Reversed-Phase High-Performance Liquid Chromatography of Oat Proteins: Application to Cultivar Comparison and Analysis of the Effect of Wet Processing

A. LAPVETELÄINEN,[1] J. A. BIETZ,[2] and F. R. HUEBNER[2]

Cereal Chem. 72(3):259-264

ABSTRACT

Oat protein fractions were characterized by reversed-phase high-performance liquid chromatography (RP-HPLC). Salt-soluble, alcohol-soluble, and alkali-soluble protein fractions were extracted with 1.0*M* NaCl, 52% ethanol, and 1% sodium dodecyl sulfate (SDS) in 0.05*M* borate buffer (pH 10), respectively. RP-HPLC analysis conditions were first optimized for column performance, concentration of ion-pairing reagent (trifluoroacetic acid [TFA]), protein reductive state, and elution temperature. These analysis conditions were used to characterize five Finnish oat cultivars (Puhti, Ryhti, Veli, Nasta, and Virma). In addition, effects of processing on oat protein composition were analyzed in high-protein oat flour and steamed oat groats derived from the oat starch process. Wet processing only slightly influenced RP-HPLC separation profiles of protein fractions. The greatest difference between high-protein oat flour and groats was the amount of salt-soluble components eluting during the first 15 min. Prolamin patterns of Puhti, Ryhti, and Virma clearly differentiate these cultivars. Prolamin patterns of cultivars Veli and Nasta were similar; half the genome in these cultivars is from the same parent. For all cultivars, RP-HPLC separations of salt- and alkali-soluble proteins were similar. However, quantities of some components differed, particularly those in the alkali-soluble fraction. RP-HPLC reproducibility was generally good, although replicate alcohol extractions revealed some components not consistently present. These were probably due to the extractant (52% ethanol). Other trials suggested that 70% ethanol may be a more reliable oat prolamin extractant for RP-HPLC analysis. These results emphasize the importance of thoroughly optimizing RP-HPLC analysis conditions for protein characterization.

Reversed-phase high-performance liquid chromatography (RP-HPLC) is a valuable method for analyzing cereal proteins (Bietz 1983). It separates proteins according to surface hydrophobicities, thus complementing analysis techniques based on size or charge differences. RP-HPLC is fast, sensitive and reproducible; it is easily automated and is amenable to computerized data analysis. The major applications of RP-HPLC include varietal identification and selection for quality factors during breeding, marketing and processing. Moreover, RP-HPLC may reveal valuable information about protein interactions, conformations, and composition (Bietz 1990).

RP-HPLC has been used to characterize oat proteins by Lookhart (1985), Lookhart and Pomeranz (1985), and Wieser and Belitz (1989). The method was applied largely to genotype identification and characterization, so analyses were performed on alcohol-soluble proteins (prolamins). However, when the aim is to determine the influence of processing on oat protein composition, globulins and glutelins also become of interest, especially since globulins are the major oat storage protein fraction, comprising 50–80% of the total nitrogen (Robert et al 1983b, 1985).

Lookhart et al (1986) studied the effect of commercial flake manufacturing on oat prolamins by polyacrylamide gel electrophoresis (PAGE). Flake production did not affect the electrophoretic patterns of the three varieties studied. Lapveteläinen and Aro (1994) analyzed the chemical composition and functionality of high-protein (close to 50%) oat flour, by-product of an oat starch process. Processing caused some changes in percentages of salt- and alkali-soluble fractions, but molecular weight distribution and amino acid compositions of extracted fractions remained fairly constant.

This study was conducted to characterize all major oat protein fractions by RP-HPLC. Analysis conditions were first optimized. The influence of wet processing on protein composition was determined by comparing separation profiles of high-protein oat flour and of oat groats, raw material for the starch process (Lapveteläinen and Aro 1994). In addition, protein fractions of five Finnish oat cultivars were analyzed by RP-HPLC and compared to gel electrophoretic separations to determine genotypic variation. The use of RP-HPLC to analyze oat globulins and glutelins has not been reported previously, though numerous articles describe characterization of corresponding protein fractions in other cereals (Bietz 1983; Burnouf and Bietz 1984, 1989; Huebner et al 1990) and in soybeans (Peterson and Wolf 1988, 1992).

MATERIALS AND METHODS

Oat Materials

The influence of wet processing on RP-HPLC protein separations was determined using materials from an oat starch process (Alko Ltd., Rajamäki Factories, Finland). High-protein oat (*Avena sativa* L.) flour is a spray-dried by-product of the process, containing 52% protein (Lapveteläinen and Aro 1994). Steamed groats from a mixture of Finnish feed cultivars was the raw material of the process. Protein content of the groats was 17% (Lapveteläinen and Aro 1994). For analysis, groats were milled in a table mill (Allen Bradley Co., Milwaukee, WI) with a 40-mesh sieve. RP-HPLC analysis conditions were optimized using the milled, steamed groats.

Samples of five Finnish oat cultivars were selected from pure seed maintained by plant breeders. Cultivars Puhti, Ryhti, Veli and Nasta (protein contents 20, 18, 18, and 18%, respectively) were from the Institute of Plant Breeding (Agricultural Research Center of Finland, Jokioinen, Finland), and the cultivar Virma (21% protein) was from the Hankkija Plant Breeding Institute (Finland). The cultivars studied account for 83% of cultivated oats in Finland (Laboratory of State Granary 1990). Grains of each cultivar were manually dehulled and then milled as described above.

