Reversed-Phase High-Performance Liquid Chromatography of Ethanol-Soluble and Ethanol-Insoluble Reduced Glutenin Fractions[1]

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ABSTRACT

Glutenin was extracted with 70% aqueous ethanol in the presence of dithioerythritol. Reversed-phase high-performance liquid chromatography (RP-HPLC) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the reduced, but underivatized material showed that the ethanol-soluble fraction consists of middle molecular weight glutenins and the major portion of low molecular weight glutenins. The ethanol-insoluble fraction contains high molecular weight subunits and the minor portion of low molecular weight glutenins. Soluble and insoluble fractions from four wheat varieties of different baking quality were compared by RP-HPLC. The HMW patterns of the insoluble fractions, which were obtained using a urea-containing system, show a relationship with baking quality. The HPLC patterns of the soluble fractions were unique for each variety. For the isolation of single glutenin components, a subfractionation of glutenin by 70% aqueous ethanol under reducing conditions is recommended before separation by RP-HPLC.

Cereal Chem. 66(1):38–41

As shown by Bietz and Wall (1973), reduced and alkylated glutenin can be fractionated by extraction with 70% aqueous ethanol at neutral pH. The ethanol-soluble fraction consists mainly of low molecular weight (LMW) components, and the ethanol-insoluble fraction contains the high molecular weight (HMW) subunits and some of lower MW.

More recently, separations of reduced and alkylated glutenin (Burnouf and Bietz 1984, Huebner and Bietz 1985) and of reduced, but not derivatized glutenin (Seilmeier et al 1987, 1988) by reversed-phase high-performance liquid chromatography (RP-HPLC) have been described. In the present paper, reduced glutenin fractions, soluble and insoluble in neutral 70% aqueous ethanol, are characterized by RP-HPLC. Four wheat varieties with different baking qualities are included in these studies.

MATERIALS AND METHODS

Preparation of Reduced Glutenin Fractions

The flours of the wheat varieties Apollo, Diplomat, Norman, and Rektor were defatted and albumins, globulins, and gliadins were removed according to the method of Wieser et al (1987). Then 5 g of the lyophilized residues was suspended under nitrogen in 50 ml of an aqueous solution containing ethanol (70%, v/v), Tris/HCl (1 mmol/L, pH 7.6), and dithioerythritol (DTE) (0.5%, w/v). After stirring at 4° C for 2 hr, the suspension was centrifuged (5,000 × g, 30 min, 22° C). The sediment was extracted a second time and the supernatants were combined. After removing the main portion of ethanol by N_2 stream, the extract and the residue were lyophilized.

RP-HPLC of Ethanol-Soluble Reduced Glutenins

The chromatography was performed with minor changes as described previously (Wieser et al 1987) under the following conditions: column material, two SynChropak RP-8 (6.5 μm, 300Å) columns were used in series; column size, 4.6 × 240 mm (each column); temperature, 60° C; sample, 1.0 mg lyophilized extract dissolved under nitrogen in 100 μl of acetic acid (0.5 mol/L); elution system A, 2-propanol (15% v,v)/trifluoroacetic acid (TFA) (0.1%, v/v)/DTE (0.01%, w/v); elution system B, acetonitrile (80% v/v)/TFA (0.1%, v/v)/DTE (0.01%, w/v); gradient, 0 min 8% B, 20 min 28% B, 60 min 36% B, 108 min 52% B; flow rate, 0.8 ml/min; detection, ultraviolet absorbance at 220 nm.

