Assessment of Potential Breadmaking Quality of Hard Spring Wheats by High-Performance Liquid Chromatography of Gliadins—Year Two

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ABSTRACT

There is a need for a quick analysis to determine breadmaking quality of small wheat samples for the breeding of new wheat varieties. Reversed-phase high-performance liquid chromatography previously revealed a relationship between a specific fraction found with gliadins and breadmaking quality of wheat varieties. This relationship was examined a second year for hard red spring wheats grown in different locations. Observed correlation coefficients between the amount of this fraction and a general quality score based on 20 variables were significant at the 5 or 1% level for wheats from individual locations. For samples combined from different locations, however, correlations decreased significantly. This method may permit analysis of breadmaking potential of new wheat genotypes using as little as one-half kernel of wheat, possibly eliminating the growing of thousands of plants (for each variety developed) during a period of two or three years.

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Assessing the breadmaking quality of wheat flour is very important to the breeder as well as the baker. Many methods other than baking are used to help determine baking quality (Bloksma 1971). Wheat baking quality depends on many factors (Nolte et al 1985), so no one method can satisfy all requirements. However, methods that use small amounts of flour, such as sedimentation (Axford et al 1979, Preston et al 1982, Moonen et al 1982), gel filtration (Huebner and Wall 1976), and gel electrophoresis (Payne et al 1979, Branlard and Rousset 1980, Wrigley et al 1981), are being used to assist in determining flour quality.

Reversed-phase high-performance liquid chromatography (RP-HPLC) can also predict breadmaking quality. The amount of a specific fraction, previously termed "BQGF" (baking-quality gliadin fraction), correlates negatively with wheat quality (Huebner and Bietz 1986). Because of the negative effect of this fraction on baking, we now propose that it be called anti-baking-quality fraction (ABQF). The 1986 study examined hard red spring wheats, mostly from Arizona and California, for which a general score was known (Nolte et al 1985). To extend our confidence in such results, it was necessary to characterize additional samples from another year, grown at more locations, especially the normal hard red spring wheat growing areas. More wheat samples were

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therefore obtained from the previous sites and from additional locations. These samples were scored for quality (Nolte et al 1985) and analyzed as before (Huebner and Bietz 1986). Also, additional RP-HPLC columns were used to determine if they would work as well or better. Analysis of this larger series of samples again demonstrates that RP-HPLC can quickly determine baking quality of wheats grown at a specific location; however, evaluation of wheats from various locations gives a less significant correlation. No data are yet available on the nature of ABQF; it may or may not be a protein.

MATERIALS AND METHODS

Materials

Hard red spring (HRS) wheat flour samples were from the USDA Spring and Durum Wheat Quality Laboratory, Fargo, ND. The varieties used were Alex, Butte, Chris, Coteau, Era, Len, Marquis, Marshall, Olaf, Waldron, Beagulita 'S', Juanillo 168, Probrand 711, Probred, Westbred 911, Yecora Rojo, Genaro, Hermosillo, Oslo, and about 60 experimental varieties. The wheats were grown during 1984 in test plots in Arizona, California, Washington, Idaho, Montana, Wyoming, North Dakota, South Dakota, Nebraska, Minnesota, and Wisconsin. Many varieties were grown at two or three locations. Samples were analyzed at Fargo for 20 variables, ranging from kernel size to loaf volume (Nolte et al 1985).

Organic solvents, including acetonitrile (ACN) and trifluoroacetic acid (TFA), were HPLC grade; distilled water was further purified with a Barnstead Nanopure system. Other chemicals were reagent grade or better (Bietz 1983, Huebner and Bietz 1984).

Sample Preparation

Flour samples (60 mg) were extracted for 30 min at room temperature with 1.5 ml of 70% (v/v) aqueous ethanol. Extractions used continuous agitation on a Buchler vortex-evaporator (Buchler Instruments, Fort Lee, NJ) and were done in 10-ml capped polypropylene centrifuge tubes (Huebner and Bietz 1984). Extracts were then centrifuged for 15 min at 20,000 × g in a Beckman model L8-70M centrifuge (Beckman, Fullerton, CA) (Bietz 1983, Bietz et al 1984).