Extraction of Protein Fractions

Extractions were performed in 10-ml capped polypropylene centrifuge tubes at room temperature. Samples (60 mg of milled groats or 30 mg of high-protein oat flour) were first defatted using *n*-butanol. After centrifugation, supernatants were discarded. The solvents then used to sequentially extract salt-soluble, alcohol-soluble, and alkali-soluble proteins were 1*M* NaCl, 52%

[1]Visiting research associate, USDA-ARS-NCAUR, Peoria, IL. Current address: University of Turku, Department of Biochemistry and Food Chemistry, FIN-20500 Turku, Finland.
[2]USDA-ARS, National Center for Agricultural Utilization Research, Peoria, IL.
The mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.

ethanol, and 1% SDS in 0.05 *M* borate buffer (pH 10), respectively. To simplify the nomenclature of extracted proteins, the final fraction is called alkali-soluble, although extractability of these proteins is greatly facilitated by SDS in the buffer. Each extraction used 1 ml of solvent and was performed for 30 min by mixing in a shaker (Lab-line Instruments, Melrose Park, IL). Slurries were centrifuged for 15 min at 27,000 × *g* and supernatants were removed to vials for HPLC analysis. Protein extracts were stored at room temperature and analyzed by RP-HPLC within four days. The first fraction, salt-soluble proteins, contained both albumins and globulins. Some components in this NaCl extract precipitated during the first 10 hr of storage at room temperature. Therefore, NaCl extracts were again centrifuged (15 min, 12,000 × *g*) to prevent HPLC column blockage. The alkali-soluble fraction was diluted 1:1 with extraction solution and reduced by adding dithiothreitol (DTT) (5 mg/ml). Mixtures were kept at 60°C for 10 min, cooled to room temperature, and then analyzed by RP-HPLC.

Chromatography

The HPLC apparatus included a Spectra-Physics (San Jose, CA) SP8700 solvent delivery system and an SP8780XR autosampler. Proteins were detected at 210 nm with an SF770 Spectroflow monitor (Kratos, Ramsey, NJ). Separation were done on two Vydac C_4-columns (150- × 4.6-mm; 214TP5415, Separations Group, Hesperia, CA), a Vydac C_{18} column (250- × 4.6-mm; 218TP54, Separations Group, Hesperia, CA), and a Supelcosil C_8 column (50- × 4.6-mm; LC-308, Supelco, Bellefonte, PA). Columns were preceded by a 20- × 2-mm guard column (C-130B, Upchurch, Oak Harbor, WA).

Chromatographic solvents A (10% acetonitrile [ACN] + 0.11% trifluoroacetic acid [TFA]) and B (90% ACN + 0.09% TFA) were deaerated by vacuum filtration (0.45 μm) and sparged with helium during use. TFA concentrations were varied in the solvents to prevent baseline drift (Huebner and Bietz 1987). Throughout the text, however, TFA concentration is designated as its average value, 0.1%. A higher TFA concentration (0.22% and 0.18% in solvents A and B, respectively; average 0.2%) was also tested. In some experiments, 0.05% SDS was added to each solvent; SDS enhances separation of some proteins (Bietz 1983). Organic solvents were HPLC grade; other chemicals were reagent grade or better.

Oat protein separations were performed at 50–72°C. Temperature was maintained with a CH-460 column heater (Fiatron, Oconomowoc, WI). Samples (5–50 μl) were analyzed at a flow rate of 0.9 ml/min. Gradients were linear, but usually had multiple steps to improve resolution. Gradients were optimized for each separation, as described in the figure captions. At the end of each run, the maximum ACN concentration for each gradient was maintained for 4 min before the column was reequilibrated (10 min) for the next sample.

Data were recorded on an Omniscribe recorder (Houston Instruments, Austin, TX) and stored in a ModComp computer system for later integration and replotting. To facilitate visual comparison of chromatograms, plotted data were normalized so that peaks of maximum absorbance had equal heights.

Electrophoresis

Molecular weight of salt-, alcohol-, and alkali-soluble polypeptides were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Extract (100 μl) was mixed with 200 μl of sample buffer (62.5 m*M* Tris-HCl, pH 6.8, containing 10% glycerol, 2% SDS, and 5% 2-mercaptoethanol), and heated for 5 min at 95°C. For electrophoresis under nonreducing conditions, 2-mercaptoethanol was omitted from the sample buffer. Buffered samples(15-40 μl) were applied to Mini-PROTEAN II Ready Gels (Bio-Rad, Richmond, CA) containing 12.0% polyacrylamide. Gels were run at 200 V for 30 min using an electrode buffer of 25 m*M* Tris, 192 m*M* glycine, 0.1% SDS, pH 8.3. Gels were stained with methanol-acetic acid-water (3:1:12, v/v/v) containing 6% trichloracetic acid and 0.024% Coomassie Blue R-250, and destained with methanol-acetic acid-water (4:1:8, v/v/v). Gels were calibrated using Bio-Rad molecular weight standard proteins.

RESULTS

Optimization of Analysis Conditions

Extracted oat protein fractions exhibited different chromatographic profiles. Figures 1–3 show chromatograms of salt-soluble, alcohol-soluble, and alkali-soluble fractions, respectively, analyzed using different columns and TFA concentrations. Gradients differed for each analysis. Plots show the best resolutions achieved.

Using 0.1% TFA, all salt-soluble proteins eluted between 18 and 47 min (Fig. 1 B,D,F). Most major components had similar hydrophobicities (eluting with 36–42% ACN). Some minor components also eluted during the first 20 min, with 30–36% ACN. When TFA concentration was increased to 0.2%, higher ACN concentrations were required to elute components. Simultaneously, resolution improved slightly (Fig. 1 C,E,G). Resolution and selectivity of different columns varied somewhat. The best separations of salt-soluble proteins were with C_{18} and C_4 columns (Fig. 1 A,F,G).

