

Prediction of Baking Quality of Bread Wheats in Breeding Programs by Size-Exclusion High-Performance Liquid Chromatography

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ABSTRACT

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Extracts of unreduced protein of wheat flour were analyzed by size-exclusion high-performance liquid chromatography. Four chromatographic fractions corresponding to different sizes of aggregates and monomers were separated from phosphate-sodium dodecyl sulfate extracts. Experimental conditions, automating sampling, and computer assistance were investigated for improving reproducibility and automation of the system and discrimination between quality classes. A three-year experiment on a large number of breeding lines grown in different locations demonstrated that this chromatographic method has potential for assessing baking quality in breeding programs. The analysis that could be used for predictive purposes was mainly based on molecular weight

distribution between excluded peak (F1) and intermediate aggregates (F2), which is essentially variety dependent and very highly correlated with baking quality data. The best indicator of the potential baking strength of genotypes (measured by alveograph *W* index, mixograph index, or gluten viscoelasticity) was generally the F1/F2 ratio, whereas loaf volume in French baking technology was more likely to depend on the amount of the F2 fraction. However, the prediction equation of baking strength must be calibrated from samples of each new crop year, and the prediction may be improved by taking into account both the F1/F2 ratio and the percentage (Fi) of proteins insoluble in the phosphate-sodium dodecyl sulfate buffer.

Baking quality is difficult to assess in a varietal breeding program. Especially in the early stages, breeders need rapid and small-scale microtests for predicting the intrinsic value of genotypes (Feillet 1980, Bietz 1987, Branlard and Autran 1986, Autran 1987). Several biochemical tests have a high potential for analyzing large series of samples from small amounts of seeds. Microtests based on protein solubility (Pomeranz 1965, Orth and Bushuk 1972, Mecham et al 1972, Jeanjean and Feillet 1978, Kobrehel and Matignon 1980, Kurowska and Bushuk 1988) or allelic variations at loci coding for high molecular weight (HMW) glutenin subunits (Payne et al 1984, 1987; Branlard and Dardevet 1985; Pogna et al 1987) yield results generally independent with regard to the agronomical record of the sample and have been reported for their suitability for screening genotypes in early generations of breeding programs.

Recently, the introduction of high-pressure liquid chromatography (HPLC) for wheat protein analyses has made it possible to consider its use routinely for large series of samples, which was not possible with low-pressure conventional liquid chromatography (Bietz 1983, 1984, 1985; Orsi and Békés 1986). In addition, HPLC exhibits high resolution and reproducibility,

its other most attractive features being automation and quantitation due to its computer capabilities (Bietz 1986).

The major advances in this field, however, have been obtained by using reversed-phase type HPLC (RP-HPLC), and several reports have shown applications to quality prediction because of the relationship of some specific peaks or specific regions of the chromatogram to quality characteristics (Huebner and Bietz 1986, Burnouf and Bietz 1987, Lookhart and Albers 1988).

Conversely, although it has been reported that baking strength is associated with the occurrence of large protein aggregates (Huebner 1970; Huebner and Wall 1976; Field et al 1983; Mifflin et al 1983; Bushuk 1985, 1987), size-exclusion HPLC (SE-HPLC) has rarely been attempted for quality prediction. Some promising relationships have been reported from limited numbers of samples. For instance, Huebner and Bietz (1985, 1986) showed that the ratio of peak 1 (size > 800 kDa) from unreduced extracts is directly related to mixing time, indicating a possible utility for breeding programs. In a previous work (Autran 1987), we also showed from 30 genotypes that the amount of fraction 2 (size 115-650 kDa) or the peak 1/peak 2 ratio are respectively related to French baking score or gluten elastic recovery.

However, because of strong effects of year and growing location on baking quality, demonstrating the real advantage of SE-HPLC method as a breeding test for quality assessment required extending the study to a much larger number of samples and assessing not only correlations with quality data, but also reproducibility of the test, ability to discriminate between

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genotypes, inheritance, and respective influence of genetics and growing location, year, or protein content.

The aim of the present paper is to present results on the fractionation of unreduced wheat protein extracts by SE-HPLC and to examine by statistical analysis the possibility of predicting breadmaking potential of breeding lines from these results.

MATERIALS AND METHODS

Chemicals and Reagents

Solvents and other chemicals were of reagent grade. Extracting buffer and eluting solvent were deaerated by vacuum filtration through a 0.22- μ m filter.

Wheat Samples

Grain samples (454) of bread wheat lines grown in 1985, 1986, and 1987 were analyzed in this study. These included 15 genotypes grown in five locations in 1985, 63 genotypes grown in three locations in 1986, and 65 genotypes grown in three locations in 1987. Growing locations were far apart geographically. Samples were supplied by the "Club des Cinq," an association of French wheat breeders. The samples comprised F7-F8 lines and five standard cultivars. Flours were milled in a Brabender Senior laboratory mill.

Protein content ($N \times 5.7$) was determined from both whole grain and flour by the Kjeldahl method. Falling number, alveograph, Zeleny, and baking (CNERNA method) tests were conducted according to the French standards (Mauzé et al 1972, Rousset and Loisel 1984, Rousset 1984). The mixograph test was conducted according to Bourdet et al (1976). Glutens were washed out and subjected to viscoelastograph measurements to determine firmness and elastic recovery (Damidaux and Feillet 1978). A baking strength index was also inferred from electrophoretic composition of HMW-glutenin subunits according to Pogna and Mellini (1986).

Sample Preparation

Flour samples (80 mg) were stirred for 2 hr at 60°C in the presence of 10 ml of 0.1M sodium phosphate buffer (pH 6.9) containing 2% sodium dodecyl sulfate (SDS). Extractions were followed by centrifugation for 30 min at 37,500 $\times g$ at 20°C in a Beckman centrifuge (model JA-221). Supernatants were directly submitted to SE-HPLC fractionation. The percentage of insoluble protein fraction of the flour, determined by nitrogen analysis of the residue, was referred to as F_i (insoluble fraction) and expressed on a total proteins basis.