RP-HPLC of Ethanol-Insoluble Reduced Glutenins

For chromatography of the ethanol-insoluble glutenin fraction, 150 mg was suspended in 5 ml of a solution containing 50 mmol/L of Tris/HCl (pH 7.8), 6 mol/L of urea, and 50 mmol/L of DTE. After stirring for 2 hr at room temperature, the suspension was centrifuged (15,000 × g, 20 min, 15° C). The extraction was repeated, the supernatants were combined, and 400 μl was analyzed by RP-HPLC using a SynChropak RP-8 column (6.5 μm, 300Å, 4.6 × 240 mm, 65° C) according to the technique of Seilmeier et al (1987). The elution system was an aqueous solution of urea (5 mol/L), TFA (1%, v/v), and DTE (0.01%, w/v) with 20% (v/v) acetonitrile in solvent A and 50% in solvent B. A linear gradient was used from 0 to 100% solvent B over 120 min at 0.5 ml/min. The effluent was monitored at 223 nm.

Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method previously described (Krause et al 1988).

RESULTS AND DISCUSSION

Wheat flour of the variety Rektor was defatted and extracted sequentially with a salt solution and 70% aqueous ethanol. The

[1]Presented at the AACC 72nd Annual Meeting, Nashville, TN, November 1987.

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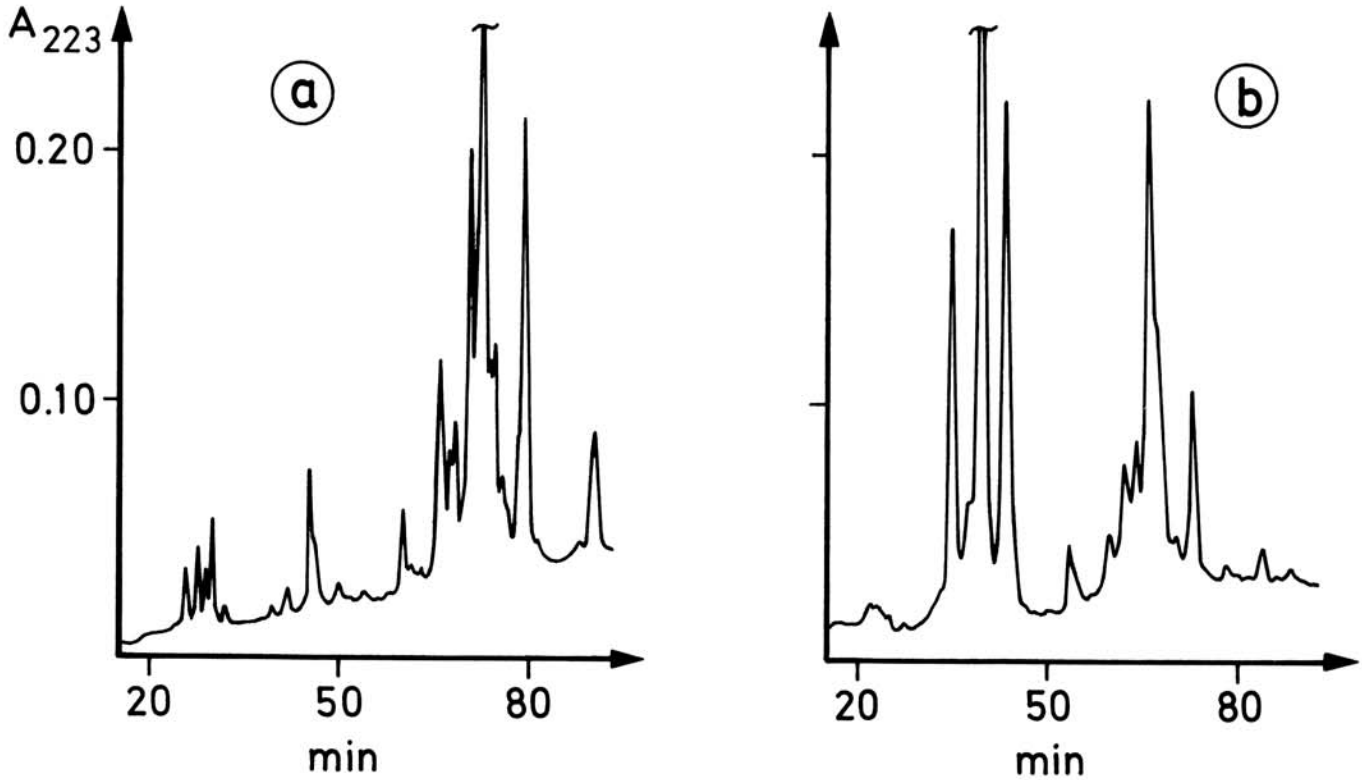