Most ethanol- and alkali-soluble proteins were eluted by ACN concentrations comparable to those used for salt-soluble components, although gradient modifications were needed to optimize separations. Ethanol-soluble components were well separated with C_4 and C_{18} columns (Fig. 2 A,D–G). Resolution with the C_8 column (Fig. 2 B,C) was, however, poor. Increased TFA concentration influenced both retention times and separation profiles of alkali-soluble proteins (e.g., Fig. 3 D,E). Alkali-soluble proteins were most susceptible to changes in gradient conditions. In some

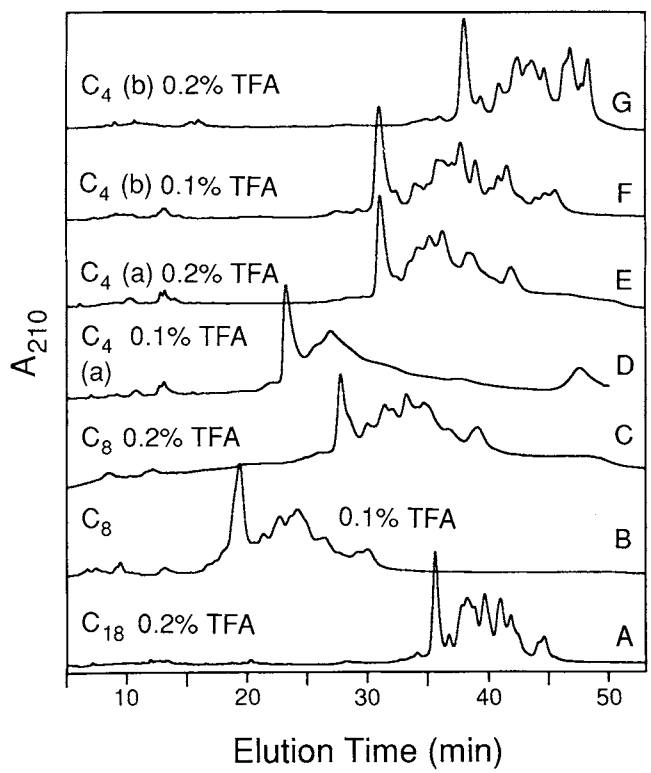

Fig. 1. Reversed-phase high-performance liquid chromatography of salt-soluble oat proteins. Elution profiles were obtained at 60°C on a Vydac 218TP54 column with 0.2% trifluoroacetic acid (TFA) (A); on a Supelcosil LC-308 column with 0.1% TFA (B) and 0.2% TFA (C); and on two Vydac 214TP5415 C_4 columns, designated as (a) and (b), with 0.1% TFA (D, F) and 0.2% TFA (E, G). Gradients: A, 26% acetonitrile (ACN) increasing linearly to 34% at 3 min, to 46.8% at 50 min, and to 48.4% at 54 min; B, C, E, G, 26% ACN, increasing linearly to 34% at 3 min, to 41.2% at 40 min, and to 43.6% at 42 min; D, 26% ACN, increasing linearly to 31.6% at 4 min, to 36.4% at 15 min, to 38% at 40 min, and to 42% at 42 min; and F, 26% ACN, increasing linearly to 34% at 3 min, to 42% at 50 min, and to 43.6% at 52 min.

cases, a difference of only 1% in initial (4–25 min) ACN concentration caused as great a change as did increased TFA concentration (results not shown). Alkali-soluble proteins were best separated with the C_4 (b) column using 0.2% TFA (Fig. 3G).

Separation efficiency of the two Vydac C_4 columns tested differed, presumably due to previous histories of the columns. For all protein fractions, separations were superior on the column designated as C_4 (b) (Figs. 1–3), which was used in all later runs.

Temperature effects. Resolution of oat protein fractions was tested at 50–72°C in 2° increments. Changes in separations due to temperature differences were fairly small, but results show the importance of careful temperature control. Temperature changes did not alter the total elution time of alcohol-soluble proteins (Fig. 4). However, retention of alcohol-soluble components that elute between 37–44 min (at 60°C) varied with temperature. Most effects of temperature on separation of these proteins were probably caused by shifts of these components. For example, at 72°C the shifting components apparently eluted with the other components at 31–36 min. At lower temperatures, elution times of shifting components increased, and at 50°C these components eluted about 10 min later than they did at 72°C. The best separation of oat alcohol-soluble proteins was at 54–60°C.

Higher temperature decreased elution times of salt- and alkali-soluble proteins by about 5–7 min. Separation profiles also varied little with temperature (results not shown). The best resolution for salt-soluble proteins was at 64°C. For alkali-soluble proteins, temperature most affected components eluting from 28–36 min at 58°C (Fig. 5C). The best resolution of earlier eluting alkali-soluble proteins was at 56–58°C; later-eluting proteins separated best at 70–72°C.

