluble, and alkali-soluble protein fractions are used instead of albumins, globulins, prolamins, and glutelins, respectively.

Total Nitrogen, Protein, and NPN Determination

The nitrogen contents of the barley protein flour, barley grains, extracts, and insoluble residues were determined by the conventional Kjeldahl method. The protein content of the freeze-dried fractions was determined using the method of Lowry et al (1951) for evaluating the amount of sample needed in the protein

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hydrolysis and gel electrophoresis. For determining the amount of NPN, 100 ml of salt-soluble fraction was mixed with 100 ml of 10% (w/w) trichloroacetic acid (TCA) (Shewry et al 1978b) and allowed to stand overnight at 4°C. The precipitated proteins were then removed by centrifugation at 16,000 × g for 25 min. The amount of NPN in a clear solution was determined with the Kjeldahl method.

Amino Acid Analysis

Samples containing approximately 5 mg of protein were hydrolyzed with 6M HCl under nitrogen in sealed tubes at 110°C for 24 hr (Gehrke et al 1985). The amino acids were determined as their pentafluoropropionyl-2-propyl esters (Frank et al 1977) by gas chromatography (Varian, model 3700) with a flame-ionization detector and Hewlett-Packard 3388A-integrator. The amino acid derivatives were separated with a SE-30 fused silica capillary column (25 m × 0.32 mm i.d., d_f 0.15 μm) using nitrogen as the carrier gas (μ = 5.7 cm/sec). The oven temperature was held for 2 min at 90°C, then raised at the rate of 4°C/min to a final temperature of 200°C, which was held for 15 min. The temperatures of the injector and detector were 190 and 280°C, respectively. The split ratio was 1:20. Together with all protein hydrolyses, two samples of an amino acid standard (A 44450 24, BDH) were treated similarly with protein samples. D-Norleucine was used as an internal standard.

SDS-Polyacrylamide Gel Electrophoresis

The molecular weights of the extracted proteins were determined by the vertical slab SDS-polyacrylamide gel electrophoresis (PAGE) system (LKB) modified from the method of Laemmli (1970). The separating gel contained 10% (w/v) acrylamide, 0.3% (w/v) N,N'-methylenebisacrylamide, and 0.1% (w/v) SDS in 0.366M Tris buffer (pH 8.8). The stacking gel contained 3% (w/v) acrylamide, 0.08% (w/v) N,N'-methylenebisacrylamide, and 0.1% (w/v) SDS in 0.119M Tris buffer (pH 6.8). The samples of the protein fractions were dissolved in a sample buffer (0.0625M Tris, 2.05% (w/v) SDS, 10% (v/v) glycerol, 5% (v/v) 2-mercaptoethanol, and 0.001% (w/v) bromophenol blue, pH 6.8), and heated in a boiling water bath for 2 min. After cooling, the samples were applied to the gels. A buffer of 0.025M Tris/0.192M glycine, pH 8.9, containing 0.1% (w/v) SDS was used as an electrode buffer. The samples were run using the LKB 2001 vertical electrophoresis system with a constant current setting of 60 mA. The proteins were fixed in the gels with 11.5% (w/v) TCA/3.5% (w/v) 5-sulfosalicylic acid and stained with Coomassie Brilliant Blue R 250 at 60°C for 20–30 min. A mixture of ethanol, acetic acid, and water (50:8:142, v/v) was used for destaining the gels.

The mobilities of the molecular weight standards of MW 14,400–94,000 (17-0446-01, Pharmacia Fine Chemicals AB, Sweden) were determined and plotted against their known molecular weights on a semilogarithmic scale. The molecular weights of the extracted protein components were estimated from

the standard plot. The gels were also scanned at 633 nm using an LKB Ultrascan Laser densitometer.

RESULTS AND DISCUSSION

Protein content of the high-protein barley flour was approximately three times that of the grain, being on average 36.1 and 11.5% on a dry matter basis, respectively. Similarly, the lipids and minerals of barley were concentrated in the proteinaceous by-product during processing. Thus, the high-protein flour contained, on average, 3.7% ash and 5.8% fat on a dry weight basis, compared with the dehulled grain, which contained 2.1% ash and 2.2% fat. Barley grain has been reported to contain, on average, 10.9% protein, 2.3% fat, and 2.7% ash (Lásztity 1985), in agreement with the results of the present work for dehulled barley grain.