Fig. 1. Reversed-phase high-performance liquid chromatography of the glutenin fractions (variety Rektor) (**a**) soluble and (**b**) insoluble in 70% ethanol/dithioerythritol. Solvent A, 1.0% trifluoroacetic acid + 5 mol/L urea + 0.01% dithioerythritol + 20% acetonitrile; solvent B, as for A but 50% acetonitrile. Gradient: linear; 0 min 0% B, 120 min 100% B.

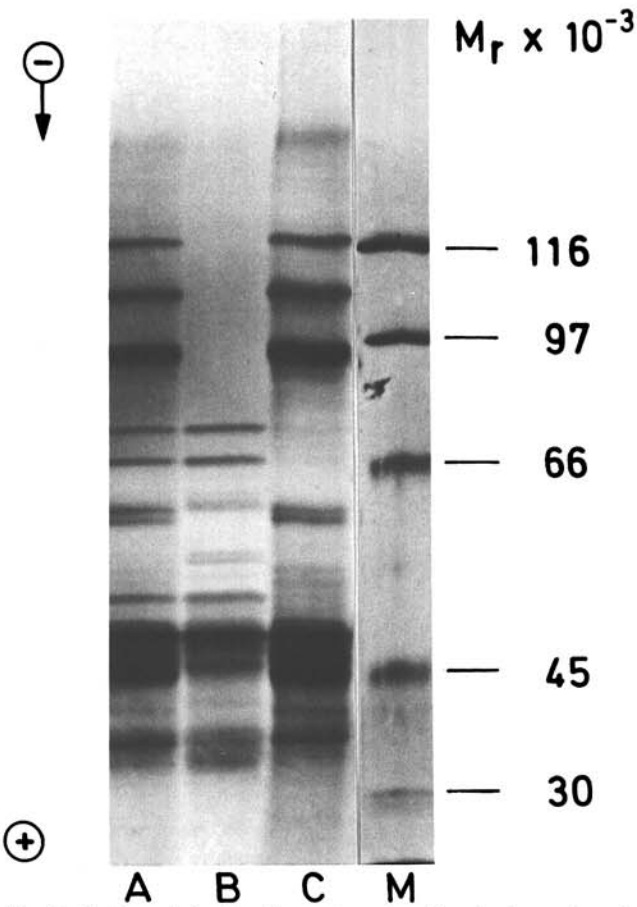

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of glutenin fractions (variety Rektor) Whole glutenin (**A**), glutenin fraction soluble in 70% ethanol/dithioerythritol (**B**), glutenin fraction insoluble in 70% ethanol/dithioerythritol (**C**). Marker proteins (**M**): β-galactosidase ($M_r \times 10^{-3}$ = **116**), phosphorylase b (**97**), bovine serum albumin (**66**), ovalbumin (**45**), and carboanhydrase (**30**).

residue (glutenin plus starch) was extracted with 70% aqueous ethanol containing DTE and Tris/HCl (pH 7.6), as done by Bietz and Wall (1973). The ethanol-soluble and insoluble fractions were characterized by RP-HPLC (Seilmeier et al 1987) and by SDS-PAGE (Krause et al 1988).

The separation of both fractions by RP-HPLC using an elution system containing urea/DTE is shown in Figure 1. The chromatograms are clearly different; according to the results obtained by RP-HPLC of whole reduced glutenin (Seilmeier et al 1987), the soluble fraction consists of middle molecular weight (MMW) glutenins (retention time: 25–32 min) and of the major portion of low molecular weight (LMW) glutenins (59–92 min). The MMW glutenins are similar to ω-gliadins in amino acid composition, but not extractable by 70% aqueous ethanol under nonreducing conditions (H. Wieser et al, *unpublished*). The insoluble fraction contains high molecular weight (HMW) subunits (33–47 min) as the major protein group and LMW glutenins in lesser amounts.

