

# The Industrial Use of EU Wheats

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A four-year coordinated wheat research programme was recently conducted with the aim of advancing understanding of wheat processing and quality under the specific conditions of the European Union. The main areas examined included milling quality, starch/gluten separation, the basis of breadmaking quality, the basis of biscuit quality, flour composition, dough development, the genetics of wheat storage proteins and sprouting resistance. The programme produced a range of results which will contribute to developments in the processing industry, wheat breeding and trade.

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Exploring and improving the industrial use of wheat produced in the European Union is of key importance. Recent research within the framework of the European ECLAIR programme, which exists to further European cooperation in research, was aimed at filling the growing gap between the industrial processes that are being developed for wheat products and the understanding of their implications for wheat quality requirements. A further objective was to stimulate the breeding of new types of wheats capable of satisfying the future demands of European industries and the export market.

The rationale behind the objectives was manifold:

1. Despite the fact that wheat is an essential crop for European agriculture and for a large processing industry (milling, bread-making, starch processing), one of the main problems limiting

the use of EU wheats is the lack of knowledge of processing requirements for specific uses, especially recent developments in gluten and starch separation, wholemeal breadmaking, biscuit manufacture, flour blends, sour doughs and sweet bakery products.

2. The milling and baking industries require wheat of a higher quality than before because of modern developments in technology, such as frozen dough, and greater understanding of these requirements will help breeders to meet them.

3. Current methods of breeding are predominantly focused on white bread-making, and its scope needs to be widened.

4. Finally, the consistency of the quality of much European wheat is poor because of excessive sensitivity to agronomic and climatic factors. In southern Europe the climate limits

successful wheat cultivation, whereas in the coastal regions of northern Europe, where the crop can be cultivated intensively, premature sprouting of the grain puts a severe strain on both yield and quality.

Improvement in industrial use was expected to result if each main processing parameter and its effects could be expressed in terms of the functional properties of the wheat and specific wheat protein constituents and their interactions. Meanwhile, combined functional, physicochemical and biological methodologies would be applied to evaluate quality determinants, thus obtaining a better understanding of variability in composition, structure and mechanism of action in the various industrial processes. Finally, as a consequence of the availability of genetic stocks and wheat samples produced in highly controlled environments in the

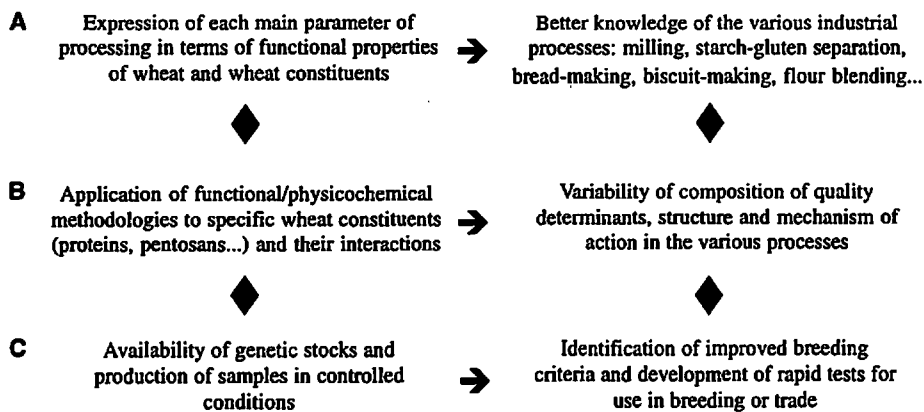


Figure 1 How to explore and improve the industrial use of EU wheats?

various EU countries, the identification of improved breeding criteria (for milling quality, bread-making or biscuit-making quality, adaptation to starch and gluten separation, sprouting resistance and so on) and the development of rapid tests for use in breeding programmes and trade could be obtained (Figure 1).

Apart from purely scientific and technical aspects, a particularly innovative element was the establishment of a multidisciplinary programme, bringing together physical chemists, biochemists, immunochemists, rheologists and geneticists and involving different industries: millers, bakers, biscuit manufacturers, starch/gluten manufacturers and breeders. The large number of participants of this programme was perhaps the price that had to be paid in order to make progress on such a complex problem as satisfying, year after year, the industrial need for quality in wheat.

