Influence of Specific Gliadins on the Breadmaking Quality of Wheat[1]

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ABSTRACT

Thirty-two wheat samples with the same high molecular weight (HMW) glutenin-A subunit composition but different gliadin compositions varied greatly in breadmaking quality. Loaf volumes ranged from 4,450 to 6,160 ml per kilogram of flour. Variation in the relative composition of four gliadin peaks, as identified and quantified by reversed-phase high-performance liquid chromatography, explained 82% of this variation. Multiple linear regression coefficients corresponding to three of these peaks (4, 10, and 33) were negative. Lactate polyacrylamide gel electrophoresis (lactate-PAGE) analysis revealed that these peaks contain gliadins migrating in the ω-gliadin region. The coefficient of peak 70, which contains gliadins migrating on lactate-PAGE in the γ-gliadin region, was positive. These results show that gliadins, as well as HMW glutenins, can strongly influence breadmaking properties of wheat.

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Hamer et al (in press) analyzed 164 wheat samples to investigate correlations among high molecular weight (HMW) glutenin-A subunit composition, sodium dodecyl sulfide sedimentation value, grain hardness, and breadmaking quality as assessed by a pan loaf test. A subset of 32 samples was identified as having the same glutenin-A subunit composition (-, 7, and 2+12) in the Payne system (Payne et al 1984) but different breadmaking quality. Loaf volumes ranged from 4,450 to 6,160 ml/kg. This variation could not be explained by differences in protein content, Zeleny sedimentation value, or grain hardness.

It is well accepted that the viscoelastic properties of a dough, which govern breadmaking quality, result not only from the interaction of glutenin polymers but also from the interaction of glutenins with the monomeric gliadin proteins (Pomeranz 1982). Although at first only the ratio of gliadins to glutenin proteins was considered important (MacRitchie 1987), other authors have claimed that gliadins have a direct effect on breadmaking quality. Sozinov and Poperelya (1980) reported that certain groups of gliadins were correlated with breadmaking quality. Wrigley et al (1981) and Branlard and Dardevet (1985) correlated individual gliadin bands (analyzed by lactate polyacrylamide gel electrophoresis [lactate-PAGE]) with functional dough parameters. They could explain 37–54% of the variation among cultivars with only seven to 12 gliadin bands.

More recently, reversed-phase high-performance liquid chromatography (RP-HPLC) techniques have been extensively developed (Bietz 1983, Bietz et al 1984, Marchylo et al 1988) and used to characterize wheat cultivars with respect to their gliadin composition. RP-HPLC was also used to find correlations between potential breadmaking quality and gliadin RP-HPLC fractions. Huebner and Bietz (1986) and Huebner (1989) identified a correlation between a specific gliadin fraction and a general breadmaking score. Because the fraction apparently has a negative effect, they recently renamed it the "anti-baking-quality fraction." Scanlon et al (1990) predicted dough extensibility from equations derived from HPLC analyses of gliadins.

In studies of variables that can influence baking quality, it is important to perform real baking tests on the material being investigated. Many of the cited papers neglected this aspect, and some ignored the HMW glutenin-A subunit composition of the cultivars studied.

We used RP-HPLC to explore correlations between gliadin fractions and breadmaking quality (as assessed by a baking test) for wheat samples of matched HMW glutenin-A subunit composition. We derived an equation that relates loaf volume for these samples to certain gliadin peaks in chromatograms. We also attempted to identify important RP-HPLC fractions by lactate-PAGE.

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MATERIALS AND METHODS

Wheat Samples

Wheat samples were selected from a set of 164 crosses of Dutch-grown wheat (1984 harvest). Based on previously determined HMW glutenin-A composition, a subset of 32 samples was identified that each contained the subunits -, 7, and 2+12. Despite their identical glutenin-A composition, these varieties varied markedly in loaf volume. The gliadin composition of the samples was determined with lactate-PAGE, pH 3.1, to ascertain that each sample had a unique gliadin composition.

