(SLIDE 1)

Size-exclusion High-Performance Liquid Chromatography of Bread Wheat and Durum Wheat Proteins: an Efficient Tool for Predicting Quality in Breeding Programs and Investigating Physico-Chemical Basis of Quality.

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Baking quality and other wheat end-product qualities are complex to assess in any varietal breeding program. (SLIDE 2) Especially in early stages, the breeders need rapid and small-scale microtests for predicting the intrinsic value of genotypes. Several biochemical tests, based on protein solubility, and suitable for screening genotypes in early generations of breeding programs have been reported by Pomeranz, Orth and Bushuk, Feillet, Jeanjean, Kobrehel and coworkers. These methods have a high potential for analyzing large series of samples from small amouts of seeds and they yield results generally independent with regard to the agronomical record of the sample.

These last years, however, much of the basis on wheat breeding through biochemical tests has resulted from electrophoretic separations. For instance, allelic variations at loci coding for HMW-glutenin subunits have been extensively investigated for selecting breeding lines with higher potential of baking strength by Payne and coworkers, Branlard and coworkers, Pogna and coworkers.

Recently, the introduction by Bietz and Huebner of high-pressure liquid chromatography (HPLC) in wheat protein analyses has made it possible to consider a routine use of this technique on large series of samples, that was not possible by low pressure conventional liquid chromatography. In addition, HPLC may exhibit high resolution and reproducibility, its other most attractive features being automatization and quantitation due to its computer capabilities.

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The major advances in this field, however, were obtained by Bietz and Huebner since 1982 and later, by Kruger and coworkers, or Lookhart and coworkers, who mainly used reversed-phase type HPLC. Due to the very high resolving power of this method, it has been proposed as an alternative to electrophoresis for a varietal identification of wheats and, on the other hand, for predicting quality because of the relationship of some specific peaks or specific regions of the graph to quality characteristics.

Conversely, although it has been reported a long time ago by Huebner, Wall, Bushuk, or Miflin, that baking strength is associated to the occurence of large protein aggregates, size-exclusion HPLC (SE-HPLC) has been more rarely attempted for quality prediction. Unlike RP-HPLC and electrophoresis, that imply a total reduction or dissociation of native protein aggregates (and a lost of the 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 physico-chemical basis of quality.

The aim of the present paper is to show and to discuss the interest and feasibility of SE-HPLC of unreduced wheat protein extracts for predicting technological quality in both bread wheat and durum wheat breeding programs and investigating physico-chemical basis of quality. It is based on the study of a large number of samples, covering a large range of technological quality: 450 bread wheat and 100 durum wheat samples consisting of breeding lines and cultivars grown in 3-5 locations and during 3 years.

RESULTS

HPLC Elution Pattern

SLIDE 3 shows a typical elution profile of a flour extract (cv. Capitole) obtained under unreduced conditions (using a 2 hours extraction at 60°C by the phosphate-SDS buffer proposed in 1985 by Huebner and Bietz). This extract was applied to a TSK 4000-SW column. The chromatogram was breaked up into 4 segments:

- Peak F1, with apparent MW above 650 KDa and a maximum of about 1 million Da, elutes at the void volume of the column and corresponds to highly aggregated material.

- Fraction F2, an intermediate fraction that does not make a real peak, elutes between 115 and 650 kDa, and is likely to consist of partly aggregated material with continuous range of molecular size.

- Peaks 3 and 4 correspond to monomeric proteins whose apparent MW agree with the bulk of gliadins (45 Da) and salt-soluble proteins (25 Da), respectively.

Processing the chromatographic curve by a Nelson Analytical Software has allowed to characterize each sample by the percentages of the different peak areas (F1 thru F4), by the ratio between some fractions (F1/F2, for instance) and by the total area under the elution curve, which corresponds to the percentage of proteins that are solubilized in the phosphate buffer. In some cases, we added to these data the percentage of proteins insoluble in the phosphate-SDS buffer determined by Kjeldahl analyses on the residue and referred to as F0.

Origine of variation of SE-HPLC data

The first thing that I would like to go into is the origine of variation of SE-HPLC data. In contrast to earlier reports that stressed the unstability of phosphate-SDS extracts, due either to a slow dissociating effect of SDS, or perhaps to some protease action, an important point to mention is the extreme stability of supernatants obtained after extraction at 60°C. SLIDE 4 shows some example of variation of the ratio of excluded peak (the most sentitive to unstability) during a 2-day storage of the extract, compare to variations due to other factors.

It was also extremely important to determine the respective influence of variety and growing location on the amount of each peak, and on the soluble and insoluble fractions. This was investigated by analysis of variance. The results concerning 1985 crop (percentage of variability assignable to variety, growing location and residual; F test) are presented on SLIDE 5. This clearly shows that the amount of peak F1 and the F1/F2 ratio are essentially varietal-dependent while the amounts of F2 or F3 are also significantly influenced by the growing location. The ratio $\sigma^2 G / \sigma^2 L$, that express the ability of a test to discriminate between genotypes, is extremely high for both % F1 and F1/F2 ratio. Conversely, the total area under the curve and the percentage of insoluble fraction (F0) are much more influenced by growing location than by genotype.

This result demonstrates that the percentage of fraction F1 or the F1/F2 ratio have a good ability to discriminate betwen genotypes and may be more reliable

parameters for breeding programs than the other criteria which seem more associated to environmental factors than to genotype.

Relationships between HPLC data and baking quality among bread wheats

Although the patterns of different samples appear similar, major differences in the percentages of the 4 fractions may be observed between samples of bread wheats known to differ widely in baking strength or between samples of durum wheats known to differ widely in gluten strength and in pasta cooking quality. These samples were primarily distinguished by the amount of peaks F1 and F2, or by the ratio: F1/F2 and also by the percentage of insoluble nitrogen material (F0). Typical separations from three different bread wheat cultivars are shown in the SLIDE 6, confirming earlier reports of Huebner and Bietz. that the elution profile of proteins upon SE-HPLC relates to technological quality.

SLIDE 7 shows that strong cultivars (high Alveograph W index) have a smaller peak 1 (4.0 - 6.5 %) and a lower F1/F2 ratio (0.30 - 0.40) while those with low W index contain larger amounts of peak 1 (over 9.0 %) and a higher F1/F2 ratio (over 0.50).

The percentages of the 4 fractions (F1 to F4), of the F1/F2 ratio. of the insoluble fraction (F0) and of the total area under the curve were calculated from all genotypes of the 3 growing years 1985, 1986 and 1987 and linear correlations were calculated between all SE-HPLC data and all technological scores available.

SLIDE 8 shows the correlations calculated between all SE-HPLC data and some of the technological scores available from the 1987 crop.

Three main observations were made:

1) The total protein content of the flour is much more associated to the total area under the elution curve ($r = 0.78^{***}$) than to any of the fractions F1 thru F4.

2) Both % F1 and % F2 are negatively correlated to W index (Alveograph), or to Mixograph index and to Zeleny volume, and, to a lesser extent to P index (Alveograph), while % F0 and % F3 are positively correlated to these data.