The main approaches were based on several recent advances in wheat studies:

- the availability of isogenic, aneuploid and translocation stocks which enable the gene products that are important in functional performance to be pinpointed;
- the introduction of original approaches based on new concepts (e.g. intrinsic quality of wheat genotypes), or new protein fractions (e.g. friabilin, low molecular weight (LMW) subunits of glutenin, HMW-albumin);
- 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, such as starch, pentosans and lipids;

- the development of modern physical and spectroscopic methods that can be used to observe the behaviour of individual components in a complex mixture, including *in situ* NMR spectroscopy, electron spin resonance, electro spray mass spectrometry, X-ray scattering and scanning tunnelling microscopy;
- 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, for instance during dough development or seed dormancy;
- the development of a range of physicochemical techniques that determine interfacial and aggregation behaviour.

The main achievements of the programme are summarized in this paper.

#### Milling quality

In contrast with the considerable effort that has been devoted to the improvement of wheats in terms of bread-making quality, milling quality has only had minor attention. It has necessarily been left out of breeding programmes until the last stages, and its physicochemical basis is still poorly understood. Nevertheless, milling quality is of great economic importance: a 1% increase in yield of milling quality wheat represents an advantage of 40 million ECU per year for the EU millers.

Accordingly, the milling quality project was aimed at developing new

ways of understanding and predicting milling yield. In particular, it concentrated on identifying the nature and relative importance of factors determining milling quality, such as endosperm hardness, bran friability and endosperm ash content; it investigated the morphological and chemical bases of milling quality; and it worked on producing a predictive test of milling quality for breeders to use.

The first investigations on European sample sets concentrated on information gained by image analysis, which was used to examine morphometric parameters of the kernel. However, image analysis did not show good predictive value except for samples containing seriously shrivelled grains. Thus, in general, endosperm content is not a factor which limits flour yield and there is no positive correlation between grain size and endosperm content (Evers, 1992). Much better correlations were detected when milling quality was described in terms of milling factors such as bran friability, endosperm content and pericarp/endosperm separability, indicating that it was possible to develop a comprehensive model describing the relative influence of both chemical and morphometric parameters on milling quality.

One discovery was that ferulic acid measurements and image analysis measurements of bran specks were more sensitive predictors of milling quality than conventional methods. For instance, ferulic acid appeared a far better marker for bran friability than ash, so that bran friability could be calculated from the difference in ferulic acid content of the pure endosperm and flour fractions (see Figure 2).

Another important finding which has drawn considerable interest from millers and milling scientists is the possibility of explaining 70–80% of the variation in milling quality in terms of the potassium content of the kernel, which allows very good prediction of ash content of the flour (and hence flour yield), bran friability and kernel width. Meanwhile, an important breakthrough was made in the understanding of the physicochemical bases of endosperm texture (hardness) after recent investigations of the proteins associated with starch granules (friabilin, puroindoline) and their status as lipid-binding proteins (see below).