Fractionation of the Proteins

The nitrogen contents of high-protein barley flour, barley grain, and their extracted fractions are shown in Table I. The content of nitrogen in each protein fraction is also expressed as a percentage of the total nitrogen in the original sample.

In the high-protein flour, the main protein fraction (45% of nitrogen) was alkali extractable, whereas in barley grain the share of the comparable glutelin fraction was clearly lower (31%). Thus, the alkali-soluble proteins seem to be more resistant to the processing conditions than the other proteins. On the other hand, the proportion of the alcohol-soluble proteins decreased from 36% in barley grains (prolamins) to 24% in high-protein flour. The relative amount of salt-soluble nitrogen compounds decreased slightly during processing. However, the amount of TCA-soluble NPN compounds was higher in the high-protein flour (16%) than in grains (8%). The proportion of NPN in the salt-soluble nitrogen was approximately 80% in the protein flour and 34% in the grains. Such a high proportion of NPN as well as a low content of albumins and globulins in the barley protein flour is presumably caused by the proteolytic activity of the grain and the processing conditions. It should be noted that the pH of the processing water of the barley slurry was 4–4.5 and that its temperature varied from 40 to 50°C. Thus, the processing conditions were optimal for the activity of endogenous aspartylproteinase and acidic carboxypeptidases II and IV in barley seeds (Mikola 1983). Therefore, the hydrolysis product in the TCA-soluble NPN compounds of the high-protein barley flour is apparently a mixture of free amino acids and small peptides. The relative amount of NPN found in barley grain corresponds closely with those given for other cereals (5–10%) (Mossé and Baudet 1983). The relative amount of insoluble residue was the same (6%) in both the high-protein flour and in barley grain. As a whole, the nitrogen recovery of the fractionation was good (Table I) and comparable with those found by Mifflin and Shewry (1977), Shewry et al (1978b), Kirkman et al (1982), Ingversen and Kjøie (1973), and El-Negoumy et al (1977) in different barley varieties.

TABLE I
Distribution of Nitrogen in the Protein Fractions of High-Protein Barley Flour and 10% Dehulled Barley Grain

Fraction	High-Protein Barley Flour			Barley Grain		
	N (mg/g) Dry Wt ^a	SD	% of Total N	N (mg/g) Dry Wt ^a	SD	% of Total N
Total N	62.0	0.3	100	19.8	0.1	100
Salt-Soluble N Compounds	12.5	0.1	20.1	4.6	0.2	23.4
Proteins ^b	2.5	...	4.1	3.1	...	15.6
(albumins + globulins)						
Nonprotein nitrogen	10.0	0.3	16.1	1.6	0.1	7.9
Alcohol-soluble proteins (prolamins)	14.7	0.7	23.8	7.1	0.4	36.0
Alkali-soluble proteins (glutelins)	28.0	0.2	45.2	6.2	0.2	31.3
Residue	3.6	0.4	5.9	1.2	0.2	5.9
Total N recovered	58.9	...	95.1	19.1	...	96.6

^aMean of three replicates.

^bObtained by subtracting the nonprotein nitrogen from the salt-soluble N.

On average, barley grain has been found to contain 3–4% albumins, 10–20% globulins, 35–45% prolamins, and 35–45% glutelins expressed as a percentage of the total nitrogen content (Konzak 1977, Lásztity 1984). This is similar to the results of this study for the partly dehulled barley grain. The distribution of the proteins among the different fractions, according to their solubility, is also known to depend on the variety, cultivation condition (Lásztity 1984), and the fractionation procedure used (Shewry et al 1978b, Byers et al 1983).

Amino Acid Composition

The amino acid compositions of the separated protein fractions are presented in Table II. Proportions of the single amino acids are expressed as mole percent of the sum of determined amino acids. However, the gas-liquid chromatography method used for the determination of amino acids as pentafluoropropionyl-2-propyl derivatives was not wholly satisfactory in that the proportions of histidine, arginine, and tryptophan could not be determined. Tryptophan decomposes during HCl hydrolysis, whereas pentafluoropropionyl-2-propyl derivatives of arginine and histidine were not formed. There were also other restrictions due to the chromatographic method. Valine and threonine were not separated sufficiently with the SE-30 column and their proportions could thus not be verified. All other amino acid derivatives were separated well in 40 min. Asparagine and glutamine are expressed within the proportions of glutamic acid and aspartic acid, because the amides will be hydrolyzed to the corresponding acids during the HCl hydrolysis. According to Belitz et al (1981) about 70% of glutamic and aspartic acids of barley proteins are in the amide form. The relative standard deviations of the contents of amino acid were below 11% except for lysine (18.6%) and proline (15.6%).

