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BIOCHEMICAL TESTS FOR THE EVALUATION OF WHEAT TECHNOLOGICAL QUALITY: THEIR POTENTIAL IN BREEDING PROGRAMS

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ABSTRACT

Much of the future improvement of wheat technological quality is likely to result from a better understanding of biochemical basis, especially aggregative characteristics and functionality of grain proteins. Biochemical tools such as electrophoresis of protein subunits or HPLC fractionations of native aggregates, particularly when computerized and applied to early generations, should contribute to improve the efficiency of breeding for high quality genotypes. Physico-chemical and structural meaning of correlations between protein components and quality traits should be investigated through more "dynamical" studies including physiological and agronomical aspects involving immunochemistry and molecular biology. The need of breeding new genotypes not necessarily with top grade quality but with a high stability of quality is stressed.

INTRODUCTION

Baking quality and other wheat end-product qualities are complex traits to predict in a varietal breeding program. There are three main reasons for it:

- a) The concept of quality varies between a miller, a baker or a breeder, and even between the earlier or the later stage of breeding.
- b) As far as breeding methodology is concerned, quality assessment requires to make difficult choices. For a long time, end-product uses had not been assigned a high priority in varietal breeding programs and, due to large amounts of grain required for carrying out classical quality tests, quality assessment was postponed to the latest stages, i.e. generally too late for significantly affecting the result. More recently, a number of small-scale quality prediction tests have been made available for screening early generations but, in that case, the cost may become limiting because thousands of lines are to be analysed.
- c) The fundamental basis of quality and of genetic differences in quality (when comparing one high quality cultivar and another one which is unsuitable for baking purposes) is still a difficult question to explain

in physico-chemical or molecular terms and remains a challenge for the scientists.

The alm of this paper is to illustrate how the application of modern science in developing breeding tools can contribute to significant advances in wheat breeding for quality and to comment on new research objectives for the near future.

COMPLEXITY OF QUALITY CONCEPT

Quality of wheat cannot be expressed in terms of specific characteristics (Feillet, 1980) and means different things to different people (Vetter, 1987). Its significance is different for millers, bakers, consumers, or for nutritionists or geneticists. From the viewpoint of millers, the best method to assess the quality of a wheat sample is to process the flour into an end-product: mixing test, bread-making test or cookie-making test. On the other hand, the breeders have a quite different objective and, more especially in early breeding stages, they need microtests for the prediction ot intrinsic value of genotypes, a quality potential which is likely to express itself differently according to environmental factors. In the latest stages of breeding, it can be useful to take into account the influence of environment, but, in the first stages, the major problem is to identify the component of technological quality that falls to genotype only. The tests that allow to assess an end-product quality may be totally unsuitable at this stage since their score is the result of genetic component and of growing conditions (e.g. fertilization, weather and disease) and of interaction between them. It turns out that the efficiency of breeding and the rapidlty of genetic improvement rely on the development and the use of microtests that are an approach of an ideal breeding test which could be described as follows:

- independance of the results with regard to the agronomical record of the sample (more especially to protein content) and ability to discriminate genotypes: high ratio of genotypic variance to environmental variance (Rousset et al., 1985) and high inheritance
- high correlation with the varietal ranking that would have resulted in considering mean values of baking tests performed on many samples of different origines and during several years

 rapidity, simplicity and potential for analysing large series of samples from small amounts of seed, preferably with automatized systems that allow computerized data acquisition and processing.

Several biochemical tests have a such potential. These last years, a certain number of tests based on the characteristics of grain proteins have been set up or adapted in order to approach these characteristics. For example: protein solubility in acetic acid (Orth and O'Brien, 1976), in 3M urea (Pomeranz, 1965) or in AUC (Huebner and Wall, 1976); determination of gluten viscoelasticity (Damidaux and Feillet, 1978; Autran et al., 1982); measurement of the amount of protein gel (Jeanjean and Feillet, 1978), SDS-sedimentation test (Axford et al., 1978); modified Zeleny test (Loisel, unpublished results); specific high-molecular-weight glutenin subunits composition (Payne et al., 1979;

TABLE 1: Comparison of different classical and biochemical tests of bread wheat quality assessment for their potential to discriminate genotypes (from 45 breeding lines grown in 7 locations in 1984)