3) The highest (negative) correlation coefficients with the different baking strength criteria are observed for the F1/F2 ratio (for instance: $r = -0.80^{***}$ with both W index and Mixograph index (n = 65 genotypes)).

Scatter diagramms of the relationship of W index and Mixograph index to F1/F2 ratio are shown on SLIDES 9 and 10, respectively.

Similar, but not identical, results were obtained when considering the different growing years (SLIDE 11). For instance, it is confirmed that both % F1 and F1/F2 ratio showed highly significant (negative) correlations with the baking strength criteria. However, the level of significance was higher some years and for some tests. For instance, the F1/F2 ratio was always very significantly associated to Mixograph index, while 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 and 1985.

Although the baking data were available in 1985 only, the results also show, that a (positive) association can be observed between % F2, loaf volume (or baking score in french baking technology).

On the other hand, when comparing the correlation coefficients within each growing location, significantly different results may be obtained (SLIDE 12) 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 between the three growing locations D, E and F. On the contrary, in 1986, the samples grown in the location A gave lower correlation coefficients with all 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 the location 1986-A (results not shown).

Partial Correlation Coefficients and Equations of Prediction

Now, let us discuss a difficult question: considering that the % of the insoluble fraction F0 was positively correlated to baking strength and negatively to F1/F2 ratio, another question was raised as to whether F1/F2 ratio directly determines baking strength, or if this association simply results from its correlation to % F0.

Accordingly, partial and multiple correlation coefficients were calculated to take into account, or to remove, the effect of the variation in % F0. SLIDE 13 shows, for instance, among genotypes of the 1986, or of the 1987 crop, that the coefficient of correlation between W index and F1/F2 ratio remains significant whether or not the effect of F0 is taken into account, indicating that a large part of the variation in W index was determined by F1/F2 ratio, independantly of the association of this ratio to the percentage of insoluble

fraction. On the other hand, the multiple correlation (F1/F2, F0) did not show any improvement compared to the simple correlation. A different result, however, was obtained in the particular case of the 1985 crop: the correlation coefficient (- 0.59*) resulted essentially from an association to the percentage of insoluble fraction since rp is no longer significant. In this case, a higher coefficient was obtained (r = - 0.80***) when using the multiple (F1/F2, F0) correlation, indicating that a more accurate prediction could be expected only when taking into account both F1/F2 ratio and % F0.

Equations of prediction of technological data were also calculated to compare the respective weights of F1/F2 and F0 parameters in the determination of baking strength. Considering a variation of one standard deviation unit of F1/F2 and of F0 around the mean values, it could be demonstrated that the variation of W index was respectively - 23.4 % and + 4.0 % and that the effect of F1/F2 ratio was therefore 6 times higher than the effect of F0. A very similar result has been observed from the samples of 1986 crop (an effect of F1/F2 ratio 7 times higher than the one of F0). A different result, however, was found in 1985 (an effect of F0 5 times higher than the one of F1/F2 ratio), confirming the above-mentioned strong influence of the percentage of insoluble protein on quality data of this crop year.

Considering for instance 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 F0 parameters, W index could be predicted by the following equation:

$$W = 370.9 - 766.1 \times (F1/F2) + 1.85 \times (F0)$$

The F1/F2 ratio, however, should be considered as a relative index rather than an absolute one. As a result of variations in the climatic conditions of each year (and in the aggregative level of the proteins that are synthesized), the MW distribution may be influenced and the F1/F2 ratio may vary within different ranges. As illustrated on the SLIDE 14, a comparison of the data obtained from 1985, 1986 and 1987 years shows that F1/F2 ratio and W index remain significantly associated, but that the coordinates of the regression line vary from year to year. Accordingly, a new equation of prediction may have to be calibrated from a set of samples of each new crop year.

Relationships between Quality Tests and SE-HPLC among durum wheats

Interestingly, different results have been obtained in the case of durum wheats. In spite of the similarity of the elution curve (SLIDE 15), a different relationship was found between the percentages of the main peaks and technological data such as gluten firmness or elasticity, SDS-sedimentation, or condition of surface of cooked pasta.

SLIDE 16 shows that, unlike in bread wheats, stronger cultivars (Amidur, Mondur, Agathe) contain higher percentages of peaks 1 and 2 and lower percentages of peak 3, while the opposite holds true for weaker cultivars (Kidur, Cando).

From 37 durum wheat genotypes, a very highly significant, and positive, correlation has been found between % P1, or % P2, or % (P1+P2) and viscoelastic characteristics of gluten (SLIDE 17). This is also illustrated by the principal component analysis printout of these different data (SLIDE 18).

Interestingly, as shown on SLIDE 19, durum cultivars belonging to the type γ-gliadin 45 have higher (27-40 %) percentages of peak 1 + peak 2 than genotypes with γ-gliadin 42 (15-30 %).

The different behaviours of bread wheats and durum wheat can be explained by further investigations on the amount of the protein fraction which is insoluble in phosphate-SDS buffer and on the polypeptide composition of the different peaks, using preparative HPLC fractionations and SDS-PAGE (SLIDE 20).

The 0.1 M sodium phosphate buffer containing 2 % SDS is likely to solubilize all single-chained polypeptides and many aggregated proteins (Bietz 1986). Since no reducing agent has been used, the extract is likely to contain some native aggregates, but not all wheat proteins are extracted. In bread wheats, extraction rates ranged between 55 and 90 %, depending on genotypes and the data from elution curves do not totally represent the least soluble aggregates, resulting in a negative correlation between the percentage of excluded peak and gluten strength.

On the contrary, among durum wheats, the protein extraction is more exhaustive and is near 90 % irrespective of the genotype. The major part of the protein aggregates are likely, therefore, to make up peaks 1 and 2. This has been confirmed by preparative SE-HPLC, as shown on the slide. HMW subunits of glutenin, and more especially LMW subunits, are obviously present in the

fractions 1 and 2, while the bulk of gliadins are essentially present in peak 3. Among durum wheats (SLIDE 21), the fact that two main genetic types have been identified (those with strong gluten and δ-gliadin 45, that contain large amounts of LMW-glutenins and those with poor gluten and δ-gliadin 42, that contain small amounts of LMW-glutenins) explain the previous results. Since most of LMW glutenins are solubilized, the former types yield larger peaks 1 and 2 than the latter, resulting, in this case, in a positive correlation between the amount of these peaks and gluten strength.

Advantages of SE-HPLC (SLIDE 22)

SLIDE gives a summary of the advantages of SE-HPLC over other methods of quality prediction:

- Ease, simplicity and reliability: the method requires less "art" than does electrophoresis.

- Excellent reproducibility and accurate quantitation of the fractions.

- Very small samples required (half-kernel of wheat).

- Automation: automated sample loading and computerized data processing.

- Preparative capabilities.

However, compared to RP-HPLC, SE-HPLC is faster (30 min.) and requires simpler equipments (1 pump, no gradient), simpler interpretation of the curve (4 peaks), and fewer reagents with less critical purity. On the other hand, SE-HPLC columns are more expansive, less resistant and have generally a more limited life time than RP-HPLC columns.

