

## Trends in Research on the Improvement of Quality of European Wheats

### Introduction

Despite the fact that wheat is an essential crop for European agriculture and for the wheat-processing industry (milling, bread-making, biscuit-making and gluten/starch industries), **European wheats are not really adapted to this wide range of applications, especially to their future developments, because the various processes have not been clearly explained in terms of process requirements and wheat quality requirements.**

Whereas Europe is deficient in good quality strong wheat, the milling and baking industries require higher and higher quality wheat because of modern developments in technology. In particular, the use of 'cold' methods in baking (refrigeration and deep-freezing of dough) makes it necessary to have available flours of higher protein content and greater and greater strength.

On the other hand, the fact that **current methods of breeding are predominantly focused on white bread-making and pasta production stands more and more in contrast to the current applications of wheat in wholemeals, biscuit manufacture, wheat/starch production, sweet leavened products and fermented products, and considering that quality is also related to flour extraction rate (the amount of white flour extractable from wheat), performance in flour blends and degree of sprout damage.**

Also, in contrast to USA or Canada, **the consistency of the quality of the greater part of existing wheat is insufficient because of too great a sensitivity to agronomic and climatic factors.** In Southern Europe, the climate is often the factor limiting both yield and quality; in the coastal regions of Northern Europe, where the crop can be cultivated intensively (with average yields of 90 bu/acre and of 150 bu/acre in many places), sprouting puts a severe strain on both yield and quality.

Based on these observations, there was a need to intensify research work aimed at exploring new outlets and developing new applications for European wheats and at improving their quality.

Therefore, the following specific objectives have been formulated :

- To stimulate breeding and development of novel wheat varieties that combine good agronomic character and excellent technological qualities which would satisfy simultaneously the farming and manufacturing industries and export markets;
- To maximize EC grown wheat quality by providing tools to minimize sprout damage and maximize milling quality;
- To further improve the economy of EC wheats by relating current processing requirements to wheat characteristics, thereby enabling traders, millers and breeders to select on these characters;
- To open new outlets for wheat by investigating and developing new applications of wheat and wheat products (flour, starch, gluten).

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Consequently, a programme aimed at **Exploring and Improving the Industrial Use of EC Wheats (*T. aestivum*)** involving 23 laboratories from 7 countries has been set up in the framework of the ECLAIR programme of the Commission of the European Communities. (ECLAIR - European Collaborative Linkage of Agriculture and Industry through Research - is one of the main precompetitive research programmes supported by the EEC, for a total contribution of 100 million ECU (120 million \$). The total cost of this wheat programme is 6.5 million ECU (7.8 million \$), including a 3.6 million ECU (4.3 million \$) support by the EEC.

Again, the major objective of this programme is to fill the growing gap between process development and its understanding in terms of processing requirements and thus wheat quality requirements. A further objective is the stimulation of breeding and development of wheats capable of satisfying the present and future demands of European industry and the export market.

Improved use will result from better knowledge of the various applications of wheat (milling, white and wholemeal bread-making, gluten/starch industry, flour blends, fermented products and biscuit manufacture). Each main parameter of processing and its effect will be expressed in terms of functional properties of the wheat and related to specific wheat protein constituents and their interactions.

Combined functional/physico-chemical and biological advanced methodologies will be applied to quality determinants, which will result in a better understanding of their variability of composition, structure, and of their mechanism of action in the various industrial processes. As a consequence of the availability of genetic stocks and wheat samples produced in highly controlled environments of the various EC countries, the identification of improved breeding criteria (for sprouting resistance, milling quality, bread-making or biscuit-making quality, adaptation to gluten/starch separation) and the development of rapid tests for use in breeding programmes and trade will be obtained.