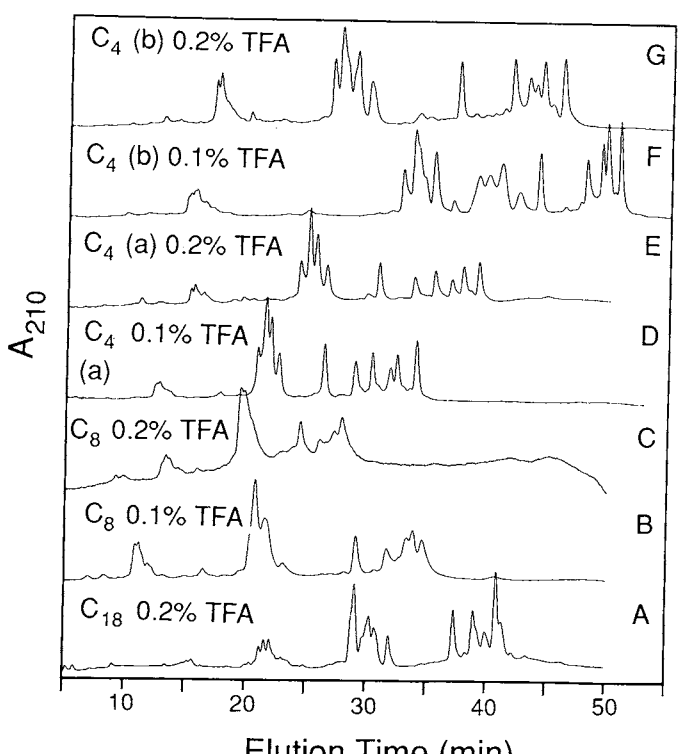

Fig. 2. Reversed-phase high-performance liquid chromatography of alcohol-soluble oat proteins. Elution profiles were obtained at 60°C on a Vydac 218TP54 column with 0.2% trifluoroacetic acid (TFA) (A); on a Supelcosil LC-308 column with 0.1% TFA (B) and 0.2% TFA (C); and on two Vydac 214TP5415 C_4 columns, designated as (a) and (b), with 0.1% TFA (D, F) and 0.2% TFA (E, G). Gradients: A, C, D, E, 26% acetonitrile (ACN), increasing linearly to 32.4% at 4 min, to 38% at 15 min, to 43.6% at 40 min, and to 44.4% at 42 min; B, 26% ACN, increasing to 32.4% at 4 min, to 35.6% at 15 min, to 41.2% at 40 min, and to 42.8% at 42 min; F, 26% ACN, increasing to 32.4% at 4 min, to 40.4% at 42 min, and to 43.6% at 46 min; and G, 26% ACN, increasing to 30% at 3 min, to 36.4% at 10 min, to 43.6% at 50 min, and to 44.4% at 52 min.

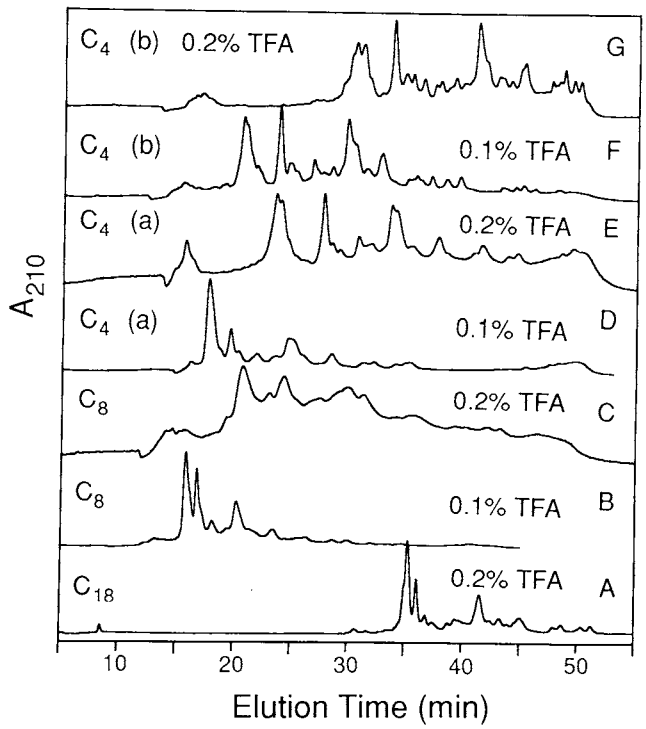

Fig. 3. Reversed-phase high-performance liquid chromatography of alkali-soluble oat proteins. Elution profiles were obtained at 60°C on a Vydac 218TP54 column with 0.2% trifluoroacetic acid (TFA) (A); on a Supelcosil LC-308 column with 0.1% TFA (B) and 0.2% TFA (C); and on two Vydac 214TP5415 C_4 columns, designated (a) and (b), with 0.1% TFA (D, F) and 0.2% TFA (E, G). In all runs, the acetonitrile (ACN) concentration was initially 26%, and increased linearly to 34% at 3 min. The remainder of the gradients were: A, increase of ACN to 43.6% at 43 min and to 50% at 54 min; B, increase of ACN to 37.2% at 15 min, to 41.2% at 35 min, and to 43.6% at 36 min; C, D, E, G, increase of ACN to 41.2% at 40 min and to 43.6% at 42 min; and F, increase of ACN to 42.8% at 40 min and to 44.4% at 42 min.

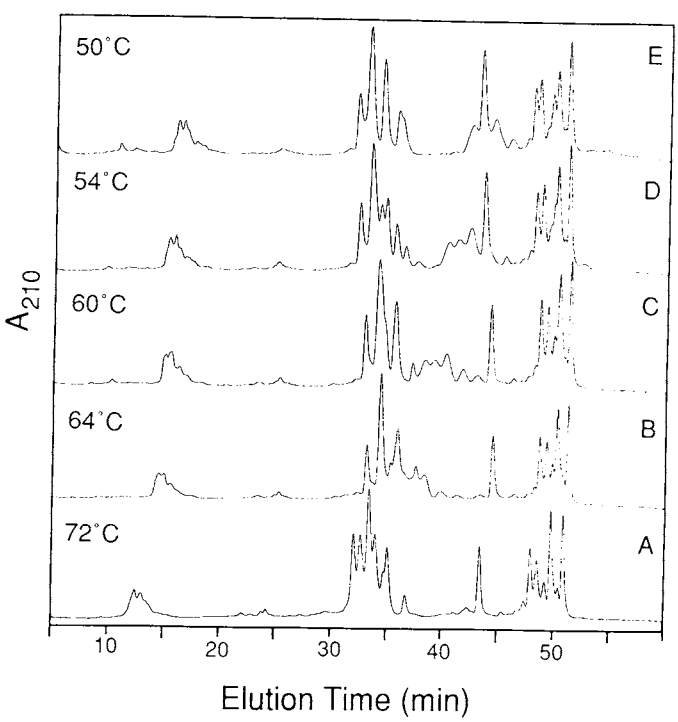

Fig. 4. Reversed-phase high-performance liquid chromatography of alcohol-soluble oat proteins at 72°C (A), 64°C (B), 60°C (C), 54°C (D), and 50°C (E). Samples were fractionated on a Vydac 214TP5415 column in the presence of 0.1% trifluoroacetic acid (TFA). Gradient conditions were as in Fig. 2F.

Reduction of proteins. Alkali-soluble protein fractions were treated with DTT to cleave disulfide bonds, permitting fractionation of subunit polypeptides (Burnouf and Bietz 1984, Bietz 1990). Reduced components (Fig. 5C) were better resolved and had a flatter baseline than did unreduced alkali-soluble proteins (Fig. 5D). Since salt- and alkali-soluble oat proteins are very similar (Brinegar and Peterson 1982; Robert et al 1983b, 1985), we also tested the effect of reduction on the salt-soluble fraction. However, reduction did not improve RP-HPLC resolution of salt-soluble proteins (results not shown).