The electrophoretic patterns obtained by SDS-PAGE are presented in Figure 2. As recently shown by Krause and coworkers (1988), the electrophoregram of whole glutenin from the variety Rektor consists of four HMW bands, several bands corresponding to MMW glutenins, and numerous LMW bands. The electrophoregrams of the ethanol-soluble and insoluble fractions differ significantly, even in the area of LMW glutenins, where the bands are closely packed to one another. The HMW subunits are present only in the insoluble fraction and the MMW glutenins only in the soluble fraction; LMW subunits are distributed among the two fractions. These results obtained for reduced, underivatized glutenin are in agreement with those of Bietz and Wall (1973) obtained for aminoethylated glutenin. Altogether, RP-HPLC and SDS-PAGE show that a separation of glutenin can be achieved by the extraction procedure with a neutral 70% ethanol/DTE solution.

For comparative purposes, the glutenin fractions of four wheat varieties with different baking quality (good quality, Diplomat and Rektor; poor quality, Apollo and Norman) were fractionated by extraction with 70% ethanol/DTE.

RP-HPLC of the insoluble fractions was performed using an

elution system containing urea/DTE (Fig. 3). In agreement with studies on whole glutenin (Seilmeier et al 1988), the chromatograms of the varieties Diplomat and Rektor are similar to one another in both HMW and LMW patterns. Their HMW patterns differ strongly from those of Apollo and Norman, which in turn are similar in their HMW patterns but differ in their LMW patterns. The LMW pattern of Norman looks like that of Diplomat or Rektor. According to peak heights, the HMW group of good and poor varieties exhibit convex and concave shapes, respectively.

RP-HPLC of the soluble fractions does not need a urea-containing elution system; a similar system previously used for the separation of gliadins (Wieser et al 1987) can be applied, in the presence of DTE as reducing agent. The chromatograms obtained are shown in Figure 4. Because of the poor resolution of peaks of intermediate retention time (50–60 min), two combined separation