SE-HPLC

The SE-HPLC apparatus was a Beckman model 332 that included a microprocessor system controller model 420, a pump model 110A, and a detector model 153 with lamp and filter for ultraviolet detection at 214 nm. Automatic injection of as many as 125 samples was realized by a Spark Holland model 125 injector with a 20- μ l sample loop.

A TSK 4000-SW (Beckman) size-exclusion analytical column (7.5 \times 300 mm) was used with a TSK 3000-SW (Beckman) guard column (7.5 \times 75 mm). The TSK 4000-SW columns have pores of 450 Å and allow the separation of proteins on a large molecular weight range (from 10,000 to 1 million Da). The columns were eluted isocratically by 0.1M sodium phosphate buffer (pH 6.9) containing 0.1% SDS as previously described by Bietz (1984). The flow rate was 0.7 ml/min at ambient temperature. The major peaks, referred to as F1 to F4, were eluted between 9 and 20 min. Samples were loaded every 30 min.

Apparent molecular weight of major peaks were estimated by calibrating the column with four unreduced protein standards: thyroglobulin (669,000), bovine serum albumin (66,000), chymotrypsinogen A (25,700), and cytochrome C (11,700).

Computation

The chromatograms were recorded on an IBM PC/XT. The positions of the main peaks of the elution curve were automatically

determined through Nelson analytical software (version 4.1) which permitted us the storage, reintegration, replotting, and comparison of data. The total area under the elution curve, which corresponds to the amount of proteins extractable by the phosphate-SDS buffer, was recorded. By calibrating the data with Kjeldahl analysis of the extract, this area was referred to as %Fs (soluble fraction) and expressed on a total protein basis. Peak areas were then calculated by setting a cursor on the base line at positions where peaks began and ended. Peak areas were expressed in percent of the soluble fraction ($\%F1 + \%F2 + \%F3 + \%F4 = \%Fs$), with $\%Fs + \%Fi = \text{total protein content}$.

Statistical Analysis

Relations between HPLC fractions and other quality tests were investigated by carrying out analysis of variance, linear correlation coefficients, partial correlation coefficients, and equation of prediction based on multivariate analysis.

RESULTS

HPLC Elution Pattern

Typical elution profiles obtained when flour extracts were applied to a TSK 4000-SW column are shown in Figure 1. There are four distinct areas of material absorbing at 214 nm. Upon calibration of the column using five molecular weight standards, the limits between peaks were estimated as indicated.

Peak F1 elutes at the void volume of the column and it is likely to correspond to highly aggregated material. Fraction F2, which elutes between 115 and 650 kDa, does not make up a real peak and it is likely to consist of smaller aggregates with a continuous range of molecular size. Peaks F3 and F4 are likely to correspond to monomeric proteins whose apparent molecular weights agree with the bulk of gliadins and salt-soluble proteins, respectively.

Reproducibility of the Method

Extracting the flour for 2 hr at 60°C resulted in good stability of the extracts at ambient temperature for at least two days (discussion below), and a good reproducibility of the percentages of the different peaks was obtained. Variations in the amount of peak F1 (in exclusion volume), which is considered the most sensitive to experimental changes (Huebner and Bietz 1985), and in the F1/F2 ratio were especially studied. For instance, when loading either the same protein extract at different times (0, 24, 36, and 48 hr after extraction), or when running different extracts of the same flour, the coefficients of variation (CV) in %F1 were very low (1-2% and 2-3%, respectively). Similar values were found for the F1/F2 ratio. These values have to be compared to the much higher variation observed when analyzing extracts from different growing locations of a given genotype, or from different genotypes (discussion below).

Origin of Variation in the Amount of SE-HPLC Fractions

The respective influences of variety and growing location on the amount of each peak of soluble (Fs) and insoluble (Fi) fractions were investigated by analysis of variance on the harvest years 1985, 1986, and 1987. Percentages of variability assignable to variety, growing location, residue, and F test are presented in Table I for the 1985 crop (very similar results were obtained for the two other years). These results clearly show that the amount in peak F1 and the F1/F2 ratio are essentially variety dependent, whereas the amounts in peaks F2, F3, and F4 are also significantly influenced by the growing location. The ratio of variances respectively attributable to genotype and to location (σ_G^2/σ_L^2), which expresses the ability of a test to discriminate between genotypes, is extremely high for both F1 and F1/F2 ratio. Further statistical analysis of the variation between different growing locations indicated, for instance, that the coefficients of variation in F1 ranged between 3 and 5% only (detailed results not shown). Conversely, the soluble fractions (Fs), the insoluble fractions (Fi) and, to a lesser extent, the amount in peak F4, were more influenced by growing location than by genotype.

These results demonstrate that the percentage of fraction F1 or the F1/F2 ratio has a good ability to discriminate between genotypes and may be more reliable parameters for breeding programs than many other criteria. Table I shows, for instance, that technological criteria such as alveograph *W* and mixograph index, although considered good indicators of intrinsic value of genotypes, are significantly more influenced by environmental factors and have lower σ^2_G/σ^2_L ratios than HPLC fraction F1 or F1/F2 ratio. These SE-HPLC criteria seem, therefore, to allow a better approach to discovering the intrinsic quality of genotypes.