CONCLUSIONS AND FUTURE RESEARCH

Our results lead to five major conclusions (SLIDES 23-24):

1) A three-year experiment on a large number of samples has allowed to demonstrate that SE-HPLC of unreduced protein extracts was a potential way for predicting either baking quality of bread wheat genotypes, or gluten strength of durum wheat genotypes in breeding programs.

2) The prediction is mainly based on MW size distribution between excluded peak (F1) and intermediate aggregates (F2), the best indicator of the potential baking strength of genotypes being generally F1/F2 ratio, while loaf

volume in french baking technology seemed to be more associated to the amount of F2 fraction.

3) The equation of prediction of baking strength should be calibrated from samples of each new crop year. 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 (F0).

4) More than SDS-PAGE or RP-HPLC, that separate monomers or protein subunits after total reduction of the native aggregates, and that are helpful for fingerprinting genotypes, SE-HPLC has a potential for investigating native aggregates and approaching some physico-chemical basis of baking strength

5) Among bread wheats, the negative relationship between excluded peak and baking strength is explained by a low extraction rate of the highly aggregated HMW fractions. Among durum wheats, the positive relationship with gluten strength derives from the preferential occurence in peaks 1 and 2. of LMW subunits, which present large quantitative differences between "type 42" and "type 45" genotypes.

At present, our investigations are aimed at solving the following problems:

- To link the two approaches of biochemical basis of quality respectly based on protein aggregates and on correlations with a subunit composition. When comparing through preparative SE-HPLC the subunit composition of F1 and F2 fractions among different cultivars differing widely in baking quality: for instance, are the HMW glutenin subunits positively associated to quality (5-10, 1, 2*), present in more highly aggregated fractions than those negatively associated to quality ?

- To further investigate inheritance of SE-HPLC characteristics from progenies of diallelic crosses and to compare the selection pressure brought by an HPLC-based breeding, compared to other conventional tests.

- To study the respective effect of genotype and environmental factors from samples with narrower ranges of technological quality and check if some of the SE-HPLC criteria could be used for quality assessment of flours in commercial deliveries.

(1)

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(2)

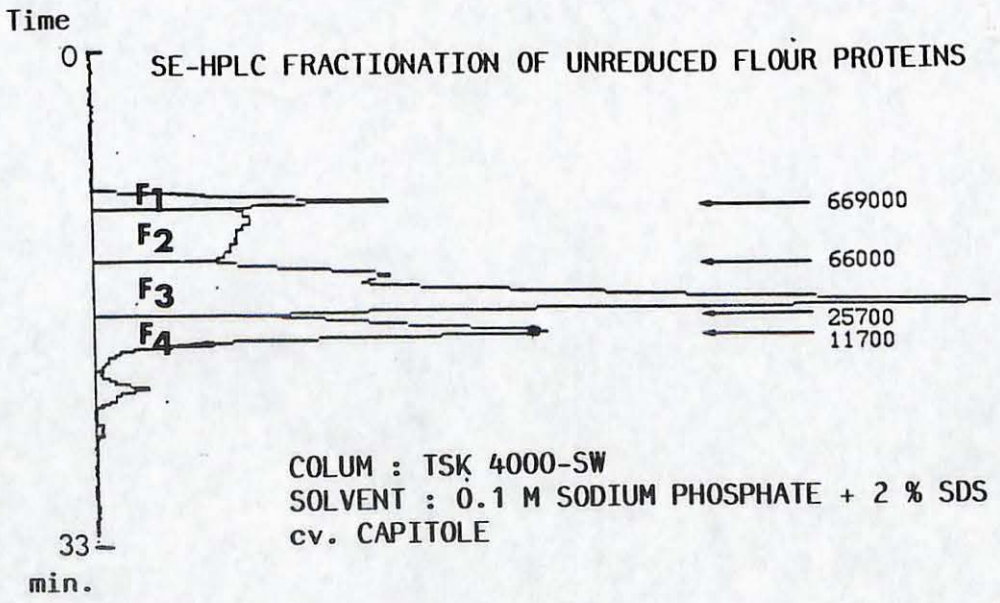
SEVERAL BIOCHEMICAL TESTS : Protein Solubility
Gel protein Content
SDS-PAGE of HMW subunits

HAVE A HIGH POTENTIAL FOR PREDICTING TECHNOLOGICAL QUALITY AT THE BREEDING STAGE :

- Large series
- Small amounts of samples
- Independent with regard to the agronomical record
- Correlated to quality potential of genotypes

QUESTION : HAS THE HPLC TECHNIQUE A HIGH POTENTIAL FOR SCREENING GENOTYPES ?

(3)



(4)

REPRODUCIBILITY

	Variation in % Peak F1	Mean (SD)
Same extract Different repeats	10.19 - 9.95 - 10.46 - 10.10	10.18 (0.18)
Different extracts	7.69 - 8.04 - 7.69 - 7.59	7.75 (0.19)
Different genotypes	11.14 - 8.90 - 12.12 - 6.76	9.73 (2.40)

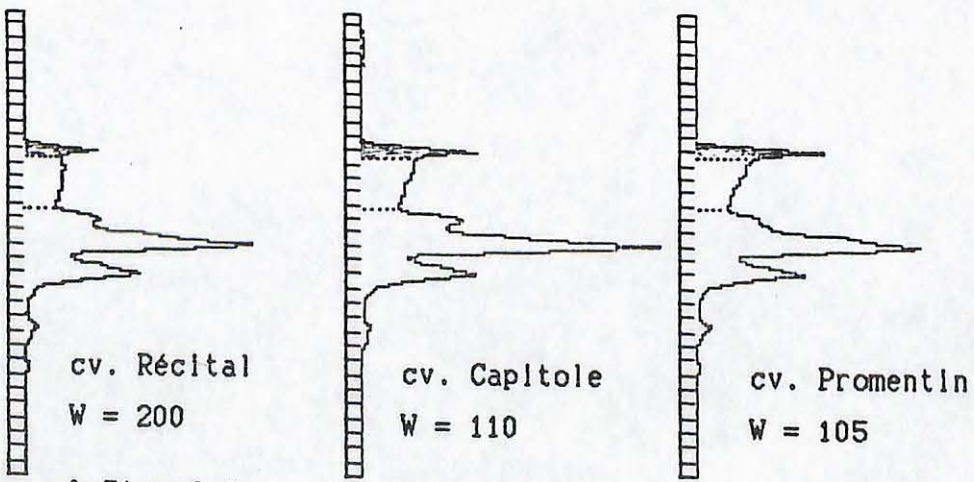
(5)

ANALYSIS OF VARIANCE

	% of variability Assignable to			
	Genotype	Growing location	Residue	$\sigma^2 G / \sigma^2 L$
% F1	76.4	2.9	20.7	26
% F2	70.4	7.8	21.8	9
% F3	67.9	14.6	17.5	5
% F4	39.5	45.4	15.1	0.8
Ratio F1/F2	62.4	2.9	34.7	21
Total Area	17.3	70.2	12.5	0.2
% insoluble F0	9.1	79.3	11.6	0.1