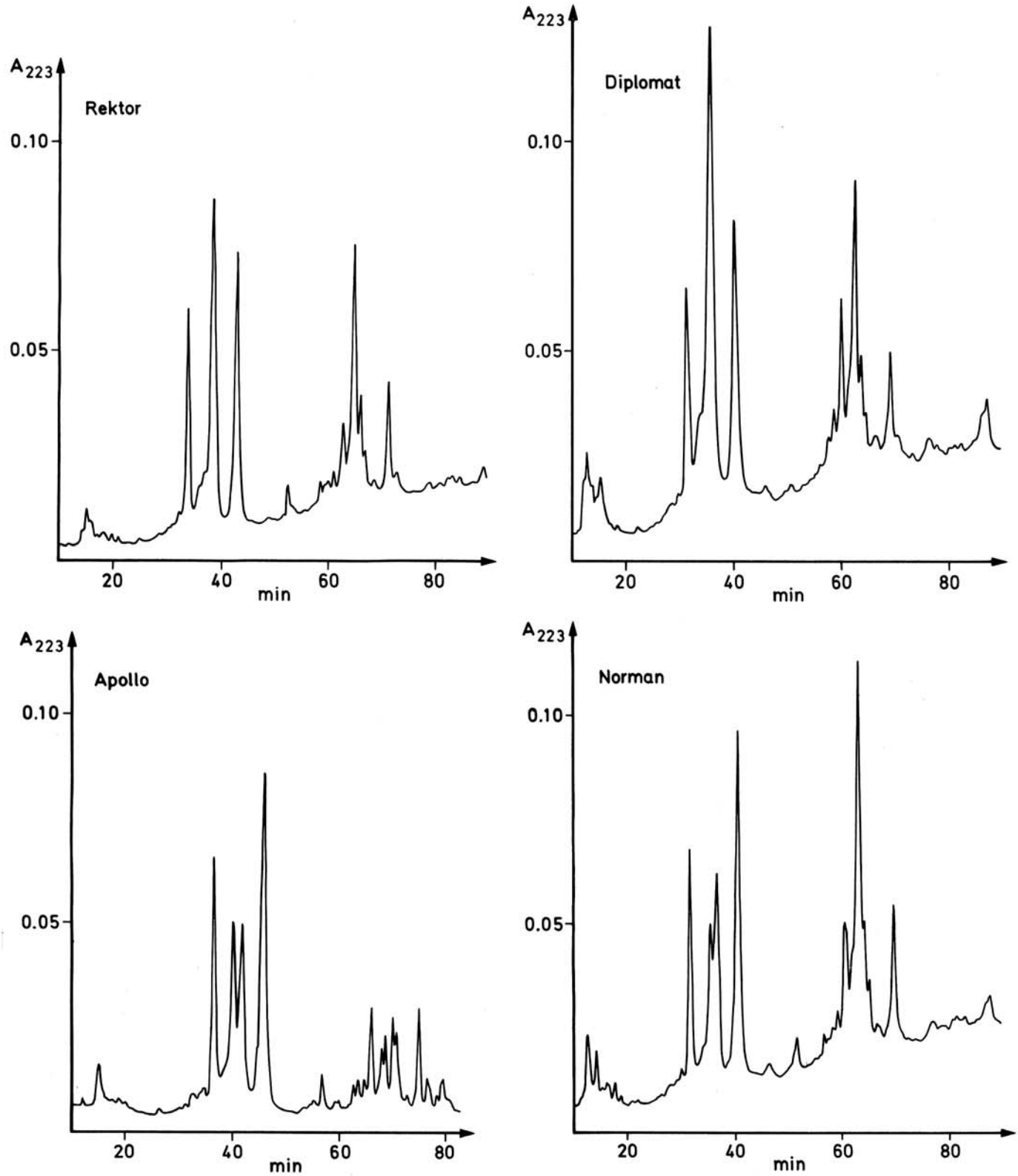

Fig. 3. Reversed-phase high-performance liquid chromatography of glutenin fractions from different wheat varieties insoluble in 70% ethanol/dithioerythritol. Solvents as in Fig. 1.