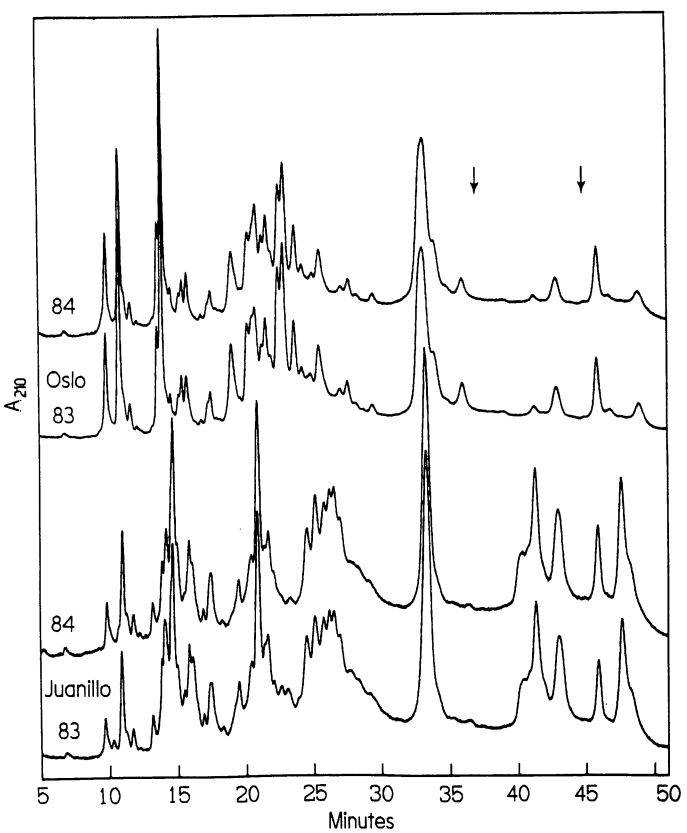

Fig. 1. Reversed-phase high-performance liquid chromatograms from a Vydac C_4 column of gliadins of Oslo and Juanillo wheats grown in the Imperial Valley, CA, during 1983 and 1984. Gradient conditions were 30–42% acetonitrile (+ 0.05% trifluoroacetic acid) during 38 min, followed by 5 min at 42% acetonitrile; temperature was 60°C. The total peak area between the arrows is defined as the anti-baking-quality fraction.

RP-HPLC

The RP-HPLC apparatus included a Spectra-Physics (San Jose, CA) solvent delivery system and SP8780XR autosampler. Proteins were detected with a SF770 Spectroflow monitor (Kratos, Ramsey, NJ). Vydac C_{18} and C_4 columns (250 × 4.1 mm; Separations Group, Hesperia, CA) and a Bio-Rad C_{18} column (250 × 4.1 mm; Bio-Rad, Richmond, CA) were used, with a 22 × 3.5 mm guard column of SynChrom RSC packing and a 0.5-μm in-line prefilter (A-103, Upchurch, Oak Harbor, WA). Eluted proteins were detected at 210 nm at 0.1 or 0.2 absorbance units full scale/10 mV.

ACN- and TFA-containing solvents were deaerated by vacuum filtration through a 0.45-μm filter and sparged with helium during use (Huebner and Bietz 1986).

Samples of 10–15 μl were analyzed at 1.0 ml/min on columns maintained at 60°C with a CH-20-C column heater (Scientific Systems, State College, PA). Gradients were nonlinear to elute the major gliadins more quickly and to better resolve peaks of BQGF (Huebner and Bietz 1986). The C_4 column gradient was 24–32% ACN from 0 to 2 min, 32–35% ACN from 2 to 8 min, 35–43% ACN from 8 to 40 min, and 43% ACN from 40 to 43 min; the column was then reequilibrated for 11 min at 24% ACN. For C_{18} columns, the gradient usually contained 2–3% more ACN. Using these conditions, a complete analysis requires 54 min. Modification of these conditions can further decrease analysis time.

Analysis of Data

Data were recorded on an Omniscribe recorder (Houston Inst., Austin, TX), and stored in a ModComp computer system (Ft. Lauderdale, FL) for subsequent integration and/or replotting. Stored chromatograms were displayed on a Tektronix (Beaverton, OR) video terminal or replotted to any suitable scale. Percentages of gliadin fractions were determined by a program (TIMCPC) that integrates chromatograms between specified time limits while correcting for baseline absorbance shifts. Data were analyzed by analysis of covariance procedures in a model that included the general score as the response variable, location as a discrete main effect, and ABQF as the covariate.

