Statistical Evaluation of Different Technological and Biochemical Tests for Quality Assessment in Durum Wheats

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ABSTRACT

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Principal component analysis was carried out on results of nine quality tests applied to 112 durum wheat cultivars and breeding lines. Rheological-type characteristics (gluten firmness and elasticity, sodium dodecyl sulfate-sedimentation) were strongly associated, typically variety-dependent, and independent of protein content. Surface characteristics (stickiness, clumping) of cooked spaghetti appeared essentially independent of rheological characteristics and were significantly influenced by protein content and growing location, but they also remained significantly influenced by genotype. Attention was focussed on the remarkable relationship between gluten rheological characteristics and the specific allele (gamma gliadin + low-molecular-weight glutenin as evidenced by polyacrylamide gel electrophoresis or sodium dodecyl sulfate-polyacrylamide gel electrophoresis) present at the *Gli-B1* chromosomic locus of durum wheat genotypes: allele "45" had a marked positive effect whereas allele "42" was deleterious as far as gluten characteristics were concerned. It was stressed, therefore, that surface characteristics of cooked pasta could not be predicted through rheological measurements or gliadin electrophoresis, making essential the separate evaluation of the two major components of durum pasta cooking quality.

Durum wheat is the cereal of choice for the manufacture of high-quality pasta products (Feillet and Abecassis 1976, Dexter and Matsuo 1980). Cooked pasta made from durum wheat semolina usually retains good rheological characteristics (firmness, elasticity) and is resistant to surface disintegration and stickiness. These characteristics depend more or less on the cultivars that are processed, which makes it necessary to breed durum wheat lines for high-quality potential, especially in countries such as France and Italy where use of pure durum wheat semolina for pasta making is required.

The ability of a cultivar to be processed into yellow-amber-color pasta is readily evaluated, but predicting the decisive criteria for cooking quality is still a critical problem (Feillet 1979, 1980; Autran 1981; Autran et al 1981; Kobrehel et al 1982). In France,

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most of the new lines submitted for registration are currently rejected on the basis of their insufficient cooking tolerance. Accordingly, breeders need fast, small-scale methods to predict the different parameters of cooking quality. Genetic improvements depend on their capacity to efficiently screen thousands of lines each year.

Cooking quality has been shown to depend on two main parameters: rheological properties (related to gluten viscoelasticity or strength) and surface characteristics (absence of surface deterioration, e.g., stickiness, mushiness, or clumping). These two parameters do not seem to be directly related (Alary et al 1979, D'Egidio et al 1979, Abecassis et al 1981, Houliaropoulos et al 1981, Feillet 1984), but their independence has never been conclusively demonstrated.

Biochemical methods (electrophoresis, high-performance liquid chromatography) have been recommended for quality assessment and prediction at the breeding stage. Such methods also allow a better understanding of quality at the molecular level. Of particular interest is the finding by Damidaux et al (1978, 1980), from a large set of homozygous cultivars, of an unbroken association between the presence of a γ-gliadin (electrophoretic

band no. 45) and strong gluten (viscoelasticity) and between the presence of another γ-gliadin (no. 42) and weak gluten. Subsequently, although some overlap was reported by Leisle et al (1981) in the progeny of some durum wheat crosses, this relationship was confirmed by Kosmolak et al (1980), Du Cros et al (1982), and more recently by Burnouf and Bietz (1984) using reversed-phase HPLC. Furthermore, the relationship was extended from γ-gliadins only to the whole allelic block, called *Gli-B1*, coding for γ-gliadins, ω-gliadins, and low-molecular-weight glutenin subunits (Payne et al 1984, Autran and Berrier 1984). These discoveries gave rise to an extensive use of electrophoresis as a very small-scale breeding tool to predict durum wheat cooking quality (gluten viscoelastic properties), especially in early generations, along with other conventional tests for quality assessment.

In this paper, we attempted to draw an evaluation of the respective usefulness of different technological and biochemical tests that are commonly used for durum wheat quality evaluation at the breeding and registration stages.

MATERIALS AND METHODS

Plant Material
The samples used for this study are representative of the main durum wheat cultivars grown in France and of the genetic material currently developed by breeders.

Twenty-six durum wheat breeding lines submitted to registration in France and five standard cultivars (Agathe, Capdur, Kidur, Mondur, and Tomclair) were grown in 1983 or 1984, either in two locations in the north (spring sowing), or two locations in the south (winter sowing), or in both regions, to produce samples (200 kg) submitted to pilot tests. On the whole, 112 samples were analyzed.