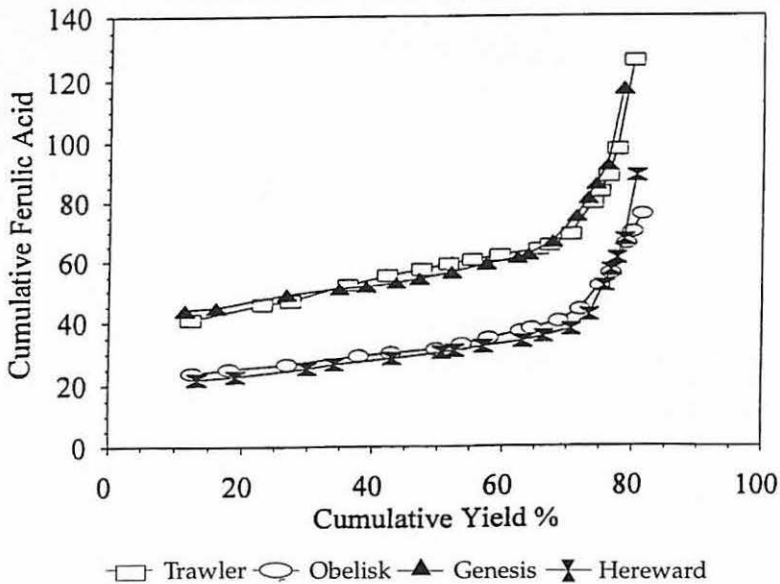
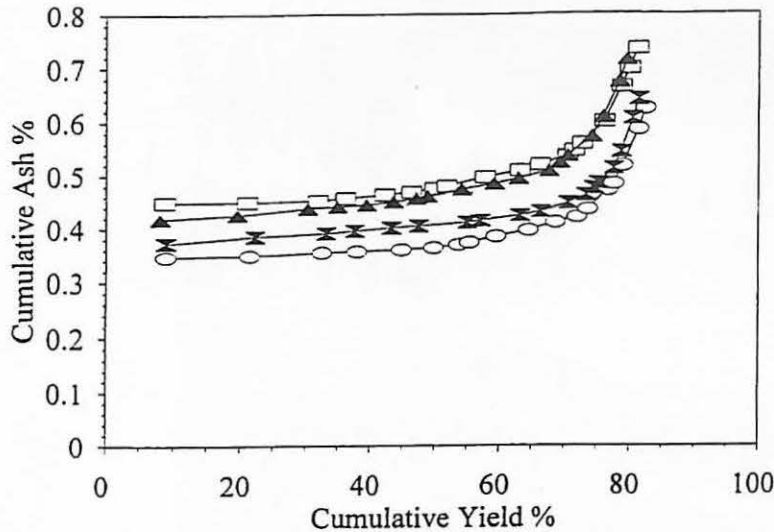


Figure 2 Ferulic acid appears to be a far better marker for bran friability than ash (from Hamer and Kelfkens, TNO Nutrition and Food Research Institute, the Netherlands).

### Starch/gluten separation

To better control the process of starch/gluten separation, to investigate the causes for differences in quality of gluten extracted from wholemeal flour compared to gluten prepared from white flour, and to study the effect of processing aids such as hemicellulases, a unique miniaturized decanter centrifuge (Figure 3) was constructed (Meuser, 1993).

The integration of this decanter into the lab scale separation system allowed

considerable reduction of the residence time of gluten in the system, which was shown to have an important effect on gluten properties and allowed gluten and starch to be obtained from all the raw materials including wholemeal flours. Glutens from wholemeal contain more LMW and fewer HMW subunits of glutenin than glutens from white flours. On the other hand, it was clearly demonstrated that hemicellulose in flours has a strong effect on gluten yield and that flour processing properties are

strongly determined by the way flour milling fractions are blended, information of great practical value for millers producing flour for the starch industry.

Differences in elastic behaviour cannot be attributed to proteolytic activities, but a strong effect of the process water (exposure to acid concentrations that are produced micro-biologically upon continuous running of the system) was observed. Furthermore, addition of 2% hemicellulose to flour decreased the gluten yield by 20%, which could be corrected for by the addition of the enzyme hemicellulase. Also, hemicellulase addition to white flour increased gluten yield.

### The basis of bread-making quality

This study was aimed at determining the underlying physicochemical reasons for differences in gluten strength and bread-making quality, thus providing feedback to plant breeding programmes and grain trading.

Much of the effort was directed at determining and understanding the mixing requirements of wheat varieties. Based on the Chorleywood Bread Process (CBP), it was shown that the work-input requirement ranged from 18 to 73 kJ/kg. Samples with work-input requirements greater than 40 kJ/kg may not achieve their full potential in a breadmaking process based upon a fixed energy input during mixing. These high work-input varieties were shown to be suitable for blending with weaker varieties, for example a 50 : 50 blend of Fresco (extra-strong) with Riband (weak) resulted in a quality similar to that of Avalon, a popular breadmaking variety during the 1980s.

One development was the definition of the concept of 'glutenin macropolymer' (GMP), which is a basis for assessing baking quality in the UK CBP test or the German Rapid Mix Test (RMT). The glutenin macropolymer, whose breakdown rate during mixing determines the baking strength, plays a key role in the process, changing from a linear polymer in flour to a three-dimensional structure in dough, and was shown to be related to the composition and incorporation rate of HMW glutenin subunits (Weegels *et al.*, 1996).