Relationships Between Quality Tests and SE-HPLC

Preliminary works (Autran 1987) showed that distinct differences in sizes of corresponding peaks were observed for samples known to differ widely in baking strength. These samples were primarily distinguished by the amount of peaks F1 and F2, or by the F1/F2 ratio, and also by the percentage of insoluble nitrogen material (Fi). Typical separations are shown in Figure 1, confirming that the elution profile of proteins upon SE-HPLC relates to baking strength (Huebner and Bietz 1985). Considering, for instance, the 1986 crop, Table II illustrates that cultivars with high *W* index (alveograph) generally have a smaller peak 1 (5.0–6.5%) and a lower F1/F2 ratio (0.30–0.40), whereas those with a low *W* index contain larger amounts of peak 1 (9.0–11.0%) and a higher F1/F2 ratio (over 0.50). This range of variation between genotypes is highly significant compared with the reproducibility of the method (CV in %F1, 1–2%) or with the variation between different growing locations (CV 3–5%).

In order to evaluate the potential and limits of application of the method for quality assessment in breeding programs, a large number of breeding lines was subjected to protein extraction and SE-HPLC. All the analyses were done singly to be in accord with approaches used in early breeding programs. The percentages of the four fractions (F1–F4), the F1/F2 ratio, and the percentages of the soluble (Fs) and insoluble (Fi) fractions were obtained for the three sets of genotypes grown in 1985, 1986, and 1987, and linear correlations were calculated between all SE-HPLC data and all technological scores available. Table III shows the correlations obtained from the entire 1987 set, considering SE-HPLC criteria and technological scores averaged per genotype.

Three main observations were made: 1) The flour protein content was more closely associated with the soluble fraction Fs ($r = 0.78$, $P < 0.001$) than with any of the fractions F1 to F4. 2) Both F1 and F2 were negatively correlated with alveograph *W* index, mixograph index, and Zeleny volume, and, to a lesser extent with alveograph *P* index, whereas Fi and F3 were positively correlated with these criteria. 3) The highest (negative) correlation coefficients for the different baking strength criteria were observed for the F1/F2 ratio (for instance $r = -0.80$, $P < 0.001$, with both *W* index and mixograph index, $n = 65$). A scatter diagram and a statistical summary of the relationship of *W* index, mixograph index, and F1/F2 ratio are shown in Figure 2.

Similar, but not identical results were obtained when considering the different growing years (Table IV). For instance, it is confirmed that both F1 and F1/F2 ratio showed significant (negative) associations with most of the baking strength criteria, including *W* and *P* indexes, Zeleny volume, mixograph index, gluten firmness, and gluten elastic recovery. They were also strongly correlated to the electrophoretic index based on the HMW-glutenin pattern (Pogna and Mellini 1986). However, the level of significance was higher some years and for some tests. For instance, the F1/F2 ratio was always highly significantly associated with mixograph index, whereas it seemed to allow a prediction of *W* index in 1986 and 1987 only. Also, an association between F1/F2 ratio and flour protein content was noticeable in 1987, but not in 1986 or 1985.

Although the baking data were available in 1985 only, Table IV also shows, interestingly, that a (positive) association can be observed between F2, loaf volume (or baking score in French baking technology), and alveograph *G* index. On the other hand, when comparing the correlation coefficients within each growing location, significantly different results may be obtained (Table V). For instance, in 1987, the correlation coefficients between either *W* index and F1, or between *W* index and F1/F2, or between mixograph index and F1/F2, were very similar among the three growing locations D, E, and F. In contrast, in 1986 the samples grown at location A gave lower correlation coefficients with baking strength criteria than those grown in B and C, which might be explained by the much lower protein content (below 10%) of the samples grown in location 1986-A (results not shown).