Effect of SDS. Peterson and Wolf (1992) added SDS to RP-HPLC solvents to enhance soybean protein separations. We did not observe a similar beneficial effect for any oat protein fraction. In each case, separations were superior without SDS. Since the extractant for alkali-soluble proteins already contained SDS, the deterioration in separation upon adding SDS to the RP-HPLC solvents was probably not as dramatic as that for the other fractions.

All later RP-HPLC separations in this study were conducted at 58°C. This temperature was a compromise between optimal separation temperatures for various protein fractions. For analysis of alcohol-soluble proteins, it was important that shifting components could be detected separately from the other components at the temperature chosen. If analyses were restricted, for instance, to salt-soluble proteins, the best separation would be at 64°C. Resolution of proteins might also be slightly enhanced by elevating TFA concentration to 0.2%. However, 0.1% TFA was used to prolong column stability and performance.

Effect of Processing and Cultivar Differences

Figure 5 shows RP-HPLC separations of salt-, alcohol-, and alkali-soluble proteins of oat groats, the raw material of the starch process. Cultivar differences and effect of processing were characterized by visual comparison of chromatograms and by calculating indicated individual areas as percentages of total integrated area.

Salt-soluble proteins. Wet processing increased the amount of salt-soluble components eluting during the first 15 min (Fig. 5A and Fig. 6 [Flour]). Thus, area 1 was 3% of the total integrated area in oat groats, but 14% for high-protein flour. Otherwise, separation profiles of groats and flour were similar. Salt-soluble proteins of oat cultivars differed slightly in HPLC profiles; the extremes were cultivars Ryhti and Nasta (Fig. 6). However, components were not sufficiently resolved to prove whether any cultivars had unique salt-soluble marker proteins. Nevertheless, quantitative differences in area percentages were detected for the samples used. For instance, Ryhti had less of areas 4 and 5 and more of area 6, possibly differentiating it from the other cultivars.

In agreement with Lookhart and Pomeranz (1985), salt-soluble proteins of cultivars were electrophoretically homogeneous. All samples had major components of 47–60 kDa under nonreducing conditions and 32–38 and 22–24 kDa under reducing conditions, corresponding to α- and β-globulins, respectively (Brinegar and Peterson 1982; Robert et al 1983b, 1985). Several minor components were also visible, especially for salt-soluble proteins from the cultivars (results not shown).

Alcohol-soluble proteins. The RP-HPLC profile of alcohol-soluble proteins from high-protein oat flour did not differ from that of oat groats (Fig. 5B). The only noticeable difference between the high-protein oat flour and groat mixture was that the percentage of area 2 was lower in the protein-rich by-product (7%) than in the raw material (13%). This agrees with results of Lookhart et al (1986), who reported that commercial processing did not cause electrophoretically detectable changes in oat prolamins. These results for alcohol-soluble proteins of oat cultivars (Fig. 7) support earlier studies using RP-HPLC for cereal cultivar identification (Bietz et al 1984; Marchylo and Kruger 1984; Lookhart 1985; Paulis and Bietz 1986; Kubiczek et al 1993). Three of five oat cultivars had characteristic different profiles. Ryhti lacked a component around 44 min that was present in all other cultivars. Puhti had a major component at 34 min. Virma had a major component at around 40 min (Fig. 7). Corresponding differences were detected in percentages of areas 9, 4, and 7, respectively. Moreover, Ryhti, Veli, and Nasta had higher percentages (19, 11, and 10%, respectively) for area 6 than did Puhti and Virma (1%). RP-HPLC profiles and area percentages for Veli and Nasta were similar. These cultivars have one common parent (Multamäki and Kaseva 1987), which may explain these similarities. Also, electrophoretic patterns of these two cultivars were similar, whereas the patterns of the other three cultivars

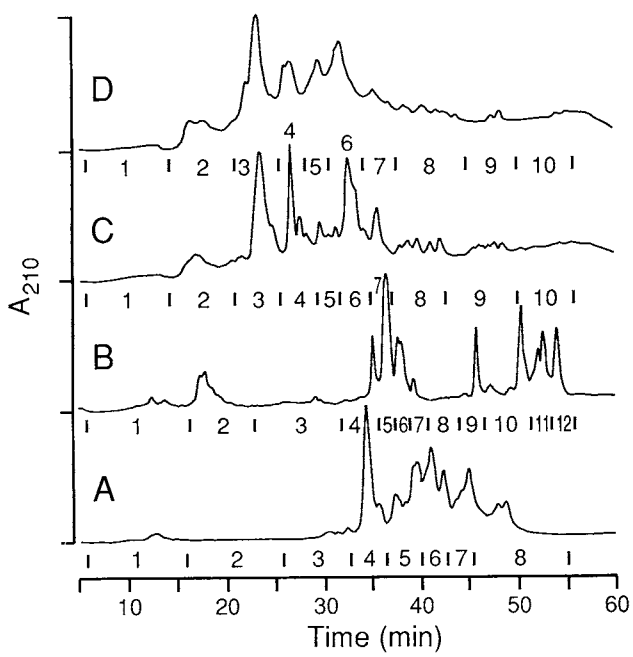

Fig. 5. Reversed-phase high-performance liquid chromatography separations of oat groat protein fractions: salt-soluble proteins (A); alcohol-soluble proteins (B); alkali-soluble proteins reduced with dithiothreitol (DTT) after extraction (C); and unreduced alkali-soluble proteins (D). Samples were fractionated at 58°C on a Vydac C_4 214TP5415 column in the presence of 0.1% trifluoroacetic acid (TFA). Dividing lines show areas integrated to calculate percentages of total area. The integration intervals were adjusted to correspond to selected troughs in the chromatograms. Gradients: A, 26% acetonitrile (ACN) increasing linearly to 34% at 3 min, to 41.2% at 45 min, to 50% at 60 min, and to 51.6% at 62 min; B, 22% ACN increasing to 32.4% at 4 min, to 40.4% at 40 min, to 43.6% at 50 min, and to 48.4% at 52 min; C, D, 26% ACN, increasing to 34% at 3 min, to 42% at 36 min, and to 50% at 48 min.