RESULTS

RP-HPLC analyses of wheat flours of poor, medium, and good baking quality were shown previously (Huebner and Bietz 1986). Typical chromatograms from this study of two wheats grown in two different years are shown in Figure 1. The area between 37 and 45 min is defined as ABQF (previously "BQGF" [Huebner and Bietz 1986]). The percentage of material in this peak area correlates significantly and negatively with "general scores" of wheats (Nolte et al 1985). The general score is a mean of "wheat score," "mill score," and "bake score." Each score ranges from 1 to 4, with 1 indicating no promise; 2, little promise; 3, some promise; and 4, promise.

In an initial analysis, a simple linear regression of ABQF to the general score was fit for each location (Table I). The regression equation ($y = mx + b$) defines a line by the point at which it crosses the vertical axis (b = intercept) and the slope it takes from there (m = slope). The probability shown in Table I is the probability associated with a zero slope. If the probability is relatively large ($P > 0.10$) then the slope may actually be zero, in which case there is no linear relationship between ABQF (x) and score (y). The R^2 listed in Table I is the coefficient of determination, which is the square of the simple correlation coefficient (r) and can be thought of as the percent of the

differences among the scores that could be explained by differences in ABQF. For the specific locations, the estimates of the slopes were all negative (P value ranged from < 0.01 to 0.14). For the general locations, only Northeast had a negative slope.

The data for specific locations were combined in a least squares analysis of covariance with location as a main effect and ABQF as the covariate (Fig. 2). In initial analyses, separate slopes were fitted for each location and for the full set of locations together. There was no improvement by fitting separate slopes compared with fitting one slope for all locations (full vs. reduced model F test at $P < 0.05$). The regression lines for the specific locations could therefore be considered to be parallel. This overall slope (-0.108 ± 0.013) was thus a reasonable estimate of the slope for each location. Differences in intercept values ($P < 0.01$) indicated that the regression lines, though parallel, were at different heights in Figure 2. The two Langdon samples had the highest score, followed by the Minot sample, which in turn was followed by the Mesa samples. The Imperial Valley and Pinal Co. scores were lower than the others.

Because wheats from different locations vary in baking quality (McGuire and McNeal 1974), general scores for varieties from other locations were compared. For example, Yecoro Rojo grown in 1984 in the Imperial Valley had a general score of 2.0, but rated at 2.3 from Pinal Co., AZ, and 2.7 from Mesa, AZ (Table II). Between years, not all varieties varied in the same manner; however, four had the same general score both years, whereas others varied by as much as 50%. Part of this variation is due to the method used to obtain this score, which was designed only to give a general, simple number for breeders so they could judge the hundreds of wheat samples with which they had to work.

Because general score is an average of three integers (Nolte et al 1985), it is a discontinuous function in which the values can only be incremental, such as 1.0, 1.3, and 1.7. This weakens the correlation between ABQF and general score (Table I), especially when sample number is small and range of general scores is narrow. To determine if slight modification of deficiency faulting values used to calculate general score (Nolte et al 1985) would alleviate this apparent problem and improve correlations with ABQF, faulting values were redefined slightly, and general scores were recalculated for wheats from Minot, ND, and Pinal Co., AZ (Table III). Regression coefficients were then recalculated from the modified data, and the R^2 values for Minot and Pinal Co. increased from 0.38 and 0.52 to 0.87 and 0.83 (Table I).

This redefinition of faulting limits does not change general scores significantly, nor does it alter classification of wheats as good or poor. It does, however, improve the fit of the regression line. For wheats from Langdon, ND, this procedure did not help, however, since most had a maximum (4.0) general score (data not shown). However, even for most wheats with high general scores, similar slight modifications of faulting limits (e.g., -0.1 for wheats with slightly lower values for variables such as loaf volumes) also increased the R^2 to over 0.90 (not shown).

All data for the 1984 wheat samples are compiled in Table I. Wheats were from five specific locations and three general areas. As noted for 1983 samples (Huebner and Bietz 1986), combining results of wheats grown at different locations decreased correlations of ABQF with general score. In addition to genotype, factors such as rainfall, sunlight during kernel development, available fertilizer, temperature, and day length also obviously influence quality. The data of Table I support the previous suggestion that correlations of ABQF with general score decrease for samples that are mixed from larger geographical areas.