Partial Correlation Coefficients and Equations of Prediction

Considering that Fi was correlated (positively) with baking

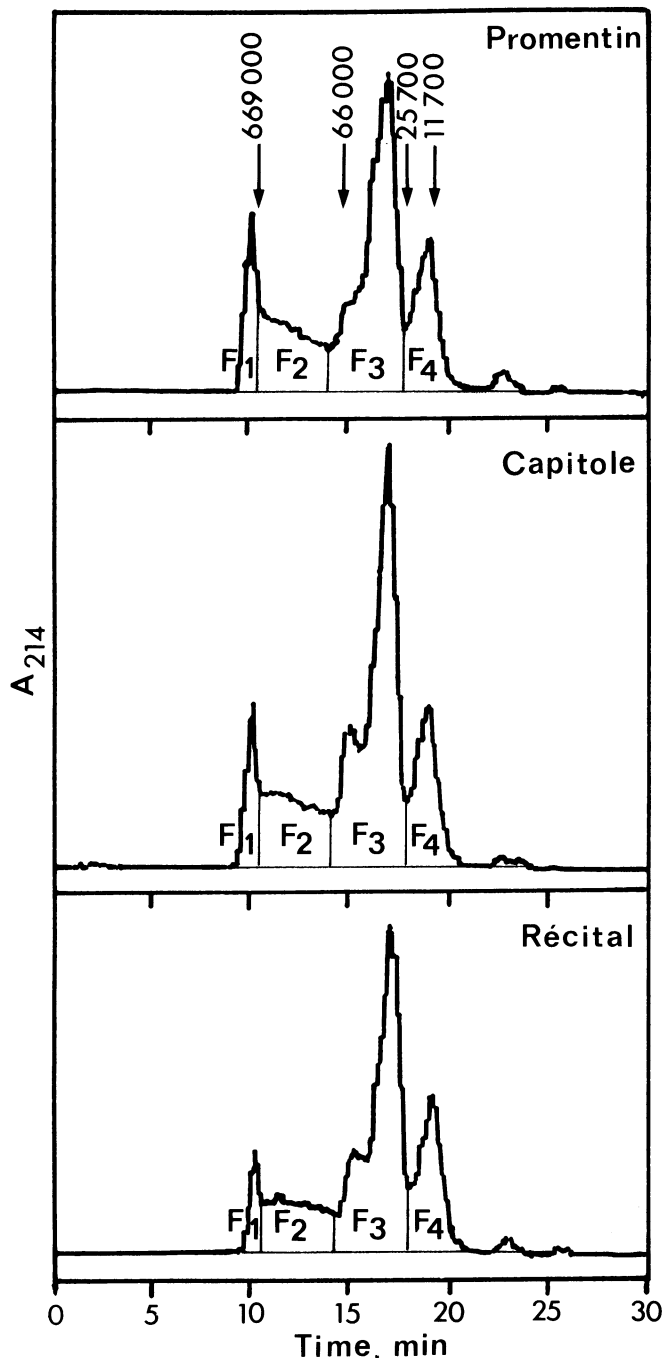


Fig. 1. Elution profiles obtained by size-exclusion high-pressure liquid chromatography on a TSK 4000-SW column of unreduced flour proteins extracted with sodium phosphate (containing 2% sodium dodecyl sulfate) from three bread wheat cultivars, Promentin, Capitole, and Récital, grown in the same location in 1987. Column solvent was 0.1M sodium phosphate, containing 0.1% sodium dodecyl sulfate. Elution positions of the molecular weight standards are indicated. Alveograph *W* indexes for the three cultivars are 105, 110, and 200, respectively. The characteristics of the chromatograms of the three cultivars, respectively, are as follows: %F1 = 11.5, 8.8, and 6.4; F1/F2 ratio = 0.49, 0.39, and 0.29; %F1 = 38.6, 37.4, and 42.8.

TABLE I
Analysis of Variance of Some Size-Exclusion High-Performance Liquid Chromatographic and Technological Characteristics
for 15 Genotypes Grown in Five Locations in 1985

Characteristic	Percentage of Variability Assignable to			F Test ^a		σ^2G/σ^2L^b
	Genotype	Growing Location	Residue	Genotype	Growing Location	
% F1	76.4	2.9	20.7	**	NS	large ^c
% FR2	70.4	7.8	21.8	**	**	9
% F3	67.9	14.6	17.5	**	**	5
% F4	39.5	45.4	15.1	**	**	0.8
F1/F2 ratio	62.4	2.9	34.7	**	NS	large ^c
% Fs	17.3	70.2	12.5	**	**	0.2
% Fi	9.1	79.3	11.6	**	**	0.1
W (Alveograph)	77.1	9.3	13.6	**	**	8
Mixograph index	70.3	18.5	11.2	**	**	4

^a** Indicates significance at the 0.01 level of probability.

^bRatio of variance assignable to genotype to variance assignable to growing location.

^cLarge number that cannot be expressed because σ^2L is not distinguishable from zero (value for the *F* test under growing location was not significant).

strength and (negatively) with F1/F2 ratio (Table III), the question was raised whether F1/F2 ratio directly determines the baking strength, or if this association resulted from its correlation to Fi. Accordingly, the relationships between Fi and F1/F2 ratio and baking strength (*W* index) were examined in greater detail by calculating partial and multiple correlation coefficients according to Payne et al (1988). The results (Table VI) show, for instance, that among genotypes of the 1987 crop 64.6% of the variation in *W* index can be accounted for by variation in F1/F2 ratio, and that after taking into account the variation in insoluble fraction Fi, the coefficient of determination was still high (42.3%), indicating that a large part of the variation in *W* index was determined by F1/F2 ratio, independently of the association of this ratio with the percentage of insoluble fraction. On the other hand, the multiple correlation (F1/F2, Fi) did not show any improvement compared with the simple correlation, since the coefficient of determination remained almost identical (64.4%). A similar conclusion was obtained for genotypes of the 1986 crop: the prediction of *W* index by F1/F2 ratio was not as good as for the 1987 data, but the correlation coefficient was still significant whether the variation in Fi was or was not taken into account (-0.53 and -0.45, respectively, both significant at $P < 0.001$) and was similar using the multiple correlation (F1/F2, Fi: -0.52, $P < 0.001$). A different result, however, was obtained from genotypes of the 1985 crop. The percentage of insoluble fraction had a highly significant effect on *W* index and the F1/F2 ratio did not determine by itself the variation in *W* index; the correlation coefficient (-0.59, $P < 0.05$) resulted essentially from an association with the percentage of insoluble fraction, which is evidenced by the nonsignificant coefficient of the partial correlation and by the strong improvement in this coefficient (-0.81, $P < 0.001$) when using a multiple (F1/F2, Fi) correlation.

Equations of prediction of technological data were also calculated. Considering for example the 1987 crop, and based on simple correlations, *W* index could be predicted as follows:

$$W = 466 - 862.5 \times (F1/F2)$$

Based on multiple correlations that take into account both F1/F2 and Fi parameters, *W* index could be predicted by the following equation:

$$W = 370.9 - 766.1 \times (F1/F2) + 1.85 \times (Fi)$$

The second equation allowed comparison of the respective weights of F1/F2 and Fi parameters in the determination of *W* index. Considering a variation of one standard deviation unit of F1/F2 and of Fi around the mean values, it could be demonstrated that the variation of *W* index was -23.4 and +4.0, respectively, and that the effect of F1/F2 ratio was therefore about six times higher than the effect of Fi. A very similar result was observed

TABLE II
Differences in Percentage of Fraction F1 and in F1/F2 Ratio
for 10 French Cultivars Known to Differ Widely in Baking Quality^a

Cultivar	% F1	F1/F2 Ratio	W (Alveograph)
Vilmorin 23	10.76	0.58	65
Talent	9.93	0.52	94
Cappelle	10.29	0.51	133
Capitole	9.08	0.46	137
Rescler	7.51	0.41	178
Moulin	8.69	0.46	224
Courtot	8.00	0.45	246
Chopin	6.08	0.37	295
Aubaine	6.35	0.39	308
Prinqual	5.41	0.30	342

^aINRA growing location; 1986 crop.

for the 1986 crop samples (the F1/F2 ratio effect about seven times higher than Fi). A different result, however, was found in 1985 (Fi effect about five times higher than F1/F2 ratio), confirming the above-mentioned strong influence of the percentage of insoluble protein on quality data of this crop year.