Meanwhile, a new impetus has been given to the 'gel protein' fraction as a

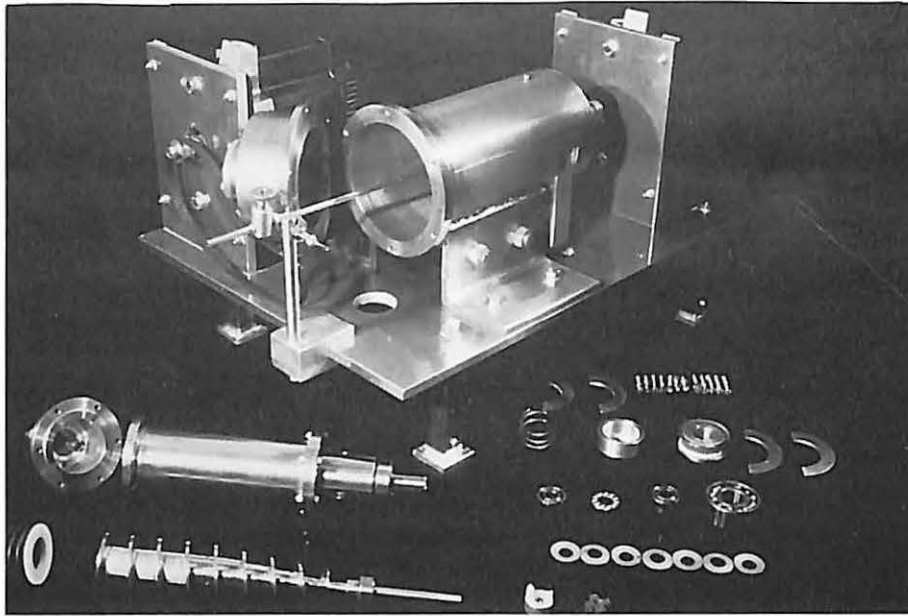


Figure 3 Miniaturized bowl and screw of the decanter centrifuge (from Meuser and Pahne, Technical University of Berlin, Germany).