Sample Preparation for HPLC

Single wheat kernels were crushed with a hammer and transferred to 1.5-ml microcentrifuge tubes. The crushed kernels were extracted with 100 μl of 70% (v/v) ethanol per 50 mg. The mixture was vortexed and left to stand for 30 min at room temperature before centrifugation in a Sigma-202 MC centrifuge (Sigma Laboratory Centrifuges, Osterode, Germany) at 10,000 rpm for 5 min. Fifty microliters of the supernatant was pipetted into a Costar Spin-X centrifuge unit with a 0.45-μm nylon filter (Costar, Cambridge, MA), diluted to 200 μl with 70% (v/v) ethanol, and centrifuged at 10,000 rpm for 30 min. One hundred microliters of the clear filtrate was transferred into a microvial for RP-HPLC; 10 μl of each sample was injected onto the column.

RP-HPLC

HPLC-grade acetonitrile and trifluoroacetic acid (TFA) were obtained from J. T. Baker, Inc. (Deventer, The Netherlands). Water was distilled and further purified in a Milli Q system (Millipore, Bedford, MA).

HPLC was performed with an LKB 2249 ternary gradient system integrated with a double pump head (Pharmacia LKB Biotechnology, Uppsala, Sweden), an LKB 2157 autosampler, and a Lambda-Max model 481 UV/VIS detector (Waters Associates, Milford, MA). The HPLC column (Bakerbond RP-7104, J. T. Baker, Inc.) was a spherical, wide-pore octadecyl analytical column (250 × 4.6 mm) with pore size 330 Å and particle size 5 μm. The column was kept at 65°C in a CX4-2 column oven (Waters Chromatography Division). Gliadins were separated on this column with a binary gradient of water containing 0.100% TFA (solvent A) and acetonitrile containing 0.087% TFA (solvent B). The percentage of solvent A was 87, 87, 79, 75, 67, and 55% at 0, 6, 12, 23, 40, and 63 min retention time, respectively. Solvent flow rate was 1.0 ml/min; eluted components were monitored at 210 nm and at 0.1 absorbance units full scale.

Solvents were degassed by vacuum filtration over a 0.45-μm filter (Millipore), then sparged with helium for 5–10 min. The solvents were kept under a slight overpressure of helium in Schott Duran bottles (Schott, Mainz, Germany) fitted with a 3220 cap assembly with valved connectors (Omnifit Ltd., Cambridge, England). Fractions were collected with an LKB 2212 Helirac fraction collector in line with the detector.

Data Analysis

The detector signal was integrated with a 3390A integrator (Hewlett-Packard Co., Avondale, PA) or with Betochrom V8 integration software (Betron Scientific, Rotterdam, The Netherlands) on an IBM-compatible personal computer. The data were analyzed statistically with the Genstat 5 statistical package, release 1.2 (Rothamsted Experimental Station, Harpenden, England), on a MicroVAX 3500 computer (Digital Equipment Corp., Maynard, MA).

Classification of RP-HPLC Peaks

In order to classify RP-HPLC peaks as α-, β-, γ-, and ω-gliadins, detector effluent was collected in 76 1-ml fractions. Identical fractions from several runs with different varieties (typically four to five) were combined to increase the amount of protein and to maximize the number of peaks. Solutions so obtained were freeze-dried. The material was then redissolved in 1 ml of 70% ethanol, and the solution was concentrated by evaporation under a stream of nitrogen until 50–200 μl was left. An equal volume of sucrose solution was added, and after mixing, 50 μl was subjected to lactate-PAGE, pH 3.1.

Electrophoresis

Glutenin composition of samples was determined as described by Hamer et al (1991). The gliadin composition of each sample was determined with lactate-PAGE, pH 3.1 (ISO 1990). The same method was used to determine the gliadin composition of RP-HPLC fractions.