The amino acid composition of the fractions of high-protein barley flour differed from that of the grain (Table II). Most distinctive were the differences in the alkali-soluble fractions, where the flour had six times the proline, three times the lysine, and twice the tyrosine of the grains. On the other hand, processing seemed to decrease to some extent, e.g., the contents of glycine, alanine, and aspartic acid in the alkali-soluble fraction. In the water-soluble fraction, the amount of glutamic acid was 73% higher and that of methionine and aspartic acid 40–50% lower in barley flour than in the grains. The salt-soluble fraction of the high-protein flour had twice as much cysteine as the grains, whereas the contents of isoleucine and methionine were approximately 45% lower than in the salt-soluble fraction of grains. The amount of glycine in the water-soluble "prolamins"

in the high-protein flour was approximately 50% lower than in the corresponding prolamin fraction of the grains. Generally, the content of alanine seemed to decrease as that of proline increased during processing, especially in the fractions of water-soluble and alkali-soluble proteins. Only slight differences between the amino acid compositions occurred in the water-insoluble prolamin fractions of the samples examined. The sum of the determined essential amino acids (not including tryptophan) varied in different protein fractions of high-protein barley flour from 20.9 to 28.2 mol% and in those of barley grain from 24.0 to 32.1 mol%.

High amounts of proline and glutamic acid and a low amount of lysine are characteristic of the amino acid composition of barley protein (Lásztity 1984), which was also recognized in this work, especially in the alcohol-soluble protein fractions.

SDS-PAGE

Changes in the molecular sizes in the protein fractions of barley grain during processing to high-protein flour were also studied with SDS-PAGE. The densitograms (Fig. 1) show that the most noticeable change occurred in the fractions of albumins and globulins. In the high-protein flour the molecular weights of the water- and salt-soluble fractions were mainly <20,000, the former having only one minor component with a molecular weight of 38,000 and the latter with two components of 37,000 and 38,000. In contrast, in barley grains the main components of albumins had molecular weights of 58,000, 36,000, 33,000, 30,500, 29,000, 21,000, and <14,000 and those of globulins 53,000, 36,000, 29,000, 28,000, 21,000, 16,500, and <14,000. These results correspond to those of Rhodes and Gill (1980), who reported that the molecular weights of barley albumins and globulins were in the range of 10,000–70,000.

The molecular weight distribution of water-insoluble prolamins was similar in barley high-protein flour (Fig. 1Da) and barley grain (Fig. 1Db). The main components had the molecular weights of 66,000, 52,000 (2–3 zones), 44,000, 41,500, 39,000, 34,000, 17,000, and <14,400. The barley grain also had proteins with molecular weights of 92,000 and 63,000, which did not occur in the corresponding fraction of the flour.

On the other hand, the water-soluble prolamins of the samples differed, the barley grain containing high amounts of proteins with a molecular weight of 59,000 (Fig. 1Eb), as already reported by Wilson et al (1981), whereas the high-protein flour consisted mainly of low molecular weight proteins (Fig. 1Ea). This partially explains the decrease in the relative amounts of alcohol-soluble proteins during the processing of barley grain. Alkali-soluble proteins (glutelins) of both samples also contained several different

TABLE II
Amino Acid Composition (mol%) of the Protein Fractions of High-Protein Barley Flour and Barley Grain^a