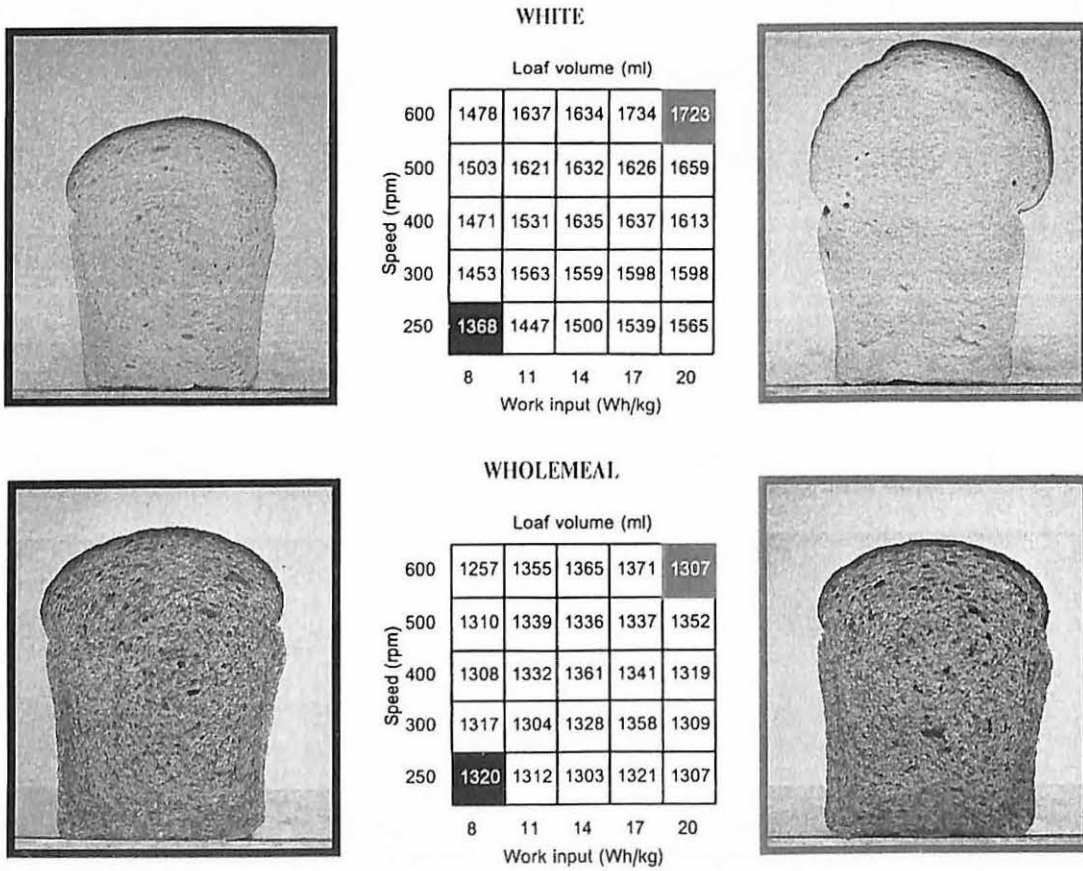


Figure 4 Test bake matrix: work input/mixing speed/loaf volume for the extra strong variety Fresco (from Pritchard, Camden and Chorleywood Food Research Association, UK).

tool in the prediction of baking quality. In fact, it was not especially the amount of gel but rather the elastic modulus or the breakdown rate of gel protein during mixing that proved to be useful for testing baking quality.

In contrast, it was not possible to predict wholemeal loaf volumes from those of white without making allowance for quality attributes such as hard or soft milling or extra-strong character. In general, protein content was more important than gluten strength for wholemeal bread performance. It was however possible to predict the wholemeal loaf volume of a test sample from measurements of the baking performance of the endosperm and bran or offal components relative to those of a control sample. This study suggested that both the endosperm and the bran or offal control the baking performance of wholemeal flours.

On the other hand, in French or southern European baking procedures, dough extensibility was often found to be a more important and critical parameter. Dough extensibility was more strongly associated with allelic variation of LMW subunits of glutenin and perhaps of gliadins than with HMW subunits (see below).

#### The basis of biscuit quality

The aims of this task were to define an optimal sweet-biscuit flour, to improve knowledge of the process, to improve productivity and quality and to create new products. The main result was a better understanding of the rheological behaviour of biscuit dough, which was found to be viscoelastic at low strains, but similar to a gel at higher strains (Contamine *et al.*, 1995).

An essential quality factor in biscuit-making is the stability of biscuit size. The weight, thickness and density of biscuits are related to the presence of constituents absorbing water, such as proteins, but also damaged starch and pentosans. For instance, insoluble pentosans limit the hydration of other constituents (which can be assessed through determination of the free water content in the dough) and have a strong negative effect on the thickness of biscuit.

On the other hand, insoluble or aggregative glutenins — the glutenin macropolymer — impart elasticity and

therefore bring about biscuit contraction, reducing biscuit width. This suggests that biscuit-type wheats should be selected on the basis of HMW composition such as is indicated by 2-7-12 or LMW allelic types 'o' or 'm', or perhaps simply on the basis of the gliadin/glutenin ratio.

#### Minor protein components associated with starch granules

Previous studies by Greenwell and Schofield (1986) showed an association between a 15 kD surface protein and a friable endosperm, but the role of starch granule protein in relation to functional properties of wheat, and the relation of this protein to the hard and soft alleles of the hardness gene, had still to be established.

In the event, it was not possible to provide a predictive test of endosperm texture in bread wheat using an anti-friabilin antibody, but a useful application for it was found in a durum wheat purity test (Durotest®). Major progress was also achieved by comparisons of the basic friabilin

components (identified through capillary electrophoresis, NEPHGE, N-terminal sequences and immunoblotting) with the lipoproteins extracted by the detergent Triton X-114.

The results obtained have considerably advanced our knowledge of the biochemical nature of friabilin and its location, and have begun to clarify the status of friabilins as lipid-binding proteins. For instance a strong homology was demonstrated between some starch granule proteins (friabilin basic 2-3) and the main lipid binding protein, named puroindoline *b* in regard to its unique tryptophan-rich domain.