(6)

SE-HPLC ELUTION PROFILES OF 3 BREAD WHEAT CULTIVARS



(7)

CULTIVARS DIFFERING WIDELY IN POTENTIAL BAKING QUALITY

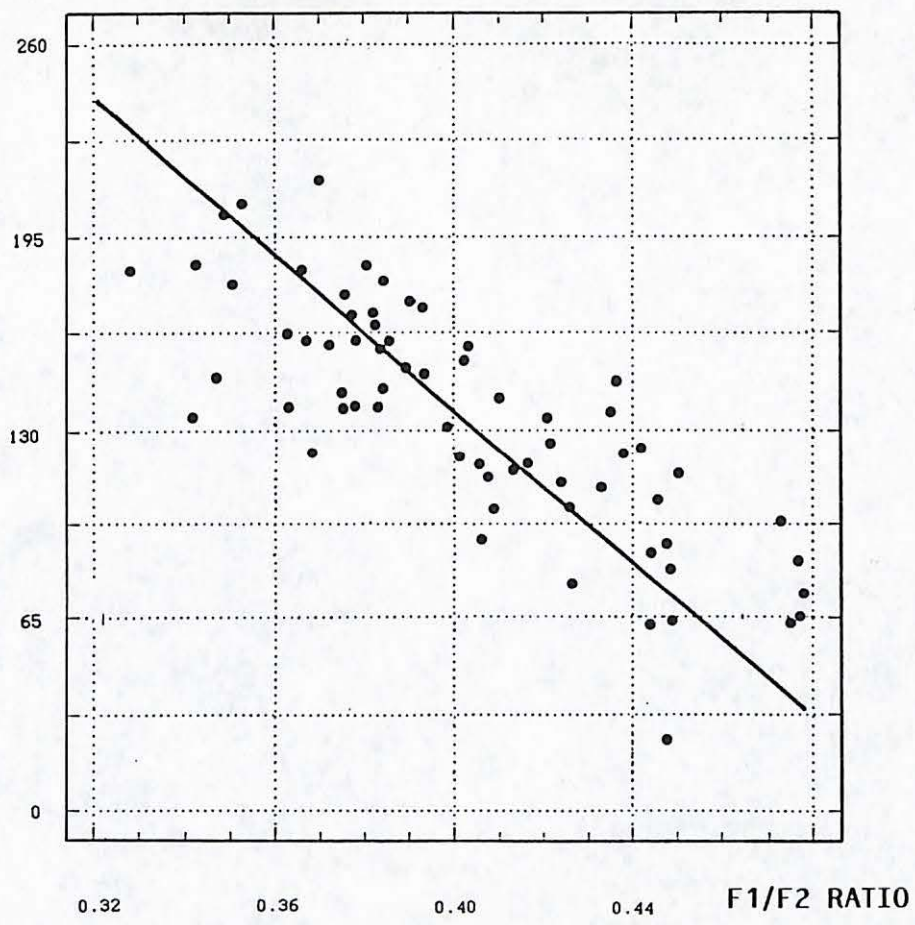
Cultivar	% F1	F1/F2/Ratio	W (Alveograph)
Talent	9.93	0.52	94
Capitole	9.08	0.46	137
Rescler	7.51	0.41	178
Chopin	6.08	0.37	295
Prinqual	5.41	0.30	342

(8)

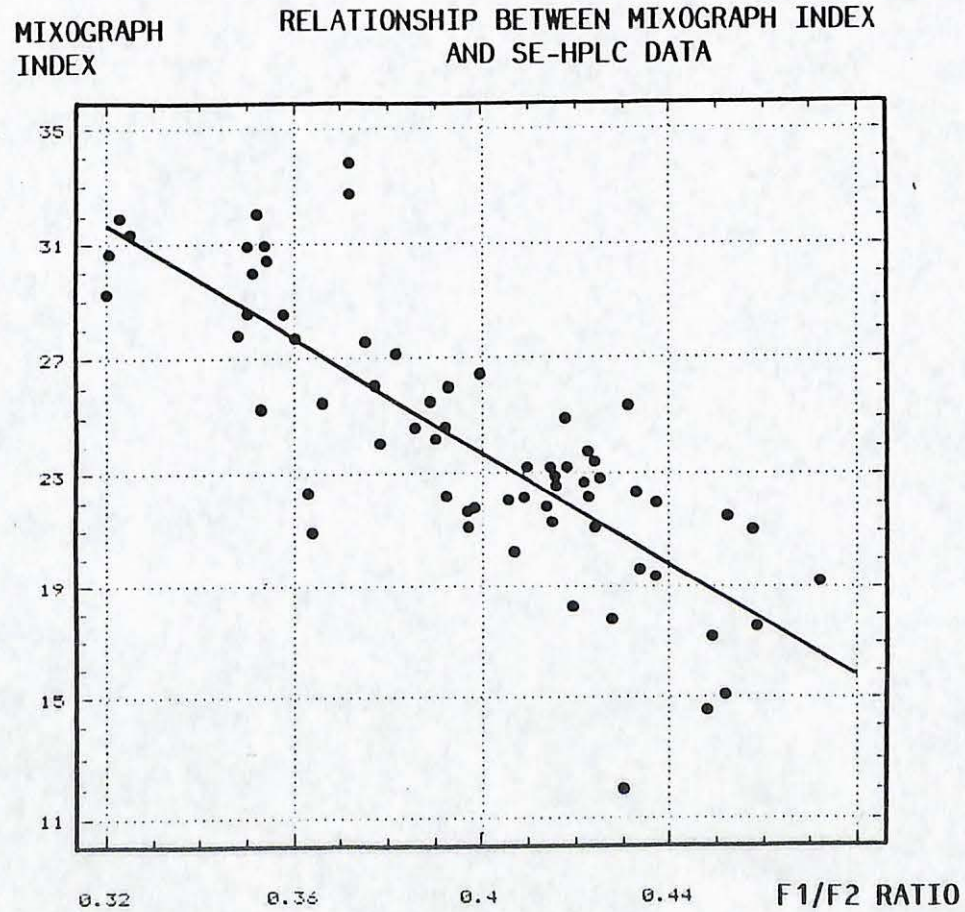
BREAD WHEATS
CORRELATION COEFFICIENTS
(65 Genotypes Grown in 3 Locations in 1987)

	Flour proteins	W (Alveograph)	I (Mixograph)
% F1	- 0.52***	- 0.72***	- 0.67***
% F2	- 0.55***	- 0.56***	- 0.44***
% F3	0.59***	0.52***	0.40**
% F4	- 0.20	0.11	0.23
F1/F2	- 0.44***	- 0.80***	- 0.80***
Total Area	0.78***	0.12	0.01
% F0	0.50***	0.63***	0.66***

(9)


W INDEX
(ALVEOGRAPH)
RELATIONSHIP BETWEEN W (ALVEOGRAPH)
AND SE-HPLC DATA
260
195
130
65
0
0.32
0.36
0.40
0.44
F1/F2 RATIO