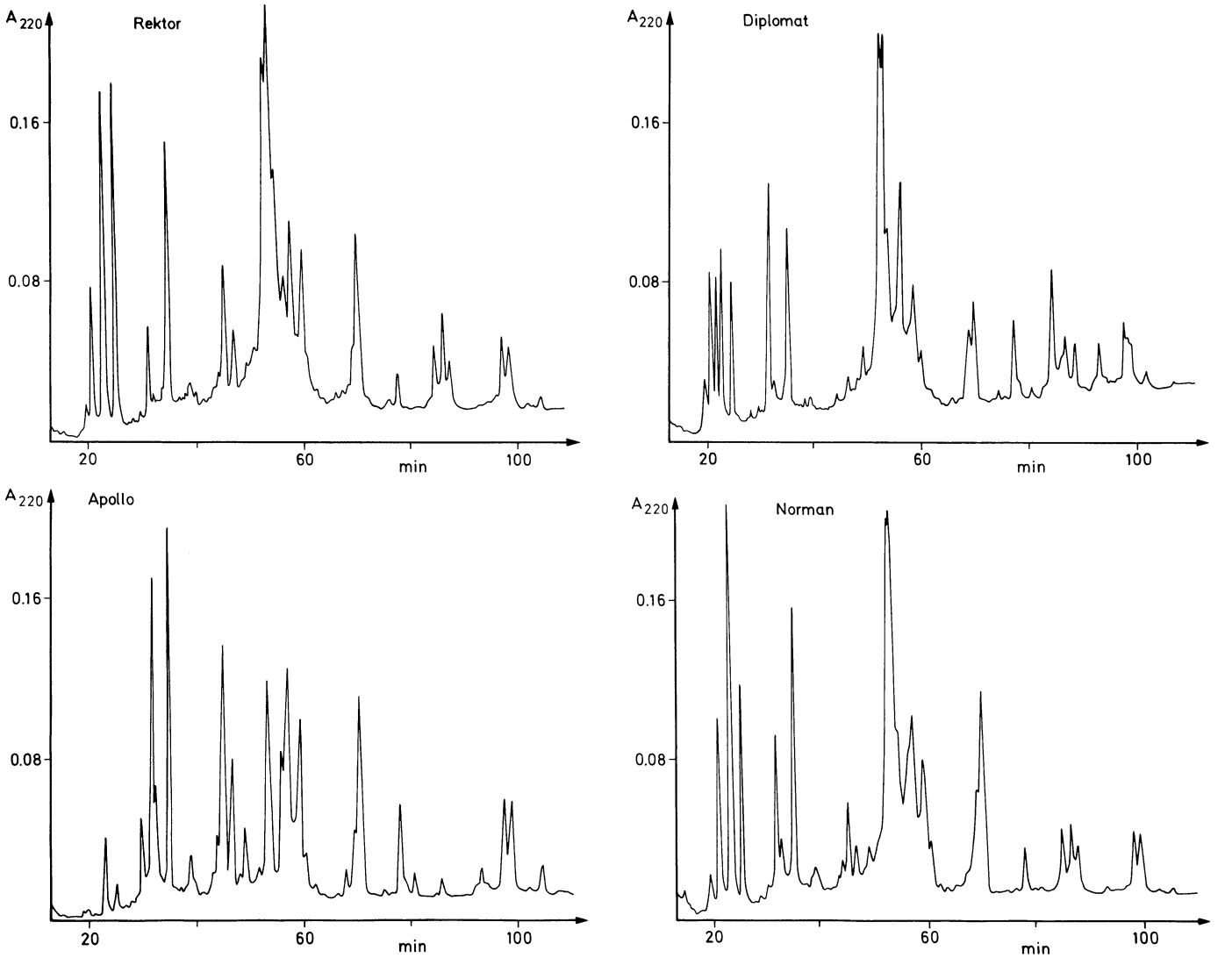

Fig. 4. Reversed-phase high-performance liquid chromatography of glutenin fractions from different wheat varieties soluble in 70% ethanol/dithioerythritol (DTE). Solvent A: 0.1% trifluoroacetic acid + 0.01% DTE + 20% 2-propanol. Solvent B: 0.1% trifluoroacetic acid + 0.01% DTE + 80% acetonitrile. Gradient: 0 min 8% B, 20 min 28% B, 60 min 36% B, 108 min 52% B.

columns had to be used. In general, the fractions consist of more than 20 components. The four varieties differ significantly, showing both qualitative and quantitative differences. A relationship between baking quality and ethanol-soluble glutenin patterns is not evident.

CONCLUSIONS

For the chemical and physical characterization of glutenin subunits, efficient preparative separations, e.g., RP-HPLC, are required. Preparative RP-HPLC of whole reduced glutenin, however, seems to be unfavorable, because of the great number of components, particularly in the LMW group (Seilmeier et al 1987). Preseparation with neutral 70% aqueous ethanol/DTE offers the possibility to obtain two clearly different fractions. RP-HPLC and SDS-PAGE show that the ethanol-soluble fraction contains the MMW glutenins and the insoluble fraction the HMW subunits. LMW glutenins were found in both fractions. Among the four varieties analyzed, the HMW patterns of the insoluble fractions are correlated to baking quality, whereas the HPLC patterns of the soluble fractions are unique for each variety.

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[Received March 21, 1988. Accepted September 19, 1988.]