DISCUSSION

Protein Solubilization and Molecular Weight Distribution in SE-HPLC

The 0.1 M sodium phosphate buffer containing 2% SDS is likely to solubilize all single-chained polypeptides and many aggregated proteins (Bietz 1986). Because no reducing agent was used in addition to SDS, we assume that no disulfide interchange could be introduced to the native glutenin complexes and consider that the extract was likely to contain native aggregates. However, not all wheat proteins were extracted, and the data from elution curves do not totally represent the least soluble aggregates that make up the Fi fraction.

In our experimental conditions (single extraction of the flour, without washing of the residue), the extraction rates ranged between 55 and 90% (%F1 ranged between 45 and 10%), on a total protein basis. The aggregated proteins are therefore likely to distribute themselves among the three fractions Fi (insoluble), F1, and F2. Our results tended to demonstrate that the baking strength of the wheat genotype is not only determined by the solubilization of the protein aggregates, which agrees with previous reports (Orth and Bushuk 1972), but also by the distribution of the aggregates between the excluded peak (apparent MW > 650 kDa) and the F2 fraction (apparent MW between 115 and 650 kDa). As previously discussed by Huebner and Bietz (1985), the question is whether stronger varieties contain less fraction F1 and have a lower F1/F2 ratio, which appears to disagree with early SE-HPLC results (Huebner and Bietz 1985) and with classical chromatography fractionations using Sepharose

gel (Huebner and Wall 1976) or controlled-pore glass matrix chromatography (Field et al 1983) which indicated that the excluded peak corresponded to large aggregates and was therefore directly related to baking strength.

The origin of these discrepancies may be explained by the conditions of protein extraction (type and efficiency of the solvent and extraction rate obtained). If a more efficient solvent were used (i.e., acetic acid, urea, and cetyltrimethylammonium

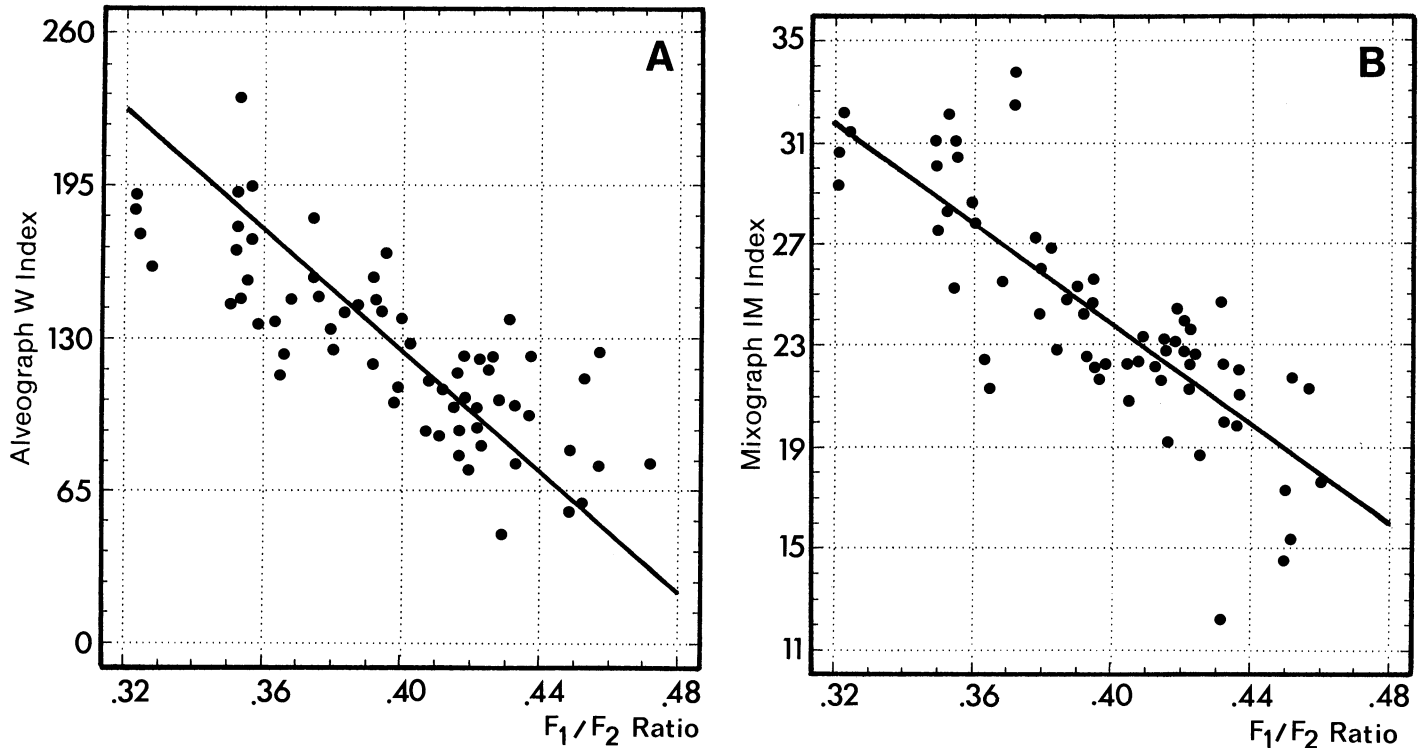


Fig. 2. Relationship between F1/F2 ratio and alveograph *W* index (A) or mixograph IM index (B) for 65 bread wheat genotypes of the 1987 crop (means of data from three growing locations).