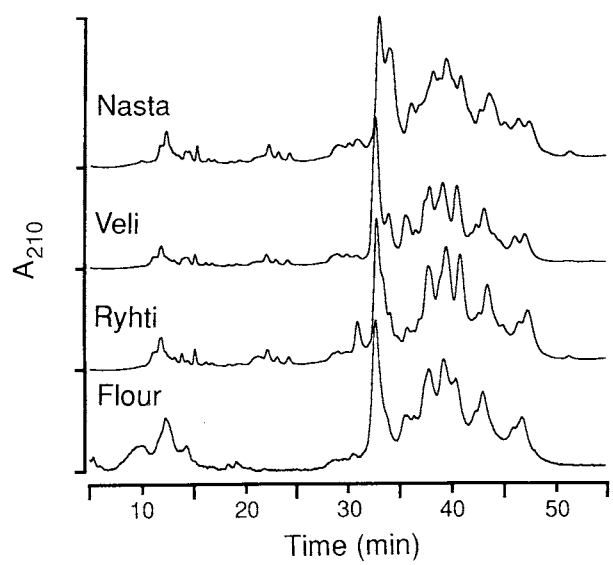

Fig. 6. Reversed-phase high-performance liquid chromatography separations of salt-soluble proteins of high-protein oat flour and oat cultivars Ryhti, Veli, and Nasta. Separation conditions were as in Fig. 5A.

had some differences (Fig. 8). Lookhart (1985) differentiated oat cultivars with similar electrophoretic patterns by RP-HPLC. In the present study, RP-HPLC did not give improved differentiation. Differences between Virma, Ryhti, and Puhti were, however, more evident upon RP-HPLC than by electrophoresis.

Alkali-soluble proteins. Groats and high-protein flour showed similar RP-HPLC and SDS-PAGE patterns for reduced alkali-soluble proteins (Fig. 5C and Fig. 9). Percentages of individual areas of these samples differed only slightly, with the largest differences being 2%. Groats used as the raw material of oat starch process had been steamed. This may be one explanation for the small amount of early eluting (at 15–20 min) alkali-soluble components in flour and groat mixture. The proportion of theses components were two to three times larger in cultivar samples (Fig. 9). Cultivars differed mainly in percentages of individual areas. If ratios of selected areas, such as 6/2 or 3/2, are calculated, Nasta clearly differs from Puhti and Virma.

Electrophoretic separations of alkali-soluble fractions had major components at the same molecular weight as in the salt-soluble fraction (results not shown), verifying that globulin polypeptides are the major components in these fractions (Robert et al 1983b, 1985). Minor bands (<20 kDa) were more apparent in cultivars than in groats or flour; these may correspond to RP-HPLC components at 15–20 min.

Reproducibility. Sample variation in RP-HPLC of oat protein fractions is generally low (coefficient of variation <10%). Some specific chromatographic areas, however, such as the early eluting (6–26 min) salt-soluble components, were highly variable. This may be associated with precipitation of some of these proteins with time, necessitating a second centrifugation. It thus appears best to analyze salt-soluble proteins by RP-HPLC as soon as possible after extraction.

Alcohol-soluble fractions varied greatly in percentages of areas 7 and 8 (38–43 min) (Figs. 4 and 5B). This variation was caused by components, previously designated as shifting, which do not appear consistently in replicate extracts. The elution times of these proteins also depend greatly on column temperature (Fig. 4). When reproducibility of extraction was tested with a groat mixture, these shifting components were visually detectable in three of ten replicate extracts. They accounted for 8–10% of the total chromatogram area. Shifting components were present in all three replicate extracts of the high-protein oat flour; they comprised 1–5% of the total area. These components were also detected in all replicate prolamin fractions of Nasta. The reason for the occasional presence and atypical behavior of these components remains unresolved. Numerous tests involving sample homogeneity, sample size, extraction time, and number of extractions were conducted to try to explain the shifting components. These studies did not, however, explain why these components are not consistently present.

DISCUSSION

Extraction conditions may greatly influence, both quantitatively and qualitatively, the composition of resulting oat protein fractions. Salt- and ethanol-soluble proteins have been extracted with

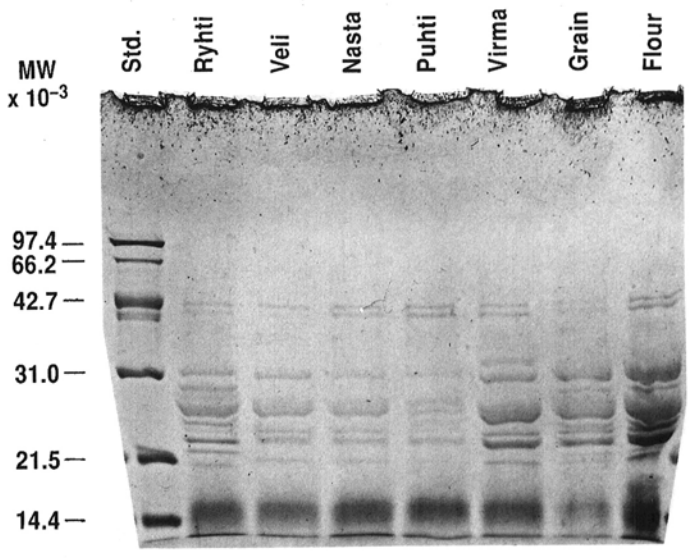

Fig. 8. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of alcohol-soluble proteins of oat cultivars Ryhti, Veli, Nasta, Puhti, and Virma, of the groat mixture (grain), and of high-protein oat flour. Samples were reduced before analysis.