Quality test	Amount of flour (g.)	Coefficient of variation	Ratio (1) $\sigma^2_{G}/\sigma^2_{L}$
Flour protein content	0.5	4.9	0.5
Baking score	1500	13.7	1.4
Zeleny test	7	14.7	3.2
Modified Zeleny test	7	5.9	6.0
W index (Alveographe)	350	18.0	13.5
Gel protein content	5	12.5	8.4
Gluten elastic recovery	10	17.0	5.2
Gluten firmness	10	20.0	10.3
Electrophoresis	0.01	-	fingerprint of the genotype

(1) Ratio of variance assignable to genotype to variance assignable to growing location

Burnouf and Bouriquet, 1980; Moonen et al., 1982; Payne et al., 1984; Branlard, 1987); distribution of gliadin or glutenin peaks in high-pressure liquid-chromatography (HPLC) (Huebner and Bietz, 1986). A comparison for their potential to discriminate genotypes in breeding programs is presented on Table 1.

WHICH BREEDING STAGES FOR APPLYING QUALITY TESTS ?

At different stages of breeding, the quality tests have not only different aims but also different constraints in terms of number of samples, amounts available, time allowed.

There is a need of quality appreciation at three different times in a breeding trial: 1) determination of the characteristics of parent genotypes, 2) prediction of quality potential by screening the lines at F3 or F4 stage, 3) assessment of quality (including the effect of environment) of advance lines at stages next before registration

- There is little difficulty to assess the quality parent genotypes. Time allowed and amount available are not limiting and heavy or time-consuming tests can be performed. Even biochemical tests as sophisticated as two-dimensional electrophoresis can be considered.
- When the aim is to assess quality on the few lines that are submitted to official registration, including an evaluation of the effect of climatic and agronomical factors, many commercial-type tests are available because these lines are stabilized (homozygous) and substantial quantities of flour are available.
- The most limiting question, therefore, arises for the screening of lines in early generations. A substantial genetic improvement depends on the possibility for the breeder to make hundreds of crosses per year and to assess the technological quality of thousands of lines. Also, these lines are heterozygous, they are available in small amounts and the results of the tests must be known in the shortest possible time, imperatively before the following sowing date.

Consequently, it would be desirable that wheat breeding takes inspiration from medical laboratories, that have managed for a long time to routinely utilize powerful, automatized and computerized blochemical tools. This is a second argument in favour of blochemical microtests, more especially as the performances of analytical methods have made and continue to make considerable progress (Table 2).

It must be concluded that, either for a better approach of the intrinsic quality of genotypes or because of methodological advantages, the blochemical tests are powerful tools that can contribute to overcome many difficulties presented by quality prediction in breeding. These different reasons urge to favourably consider blochemical methods, the interest of which can be summarized as follows:

TABLE 2: Some examples of considerable progress in methods for proteins fractionation

Electrophoresis		Chromatography	
1970	- Starch gel - 24 hours - 15 bands in a gliadin - 10 samples per day	- Classical IE or SE big columns - 1 sample run in 36-48 hours	
1980	Vertical fastpolyacrylamide gel25-30 bands in a gliadin30 samples in 3 hours	- RP-HPLC: 60 min. SE-HPLC: 30 min. - 100 % automatized and computerized	
1986	 2-dimensional systems (IEF x SDS-PAGE): 100 spots in a gliadin Automatized "Phast System 24 samples every 30 min. 	- IE-FPLC: 15 min. - 100% automatized	

- Potential of analysing minute amounts and large series of samples
- Possibilities of automatisation and of computerization of data acquisition and processing
- Continuous progress in biochemical tools
- Involvement of elementary components of quality and, therefore, of quality traits that present a more simple inheritance than the scores of end-product tests
- Also, as will be developed below, contribution to a better understanding of the physico-chemical basis of quality

WHICH BIOCHEMICAL COMPONENTS ?

Most of the blochemical components of the flour are necessary for its processing into an end-product with usual quality characteristics: starch, proteins, lipids... Only some of them, however, are likely to impart genetic differences in quality and can be used successfully in breeding programs.