Replicate analyses of ABQF on many samples (such as for Langdon in Table I) showed acceptable repeatability for the RP-HPLC method. Analyses under varying conditions gave

TABLE I
Anti-Baking-Quality Fraction Regressed on General Score for Each Location

Location	n	Correlation Coefficient		R^2	Prob[a]
		Intercept	Slope		
Mesa, AZ	16[b]	4.96	−0.148	0.51	<0.01
	16[c]	4.34	−0.131	0.44	<0.01
Pinal Co., AZ	7[b]	3.14	−0.132	0.52	0.07
	7[c]	3.69	−0.148	0.55	0.06
	7[d]	4.43	−0.168	0.55	0.05
Imperial Valley, CA	11[c]	3.00	−0.090	0.43	0.03
	11[d]	3.06	−0.086	0.62	<0.01
Langdon, ND	6[b]	4.45	−0.033	0.56	0.09
	6[d]	4.61	−0.046	0.74	0.03
Minot, ND	7[d]	4.68	−0.080	0.38	0.14
Specific modified[e]					
Minot, ND	7[d]	5.61	−0.131	0.87	<0.01
Pinal Co., AZ	7[b]	3.67	−0.153	0.83	<0.01
General					
Northeast[f]	23[d]	4.31	−0.068	0.11	0.11
Southeast[f]	31[d]	1.95	0.021	0.01	0.61
Western[f]	30[d]	2.79	0.066	0.13	0.05

[a]Probability associated with a zero slope.
[b]Analyzed on a Vydac C_4 column.
[c]Analyzed on a Bio-Rad C_{18} column.
[d]Analyzed on a Vydac C_{18} column.
[e]See text.
[f]The northeast area included the eastern three-fourths of North Dakota, the north half of Minnesota, and the north one-fourth of Wisconsin. The southeast area included the eastern three-fourths of South Dakota and Nebraska, the south half of Minnesota, and the southern three-fourths of Wisconsin. The western area included Washington, Montana, Idaho, Wyoming, and the western one-fourth of North Dakota, South Dakota, and Nebraska.

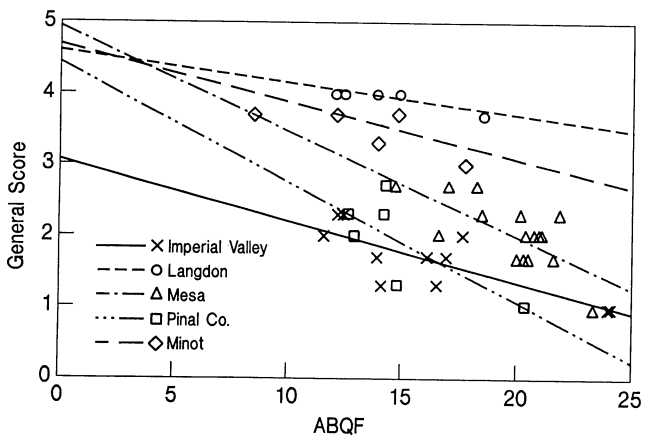

Fig. 2. Graphical representation of some results of Table I, demonstrating slope and intercept. Anti-baking-quality fraction (**ABQF**) is the area between arrows in Fig. 1. General score is a measure of wheat quality, as explained in the text.

TABLE II
Comparison of Wheats Grown in 1983 and 1984

Location/ Variety	General Score		ABQF[a]		% Protein		Amount of Gliadin[b]	
	83	84	83	84	83	84	83	84
Imperial Valley, CA								
Juanillo	1.0	1.0	17.9	19.2	10.5	10.4	165	146
Proband	3.0	2.3	7.5	9.0	12.4	11.3	210	184
Probred	3.3	1.7	7.6	8.9	12.9	11.3	216	194
Westbred	1.7	1.7	9.0	10.9	12.3	10.6	204	174
Yecoro Rojo	3.0	2.0	7.6	8.6	12.9	12.0	209	191
Pinal Co., AZ								
Hermosillo	2.3	2.3	11.9	11.5	12.1	10.5	230	176
Oslo	2.0	2.7	9.4	9.6	11.0	10.0	190	152
Yecoro Rojo	2.7	2.3	8.9	8.8	11.5	10.2	210	155
Mesa, AZ								
Yecoro Rojo	2.7	2.7	8.6	9.7	11.5	11.7	200	200

[a]Anti-baking-quality fraction.
[b]As recovered upon chromatography; expressed as area under the peaks (10^4).

correlations no better than those used in Figure 1, which appear optimal. Figure 1 also shows RP-HPLC chromatograms of gliadins from two wheat varieties grown at the same location in 1983 and 1984. Qualitative reproducibility of these (and other) RP-HPLC patterns between years is excellent.