TABLE III
Correlation Coefficients^a Between Size-Exclusion High-Performance Liquid Chromatographic Characteristics and Data from Mean Values for Each of the 65 Genotypes Grown in Three Locations in 1987

Parameter	Flour Proteins	Alveograph		Zelny Volume	Mixograph Index
		<i>W</i>	<i>P</i>		
% F1	-0.52***	-0.72***	-0.49***	-0.55***	-0.67***
% F2	-0.55***	-0.56***	-0.38**	-0.52***	-0.44***
% F3	0.59***	0.52***	0.43**	0.34**	0.40**
% F4	-0.20	0.11	-0.05	0.19	0.23
F1/F2	-0.44***	-0.80***	-0.53***	-0.65***	-0.80***
% Fs	0.78***	0.12	0.26*	0.29*	0.01
% Fi	0.50***	0.63***	0.41**	0.67***	0.66***

^aCorrelation coefficients above 0.45 are very highly significant (***) $P < 0.001$, those over 0.31 are highly significant (**) $P < 0.01$, and those over 0.24 are significant (*) $P < 0.05$.

TABLE IV
Summary of the Correlation Coefficients for Relationships between HPLC and Technological Data from Genotypes Grown in 1985, 1986, and 1987^a

Flour Protein	Alveograph			Zelny Volume	Mixograph Index	Gluten Elastic Recovery	Gluten Firmness	Loaf Volume	ELEC ^b	
	<i>W</i>	<i>P</i>	<i>G</i>							
1985 (<i>n</i> = 15)										
% F1	-0.34	-0.19	-0.03	-0.06	0.00	-0.38	-0.11	-0.15	0.13	-0.65**
% F2	-0.09	0.31	0.24	0.56*	0.48*	0.28	0.44	0.46	0.70**	-0.13
F1/F2	-0.32	-0.59*	-0.27	-0.51*	-0.61**	-0.86**	-0.56*	-0.64**	-0.54*	-0.34**
1986 (<i>n</i> = 63)										
% F1	-0.10	-0.51***	-0.29*	0.16	-0.24*	-0.68***	-0.26*	-0.47***	NA ^c	NA
F1/F2	-0.06	-0.53***	-0.33**	0.17	-0.25*	-0.66***	-0.30*	-0.51***	NA	NA
1987 (<i>n</i> = 65)										
% F1	-0.52***	-0.72***	-0.49***	-0.25*	-0.55***	-0.67***	NA	NA	NA	NA
F1/F2	-0.44**	-0.80***	-0.53***	-0.38**	-0.65***	-0.80***	NA	NA	NA	NA

^aData from the different growing locations were averaged. Correlation coefficients above 0.85 (*n* = 15) or 0.45 (*n* = 65) are very highly significant (***) $P < 0.001$; those over 0.60 (*n* = 15) or 0.31 (*n* = 65) are highly significant (**) $P < 0.01$; those over 0.47 (*n* = 15) or 0.24 (*n* = 65) are significant (*) $P < 0.05$.

^bELEC, Baking strength index based on electrophoresis of high molecular weight glutenin subunits.

^cNA, Technological data not available.

TABLE V
Correlation Coefficients Between SE-HPLC^a Criteria and Mixograph Index or Alveograph *W* Index by Growing Location^b

Parameter	Growing Locations (1986)			Growing Locations (1987)		
	A	B	C	D	E	F
Correlations with mixograph index						
% F1	-0.39** ^c	-0.61***	-0.66***	-0.64***	-0.67***	-0.60***
% F2	-0.31*	-0.60***	-0.49***	-0.31*	-0.38**	-0.31*
% F3	0.45***	0.54***	0.25*	0.33**	0.14	0.09
% F4	-0.11	-0.23	-0.38**	-0.34**	-0.02	-0.20
F1/F2	-0.36**	-0.56***	-0.64***	-0.72***	-0.78***	-0.68***
Correlations with alveograph <i>W</i> index						
% F1	-0.36**	-0.43**	-0.50***	-0.64***	-0.58***	-0.62***
% F2	-0.19	-0.43**	-0.30*	-0.39**	-0.28*	-0.34**
% F3	0.31*	0.54***	0.29*	0.38**	0.11	-0.06
% F4	-0.06	-0.02	-0.14	-0.33**	-0.17	-0.06
F1/F2	-0.32**	-0.39**	-0.53***	-0.67***	-0.63***	-0.69***

^aSize-exclusion high-pressure liquid chromatography.

^bSixty-three genotypes were grown in three locations in 1986 and 65 genotypes in three locations in 1987.

^cCorrelation coefficients above 0.45 are very highly significant (***P* < 0.001), those over 0.31 are highly significant (***P* < 0.01), and those over 0.24 are significant (**P* < 0.05).

TABLE VI
Correlation Coefficients, Partial Correlation Coefficients, and Multiple Correlation Coefficients Between Alveograph *W* Index and F1/F2 Ratio for 1985, 1986, and 1987^a

Variate	<i>r</i>	<i>rp</i>	<i>R</i> ² (%)	<i>R</i> ² <i>p</i> (%)
1985 (<i>n</i> = 15)				
F1/F2	-0.59*	0.11 NS	29.5	1.2
Fi	0.84**	...	70.6	...
F1/F2,Fi	-0.81**	...	65.3	...
1986 (<i>n</i> = 63)				
F1/F2	-0.53***	-0.45***	27.3	28.5
Fi	0.33**	...	10.9	...
F1/F2,Fi	-0.52***	...	26.6	...
1987 (<i>n</i> = 65)				
F1/F2	-0.80***	-0.65***	64.6	42.3
Fi	0.63***	...	39.8	...
F1/F2,Fi	-0.80***	...	64.4	...