For instance, starch is necessary to bread-making processes, but the absence of known genetic variability prevents any breeding program for a specific starch composition which would be favourable to baking quality. A similar situation prevails with lipids for which, in spite of recent reports about relationships between specific lipid classes and baking

quality (Zawistowska et al., 1984), inheritance is still controversial (Chung, 1985), so that it is still difficult to consider an improvement of baking quality through recommendations to breeders on specific lipids composition.

Conversely, gluten proteins (storage- and structure-type proteins) are closely associated to genetic differences in quality. They are called biochemical markers of technological quality.

Among these, there are components the amount of which is correlated to quality data but without cause and effect relationship, simply because of a genetic linkage i.e. a close proximity of genes that code for them with genes that confer quality. On the other hand, some other components are molecules or specific structures that directly impart quality because of particular functional properties (Wrigley, 1982)

MOLECULAR APPROACH TO BREADMAKING

A prerequisite of the quality improvements to come is a better understanding of the fundamental basis of quality: physico-chemical and molecular mechanisms and inheritance.

Since 10-12 years are needed to develop and release a new cultivar, breeders are bound to work on perennial objectives. They meet difficulties when technologies change too rapidly or when other segments of wheat production and usage industry appear, which is presently the case with the increasing use of industrial gluten in baking flours and with the development of new exportation markets towards countries having different quality specifications. Therefore, there is a need for identifying the elementary (physico-chemical) components that combine to give the whole expression of a given quality. For example, it can be assumed that different baking technologies (french, english, egyptian, ...) involve the same basic components but with different contributions in each. When we know these elementary components we can provide the breeders with universal guidelines for improving quality whatever the technology may be, no matter of the particular problem they are faced with.

A such approach has been quite successful in durum wheats: the resolution of cooking quality into two intermediate components (respectively gluten elastic recovery and state of surface of cooked pasta) has enabled to develop efficient breeding tests of intrinsic quality and to make progress in the understanding of physico-chemical and

molecular basis of cooking quality, which has resulted, within a few years, in a considerable genetic improvement of french durum wheat genotypes. Although the nature of the problems is different in bread wheat (hexaploidy, genetic variability, multiple end-uses, complexity of baking process), it can be assumed that, for bread wheat breeding, inspiration should be drawn from that approach which consists in resolving a complex quality problem into intermediate components, which, individually considered, are easier to deal with in terms of inheritance, physico-chemistry or functionality.

Advances based on this approach have been made by tests involving gel protein determination (Jeanjean and Feillet, 1978), measurement of gluten firmness and elastic recovery (Autran et al., 1982), protein solubility in soaps (Kobrehel and Matignon, 1980). But further investigations into basic problems are necessary.

Broadly speaking, an essential element of bread wheat quality is certainly an ability of its protein components to interact and to give insoluble aggregates when grain development proceeds and to form, upon flour and dough processing, viscoelastic complexes and continuous networks able to retain CO₂ and to raise during fermentation. The key role of gluten proteins during dough mixing - in a possible relationship to other components such as specific carbohydrates and lipids - has been recently reviewed (Miflin et al., 1983; Bushuk, 1984). Although rheological characteristics of gluten or dough do not cover all the areas relevant to achieving the whole baking quality, they certainly make up an essential basis of it, a link between all types of baking technologies so that controlling a physico-chemical trait such as the capacity of proteins to aggregate could be taken as a basic and perennial guidelines for the breeders.

Several molecular mechanisms of protein aggregation have been proposed for explaining functionality in dough: S-S bonds on which a very stable and strong gluten matrix could be based and that could determine elasticity by reacting with free SH groups; high ratios of glycine in HMW glutenin subunits that could also impart elasticity (Miflin et al., 1983); weak but numerous hydrogen and hydrophobic bonds that could determine the development of the dough structure,...*Most of these properties are likely to depend on the amount (or on the ratio) of those specific polypeptides or "subunits" that combine into long polymeric chalms (glutenin-type) that are both flexible and highly interactive. On

the other hand, the frequently observed associations between the phospholipids and the proteins in flour and the interspersion of membranes within and between aggregates (Miflin et al., 1983) seem to indicate that protein aggregation may have something to do with superstructures related to (or deriving from) membrane-type lipoproteic complexes.

. . . .