Table II lists all data for two years for seven wheat varieties grown at two or three locations. In the Imperial Valley samples, protein percentages in 1984 were lower than those in 1983 for all wheats but did not decrease equally for all varieties. The ABQF is higher for the 1984 Imperial Valley samples, suggesting that synthesis of this fraction may have remained constant while gliadins decreased. For the Imperial Valley samples, most general scores varied considerably between the two years, however. At Pinal Co., AZ, three varieties contained less protein in 1984, but the ABQF was nearly the same; general scores were also nearly identical.

Table II also contains estimates of relative amounts of total gliadin in wheat samples, as determined by integration of RP-HPLC data. Because extraction and HPLC conditions were the same, the ratio of these amounts to total protein percentage is a measure of nonuniform environmental effect on synthesis of specific protein classes (Huebner and Bietz 1988). For example, wheats from both the Imperial Valley and Pinal Co. appeared to contain a lower percentage of gliadin in 1983 than 1984. Environmental variations (moisture availability) during kernel development may cause such slight differences in protein amounts, which could greatly influence general scores. Also, general scores could be affected by year-to-year differences in flour baking procedure and personnel (*personal communication,* M. Shogren). The difference in the percentage of BQGF (Huebner and Bietz 1986) and of ABQF (this work) for specific varieties may be partially due to slight changes in chromatographic areas and minor differences in baselines due mainly to TFA absorbance at 210 nm.

DISCUSSION

This study confirmed the previous report (Huebner and Bietz 1986) concerning hard spring wheats grown mostly in California and Arizona during 1983. Larger groups of samples, grown during 1984 in areas typical for hard red spring wheats, were examined. Correlations between ABQF and general score are similar to those of the previous study.

When varieties from different areas are considered together, however, correlations decrease as geographic area increases, to the point of no significant correlation (Table I). Estimation of baking quality by quantitation of ABQF, as separated by HPLC, may therefore best compare flours from wheats grown in a common area along with a few well-known good and poor baking wheats to establish a range for ABQF for that year at that locality. The method could be very useful for estimating quality during breeding, because single kernels can be analyzed, thus saving years of breeding time necessary for increasing experimental lines before they are tested.

Our results clearly show that general scores of wheat varieties may vary significantly from year to year. The method used to assign general scores to samples of wheat is, of course, somewhat subjective. Relatively small deviations in critical variables are defined as unacceptable and may markedly influence resulting assigned scores. Such a procedure is valuable to indicate genotype stability or environment-genotype interaction. Our results suggest that ABQF may be a better indicator of the genetic component of quality, so that varieties may need to be tested in fewer locations.

ABQF may be a better predictor of breadmaking quality than the general score, because general score is based on numerous additional factors, some unrelated to protein content or composition. We realize that eventually most other factors must also be determined, since good breadmaking wheats must also have good growing and milling characteristics. Using HPLC first, however, may reduce the number of poor lines that are later discarded. Nevertheless, the correlation between ABQF and overall general score is better than that of ABQF to loaf volume or mixing time. Mixing time seems more related to the ratio of high to low molecular weight glutenin subunits (Huebner and Bietz 1985), whereas loaf volume is influenced by several factors, including the ratio of gliadins to lipids (Hoseney and Finney 1971) and pentosans (Shogren et al 1987). So far we have observed no specific gliadins that correlate positively with general score, mixing time, or loaf volume. The negative correlations suggest that ABQF components interfere with determinants of flour quality. The nature of the ABQF, and its relationship to flour quality, are being further explored.

TABLE III
Old and New Faulting Values Used to Determine General Score[a,b]

	Faulting Limits				
	Minor		Medium	Major	
Variables	Old (−1.0)	New (−0.5)	New (−1.0)	Old (−2.0)	New (−1.5)
Wheat protein	13.9	14.1	13.4	12.9	12.7
Flour extracted	67.2	67.8	66.8	65.2	65.4
Flour protein	12.9	13.2	12.6	12.4	12.2
Bake absorption	61.9	62.5	61.5	60.4	60.5
Mixing time	5.75–8	5.7–7.5	7.5–8.5	<2	<1.7
	2–2.75	2.2–3.0	1.7–2.2	>8	>8.5
Crumb color	3	5	3	...	...
Crumb grain	3	6	4	...	...
Loaf volume	795	830	760	745	730

[a]For further details see Nolte et al 1985.
[b]The author does not suggest that these values be changed for further work; readjusting the faulting limits only demonstrates that correlations of some samples can be improved by a more gradual change in general score values.

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