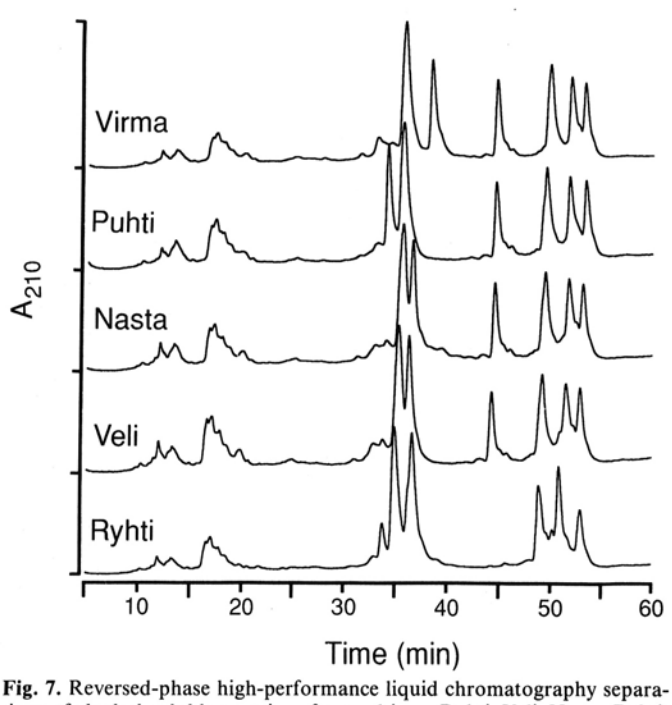

Fig. 7. Reversed-phase high-performance liquid chromatography separations of alcohol-soluble proteins of oat cultivars Ryhti, Veli, Nasta, Puhti, and Virma. Fractionation conditions were as in Fig. 5B.

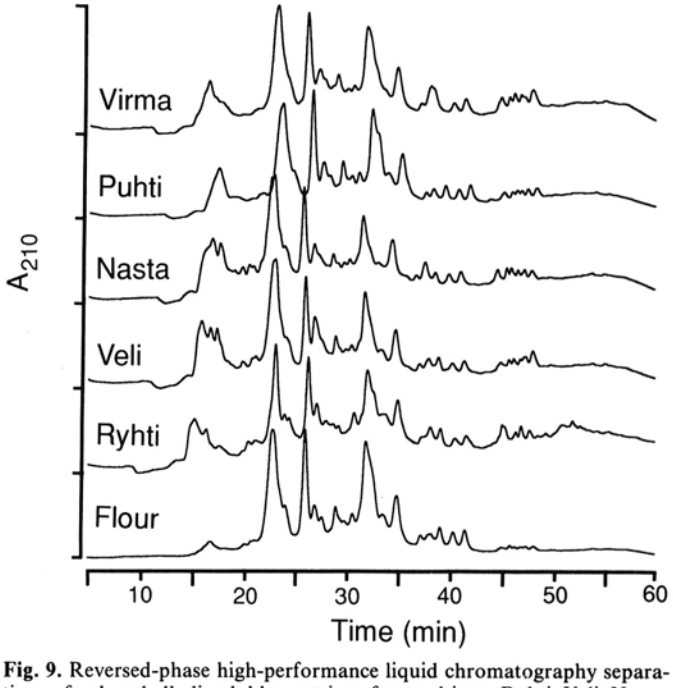

Fig. 9. Reversed-phase high-performance liquid chromatography separations of reduced alkali-soluble proteins of oat cultivars Ryhti, Veli, Nasta, Puhti, and Virma, and of high-protein oat flour. Fractionation conditions were as in Fig. 5C.

1.0*M* NaCl in 0.05*M* Tris, pH 8.5, and with 52% ethanol, respectively (Peterson 1978, Robert et al 1983b, Robert 1985). In the present study, salt-soluble proteins were solubilized with unbuffered 1.0*M* NaCl. We found that the composition of proteins extracted with buffered and unbuffered 1.0*M* NaCl were qualitatively identical, but quantities of extracted salt- and alkali-soluble proteins differed.

In a previous study of the same oat samples (groat mixture and high-protein flour), alcohol-soluble proteins were extracted with 55% 2-propanol containing 1% 2-mercaptoethanol (Lapveteläinen and Aro 1994). In the present study, however, reducing agent was omitted from this extractant, since preliminary trials showed that it had an unfavorable effect on RP-HPLC separations.

We also compared extraction performance of 55% 2-propanol and 52% ethanol. Resulting fractions differed mostly in total quantities extracted. Because 52% ethanol extracted more protein and is the usual oat prolamin extractant (Kim et al 1978; Robert et al 1983a,b, 1985), it was used in these studies. Seventy percent ethanol has also been used to extract oat prolamins (Ma 1983, Ma and Harwalkar 1984, Lookhart 1985, Lookhart and Pomeranz 1985, Wieser and Belitz 1989). This solvent seems to extract as much protein as 52% ethanol, and the two extracts appear qualitatively identical. Moreover, six replicate extractions with 70% ethanol did not display shifting RP-HPLC peaks, suggesting that 70% ethanol may extract oat prolamins more consistently than 52% ethanol.

Bietz et al (1984) reported that 70% ethanol extracts of wheat proteins remain stable for more than 20 days at room temperature. Salt- and alkali-soluble protein extracts may, however, change in composition due to proteolysis, aggregation, or changes in disulfide bond oxidative state or cross-linking. Our oat protein extracts remained unchanged for 7–10 days at room temperature, so HPLC analyses were done within four days after extraction.

CONCLUSIONS

RP-HPLC separations of oat protein fractions were affected by several factors, including concentration of ion-pairing reagent, column temperature, and protein reductive state. Results emphasize the importance of carefully optimizing analytical RP-HPLC conditions.

Our results confirm that RP-HPLC is an excellent technique for oat cultivar differentiation. We also show, however, that until the inconsistent presence of certain prolamin components is understood, the significance of minor chromatographic differences must be viewed with caution. Marker prolamins for varietal identification in this study were evident, so inconsistent components did not influence differentiation.