Accordingly, it can be distinguished three structural levels involved in technological quality, that gave rise to three "generations" of blochemical or physico-chemical tests:

- Individual polypeptides or protein subunits
- protein aggregates
- membrane-like superstructures

SUCCESSIVE GENERATIONS OF BIOCHEMICAL TESTS: SOME EXAMPLES

Relating individual subunits to quality data

Since all protein fractions of gluten are heterogeneous and enable to fingerprint the wheat genotypes, and since quality depends on the presence of specific polypeptide or subunit, any high-resolution technique may yield some qualitative or quantitative information related with intrinsic bread-making quality. Among bread wheats, such relationships have been reported with electrophoretic patterns of gliadins (Branlard and Rousset, 1980) and especially of HMW glutenin subunits (Payne et al., 1979; Burnouf and Bouriquet, 1980; Moonen et al., 1982; Payne et al., 1984; Payne, 1986; Branlard, 1987). To day, there is a large agreement about the relationship between the allelic variation in Glu-A1, Glu-B1 and Glu-D1 loci (Figure 1) and bread-making quality scores. We have recently found that the grading of the major HMW subunits was essentially the same than previously reported when intermediate components of quality (e.g. gluten firmness or elasticity, gel protein content) were considered (Figure 2). Although the allelic variation of LMW glutenin subunits is not entirely elucidated, it seems now confirmed that the relative importance of the different storage protein loci is: Glu-1 > Gli-1 > Gli-2 (unlike in durum wheats where the effect of Gli-B1 locus on gluten quality seems to prevail over those of Glu-1 loci).

Consequently, electrophoresis of protein components, especially HMW glutenin subunits, has a great potential to predict the intrinsic value of bread wheat genotypes. A such small-scale tool can be very efficient

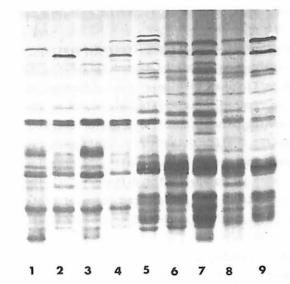


Fig. 1: SDS-PAGE patterns of total reduced proteins from different bread wheat and durum wheat cultivars evidencing the polymorphism of HMW subunits of glutenin. (1) Mondur; (2) Tomclair; (3) Agathe; (4) Durtal; (5) Avalon; (6) Castan; (7) Fidel; (8) Capitole; (9) Camp Rémy.

	Gel protein content	Gluten elastic recovery
Glu-A1		2*nul
Gl	b	a ≃ b > c
Glu-B1	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	-7-7-7-6 -8-8 -8-8 -9 - 2 - b > d
Glu-D1		$\begin{array}{c} \longrightarrow 5 \\ \longrightarrow 5 \\ \longrightarrow 0 \\$
	Quality	Quality

Fig. 2: Allelic variation in HMW glutenin subunits and its relationship to intermediate components of bread-making quality: gel protein content, and gluten elastic recovery. Mean values of 45 breeding lines grown in 7 locations in 1984. Subunit nomenclature from Payne (1986).

for screening very small samples (half-kernel) of segregants at early stages of breeding programs.

Using a small-scale totally computerized reversed-phase high-pressure liquid chromatography system (RP-HPLC), another approach of the protein composition has been developed by Huebner and Bietz (1985, 1986) either from reduced and alkylated glutenins or from gliadins. Similar relationships between specific peaks or specific regions of the graph (BQGF: "Baking Quality Gliadin Fraction") were reported and proposed for assessing the potential quality of breeding lines.

In return, when using any of the different techniques of fractionation involving a preliminary reduction of proteins into polypeptides or subunits (e.g. SDS-PAGE or RP-HPLC), it is essential to realize that:

- 1) Since gliadin or reduced glutenin electrophoretic patterns or RP-HPLC graphs are fingerprints of genotypes, the only piece of information about quality that they can predict is a potential of quality of the genotype. These techniques can neither inform about the phenotypic quality of a sample (they are unsuitable, therefore, for evaluating quality at commercial stages), nor about the quality fluctuation of the within a genotype.
- 2) The observed associations may be due to a genetic linkage between gluten protein markers with genes that impart quality. They do not demonstrate a direct contribution of the polypeptides or the subunits to quality.
- 3) Electrophoretic techniques may not be the best suitable methods for investigating the physico-chemical basis of quality because they can operate on individual molecules only. The protein aggregates (which are likely to play a key role in baking quality) have to be destroyed prior to migration and fractionation in media with an inevitable lost of the information concerning the structure, the interactive aspects and the stability of protein complexes.