Technological Tests
The wheats (150 kg) were tempered to 17% moisture and milled in a semolina pilot plant as described in Houliaropoulos et al (1981). The yield of purified semolina was 72–77%. Semolina was processed into spaghetti in a Bassano laboratory-scale extrusion press and dried for 24 hr at 37°C according to the procedure officially used in France for durum wheat registration (Abecassis et al 1984, Alary et al 1985).

Spaghetti was scored for cooking quality: samples of 100 g were cooked and overcooked in mineral water, pH 7.3 ± 0.1 (Alary et al 1979), for T + 6 and T + 11 min, respectively (T = minimum cooking time, checked by the disappearance of ungelatinized starch at the center of spaghetti strands [Feillet 1977]). Surface texture scores (from 1, very poor, to 9, excellent) of cooked and overcooked spaghetti were determined by a four-person panel considering all stickiness and surface deterioration characteristics. The rheological aspect of cooking quality was measured by submitting pilot semolina to microdisk (7-mm diameter) processing and Viscoelastograph evaluation as previously described (Feillet 1977; Feillet et al 1977a,b; Alary et al 1978). A cooking index was then inferred (score range: from 1, very soft, to

12, very strong) from the different viscoelasticity values at increased cooking times (Alary et al 1985).

Protein Electrophoresis
Gliadins were extracted and fractionated in 6% polyacrylamide gels (PAGE) according to Bushuk and Zillman (1978). Total reduced proteins were fractionated in 13% sodium dodecyl sulfate (SDS)-PAGE by the method of Payne et al (1979) slightly modified by Autran and Berrier (1984).

Other Laboratory Tests
Protein content (% N × 5.7) was determined by the Kjeldahl method. Pasta color (yellow index and brown index) was determined according to Alary et al (1985). SDS-sedimentation test was performed according to Axford et al (1978).

Glutens were extracted and submitted to Viscoelastograph measurements to determine firmness and elastic recovery according to Damidaux and Feillet (1978) and Damidaux (1979).

Statistical Analysis: Principal Component Analysis
Principal component analysis (PCA) is a method of describing data on individuals and numerical values from a table. In this work, the individuals were the durum wheat varieties, and the parameters were the scores of different technological tests.

When there are only two parameters, it is easy to plot the data. Also, with three parameters, a visual study is still possible using solid geometry. But if there are more than three parameters, for example nine technological scores, a visual representation is no longer possible because a nine-dimensional space would be required.

However, a planar representation of the data can be obtained through projections on a plan. This procedure obviously involves distortions, but it is possible, using a computer, to determine the projection plan on which the distances will be best retained, on average. This particular projection plan is called a principal plan. Through this calculation, new coordinates of the points are obtained, called principal components. PCA is therefore intended to reduce the number of numerical parameters, which allows graphical representation of even complex data. This reduction is not done by selection of some of the parameters but by creation of new synthetic variables, the principal components, that are linear combinations of the initial ones and not correlated together. The more flattened the set of points compared to the principal plan, the more valid the representation of the data on this plan. The percentage of total inertia of the principal plan gives a measure of the flatness of the set of points and therefore of the significance of the representation on this plan.

The PCA graphic is automatically drawn by the computer printer. It consists of two orthogonal axes that determine the principal plan, with one set of sample points and one set of test arrows.

PCA graphics must be interpreted with care. For example, the proximity of sample points and test points has no meaning, because the scales are different. However, each test arrow defines an axis on which the different sample points can be projected and

TABLE I
Summary of Quality Data for Durum Wheat Lines and Named Varieties (Pilot Samples)

Wheat Characteristic	1983 (n = 60)			1984 (n = 52)		
	Mean	Range	SD	Mean	Range	SD
Protein content (% wheat dry matter)	14.4	10.7–20.2	1.94	14.7	12.3–16.8	0.92
Spaghetti surface score after T+6 min cooking time[a]	4.5	2.4–6.6	0.92	5.3	3.6–6.9	0.80
Spaghetti surface score after T+11 min cooking time[a]	2.9	1.0–5.2	1.02	3.7	1.8–5.9	0.92
SDS-sedimentation volume (ml)	20.0	12.0–42.0	6.67	18.0	11.0–26.0	3.43
Gluten firmness (mm)	2.07	1.55–2.87	0.36	2.05	1.5–2.62	0.32
Gluten elastic recovery (mm)	1.26	0.64–1.79	0.35	1.45	0.65–1.81	0.31
Pasta microdisks cooking index	9.9	7.0–12.0	1.83	7.8	4.0–12.0	2.19
Spaghetti brown index	14.0	10.4–17.3	1.45	17.6	14.6–19.8	1.30
Spaghetti yellow index	44.1	33.5–56.8	4.80	41.2	33.7–51.5	4.80

[a] T = Minimum cooking time for spaghetti.