^a*r* = correlation coefficient; *rp* = partial correlation coefficient, taking into account insoluble fraction (Fi); *R*² = coefficient of determination. The multiple correlation coefficients are in italic print. Correlation coefficients above 0.85 (*n* = 15) or 0.45 (*n* = 65) are very highly significant (***P* < 0.001), those over 0.60 (*n* = 15) or 0.31 (*n* = 65) are highly significant (***P* < 0.01), and those over 0.47 (*n* = 15) or 0.24 (*n* = 15) or 0.24 (*n* = 65) are significant (**P* < 0.05).

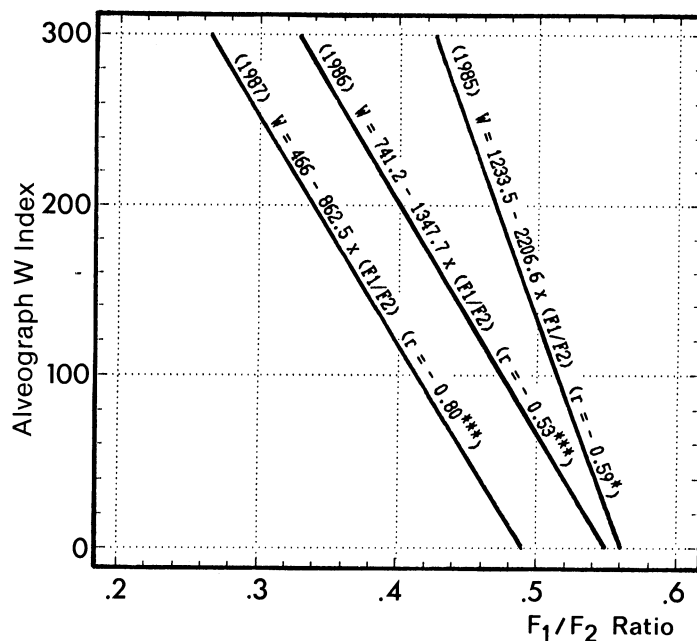


Fig. 3: Comparison between regression lines of alveograph *W* index and F1/F2 ratio for the 1985 (*n* = 15), 1986 (*n* = 63), and 1987 (*n* = 65) crops.

bromide), resulting in an extraction rate of about 85–90 for all samples and low values of F1 (results not shown), the most highly aggregated fractions would fall into the excluded peak. Since stronger genotypes contain more high molecular weight aggregates, the amount of fraction F1 would be positively correlated with baking strength. On the contrary, when a mild solvent is used (this work), the extraction rate is relatively low, but a much higher percentage of F1 fraction is observed in strong samples (35–45%) than in the weak ones (10–15%), which results in a significantly negative correlation between the amount of fraction F1 and baking strength. Interestingly, when intermediate conditions (i.e., acetic acid solvent) were used for the same genotypes, with an extraction rate ranging from 70–80 (results not shown), no significant correlation was found between SE-HPLC data and baking quality.

Since this work was aimed at investigating the different levels of aggregation of the proteins and their relationship to baking quality, it was not suitable to use very dissociating or reducing solvents, which would have resulted in a total reduction of glutenin molecules into polypeptides and in information similar to that obtained by SDS-PAGE. On the other hand, unlike Sephadex G20, or Sepharose gel, or pore-controlled glass matrix, that can stand with various solvents and pHs, SE-HPLC columns are more

fragile, because pHs over 7.5 can destroy them and high viscosities can clog them. There was, therefore, a balance to determine between solvent conditions that allow a relatively high protein extraction while remaining compatible with the use of SE-HPLC columns. The sodium phosphate (pH 6.9) containing 2% SDS seemed to be the best compromise between a relatively high extraction rate of unreduced proteins and conditions required by size-exclusion columns as far as pH and viscosity were concerned.

Also, a type of column adapted to the fractionation of relatively large protein aggregates was required. The TSK 4000-SW columns, which allow the separation of proteins on a large molecular-weight range (from 10,000 to 1 million Da) seem to be the only type adapted to this work, unlike TSK 3000-SW, whose use has been reported by Orsi and Békés (1986) and Orsi et al (1987), but that theoretically allows fractionation in the range of MW 5,000–100,000 only.

Stability of Protein Extracts and Reproducibility of Molecular Weight Distribution

In earlier SE-HPLC fractionations, a dramatic instability of the protein extracts was noticed, resulting in a continuous decrease of the percentage of excluded peak during the first hours after extraction. Reproducible and comparable results could be obtained only upon storage of the extracts for one day, which allowed an equivalent stability of the molecular weight distribution for samples extracted at different times. This led Huebner and Bietz (1985) to the conclusion that further studies were necessary to improve the method's accuracy.

It may be speculated that this instability is related to the dissociating effect of SDS, resulting in a relatively slow disruption of large noncovalently bound aggregates until the extract contains more stable disulfide-bonded complexes only. Alternatively, as proposed by Huebner and Bietz (1985), proteases may remain active in phosphate-SDS buffer and may be involved in the decrease of the excluded peak. Until this problem is understood and solved by using, for instance, enzyme inhibitors, it has been noticed that the higher extraction temperature (60°C for 2 hr) proposed in this paper could totally overcome the problem of instability and make the extracts ready for SE-HPLC without any equilibration or other treatment. Such conditions yield an extremely stable elution curve, even with supernatants that have been stored for 48 hr after extraction.

Quality Prediction

It is well known that protein content influences quality scores and that reliability of a test of quality assessment depends on origin of variation of protein content (Rousset et al 1985). When developing a breeding test, it is necessary that the variation of protein content has a genetic origin only. Accordingly, the correlations have been calculated considering, for each genotype, the mean values of the technological data obtained from the different growing locations (Tables III and IV). Correlations calculated from the whole set of data (results not shown) were not basically different but had a lower level of significance due to the confusion of genetic and environmental variations of the protein content of the samples.