(10)


MIXOGRAPH
INDEX
RELATIONSHIP BETWEEN MIXOGRAPH INDEX
AND SE-HPLC DATA
35
31
27
23
19
15
11
0.32
0.36
0.4
0.44
F1/F2 RATIO

(11)

BREAD WHEATS
CORRELATION COEFFICIENTS

		W(Alveograph)	I(Mixograph)	Gluten Firmness	Loaf Volume
1985 n=15	% F1	- 0.19	- 0.38	- 0.15	0.13
	% F2	0.31	- 0.28	0.46	0.70**
	F1/F2	- 0.59*	- 0.86**	- 0.64**	- 0.54
1986 n=63	% F1	- 0.51***	- 0.68***	- 0.47***	-
	F1/F2	- 0.53***	- 0.66***	- 0.51***	-
1987 n=65	% F1	- 0.72***	- 0.67***	-	-
	F1/F2	- 0.80***	- 0.80***	-	-

(12)

CORRELATION COEFFICIENTS

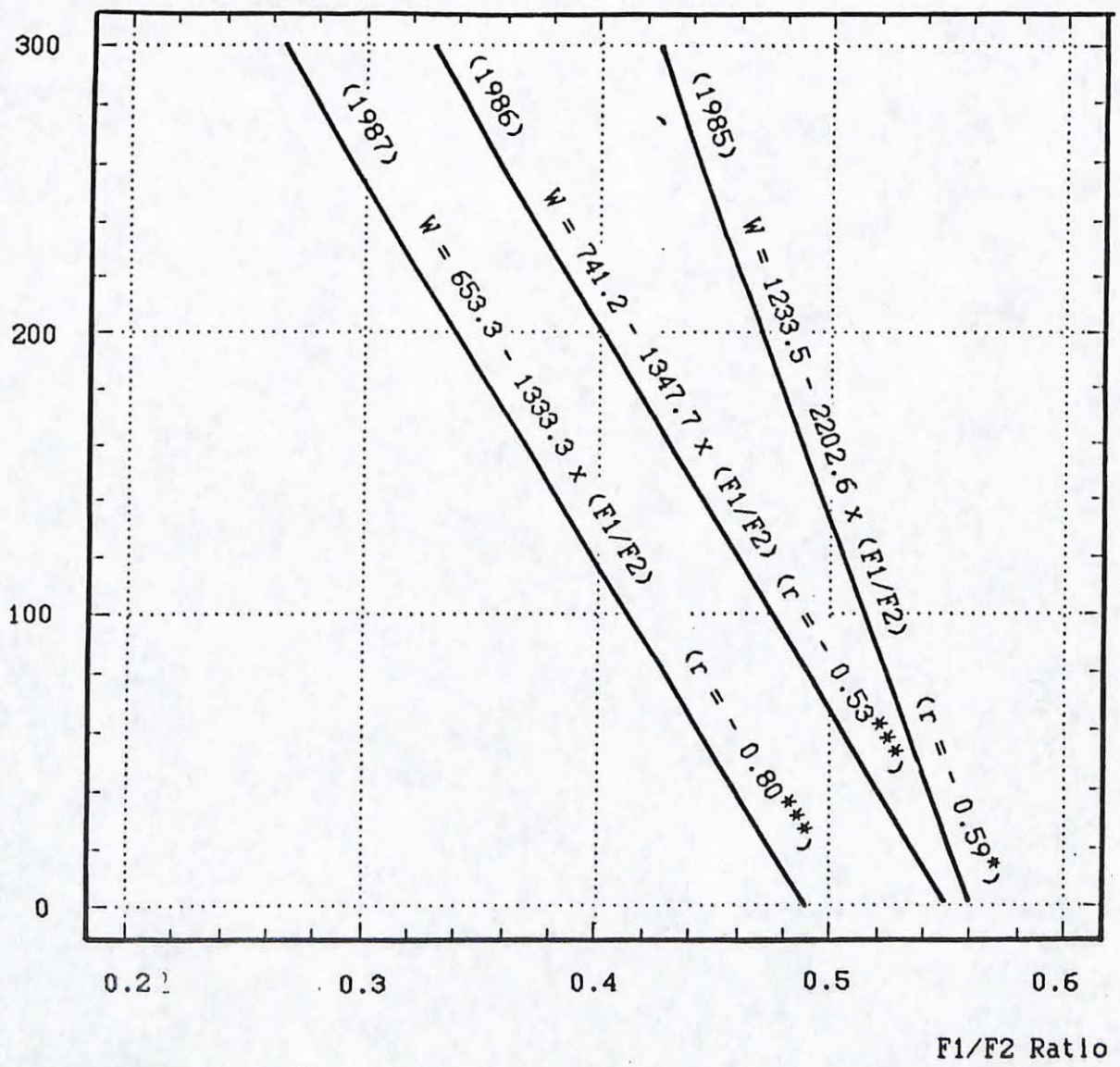
With mixograph index	Growing Locations(1985)		Growing Locations(1987)	
	A	B	A	B
% F1	- 0.39**	- 0.61***	- 0.64***	- 0.67***
% F2	- 0.31*	- 0.60***	- 0.31*	- 0.38**
% F3	0.45***	0.54***	0.33**	0.14
F1/F2 Ratio	- 0.36**	- 0.56***	- 0.72***	- 0.78***

(13)

PARTIAL AND MULTIPLE CORRELATION COEFFICIENTS
BETWEEN W (ALVEOGRAPH) AND F1/F2 RATIO

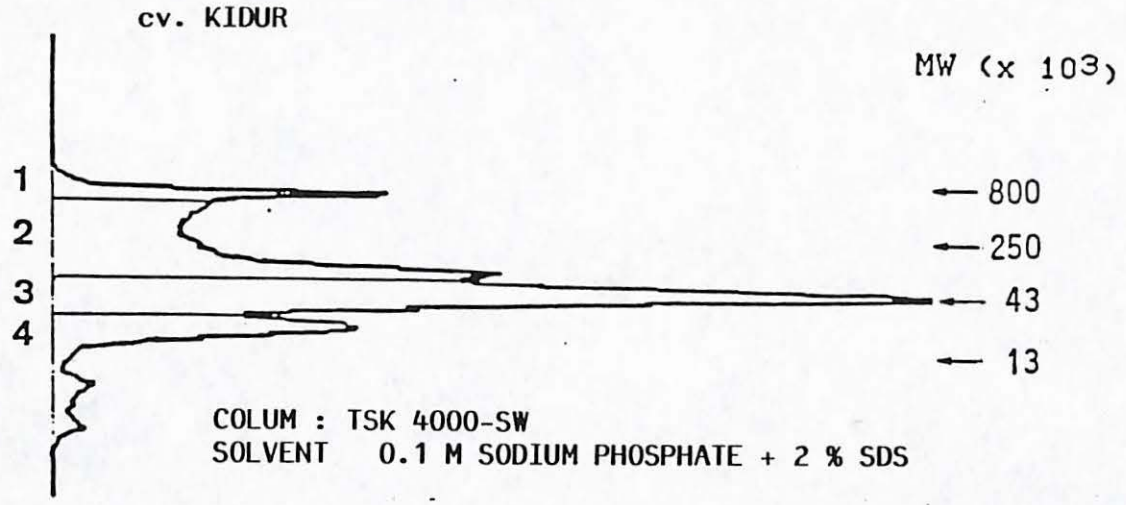
	Variate	r	rp.
1985 n=15	F1/F2	-0.59*	0.11 NS
	F0	0.84**	-
	F1/F2, F0	-0.81**	-
1986 n=63	F1/F2	-0.53***	-0.45***
	F0	0.33**	-
	F1/F2, F0	-0.52***	-
1987 n=65	F1/F2	-0.80***	-0.65***
	F0	0.63***	-
	F1/F2, F0	-0.80***	-