Amino Acid ^b	Water-Soluble Proteins (Albumins)				Salt-Soluble Proteins (Globulins)				Alcohol-Soluble Proteins (Prolamins)								Alkali-Soluble Proteins (Glutelins)			
	Flour		Grain		Flour		Grain		Insoluble in Water				Soluble in Water				Flour		Grain	
	mol%	SD	mol%	SD	mol%	SD	mol%	SD	mol%	SD	mol%	SD	mol%	SD	mol%	SD	mol%	SD	mol%	SD
Ala	7.0	8.4	9.3	7.6	6.0	9.6	7.9	7.9	2.4	7.0	2.6	7.4	3.7	11.0	6.6	11.9	6.2	9.2	10.0	7.8
Asp	6.3	12.4	10.6	3.3	7.9	7.9	10.5	8.0	2.6	7.3	1.8	7.7	3.8	5.3	5.2	9.3	7.7	3.9	11.5	6.5
Cys	5.5	14.7	6.5	2.4	12.4	5.3	5.6	6.8	1.6	13.0	2.4	26.5	2.6	8.2	tr ^c	...	1.3	5.7	tr	...
Glu	33.1	3.9	19.2	11.2	11.2	5.3	14.5	3.4	34.1	5.3	35.0	4.5	29.1	6.5	30.2	10.1	26.3	4.3	21.0	6.3
Gly	10.3	7.1	10.8	3.2	13.5	6.6	12.7	4.0	2.6	7.9	3.1	6.8	5.1	10.2	10.1	8.7	8.1	7.4	12.5	5.0
Ile	2.7	7.9	3.8	10.8	1.6	2.0	3.0	3.4	4.6	12.3	5.3	6.7	3.8	11.8	3.2	17.0	4.7	5.3	6.0	10.2
Leu	5.6	7.7	6.8	9.6	10.1	3.1	8.4	3.5	6.4	7.6	7.8	6.1	6.7	8.8	8.0	8.5	8.7	2.1	10.9	4.3
Lys	2.3	38.2	3.4	30.1	6.3	8.8	7.8	6.3	0.8	23.7	0.6	9.4	1.1	8.2	1.5	36.2	2.7	5.4	0.8	19.7
Met	1.3	11.5	2.5	8.0	0.9	8.4	1.6	4.6	0.7	16.9	1.2	8.3	1.1	21.2	1.6	11.8	1.8	2.9	1.9	15.4
Phe	3.7	4.4	3.6	8.8	3.2	5.9	4.4	2.8	8.4	5.6	6.8	5.6	5.4	7.9	4.9	12.4	5.0	4.3	5.3	8.4
Pro	6.5	29.2	6.1	23.7	7.4	6.0	7.3	4.5	24.7	10.8	20.1	12.5	26.6	16.2	13.8	30.0	12.5	6.3	2.1	17.3
Ser	7.5	6.8	6.7	10.3	10.4	4.1	7.9	2.8	4.9	6.0	5.8	7.6	4.9	6.6	7.1	7.6	7.4	2.5	9.7	7.3
Tyr	3.0	14.7	4.4	8.0	2.3	4.8	3.0	4.3	3.2	9.6	3.3	8.3	2.7	9.1	3.1	10.7	2.3	2.8	1.1	9.2
Val + Thr	5.3	7.7	6.4	9.8	6.8	5.3	5.5	3.7	3.1	8.2	4.3	6.4	3.5	10.3	4.8	11.6	5.3	6.5	7.2	10.1

^a Mean of four replicates. Standard deviations are expressed in terms of percentage.

^b Ala = alanine; Asp = asparagine + aspartic acid; Cys = cysteine; Glu = glutamine + glutamic acid; Gly = glycine; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; Phe = phenylalanine; Pro = proline; Ser = serine; Tyr = tyrosine; Val + Thr = valine + threonine.

^c tr = <0.1 mol%.

proteins with a molecular weight range of 14,000–90,000 (Fig. 1C). Clear differences between the glutelin densitograms of the samples could not be found because the fractions of both samples were too heavily stained.

There were huge differences in the amino acid and protein compositions between barley grain and the protein-rich flour.

This was especially evident in the fractions of albumins, globulins, and water-soluble prolamins. This was apparently due to proteolytic activity during processing. On the other hand, the severe drying process may partly have denatured the proteins. These two phenomena may explain the differences in the solubility and electrophoretic patterns of the proteins isolated from grains.