Assessing agregative characteristics of proteins

Consequently, a complementary approach of the major physico-chemical basis of baking quality should involve methods that preserve as much as possible the integrity of the native protein complexes. Schematically, exploring gluten "quality" could amount to investigating the aggregation level of gluten proteins through methods that can account for structural

aspects such as specific solubility fractionations and size-exclusion chromatography or HPLC.

Solubility fractionations can be valuable tools in this way (Orth and O'Brien, 1976; Jeanjean and Feillet, 1978; Kobrehel and Matignon, 1980; Graveland et al., 1980). The conventional Osborne fractionations, however, have become manifestly unsuitable for quality studies because of the occurence of at least two different protein classes in the glutenin:

LMW- and HMW-subunits. Moreover, these two classes, that are both giving rise to large aggregates, distribute themselves partly in ethanol-soluble (in mixture with gliadins) and partly in ethanol-insoluble fractions according to their respective aggregation level. Instead of the classical glutenin/gliadin ratio, the determination of a aggregated/monomeric ratio could be recommended (Autran et al., 1987).

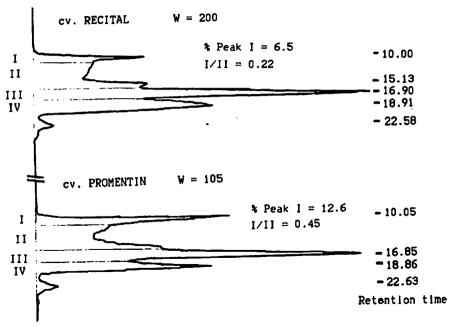


Fig. 3: SE-HPLC (TSK SW 4000 column) of unreduced proteins, extracted with 0.1 M sodium phosphate (pH 6.9), containing 2 % SDS, from the wheat cultivars Recital and Promentin. Column solvent was 0.1 M sodium phosphate (pH 6.9) containing 0.1 % SDS. Flow rate was 1.0 ml/min. Divisions of graph that indicate areas used to calculate percentages of fractions were based on elution positions of unreduced protein standards. fraction 1, MW > 750 000; fraction 2, MW 90 000-750 000; fraction 3, MW 25 000-90 000; fraction 4, MW 8 000- 25 000.

Size-exclusion chromatography makes another approach of the aggregation profile of wheat proteins. Miflin et al. (1980) reported a fractionation of gluten proteins in an AUC solvent on controlled pore glass and found a strong correlation between the amount of fraction in excluded volume and baking quality of varieties. To be used in breeding programs, a such method had to be scaled down and to be performed in a shorter time, what has been enabled by HPLC techniques. Huebner and Bietz (1985, 1986) analysed unreduced wheat proteins by SE-HPLC and showed that the percentage of the first peak was related to mixing time. However, depending if the highest molecular weight aggregates were or not largely extracted, the relationship was positive or inverse.

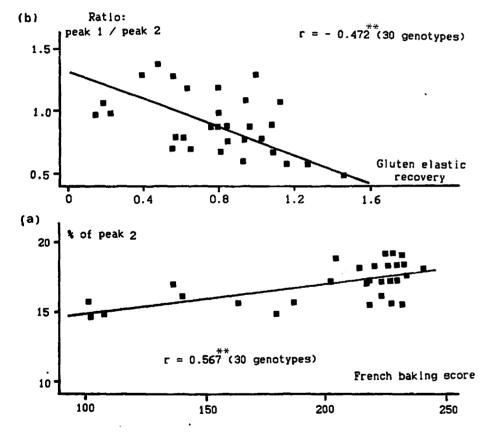


Fig. 4: Relationship between (a) french baking score and percentage of SE-HPLC peak n° 2 and (b) gluten elastic recovery (Viscoelastographe) and ratio: area of peak 1 / area of peak 2.

In recent works, we confirmed the existence of a significant genetic variability of the percentages in peaks 1 and 2 by a similar SE-HPLC fractionation and the potential of this technique for a baking quality screening in breeding programs (Figures 3 and 4).