RELATIONSHIP BETWEEN W INDEX (ALVEOGRAPH)
AND F1/F2 RATIO FOR 3 DIFFERENT CROP YEARS



SE-HPLC FRACTIONATION OF UNREDUCED DURUM WHEAT PROTEINS

(15)



DURUM WHEAT
CULTIVARS DIFFERING WIDELY IN POTENTIAL
COOKING QUALITY

(16)

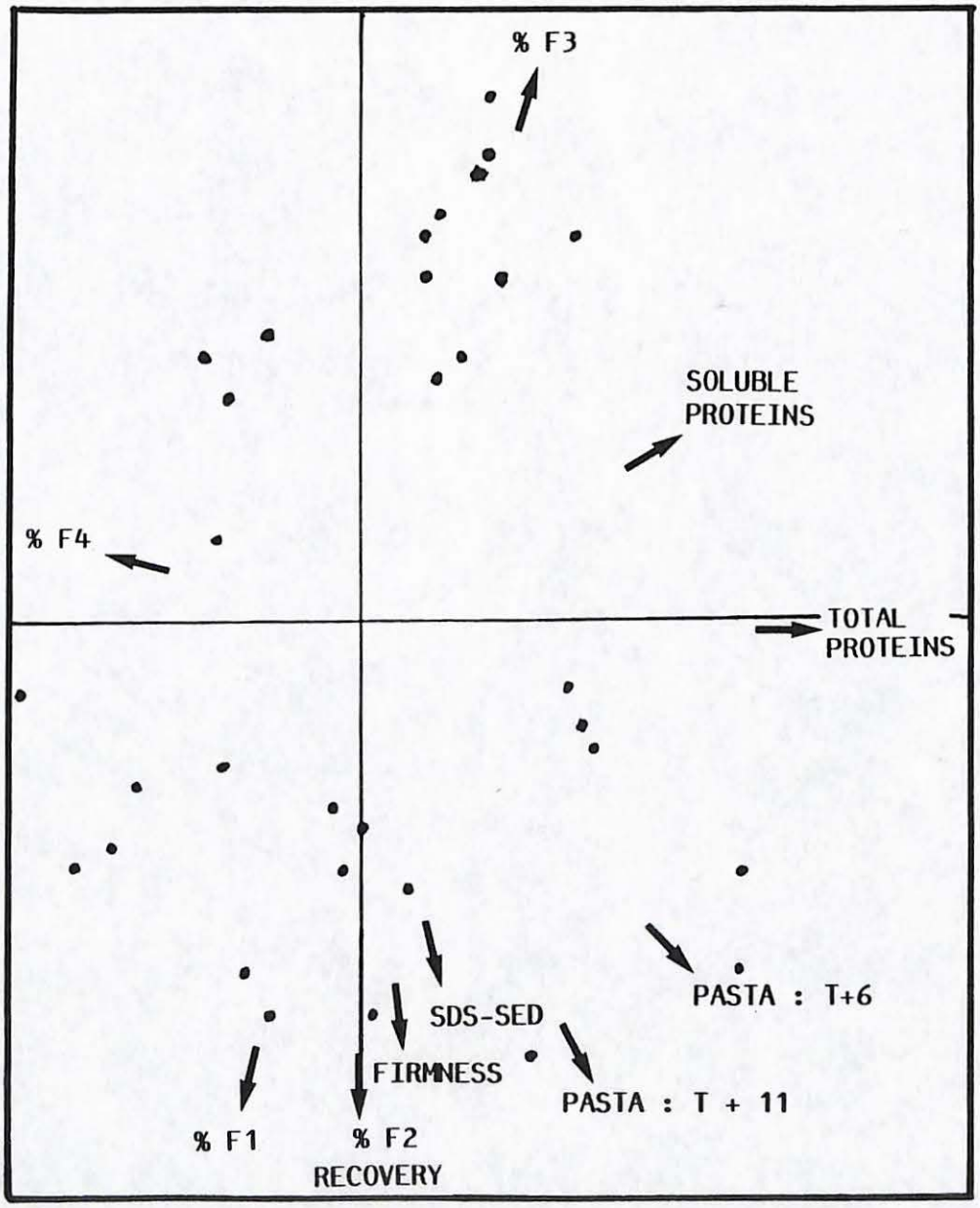
Cultivar	% F1	% F2	% F3	Gluten Elasticity	Pasta Score
Kidur	6.5	16.0	62.1	0.60	4.3
Cando	8.4	17.7	59.8	1.19	4.5
Agathe	10.1	18.5	54.9	1.35	6.0
Mondur	11.1	21.8	53.3	1.41	6.8
Amidur	14.4	23.6	48.9	1.47	5.1