	PROT	T+6	T+11	SDSS	FIRM	REC	CI	BI	YI
Protein content (PROT)	1.0	0.62**[b]	0.55**	−0.03	−0.29*	−0.03	0.35**	0.47**	−0.09
State of surface at T+6 min (T+6)[c]		1.00	0.94**	0.21*	−0.02	0.25*	0.21*	0.53**	−0.15
State of surface at T+11 min (T+11)[c]			1.00	0.30**	0.05	0.35**	0.24*	0.43**	−0.11
SDS-sedimentation volume (SDSS)				1.00	0.72**	0.78**	0.30**	0.03	−0.17
Gluten firmness (FIRM)					1.00	0.75**	0.31**	−0.11	−0.29*
Gluten elastic recovery (REC)						1.00	0.32**	0.02	−0.23*
Pasta microdisks cooking index (CI)							1.00	−0.34**	−0.02
Spaghetti brown index (BI)								1.00	−0.03
Spaghetti yellow index (YI)									1.00

[a] 1983 and 1984 data from pilot type semolina; 32 genotypes × 2 or 4 growing locations each; total number of samples: 112.
[b] *, ** Correlation is significantly different from zero at the 0.05 and 0.01 level of probability, respectively.
[c] T = Minimum cooking time for spaghetti.

TABLE III
Analysis of Variance for Each Characteristic Studied

	Percentage of Variability Assignable to			F Test[a]	
Characteristic	Variety	Growing Location	Residue	Variety	Growing Location
Protein content (PROT)	33.1	17.6	49.3	*	**
Spaghetti surface after T+6 min. (T+6)[b]	52.1	20.6	27.3	**	**
Spaghetti surface after T+11 min. (T+11)[b]	65.4	14.4	20.2	**	**
SDS-sedimentation volume (SDSS)	96.0	0.1	3.9	**	NS
Gluten firmness (FIRM)	86.4	3.9	9.7	**	*
Gluten elastic recovery (REC)	95.4	0.1	4.5	**	NS
Pasta microdisks cooking index (CI)	43.2	36.1	20.7	**	**
Pasta brown index (BI)	12.6	67.9	19.5	*	**
Pasta yellow index (YI)	86.6	8.5	4.9	**	**

[a] *, ** Significant at the 0.05 and 0.01 level of probability, respectively.
[b] T = Minimum cooking time for spaghetti.

compared according to their values for the corresponding test. On the other hand, the proximity of two test arrows means that the values of the tests are correlated, the level of correlation being relative to the distance of the test points from the origin.

RESULTS AND DISCUSSION

Correlations Between Quality Tests

Quality data (mean, range, and SD) obtained on the 112 samples grown in 1983 and 1984 are summarized in Table I. The diversity of wheat samples resulted in very wide ranges for all quality measurements. Also, sample distribution for most tests was relatively close to a normal distribution, except for gluten characteristics, which essentially followed a bimodal distribution.

Simple correlation coefficients, calculated between all possible quality traits for the entire 1983–1984 data set, are presented in Table II. Four main observations were made:

1) The two gluten rheological characteristics were associated ($r = 0.75$), as previously shown by Damidaux (1979). In addition, both firmness and elastic recovery correlated ($r = 0.72$ and 0.78, respectively) to SDS-sedimentation volume, supporting previous reports from Dexter et al (1981), who demonstrated that SDS-sedimentation volume gave a good prediction of gluten strength. On the other hand, no correlation was found between gluten strength and semolina protein content, which is in total agreement with previous reports of Damidaux and Feillet (1978) and Matsuo

et al (1982).