As stated before, this represents a completely new strategy not only innovative in this respect, but also in the advanced methodologies used to tackle the often complex problems. The general economic benefit from this is evident. Of the about 75 million tons of wheat grown annually in the EC, 15-16 million tons is surplus. Decreasing this surplus with minimizing the need to apply costly intervention regulations will save the EC millions ECU. In the following the objectives of the programme will be explained in more detail, using different research topics of the project.

#### Processing requirements and their application to wheat selection and quality definition

As stated earlier most of today's research is focused on relations between protein content and composition and white bread-making quality. Modern wheat breeding for example exploits the relation between certain HMW glutenin subunits and bread-making quality. Recent data both from applied studies as from fundamental studies have indicated that knowledge of the interaction between flour components is lacking at this moment. The proposed project will focus on this approach using advanced biochemical and physico-chemical methodology (combination between subprogrammes A and B). Furthermore, the proposed project will try to fill the gap between wheat quality requirements and present wheat applications ('cold' methods in baking, wholemeal bread-making, starch/gluten separation, biscuit-making, sweet leavened products, and fermented products) by studying the suitability of wheats for these applications on an applied level. This will lead to a better understanding of different quality characters required, to rapid selection tools for use in breeding and trade and thereby to a better exploitation of EC grown wheats. Furthermore these studies will enable increased quality assurance, improved products and the development of new products and/or new processes. The economic benefits from this (eliminating the need for wheat imports, better use of EC wheats, improved quality through selection) are evident but not easy to quantify.



### Milling quality

Milling quality is an aspect of wheat which has been necessarily left out of selection programmes until the last stages. Nevertheless, taking the amount of wheat produced annually in the EC, one percent increase in milling yield represents an advantage of 40 million ECU per year. The approach followed to tackle this problem is innovative through the use of image analysis techniques in combination with sensitive chemical assays. Strong cost reduction of image analysis equipment now enable the development of rapid test based on this equipment offering both a technical advantage as a economical advantage (decrease in labour costs, ability to select wheats on milling quality on intake or in early stages of breeding programmes).

### Starch/gluten separation

The application of wheat as a raw material for starch/gluten separation is relatively new. Using pilot scale equipment recently developed at the participating laboratories it is now possible to improve process economy by using enzyme methodology and by enabling the use of wholemeal flours in the new separation processes. Enzymes can be used to tackle the problem of variation in processing properties, allowing economical benefits in using locally grown cheaper wheats. The use of wholemeal enables a higher yield of starch/ton of wheat. The new processing enables reduced losses in terms of wastage and costs of waste water treatment.

### Sprout damage

Prevention of sprout damage is an objective long yearned for in the EC. The average costs of sprout damage once in every five years (leading to 10 % loss in yield and reduction of the amount of bread-making quality by 50 %) is 50-60 million ECU per year. The approach envisaged in this project is entirely new in both concept as methodology. Instead of detecting levels of amylase work will focus on developing for example immunoassay-based fluorescence tests for factors related to dormancy. This will enable rapid detection at an early stage (technical advantage), prevention (economic advantage) and selection of sprouting resistance in breeding programmes.

*Several recent advances provide the potential to make a significant step forward in both more effective utilisation and in the development of better European wheat varieties for the future.*

1. The availability of isogenic, aneuploid and translocation stocks which enable to pinpoint the gene products that are important in functional performance.
2. The introduction of original approaches based on new concepts (e.g. intrinsic quality of wheat genotypes), or new protein fractions (e.g. friabilin, HMW-albumin, S-protein,...), that stand out clearly against the old classical Osborne's scheme.
3. The acknowledgement that quality is not determined (and cannot be predicted) solely by protein composition, but also by interaction of the proteins with various flour components: starch, pentosans, lipids.
4. The development of modern physical and spectroscopic methods that can observe the behaviour of individual components (e.g. proteins, lipids) in a complex mixture (*in situ* NMR spectroscopy, rheological measurements).
5. The demonstration of the potential of monoclonal antibodies to quantify specific components in a mixture and to probe their dynamics and distribution within various systems (dough development, seed dormancy).