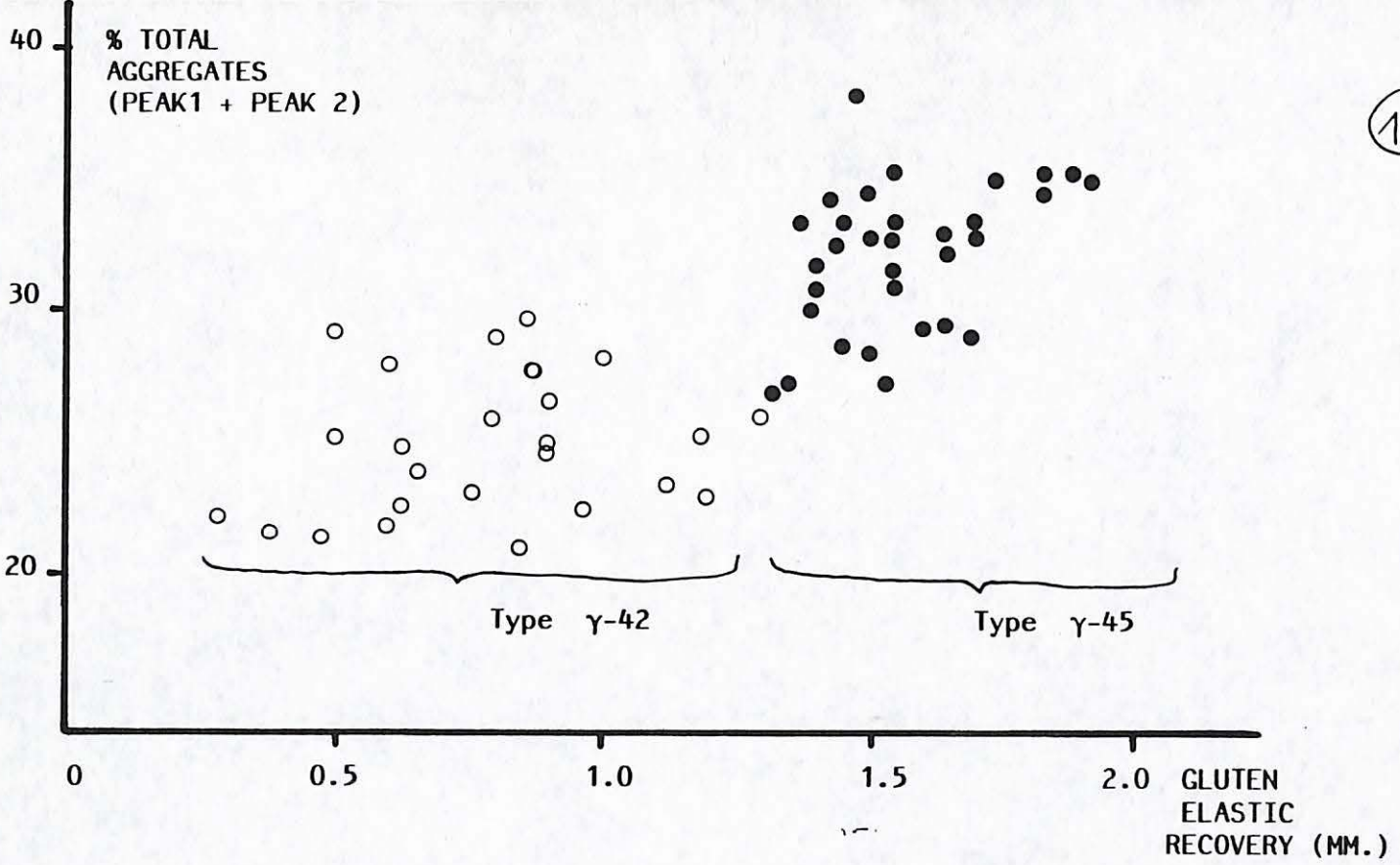
DURUM WHEATS
CORRELATION COEFFICIENTS
(37 Genotypes)

(17)

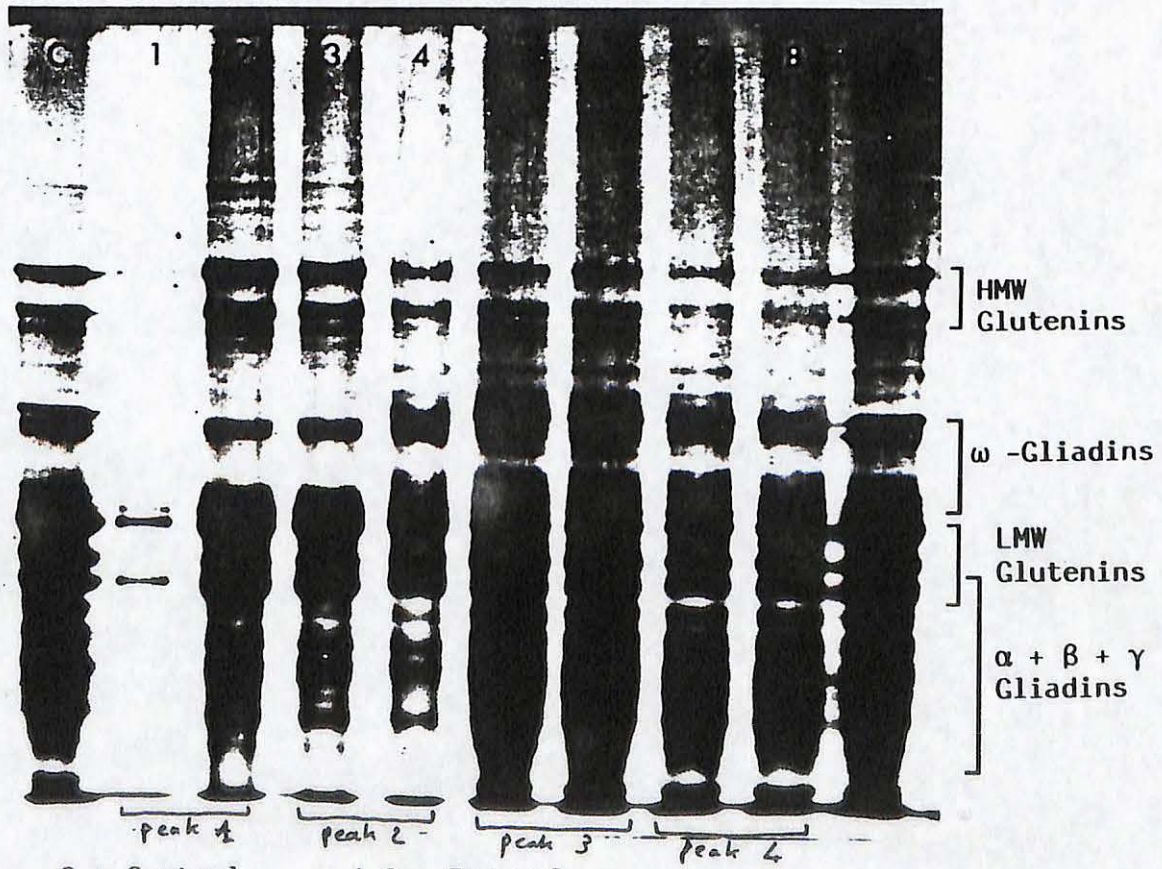
	Semolina Proteins	Gluten		Pasta condition of surface
		Firmness	Recovery	
% F1	-0.05	0.44**	0.65***	0.44**
% F2	-0.02	0.69***	0.67***	0.42**
% F3	0.15	-0.62***	-0.72***	-0.51**
% F4	-0.39	-0.08	-0.10	0.08
Total Area	0.42**	-0.11	-0.32	-0.20

PRINCIPAL COMPONENT ANALYSIS OF SE-HPLC
AND TECHNOLOGICAL CRITERIA IN DURUM WHEATS





SDS-PAGE PATTERNS
OF SE-HPLC FRACTIONS



C : Control 1-8 : Peak n°

(21)

RELATIONSHIP BETWEEN GLUTEN STRENGTH AND % OF PROTEIN AGGREGATES IN SE-HPLC

		% OF EXTRACTED PROTEINS	% OF LARGE AGGREGATES (EXCLUDED PEAK)	% OF TOTAL AGGREGATES (PEAKS F1+F2)	CORRELATION % F1/STRENGTH
BREAD WHEATS	Good	65-80	4-8	15-20	< 0
	Poor	75-90	7-12	18-25	
DURUM WHEATS	Good(*)	85-90	9-15	25-40(*)	> 0
	Poor(**)	90-95	6-10	15-30(**)	