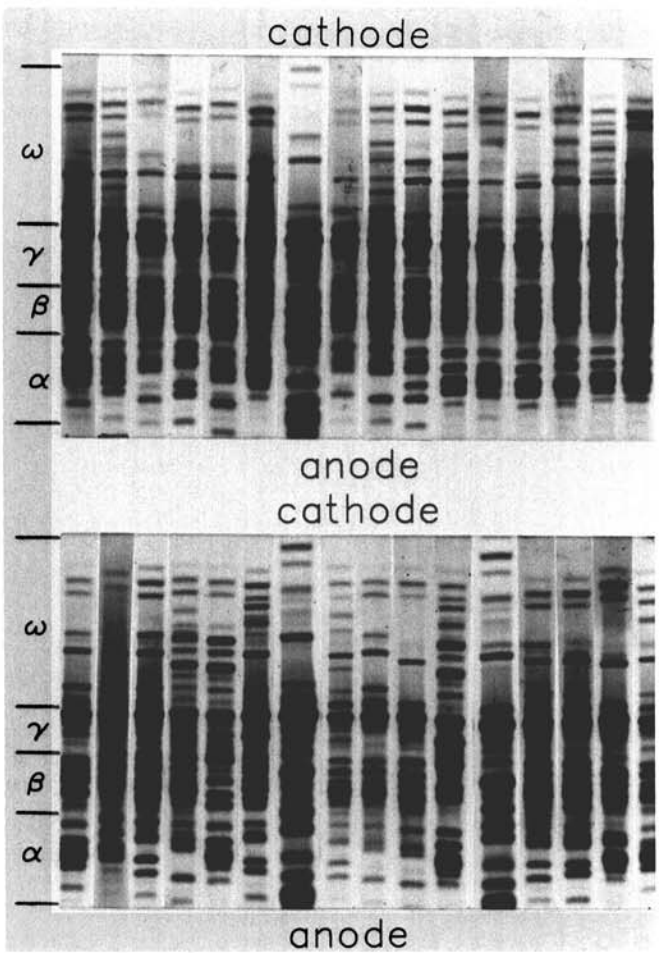

Fig. 1. Lactate-polyacrylamide gel electrophoresis of 32 wheat samples shows the unique gliadin composition of each sample. The origin is at the top; the approximate classification of the gliadin groups is indicated at the left.

Baking Test

Loaves were baked following the standard baking protocol of the TNO Food Technology Institute (Wageningen, The Netherlands). To prepare bread dough, flour, yeast (2%), salt (2%), sugar (0.5%), ascorbic acid (30 ppm), and water were mixed for 2 min at 50 rpm and 8 min at 100 rpm in an Artofex mixer (Aeschag, Aarau, Switzerland). The amount of water added was chosen to give a consistency of 450 farinograph units (ICC 1980, standard 115). Dough was fermented at 30° C and 85–90% relative humidity for two periods of 35 min, then proofed under the same conditions. The duration of the final proof was previously determined in an SJA fermentograph (Aktiebolaget S.J.A., Stockholm, Sweden) as the time taken to reach a carbon dioxide production per loaf of 900 ml. Loaves were baked in triplicate for 30 min at 230° C in a carousel-type oven. Loaf volume was determined by rapeseed displacement and expressed as milliliters per kilogram of flour.

Analyses

Chemical composition and quality parameters (moisture, protein, falling number, and Zeleny sedimentation value) were determined according to ICC standards (ICC 1980, standards 110/1, 105/1, 107, and 116).