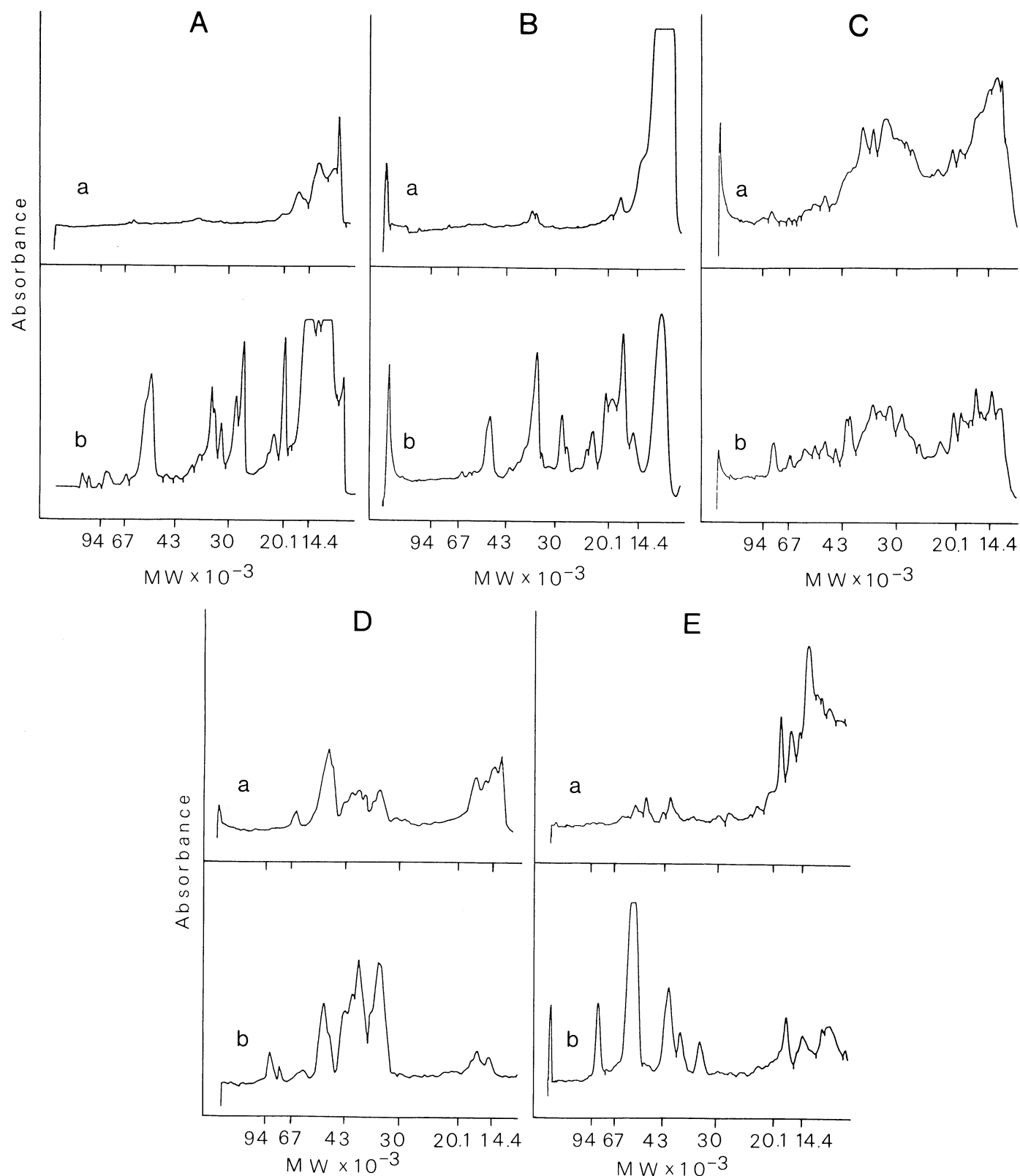


Fig. 1. Superimposed densitometric tracings (after sodium dodecyl sulfate-polyacrylamide gel electrophoresis) for the protein fractions of the high-protein barley flour (a) and 10% dehulled barley grain (b), measured at a wavelength of 633 nm. MW = molecular weight. A = water-soluble albumins; B = salt-soluble globulins; C = alkali-soluble glutelins; D = alcohol-soluble prolamins, insoluble in water; and E = prolamins, soluble in water.

and from the protein-rich flour. In conclusion, the composition of the barley protein changed dramatically during processing, e.g., the relative share of glutelin fraction increased as that of prolamin fraction decreased. Presumably, the albumin and globulin proteins of barley dissolve in the processing water, since the amount of those fractions was clearly lower in the proteinaceous by-product than in the raw material. The heat treatment during drying and the endogenous proteases of barley grain may also have caused decomposition of the proteins, which could explain the high amount of NPN (80% of the salt-soluble nitrogen) in the barley high-protein flour. During the protein fractionation procedure, the shorter polypeptides formed may also be extracted in the albumin and globulin fractions. Gel electrophoresis revealed that albumins and globulins of high-protein flour contained mainly short polypeptides with molecular weights of <20,000. The processing reduced the amount of essential amino acids in all protein fractions. Näsi showed earlier (1986) that the high-protein barley flour produced by the integrated process of Alko Ltd. could effectively be used as a feedstuff for pigs and ruminants. However, the present results clearly indicate that the process caused severe damage to the proteins as far as using the protein flour as a food ingredient is concerned. Therefore, the process has been improved further, especially in regard to process delays and drying operations.

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