2) Spaghetti surface scores after $T + 6$ min and $T + 11$ min of cooking time strongly correlated ($r = 0.94$) with each other, and both tended to be significantly associated with protein content ($r = 0.62$ and 0.55, respectively). There appeared to be no correlation between surface score and gluten firmness, and only little correlation between surface score and gluten elastic recovery or SDS-sedimentation, which is in agreement with stickiness measurements reported by Dexter et al (1983).

3) Cooking index showed no highly significant correlation with the other cooking quality tests but did show a low correlation (0.30–0.35) with protein content, gluten firmness, gluten elastic recovery, and SDS-sedimentation. This confirmed previous investigations (Feillet and Abecassis 1976, Damidaux and Feillet 1978, Dexter and Matsuo 1980, Matsuo et al 1982, Kobrehel et al 1982), which conclusively demonstrated that cooking quality depends on both gluten strength and protein content.

4) No correlation was found between yellow index and any of the other characteristics. For brown index, there was evidence of a slight correlation with protein content (as reported by Kobrehel et al [1974]), and to a certain extent with surface characteristics of cooked spaghetti ($T + 6$ and $T + 11$).

Influence of Variety and Growing Location

Analysis of variance was carried out on all genotypes grown in four locations. The results (percentage of variability assignable to variety, growing location, and residual; F test) are presented in Table III. These results clearly confirmed that tests such as gluten elastic recovery, gluten firmness, SDS-sedimentation volume, and yellow index were essentially varietal-dependent (Damidaux 1979, Dexter et al 1981, Laignelet et al 1972). On the other hand, characteristics such as protein content and brown index were much more influenced by growing location than by genotype (Abecassis and Alause 1979). Concerning cooking characteristics, the analysis of variance confirmed that both variety and growing location had a significant effect on pasta microdisks cooking index (Damidaux and Feillet 1978). However, variety seemed to explain a higher percentage of variability than growing location for surface characteristics of cooked spaghetti at $T + 6$ and $T + 11$ min, both factors having a significant effect. To our knowledge, it was the first time a significant varietal effect was evidenced for this characteristic. Although percentages of variability assignable to growing location and to residue (the latter probably originating from genotype × growing location interaction and from the lack of precision of tests using discontinuous scores) could not be neglected, these effects appeared much lower than in protein content or brown index, so that a genetic improvement of surface characteristics of cooked spaghetti is certainly possible.

PCA

To evaluate the respective importance of the technological tests, principal components were analyzed using the results of the nine tests as numerical parameters and varieties or lines as individuals. Figure 1 shows the first principal plan of the PCA graphic from the 1983–1984 set of data. This first principal plan contained 69% of

the total inertia calculated from the nine quality tests, or 83% of the total inertia when calculated from the cooking quality tests only.

From this set of 112 sample points, the most consistent trends were the following: 1) The three rheological traits (gluten firmness, gluten elastic recovery, and SDS-sedimentation) were strongly linked, which was consistent with the above-mentioned correlations and clearly defined a "rheological-quality" component or axis. 2) Protein content corresponded to a second axis, roughly orthogonal to the rheological one, corroborating the independence of the two types of characteristics. This is in agreement with the above-mentioned correlations and those published by Matsuo et al (1982). 3) Spaghetti surface at T + 6 and T + 11 min were strongly correlated and relatively well correlated with protein content, but no major trend between spaghetti surface characteristics and rheological characteristics was apparent. 4) Cooking index appeared to be intermediate between rheological characteristics and surface texture of spaghetti, as shown in the correlations results.

Relationships Between Quality Tests and Protein Electrophoretic Patterns

The most striking result was the distribution of the samples in PCA graphics according to their electrophoretic composition. In the first principal plan, all varieties belonging to the "gliadin 42 type" (which had lower scores in gluten characteristics) were clearly separated from those belonging to the "gliadin 45 type" (which had higher scores in gluten characteristics): the border between the two groups (diagonal dotted line in Fig. 1) appeared to be roughly orthogonal to the rheological axis.