6. The development of a range of physico-chemical techniques that determine interfacial and aggregation behaviour.

### Organisation of the Project

The organisation of this programme as *three interdependent subprogrammes*, each one of which will benefit from the results of the other two, will greatly facilitate the scientific direction, and thus the chances of success, of the programme as a whole. All subprogrammes are to be spearheaded by combining the expertise of the laboratories that have taken a major part in the developments cited above, with the experience of industrial laboratories in controlling dough development and of private breeders. As a matter of fact, twenty participants from seven countries are involved in this four-year programme (1991-1994). It is also clear that the role of coordinator is essential for making sure of good coordination between the participants

Each subprogramme is under the direction of a scientific programme manager :

A. Industrial Processes : (Dr. R.J. Hamer, TNO-CIVO Institutes, Zeist, The Netherlands).

B. Functional Components and their Interactions : (Dr. J.J. Plijter, Gist Brocades, Delft, The Netherlands).

C. Biochemical-Genetics and Physiology : (Dr. N.E. Pogna, Istituto Sperimentale per la Cerealicoltura, Milano, Italy)

Dr. J.C. Autran (INRA-IRTAC, Montpellier, France) will be the scientific coordinator. Ms M. Richard (I.R.T.A.C., Paris, France) will be in charge of the administrative aspects.

### Subprogramme A - Industrial Processes.

A - Subprogramme A is aimed at *improving the industrial use of EC wheats*. This aim is approached along two broad lines of research.

1. Tools are developed in order to *maximize EC grown wheat milling quality*. Using image analysis and sensitive biochemical assays tests will be developed to predict milling quality.

2. A concerted effort will be made by laboratories from six EC member countries to *fill the gap between current wheat selection in breeding programmes and trade on one hand and current applications of wheat on the other*. Applications of wheat in the wheat starch industry, in wholemeal bread-making, flour blends, fermented products (sour doughs) and biscuit manufacture will be studied on an applied level (in connection with subprogramme B which studies processes on a fundamental level). This includes both the use of advanced biochemical and physico-chemical methodology as well as recently developed process technology. Studies are aimed at understanding processing requirements and their underlying physico-chemical/biochemical causes. This will lead to the identification of process customized *selection criteria*. This in turn will enable an improved use of EC wheats, improved guidelines and criteria for breeding and improved products and processing of wheat.



## **Subprogramme B - Functional Components and their Interactions.**

The study of the *interactions and the development of dough* forms the objective of the Subprogramme B, which has the following two main themes :

1. Component interactions: Proteins from glutenin and gliadin fractions which are linked to performance attributes will be prepared in sufficient quantities to study their water-binding by NMR), their aggregation with each other or with other components by NMR, by equilibrium sedimentation, ultracentrifugation, turbidimetry, SE-HPLC, etc.) and their hydrophobicity by RP-HPLC and TNS binding). These properties will be linked to performance tests in dough development and to associated indices of rheology. Study in lipids focus on the polar and protein-binding fractions using phosphorus NMR and fluorescence spectroscopy). The role of protein and lipid fractions in stabilizing the dough-gas bubble) interface will be determined by static and dynamic interfacial techniques. *The minor protein components associated with starch granules* will be also investigated to establish their role(s) in relation to functional properties of wheat, flour and isolated starch, to extend research on the role of starch granule protein Friabilin in controlling endosperm texture in wheat and to devise a predictive test of endosperm texture for use in plant breeding as a selection tool with single seeds (in connection with subprogramme C) and as a quality test at flour mill intake (subprogramme A).

2. Dynamics of dough development: The effect of heat and mechanical treatment on the distribution and mobility of protein components will be studied by NMR spectroscopy and linked with changes in dough rheology. Monoclonal antibodies will be used to label specific proteins and hemicelluloses to determine the dynamics of their distribution by immuno-gold labelling) within the developing dough particularly in relation to swelling and the formation of the biopolymer-gas interface. New oscillatory measurement techniques will be used to distinguish between two fundamental liquid and elastic contributions to the overall viscoelastic response.