RESULTS AND DISCUSSION

Characteristics of Wheat Samples

The 32 varieties investigated were identical in HMW glutenin-A subunit composition (-, 7, and 2+12) but varied in baking quality as determined by the Institute's standard baking test. Loaf volumes ranged from 4,450 to 6,160 ml per kilogram of wheat flour. It is well known that within a single wheat variety, loaf volume increases with protein content of the flour, as has been observed in nitrogen fertilization trials. However, among the varieties we investigated, there was no relation between protein content (11.7–15.3%, dry-matter basis) and loaf volume; the correlation coefficient ($R = 0.03$) was not statistically different from zero. Zeleny sedimentation values (13–43 ml), on the other hand, were linearly related to observed loaf volumes ($R = 0.768$). These observations confirm that protein quality and composition are much more important than protein content. The rather high values for the Hagberg falling numbers (182–410) indicate that α-amylase activity of the samples was not high enough to influence baking characteristics.

Lactate-PAGE revealed that each of the 32 samples had a unique gliadin composition (Fig. 1), indicating that they were different varieties.

RP-HPLC of Gliadins

We obtained good separations of gliadins with the HPLC con-

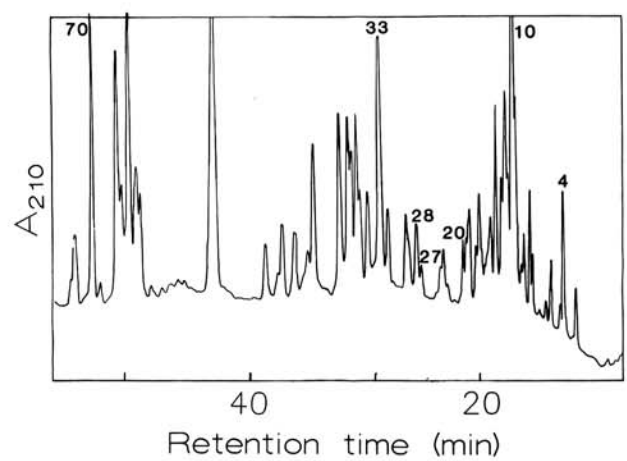

Fig. 2. A typical reversed-phase high-performance liquid chromatogram of the gliadin composition of one sample. The peaks are numbered sequentially in order of elution; those that were found to be related to quality are indicated.

figuration described. Each chromatogram had 60–70 peaks (Fig. 2). RP-HPLC has been used to identify cereal varieties (Bietz et al 1984) based on variation among chromatograms. We also observed large variation among chromatograms; no two were exactly the same.

Analysis of Chromatographic Data

To evaluate the HPLC results, we first normalized the peak areas by setting the total area of the chromatogram to 100 and calculating the relative area of each peak. This normalization corrected for differences among samples in gliadin concentration.

We then tested the ability of multiple linear regression to relate observed loaf volumes to peak areas. We selected a group of 20 samples with loaf volumes evenly spread over the whole range and calculated correlations between all possible subsets of peaks and loaf volume. Up to seven peaks in the regression equation gave statistically significant results (Table I). A correlation coefficient of 0.97 resulted when all seven peaks were considered.

However, statistical analyses revealed that peaks 29 and 30, which are very close to peaks 27 and 28, are rather strongly correlated ($R = 0.6$) with peak 70, the most important peak. Such intercorrelation is not surprising, since Sozinov (1984) reported that gliadins are inherited in stable, linked groups, which he named blocks. It thus seems wise to omit peaks 27 and 28 from the regression equation. Another significant intercorrelation was observed between peaks 33 and 42 ($R = 0.7$), but this did not influence the regression equation since peak 42 was not selected by the statistical program. All other possible intercorrelations appeared negligible. The introduction of peak 20 did not substantially improve the correlation (R changed from 0.95 to 0.97), so this peak was also not considered. In this way we established the following regression equation, which accounts for 82% of the variance in loaf volume:

$$LV = 5{,}615 + 346A(70) - 303A(33) - 564A(10) - 506A(4)\,,$$

where LV is loaf volume in milliliters, $A(70)$ is the normalized area of peak 70, $A(33)$ is the normalized area of peak 33, and so on.