When the different growing locations of each genotype were identified on the PCA graphic (details not shown), it was noticed that they were generally not superimposed. They essentially differ from protein content and surface characteristics of cooked spaghetti so that their junction line tended to be parallel to the protein content principal component and therefore also roughly orthogonal to the rheological axis. This result certainly meant that surface texture of cooked spaghetti was highly influenced by growing location and protein content. It did not, however, negate a varietal basis for this parameter as indicated in the analysis of
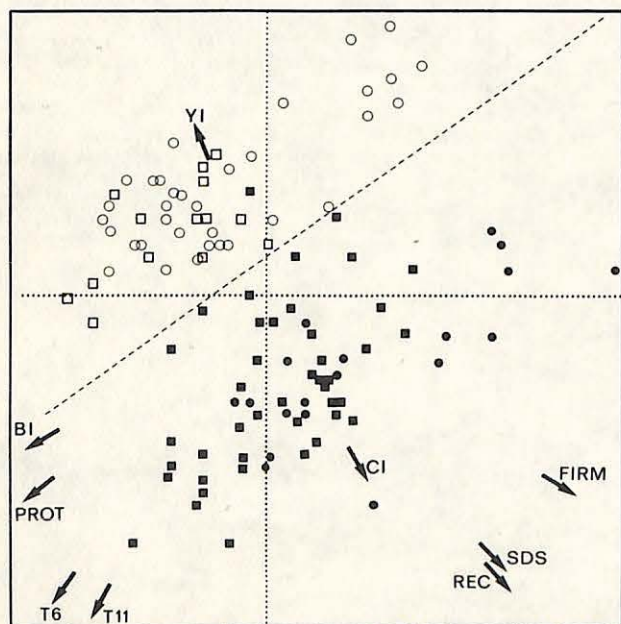
variance. On the other hand, the distribution of different sample points of the same genotype on the PCA graphic corroborated conclusions (Damidaux et al 1978, Feillet 1979) that gluten firmness and elasticity were essentially varietal dependent, with very little influence from protein content and growing location. Accordingly, PCA clearly demonstrated the independence between the two parameters of cooking quality: rheological characteristics and surface characteristics. It must be emphasized, therefore, that surface characteristics of cooked spaghetti cannot be predicted only through rheological measurements on gluten or gliadin electrophoresis. Good gluten quality appears to be a necessary but not a sufficient condition for having high-quality pasta.

CONCLUSIONS

PCA allowed a new approach toward assessing the respective importance and usefulness of some of the most commonly used small-scale tests for durum wheat cooking quality and made it possible to present a very synthetic distribution of the samples relative to different technological and biochemical tests.

From the new and large set of samples used in this study, the most conclusive result was a very strong confirmation of the genetic basis of gluten rheological characteristics. There was practically no exception to the relationship between the type of proteins coded by the *Gli-B1* locus and gluten viscoelasticity. When the "type γ-gliadin 45" allelic block (which also contains low-molecular-weight [LMW]-2 glutenins) was present, gluten firmness, gluten elastic recovery, and SDS-sedimentation volume were significantly much higher than when "type γ-gliadin 42" (with LMW-1 glutenins) was present. More than ever, electrophoresis can be regarded as a very powerful breeding tool for the prediction of the rheological component of durum wheat cooking quality. Future investigations in this field should concern the molecular basis of the relationship between the different proteins coded by the *Gli-B1* locus and gluten viscoelasticity, especially the functionality of the LMW-glutenins group.

On the other hand, the second main component of durum wheat cooking quality (surface texture of cooked pasta) turned out to be highly influenced by growing location and protein content but still had a significant varietal basis, as shown by the analysis of variance.

The quasi orthogonality of rheological characteristics and surface characteristics axis in PCA graphics was the first clear demonstration of the relative independence of the two main components of durum wheat cooking quality. Breeders must know that they cannot breed efficiently for good surface characteristics of cooked spaghetti through gluten rheological tests or gliadin electrophoresis. The two parameters must be estimated separately. For this reason we are currently developing a new microtest based on small-scale milling, pasta making, and pasta cooking operations in order to be able to screen a larger number of growing locations for each genotype, and to be able to specify the heritability of this technological trait and the possibility to include it in durum wheat breeding programs.

Fig. 1. Principal component analysis: distribution of 112 durum wheat samples and 9 quality tests in the first principal plan. □ = Type 42 winter wheat; ■ = type 45 winter wheat; o = type 42 spring wheat; ● = type 45 spring wheat. **YI,** Spaghetti yellow index; **BI,** spaghetti brown index; **PROT,** protein content; **T6,** minimum cooking time + 6 min; **T11,** minimum cooking time + 11 min; **CI,** microdisks cooking index; **REC,** gluten elastic recovery; **SDS,** sedimentation volume; and **FIRM,** gluten firmness.

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