## **C - Biochemical-Genetics and Physiology.**

The objectives of Subprogramme C *are to analyse the biochemical, genetic and physiological bases of technological quality.*

1. Production of wheat samples in controlled conditions that are necessary to carry out studies of subprogrammes A and B. Evaluation of these wheats in various environments for yield potential and quality attributes. Southern network (France, Italy, Spain, Portugal: 25 cultivars x 2-3 locations per country). North-Western network (France, North-Italy: 15 cultivars x 2-3 locations per country)

2. Determination of the agronomical, physiological, genetic and biochemical factors affecting the technological quality and its stability of expression. They will include predictive values of biochemical tests.

3. Allelic composition, chromosomal location and genetic links of genes coding for the storage proteins subunits of HMW and LMW glutenins and gliadins, for certain albumins and S-proteins by analysing the lineage and chromosomal substitution lines between varieties. This study will be both qualitative (presence or absence of constituents) and quantitative contribution of LMW and HMW glutenin subunits to the total pool of wheat proteins).

4. Statistical analysis of a large collection of wheat cultivars in view to determine the relationships between allelic composition and baking quality. The protein fractions which appear to be correlated with qualitative characteristics (notably in view of trials



carried out in industrial laboratories) will subsequently be purified so that their physico-chemical character can be determined within the framework of subprogramme B.

5. Development of rapid tests for dormancy and for initial stages of sprouting related to kernel constituents which will be used to produce wheat with a higher degree of sprouting resistance and early detection of sprouting damage in the field.

#### Some examples of tasks (SLIDES)

Because it is not possible to give a full description of the various tasks, only a few significant examples of the various approaches that are going to be followed will be given now.

#### IMPROVING MILLING QUALITY

- Establish the factors of milling in terms of bran friability and other milling factors. Potential markers are phytic acid, ferulic acid and lignin, to be determined with HPLC or colorimetric methods.
- Estimation of the contribution of kernel features to milling quality by means of image analysis (e.g. from 300 grains).
- Objective: development of a predictive test for milling quality

#### IMPROVING STARCH/GLUTEN SEPARATION THROUGH THE USE OF ENZYMES

- Development of a small-scale apparatus to investigate all process parameters and test the effect of purified protein fractions.
- Use of hemicellulases as processing aids.
- Application to the separation of gluten from wholemeal.

#### PREDICTION OF PROPERTIES OF FLOUR BLENDS

- Fractionations, purifications, reconstitutions.
- To identify which biochemical features (storage proteins, polysaccharides, enzymes, etc.) control processing properties
- Objective: to design a predictive model in view to compensate the year-to-year variations in flour quality.

#### PHYSICOCHEMISTRY AND FUNCTIONALITY OF GLUTEN SUBFRACTIONS

- Purification and characterization of gluten subfractions and protein subunits from different genotypes
- Study of the conformational and functional properties of the individual components and the interaction of the different proteins and other wheat components.



- Special consideration of LMW subunits of glutenin that are likely to impart dough extensibility, a critical parameter in French and South-Western European baking formulas.

### LIPID INTERACTIONS

- Study on the mechanisms which play a role at the interaction processes between lipids and other components.
- Study of the interfacial behaviour of various wheat flour components (gluten, lipid, hemicellulose fractions) at the dough-air interface in the gas bubble present in the dough.
- Purification of lipid-binding proteins, especially phospholipid transfer protein: homologies with other low molecular weight cystein rich proteins.

### MINOR PROTEIN COMPONENTS ASSOCIATED WITH STARCH GRANULES

- One of the surface proteins (Friabilin) appeared to be responsible for differences in endosperm texture (hardness or softness) among wheat varieties.
- It is attempted to establish the role(s) of starch granule protein in relation to functional properties of wheat, flour and isolated starch: development of monoclonal antibodies against Friabilin, physical studies (DSC, Infra-red reflectance, circular dichroism).