Table I gives the statistical characteristics of the seven peaks that were shown to be important in predicting breadmaking properties. The regression equation appears to be stable, regardless of the number of peaks considered: coefficients vary little, and the signs do not change. These results suggest that the gliadins that elute as peak 70 contribute positively to breadmaking quality, whereas the others may have a negative effect. Because these conclusions are based only on statistical correlations, they need to be confirmed by baking tests on reconstituted doughs with added isolated gliadins.

Characteristics of the four important peaks are given in Table II. The most important peak (peak 70) elutes under relatively hydrophobic conditions (42.3% solvent B); gliadins in this peak are relatively apolar. The other peaks elute at much lower acetonitrile concentration and are therefore more polar.

Table II also presents the average, maximum, and minimum relative areas of these peaks, along with the standard deviations of the areas. Peak 70 has the largest standard deviation, reflecting its importance. Standard deviations decrease as peaks become less important.

Scanlon et al (1990) used an analogous approach to relate breadmaking quality parameters to gliadin composition. They divided HPLC chromatograms of Canadian wheat varieties into 21 regions, most comprising two or more peaks. A strong correlation was found with dough extensibility, but loaf volume was poorly correlated. This difference between their results and ours may be the result of differences in glutenin composition, which could mask the effects of gliadins; unfortunately, Scanlon et al (1990) did not report glutenin compositions for their samples.

Lactate-PAGE of HPLC Fractions

RP-HPLC separates proteins on the basis of hydrophobicity, whereas lactate-PAGE separates on the basis of size and charge. To classify the gliadins in the HPLC peaks, HPLC fractions were electrophoresed. Because it is difficult to collect each HPLC peak individually, we collected 1-ml portions of the effluent ("groups").

Figure 3 shows a typical chromatogram. The first 16 groups contained no gliadins and were therefore discarded. Some groups, such as 32 and 33, gave faint or no lactate-PAGE bands because of lack of protein. Other groups (22, 40, 45, 50, 61, 71, and 73) gave pronounced bands.

PAGE results for these groups are reproduced in Figure 4. All HPLC groups gave more than one gliadin band, and adjoining groups frequently had nearly identical gliadin compositions. The hydrophilic gliadins (groups 17–26) appear to be ω-gliadins. Peaks of moderate hydrophobicity (groups 38–47) are α- and β-gliadins.

TABLE I
Results of Regression Analysis with All Possible Subsets of Gliadin Peaks[a]

Peak Selected[b]	Number of Peaks in Regression Equation				
	3	4	5	6	7
70	308	346	356	356	332
33	−300	−303	−341	−320	−324
10	−464	−564	−470	−440	−432
4		−506	−500	−605	−574
28			174	195	216
27				128	139
20					−134
Constant	5,508	5,615	5,517	5,401	5,513
Correlation coefficient	0.860	0.904	0.937	0.952	0.969
Percentage of variance accounted for	74	82	88	91	94

[a] Data are regression coefficients.
[b] Individual peaks were selected by the Genstat statistical regression program.

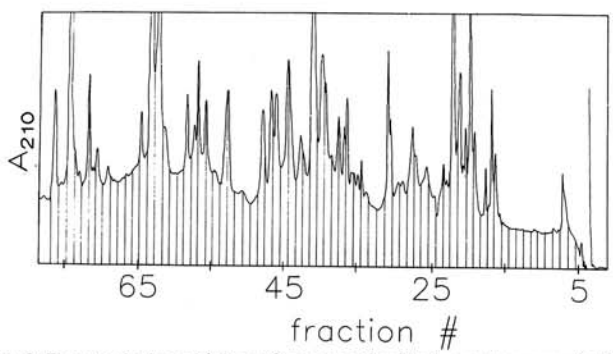

Fig. 3. Reversed-phase high-performance liquid chromatogram of gliadin fractions, indicating the groups of peaks collected for lactate-polyacrylamide gel electrophoresis. The horizontal line does not represent the baseline used in integration.