Aggregation level of storage proteins is also likely to be influenced by growing conditions. Little is known about the changes that wheat storage polypeptides undergo after their synthesis. The stage taking place immediatly before maturity, especially the factors (climate, temperature, humidity) that affect the kinetic of grain drying and kernel hardness, could strongly influence the aggregation process and could have a great importance in determining the phenotypic quality within a given genotype (i.e. from a given pool of monomeric proteins or subunits) and the ability for a genotype to yield or not a constant quality with respects to environmental changes. Another factor influencing protein aggregation is technology. For instance, type of milling, mill stream composition of flours, hydratation of the dough,... are likely to influence the result of a test, either because they contribute to build up new associations between some protein fractions and some lipid classes, or because they give rise to different conditions of competition between gluten proteins and starch. A special attention must be paid to these factors when developing a breeding test supposed to predict the genotypic component of quality.

Exploring new superstructures

According to Marion et al. (1987), NMR spectroscopy and freeze-fracture electron microscopy studies of gluten evidenced multilamellar vesicles that are likely to come from a rearrangement of cell membranes. Whereas no specific complex between storage proteins and lipids seemed to occur during dough and gluten formation, there could be a co-extraction of these components, resulting in a micro-emulsion through vesicular structures, what would explain the effects of lipids on viscoelastic properties of gluten. On the other hand, mechanical treatments seemed to shift the vesicles towards microscopic water bags, this phenomenon being highly temperature and variety dependent. It results that physical tests based on ³¹P NMR could be worked out in order to explain how lipo-proteic structures can impart quality and (especially proton NMR) to predict gluten characteristics at the breeding stage.

Several other approaches on lipid-binding proteins in gluten, on very low molecular weight glutenin subunits, or on phospholipid-transport proteins, could be also relevant to the question of membrane-like structures in gluten.

CONCLUSIONS AND NEW PERSPECTIVES

Improvements in analytical methods and in understanding of fundamental basis of quality have contributed to identify several specific proteins affecting bread-making quality and to supply breeders in a broadening array of tools available to develop new genotypes that meet the needs of producers, millers and bakers. This strain in such a strategical domain, must not be stopped because of the intense international competition and of the rapid turnover of top-level cultivars.

In addition of all the above mentioned studies, a closer attention should be paid on the following important topics:

- To think about the best possible incorporation of the present blochemical tests into breeding programs. Many breeding tests have been set up from registrated varieties or homozygous lines. Are these tests adjusted for screening segregants at very, early generations? Does the origine (genotypic or environmental) of the quantitative variation in proteins in the breeding trial will allow to draw conclusions about intrinsic quality of genotypes?
- To be careful of the decreasing percentage of wheats and flours that swings from traditional baking to new baking technologies or to other end-products. Until now, the wheats that satisfy to traditional baking generally satisfy to those of importing countries, but it cannot be excluded that different quality requirements arise which lead the evaluation of breeding lines to be shifted to these new products. This also reinforces the idea that research scientists must further improve their understanding of physico-chemical basis of quality, must identify elementary components of wheat or flour quality in order to predict any specific end-use characteristic.
- To develop breeding programs, not only for predicting an intrinsic quality of the genotypes but for estimating the quality variation within a genotype. For many industries, it is much easier to adjust to a medium (but constant) level of quality than to cope with daily fluctuations in quality of the raw materials. So, an important

objective should be to breed those genotypes that do not belong necessarily to a top grade but that have the minimum tendancy to fluctuate with regards to environmental factors.

This requires:

- 1) To develop advance methodologies: HPLC, FPLC, 2-dimensional electrophoresis that are, as far as possible, connected with computerized data acquisition and processing systems. Considerable progress can be expected as soon as the breeder can afford to assess technological quality, by automatized and computerized screening tools, on thousands of lines per year.
- 2) To search for new components (other than proteins) or other types of structures (possibly involved in membrane-like rearrangements) that may impart genetic differences in quality. The possibility that minor differences in components such as specific lipid may influence quality in complement to proteins functionality cannot be ruled out.

 Demonstrating that compositional differences are inherent in the genotypes is however essential before providing the breeders with new biochemical tools.
- 3) To emphasize the use of more "dynamical methods", such as the study of the evolution of protein complexes during grain development, or under the influence of agronomical factors. It is essential to understand how and when such complexes are formed and which are the physiological and agronomical factors that can affect their building-up. Of special importance would be to follow the aggregation process from the synthesis step, to the migration to protein body, and then to flour, dough and gluten. The modifications that these complexes undergo during the last step of grain development might be essential for imparting the phenotypic expression of the quality in a given genotype and for explaining the particular inconstancy of quality of some genotypes. Anatomic problems related to the interaction between the protein gradient in the different grain layers and the milling technology must not be neglected.