Considering the data within each growing location (Table V) was another way to approach the genetic basis of the variation and had the advantage of identifying the locations with uneven behavior in the SE-HPLC test. As for any kind of breeding test, certain growing locations may have a better efficiency for discriminating between genotypes and predicting intrinsic quality through SE-HPLC, while certain other locations (e.g., 1986 A in Table V) should be excluded because the data show a strong genotype \times environment interaction. On the whole, the data suggest that the MW distribution of protein aggregates is a good predictor of baking strength. The percentage of excluded peak (F1) and, more especially the F1/F2 ratio, are highly variety dependent (Table II). The very good reproducibility of these criteria and their respective standard deviations within the same genotype and between genotypes lend evidence to their reliability for assessing the potential baking strength of genotypes in breeding programs.

Conversely, the major peak (F3), which is likely to consist of monomeric gliadinlike proteins, is significantly influenced by the growing location, and it is generally a much poorer indicator of baking strength than the fractions F1 or F2.

The F1/F2 ratio, however, should be considered as a relative index rather than an absolute one. As a result of different weather conditions from year to year (and in the aggregative level of the proteins that are synthesized), the molecular weight distribution may be influenced and the F1/F2 ratio may vary within different ranges. As illustrated in Figure 3, a comparison of the data obtained from 1985, 1986, and 1987 shows that F1/F2 ratio and *W* index remain closely associated but that the coordinates of the regression line vary from year to year. Accordingly, a new equation of prediction must be calibrated from a new set of samples each crop year. In addition, because different weather patterns may characterize the various growing locations, further

studies are necessary to determine if separate calibrations are or are not required for locations far apart geographically.

The percentage of the least soluble proteins of the flour (Fi) may give some indication of the baking strength, but as shown in Tables IV and VI, Fi is generally more closely associated with the effect of growing conditions and protein content of the samples than with the intrinsic quality of the genotypes, so that a prediction based on Fi is generally poorer than one based on F1/F2 ratio. However, according to the specific conditions of each year, and for better accuracy of the prediction, it may be useful to add the variate Fi to the equation. As a general rule, the higher the baking strength, the higher the percentage of insoluble fraction (Fi) and the lower the values of F1 and F1/F2 parameters. However, the percentage of F2 seems more specifically associated to dough extensibility, as measured by the *G* index, and to loaf volume in French baking technology. These results confirm that the molecular weight distribution of glutenin aggregates determines the balance between elasticity and extensibility (Fullington et al 1987) but tend to show that the intermediate class of aggregates (115–650 kDa) is likely to influence dough extensibility more specifically. Should it be confirmed that F2 indicates dough extensibility and loaf volume or French baking score, an extremely useful breeding test would be developed. Unlike baking strength or mixing requirements for which several biochemical markers are already available, loaf volume and baking score in the particular French technology (which are known to strongly depend on the extensibility component, *G*, of the alveographic curve) are still extremely difficult to predict in breeding programs.

Advantages of SE-HPLC

As recently reviewed by Mifflin et al (1983) and Bushuk (1987), wheat proteins differ in solubility and in molecular weight (Huebner and Wall 1976). In bread wheats, baking quality and mixing requirements are determined by amounts or ratios of these classes (Huebner and Wall 1976, Huebner and Bietz 1985). The methods used to quantitate amounts of wheat protein classes have employed sequential extraction or size-exclusion chromatography, which are tedious, lengthy, and difficult to reproduce or to quantitate. SE-HPLC has the potential of greatly reducing sample size and analysis time (30 min per analysis) while increasing reproducibility and giving better quantitative data.

Although SE-HPLC columns are more expensive, SE-HPLC may have some advantages over RP-HPLC: SE-HPLC equipment is much simpler, a single pump and no gradient controller are needed since an isocratic elution is used; the time of analysis is shorter (55 min in RP-HPLC); fewer reagents are necessary (phosphate buffer and SDS), and their purity seems to be less critical. Also, in view of a routine use for predicting baking quality in breeding programs, the interpretation of the elution curve is simpler since it consists of four major fractions very easy to identify (instead of 20–30 in RP-HPLC), two of them related to quality.

In addition, unlike RP-HPLC (and electrophoresis), that imply a total reduction or dissociation of native protein aggregates and a loss of information concerning structure, interactive aspects, and stability of the protein complexes, a major advantage of SE-HPLC is its potential to work with relatively large aggregates and to be more likely to approach the physicochemical and structural basis of wheat quality.

CONCLUSIONS

Our results lead to four major conclusions:

1) A three-year experiment on a large number of samples allowed us to demonstrate that SE-HPLC of unreduced protein extracts has a potential for assessing baking quality of bread wheat genotypes in breeding programs.

2) The analysis that could be used for predictive purposes is mainly based on molecular weight distribution between the excluded peak (F1) and intermediate aggregates (F2). The best indicators of the potential baking strength of genotypes are generally %F1 or F1/F2 ratio, whereas loaf volume in French

baking technology seems more associated with the percentage of the F2 fraction.

3) Despite a strong effect of the year on absolute values of HPLC data, making it necessary to calibrate the prediction equation of baking strength from samples of each new crop year, the percentage of fraction F1, or the F1/F2 ratio, seems to allow a better measure of intrinsic quality than other technological tests. In spite of the extra work involved for determining SE-HPLC criteria, a much larger part of the variation in *W* index was determined by F1/F2 ratio than by protein solubility. However, for improving the accuracy of the prediction, it may be useful to take into account not only the MW distribution of protein aggregates but also the percentage of insoluble proteins (Fi).

4) Unlike SDS-PAGE or RP-HPLC that can separate monomers or protein subunits useful for fingerprinting genotypes, SE-HPLC is a powerful tool for studying native protein aggregates and the physicochemical basis of baking strength.

At present, our studies are investigating subunit composition of SE-HPLC peaks, inheritance of the various SE-HPLC parameters, and effect of an HPLC-based selection in actual breeding programs.

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