TABLE II
Characteristics of Gliadin Peaks Correlated with Loaf Volume

Peak Number	Solvent B (%)	Average Area	Maximum Area	Minimum Area	Standard Deviation	Classification
70	42.3	2.848	1.563	0	1.196	γ
33	26.2	2.062	3.885	0.764	0.898	β
10	21.5	0.697	1.563	0	0.432	ω
4	17.7	0.272	1.140	0	0.330	ω

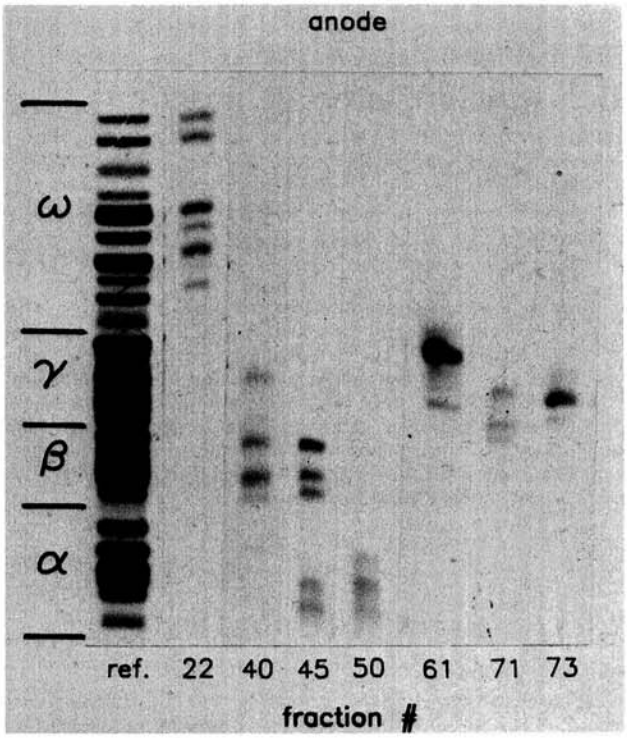

Fig. 4. Lactate-polyacrylamide gel electrophoresis of the collected groups indicated in Figure 3. The origin is at the top; the approximate classification of the gliadin groups is indicated at the left.

Groups 48–58 contain mainly α-gliadins, and the most hydrophobic gliadins (groups 61–75) are γ-gliadins.

The most important peak, peak 70, elutes in group 73 and clearly belongs to the class of γ-gliadins. Its positive regression coefficient suggests a positive contribution to breadmaking. This hypothesis agrees with findings of Branlard and Dardevet (1985), who showed that individual γ-gliadins, as analyzed with PAGE, correlate positively with certain technological criteria, such as Zeleny sedimentation value. Our findings strongly support these observations. We found good correlation between loaf volume and Zeleny sedimentation value for samples with the same HMW glutenin-A subunit composition but different gliadin composition. We also showed a good correlation between loaf volume and composition of γ-gliadins, which indicates that γ-gliadins could be responsible for the correlation between Zeleny value and loaf volume. Glutenins, having the same composition in these samples, swell to the same extent in the Zeleny test, whereas γ-gliadins can unfold and form a more or less voluminous network, in proportion to their amount.

Two of our other peaks related to loaf volume (4 and 10) are ω-gliadins, with negative coefficients in the regression equation. Branlard and Dardevet (1985) also showed negative coefficients for these gliadins.

CONCLUSIONS

We have demonstrated a strong statistical relation between breadmaking quality and the relative content of certain types of gliadins in samples of wheat varieties with a constant HMW glutenin-A subunit composition (-, 7, and 2+12). Amounts of only four gliadin peaks explained 82% of the variance in loaf volume. Reconstitution experiments with purified gliadins are needed to prove these relations. To rule out the possibility that the relationship is valid only for a particular HMW glutenin-A subunit composition, similar studies are also necessary for wheat varieties varying in HMW glutenin-A subunit composition.

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