4) To intensify collaborative studies

- between geneticists and physico-chemists in order to strengthen the conclusions that often derive from correlation studies and to demonstrate the physico-chemical basis of such correlations. High priority must be assigned to the demonstration that, among subunits previously found correlated to quality data, just occur those having

specific physico-chemical characteristics: a better ability to interact, to form more stable and/or more numerous hydrophobic interactions or disulfide bonds due to a particular location of hydrophobic domains or cysteine residues.

- between biochemists and molecular biologists (regardless of any future genetic engineering consideration) in order to clone and to sequence those particular genes that code for proteins directly involved in technological quality (Joudrier et al., 1987). This approach is certainly the most powerful for revealing new insights into the structure of gluten proteins, for giving ultimate physico-chemical explanations to their functional properties and in view to contribute to future improvements of bread wheat quality through genetics and breeding.

REFERENCES

- Autran, J.C., Houliaropoulos, E. and Laignelet, B. 1982. Viscoelastic properties of bread wheat gluten: measurements, use in breeding and biochemical basis. Cereal Foods World, 27, 8, 469, abstract n° 177.
- Autran, J.C., Abecassis, J. and Feillet, P. 1986. Statistical evaluation of different technological and biochemical tests for quality assessment in durum wheats. Cereal Chem., 63, 5, 390-394.
- Autran, J.C., Laignelet, B. and Morel, M.H. 1987. Characterization and quantification of low-molecular-weight glutenins in durum wheats. Biochimie (in press).
- Axford, D.W.E., McDermott, E.E. and Redman, D.G. 1978. Small-scale tests of breadmaking quality. Mill. Feed Fert., 161, 18.
- Branlard, G. 1987. Prediction of bread wheat quality from HMW glutenin subunits and gliadins. In: Proc. 3rd Workshop on Gluten Proteins, Budapest, 6-9 may (in press).
- Branlard, G. et Rousset, M. 1980. Les caractéristiques électrophorétiques des gliadines et la valeur en panification du blé tendre. Ann. Amélior. Plantes, 30, 2, 133-149.
- Burnouf, T. and Bouriquet, R. 1980. Glutenin subunits of genetically related hexaploid wheat cultivars: their relation to bread-making quality. Theor. Appl. Genet., 58, 107-111.
- Bushuk, W. 1984. Functionality of wheat proteins in dough. Cereal Foods World, 29, 2, 162-164.
- Chung, O.K. 1986. Lipid-protein interactions in wheat flour, dough, gluten and protein fractions. Cereal Foods World, 31, 3, 242-256
- Damidaux, R. et Feillet, P. 1978. Relation entre les propriétés viscoélastiques du gluten cuit, la teneur en protéines et la qualité culinaire des blés durs (<u>T. durum</u>). Ann. Technol. agric., 28, 4, 799-808.
- Feillet, P. 1980. Wheat proteins: Evaluation and measurements of wheat quality. In: Cereal for Foods and Beverages, Acad. Press (G. Inglett and G. Charalambous. Ed.). 183-200.