(*) Type Gliadin 45 : Large Ratio of LMW-Glutenin Subunits
(**) Type Gliadin 42 : Small Ratio of LMW-Glutenin Subunits

(22)

AVANTAGES OF SE-HPLC

- Ease, Simplicity and Reliability (Requires less Art than does Electrophoresis).
- Speed (30 min.)
- Excellent Reproductibility
- Very Small Samples Required (Half-Kernel of wheat)
- Automation (Automated Sample Injector and Data Processing)
- Accurate Quantation of Fractions
- Preparative Capabilities
- Equipment : Simpler than for RP-HPLC
- Fewer reagents with less critical purity than for RP-HPLC

CONCLUSIONS

(23)

1) SE-HPLC of unreduced protein extracts is a potential way for predicting baking quality of bread wheats or gluten strength of durum wheats in breeding programs

2) The prediction is mainly based on MW size distribution between excluded peak (F1) and intermediate aggregates (F2); F1/F2 ratio is highly varietal-dependent

3) The equation of prediction may have to be calibrated each new year crop and may have to take into account the percentage (F0) of insoluble proteins

(24)

4) More than RP-HPLC, or SDS-PAGE, that essentially separate monomers and are helpful for fingerprinting. genotypes, SE-HPLC has a potential for investigations native aggregates and approaching some physico-chemical basis of gluten strength.

5) Among <u>bread wheats</u>, the positive relationship between excluded peak and baking strength is explained by a low extraction rate of the highly aggregated HMW fractions. Among <u>durum wheats</u>, the positive relationship with gluten strength derives from the preferential occurence in peaks F1 and F2 of LMW-glutenins, which present large quantitative differences between "type-42" and "type-45" genotypes.

184 Size-exclusion High-Performance Liquid Chromatography of Bread Wheat and Durum Wheat Proteins : an Efficient Tool for Predicting Quality in Breeding Programs and Investigating Physico-Chemical Basis of Quality.

J.C. Autran, T. Dachkévitch and P. Feillet, Laboratoire de Technologie des Céréales, I.N.R.A., 9 place Viala, 34060, Montpellier, France.

Bread wheat and durum proteins have been analysed by SE-HPLC. Experimental conditions were investigated for extracting aggregates and improving reproducibility and discrimination between quality classes. Five chromatographic fractions corresponding to different size of aggregates and monomers were separated from phosphate-SDS extracts. A negative and highly significant relationship was found between the ratio of the area of either of the two first peaks to the whole elution area and baking strength (W value), or gluten viscoelasticity, in bread wheats. Interestingly, a positive relationship was found between the same ratios and gluten viscoelasticity in durum wheats. Computer-assisted processing of the data allowed to propose an efficient system for the prediction of gluten strength in breeding. Preparative SE-HPLC and SDS-PAGE analyses of the fractions allowed to give new insights in the basis of the relationship between aggregative level of the proteins and gluten characteristics. In bread wheats, the negative relationship was explained by a low extraction rate of the highly aggregated HMW fractions, while the positive relationship observed in durum wheats derived from the preferential occurence of LMW glutenins in peaks 1 and 2.

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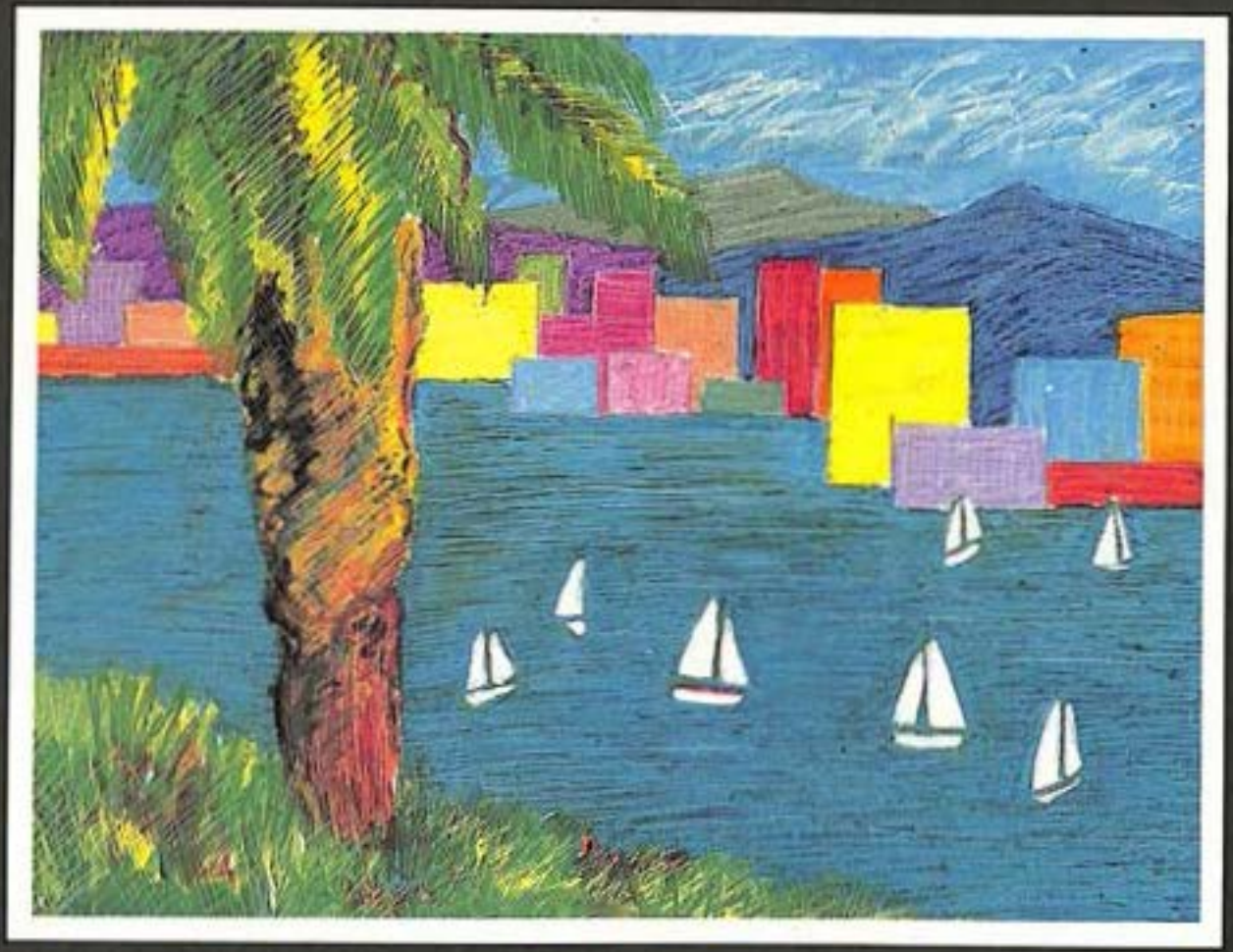

SAN DIEGO

AACC 73rd Annual Meeting

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