Analyses of salt- and alkali-soluble oat proteins may be useful for assessing effects of processing on resulting products. Changes in protein composition and distribution resulting from processing can be quickly and conveniently determined by RP-HPLC. In this study, however, qualitative changes in protein composition of the products we examined, as detected by RP-HPLC, were small.

RP-HPLC separates proteins primarily on the basis of surface hydrophobicities. It will be valuable to relate this information to molecular weight of separated components to further assess the utility of information resulting from this technique.

LITERATURE CITED

BIETZ, J. A. 1983. Separation of cereal proteins by reversed-phase high-performance liquid chromatography. J. Chromatogr. 255:219.

BIETZ, J. A. 1990. HPLC of cereal endosperm storage proteins. Page 429 in: HPLC of Biological Macromolecules: Methods and Applications. K. M. Gooding and F. E. Regnier, eds. Marcel Dekker: New York.

BIETZ, J. A., BURNOUF, T., COBB, L. A., and WALL, J. S. 1984. Wheat varietal identification and genetic analysis by reversed-phase high-performance liquid chromatography. Cereal Chem. 61:129.

BRINEGAR, A. C., and PETERSON, D. M. 1982. Separation and characterization of oat globulin polypeptides. Arch. Biochem. Biophys. 219:71.

BURNOUF, T., and BIETZ, J. A. 1984. Reversed-phase high-performance liquid chromatography of reduced glutenin, a disulfide-bonded protein of wheat endosperm. J. Chromatogr. 299:185.

BURNOUF, T., and BIETZ, J. A. 1989. Rapid purification of wheat glutenin for reversed-phase high-performance liquid chromatography: Comparison of dimethyl sulfoxide with traditional solvents. Cereal Chem. 66:121.

HUEBNER, F. R., and BIETZ, J. A. 1987. Improvements in wheat protein analysis and quality prediction by reversed-phase high-performance liquid chromatography. Cereal Chem. 64:15.

HUEBNER, F. R., BIETZ, J. A., WEBB, B. D., and JULIANO, B. O. 1990. Rice cultivar identification by high-performance liquid chromatography of endosperm proteins. Cereal Chem. 67:129.

KIM, S. I., CHARBONNIER, L., and MOSSÉ, J. 1978. Heterogeneity of avenin, the oat prolamin. Fractionation, molecular weight and amino acid composition. Biochim. Biophys. Acta 537:22.

KUBICZEK, R. P., HUEBNER, F. R., and BIETZ, J. A. 1993. Reversed-phase high-performance liquid chromatography of secalins: Application to rye cultivar identification. J. Cereal Sci. 17:191.

LABORATORY OF STATE GRANARY. 1990. Research Reports: Sample Survey of Cereals 1990. Helsinki, Finland.

LAPVETELÄINEN, A., and ARO, T. 1994. Protein composition and functionality of high-protein oat flour derived from integrated starch-ethanol process. Cereal Chem. 71:133.

LOOKHART, G. L. 1985. Identification of oat cultivars by combining polyacrylamide gel electrophoresis and reversed-phase high-performance liquid chromatography. Cereal Chem. 62:345.

LOOKHART, G. L., ALBERS, L., and POMERANZ, Y. 1986. The effect of commercial processing on some chemical and physical properties of oat groats. Cereal Chem. 63:280.

LOOKHART, G. L., and POMERANZ, Y. 1985. Characterization of oat species by polyacrylamide gel electrophoresis and high-performance liquid chromatography of their prolamin proteins. Cereal Chem. 62:162.

MA, C.-Y. 1983. Chemical characterization and functionality assessment of protein concentrates from oats. Cereal Chem. 60:36.

MA, C.-Y., and HARWALKAR, V. R. 1984. Chemical characterization and functionality assessment of oat protein fractions. J. Agric. Food Chem. 32:144.

MARCHYLO, B. A., and KRUGER, J. E. 1984. Identification of Canadian barley cultivars by reversed-phase high-performance liquid chromatography. Cereal Chem. 61:295.

MULTAMÄKI, K., and KASEVA, A. 1987. Domestic Varieties. Report 11/87. Agricultural Research Center: Jokioinen, Finland.

PAULIS, J. W., and BIETZ, J. A. 1986. Separation of alcohol-soluble maize proteins by reversed-phase high-performance liquid chromatography. J. Cereal Sci. 4:205.

PETERSON, D. M. 1978. Subunit structure and composition of oat seed globulin. Plant Physiol. 62:506.

PETERSON, R. E., and WOLF, W. J. 1988. Scale-up from analytical to preparative reversed-phase high-performance liquid chromatography of soybean glycinin. J. Chromatogr. 477:263.

PETERSON, R. E., and WOLF, W. J. 1992. Enhancement of high-performance liquid chromatography of soybean proteins by addition of sodium dodecyl sulfate. Cereal Chem. 69:101.

ROBERT, L. S., NOZZOLILLO, C., and ALTOSAAR, I. 1983a. Molecular weight and charge heterogeneity of prolamins (avenins) from nine oat (*Avena sativa* L.) cultivars of different protein content and from developing seeds. Cereal Chem. 60:438.

ROBERT, L. S., NOZZOLILLO, C., and ALTOSAAR, I. 1985. Characterization of oat (*Avena sativa* L.) residual proteins. Cereal Chem. 62:276.

ROBERT, L. S., NOZZOLILLO, C., CUDJOE, A., and ALTOSAAR, I. 1983b. Total solubilization of groat proteins in high protein oat (*Avena sativa* L. cv. Hinoat): Evidence that glutelins are a minor component. Can. Inst. Food Sci. Technol. J. 16:196.

WIESER, H., and BELITZ, H.-D. 1989. Amino acid compositions of avenins separated by reversed-phase high-performance liquid chromatography. J. Cereal Sci. 9:221.

[Received April 11, 1994. Accepted February 23, 1995.]