- Feillet, P. 1984. The biochemical basis of pasta cooking quality: its consequences for durum wheat breeders. Sciences des Aliments, 4, 551-566.
- Graveland, A., Bosveld, P. and Lichtendonk, W.J. 1980. The functionality of wheat proteins. In: Cereal for Foods and Beverages, Acad. Press (G. Inglett and G. Charalambous, Ed.), 171-181.
- Huebner, F.R. and Wall, J.S. 1976. Fractionation and quantitative differences of glutenin from wheat varieties varying in baking quality. Cereal Chem., 53, 258-269.
- Huebner, F.R. and Bletz, J.A. 1985. Detection of quality differences among wheats by high-performace liquid chromatography. Journal of Chromatography. 327. 333-342.
- Huebner, F.R. and Bietz, J.A. 1986. Assessment of potential breadmaking quality of hard wheats by reversed-phase high-performance liquid chromatography of gliadins. Journal of Cereal Science, 4, 379-388.
- Jeanjean, M.F. and Feillet, P. 1978. Nouveau test biochimique d'appréciation de la qualité boulangère des blés tendres. 6° Congrès International des Céréales et du Pain, Winnipeg, septembre.
- Joudrier, P., Autran, J.C. et Gautier, M.F. 1987. Pourquoi cloner les gènes des protéines de réserve du blé ? Biofutur (in press).
- Kobrehel, K. and Matignon, B. 1980. Solubilization of proteins with soaps in relation to the breadmaking properties of wheat flours. Gereal Chem., 55, 1060-1064.
- Marion, D., Le Roux, C., Akoka, S., Tellier, C. and Galland, D. 1987 Lipid-protein interactions in wheat gluten: a phosphorus nuclear magnetic resonance spectroscopy and freeze-fracture electron microscopy study. Journal of Cereal Science, 5, 2, 101-115.
- Miflin, B.J., Fleld, J.M. and Shewry, P.R. 1983. Cereal storage proteins and their effect on technological properties. In: Seed Proteins, Acad. Press (J. Daussant, J. Mossé and J. Vaughan, Ed.), pp. 255-319.
- Moonen, J.H.E., Scheepstra, A. and Graveland, A. 1982. Use of the SDS-sedimentation test and SDS-PAGE for screening breeder's samples of wheat for bread-making quality. Euphytica, 31, 677-690.
- Orth, R.A. and O'Brien, L. 1976. A new biochemical test of dough strength of wheat flour. The Journal of the Australian Institute of Agricultural Science, june, 122-124.
- Payne, P.I. 1986. Varietal improvement in the bread-making quality of wheat: Contributions from blochemistry and genetics, and future prospects from molecular biology. In: Biotechnology and Crop Improvement and Protection, N° 34, pp. 69-81.
- Payne, P.I., Holt, L.M., Jackson, E.A. and Law C.N. 1984. Wheat storage proteins: their genetics and their potential for manipulation by plant breeding. Phil. Trans. R. Soc. Lond., B 304, 359-371.
- Payne, P.I., Corfield, K.G. and Blackman, J.A. 1979. Identification of a high-molecular-weight subunit of glutenin whose presence correlates with bread-making quality in wheats of related pedigree. Theor. Appl. Genet., 55, 153-159.
- Pogna, N. e Mellini, F. 1986. Alla ricerca delle basi biochimiche e genetiche della qualita del glutine. L'Informatore Agraria, Verona XLII. 35, 65-66.
- Pomeranz, Y. 1965. Dispersibility of wheat proteins in aqueous urea solutions a new parameter to evaluate breadmaking potentialities of wheat flours. J. Sci. Food Agric., 16, 586-593.
- Rousset, M., Tribol, E., Branlard, G. and Godon, B. 1985. Influence du génotype et du milleu sur les tests d'appréciation de la valeur

d'utilisation du blé tendre (<u>Triticum aestivum</u> em. Thell.) dans les industries de cuisson. Agronomie, 5, 7, 653-663.

Vetter J.I. 1987. Wheat quality: can everyone be satisfied? Cereal Foods World, 32, 4, 305.

Wrigley, C.W. 1982. The use of genetics in understanding protein composition and grain quality in wheat. Qual. Plant Foods Hum. Nutr., 31, 205-227.

Zawistowska, U., Bekes, F. and Bushuk, W. 1984. Intercultivar variations in lipid content, composition and distribution and their relation to baking quality. Cereal Chem., 61, 6, 527-531.



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Perenzin M., Corbellini M. and B. Borghi. 1987. Growth analysis of ten bread wheat hybrids (T. aestivum) produced with a chemical hybridizing agent. Genetica Agraria 41, 163-172.

Shebeski L.H. 1966. Quality and yield studies in hybrid wheat (Triticum aestivum L.). Can. J. Genet. Cytol. 8, 375-386.
Wilson J.A. and E. Villegas. 1966. Genetic interactions for the hybridization of wheat and their effect upon quality. Cereal Sci. Today. 11, 306-309.



Dr Autran in discussion with Dr Edwards (Pioneer, UK)

Visit to the S.Angelo Lodigiano's Castle



Meeting participants in front of the S.Angelo Lodigiano's Castle