### DYNAMICS OF DOUGH DEVELOPMENT

- Development of microscopic techniques and anti-gliadin monoclonal antibodies for examining bread doughs.
- Characterization of polyclonal and monoclonal antibodies to wheat pentosans. and study of the role of pentosans in the structure of dough and baked products.
- Effect of heat and mechanical work (NMR spectroscopy).

### GENETIC AND TECHNOLOGICAL ASPECTS OF HMW and LMW SUBUNITS OF GLUTENIN, HMW-ALBUMINS AND S-PROTEINS

- Screening of lines and populations of *Triticum* species for novel subunits.
- Development of near-isogenic lines with contrasting HMW-glutenin subunits and gliadins, and of null forms lacking *Gli-A1*-, *Gli-B1*- and *Gli-D1*- encoded gliadins and LMW-glutenin subunits.
- Transfer of good quality LMW-2 subunits from type-45 wheat cultivars into the genome of common wheats: effects on bread-making quality.
- Development of biochemical markers for screening early generations for higher values of bread-making parameters (quality indexes based on storage protein composition: HMW, LMW, gliadins).



## CHROMOSOME INTERACTION ON PROTEIN SYNTHESIS

- Development of 36 intervarietal chromosome substitution lines in which individual chromosome pairs of the homeologous groups 1 and 6 of cv. Courtot have been replaced by their homologues of 6 donor cvs, selected on the basis on their protein subunit composition.
- Study of the genetics of endosperm proteins and of the regulation of genes expression with chromosome interactions.

## SPROUTING RESISTANCE

- Development a new methodology based on factors related to dormancy (e.g. monoclonal antibodies against abscissic acid and wheat germ agglutinin).
- Identification of biochemical markers for dormancy in view to design (i) a reliable test (e.g. fast immunochemical procedure) for screening breeding stocks with sprouting resistance and (ii) a rapid test (e.g. immunofluorescent detection on single seeds plus fast detection by image analysis) to monitor the early changes of sprouting in the field or to ascertain the extent of sprouting damage of wheat.

### **Conclusion**

Apart from these purely scientific and technical aspects, *a particularly innovative element of this project is therefore the establishment of a multidisciplinary programme (bringing together physical chemists, biochemists, immunochemists, rheologists and geneticists) and involving different industries (millers, bakers, biscuit manufacturers, gluten/starch manufacturers and breeders).*

The large number of participants of this programme is without doubt the price one must pay in order to make progress on such a complex problem as satisfying, year after year, the industrial need for quality in wheat.



## LIST OF PARTICIPANTS

CODE	PARTICIPANTS	COUNTRY	ROLE
01	I.R.T.A.C., Paris	F	Coord
02	Produttori Sementi, Bologna	I	P
07	I.N.R.A. (Clermont-Ferrand + Montpellier + Nantes)	F	P
15	Gist Brocades, Delft	NL	P
17	TNO, Wageningen/Zeist	NL	P
03	I.S.C., S. Angelo Lodigiano	I	A
04	Nuovo Crai, Caserta	I	A
05	Champagne Céréales, Reims	F	A
06	Roquette Frères, Lestrem	F	A
08	B.S.N., Biscuit, Athis Mons	F	A
09	I.T.C.F., Paris	F	A
12	I.A.T.A., Valencia	ES	A
13	T.U. Berlin	DE	A
14	F.M.B.R.A., Chorleywood	UK	A
16	A.F.R.C. - I.F.R., Norwich	UK	A
18	Club des 5, Paris	F	A
19	A.F.R.C. - I.A.C.R., Bristol	UK	A
22	Università di Padova	I	SC
23	Università di Viterbo	I	SC
24	E.E.R.M., Cadiz	ES	SC
25	E.M.P., Elvas	PO	SC