Thus, friabilins are involved in some way with endosperm texture, but it has not so far been possible to use them to develop a rapid diagnostic test for this important quality parameter of bread wheat. Friabilin might be found during starch purification and further work is needed to explain the true molecular basis of friabilin-starch interaction. Moreover, it is more likely that hardness is due to a lipid-like factor binding friabilin to starch on the surface of the granules.

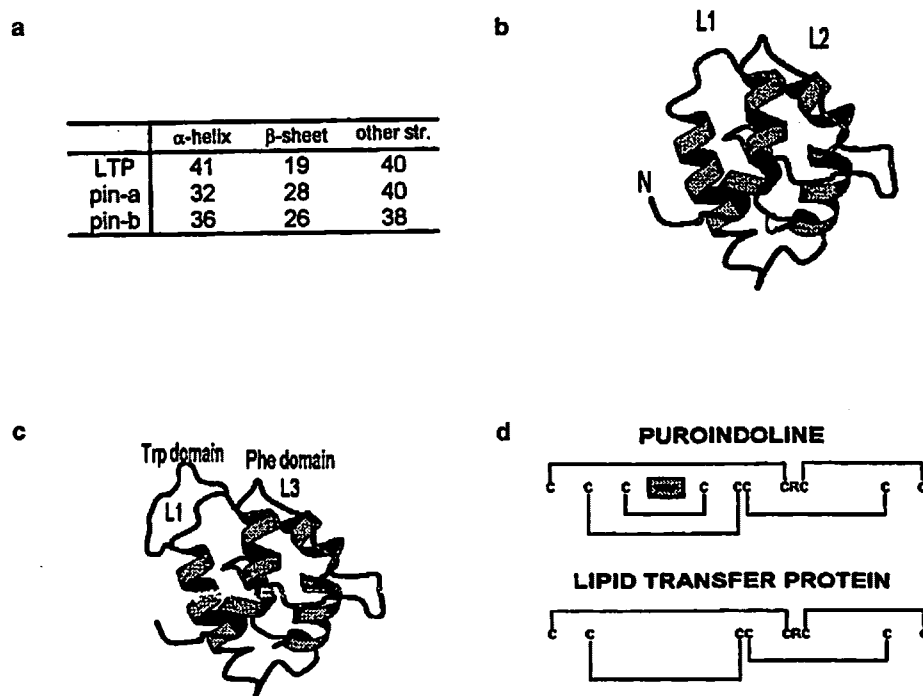


Figure 5 Structure of puroindoline and lipid transfer protein (from Marion, Institut National de la Recherche Agronomique, Nantes, France): a. Secondary structure of wheat lipid transfer protein (LTP) and puroindolines determined by Fourier transform infrared spectroscopy. b. Structure of wheat LTP from multidimensional  $^1\text{H}$  NMR data. c. 3D model of puroindolines deduced from the 3D structure of wheat LTP. d. Disulphide bond pattern of puroindolines and lipid transfer proteins.



### Lipid-protein interactions

Lipid-binding proteins, which are mostly recovered in the detergent-rich phase after phase partitioning using Triton X-114, have been examined in studies aimed at describing the mechanisms of the interaction between lipids and other components.

It was shown that at pH 4 the puroindoline protein was mainly composed of helices, that it strongly interacted with anionic phospholipids and that it was stabilized by five disulphide bridges (Figure 5). It is this structural flexibility that controls the lipid-binding specificity. Good foaming properties were also found for puroindoline due to the formation of a highly stable lipoprotein film at the air-water interfaces. Such a mechanism is probably important in gas phase expansion during the proving and baking of bread doughs. The phospholipid-puroindoline interactions observed in model systems were similar to the behaviour of different membrane-invading or membranotoxic proteins (Marion *et al.*, 1994).

Using monoclonal antibodies, the main puroindoline (puro-*a*) could also be mainly located in the aleurone layer while puro-*b* was located mainly in the starchy endosperm.

### Characterization and purification of gluten subfractions

Purification of gluten subfractions is an essential step in studying their functional, rheological and physicochemical properties. But there is great difficulty in obtaining pure subunits (especially LMW glutenins, which are closely linked fractions with molecular weights similar to those of gliadins). Their aggregative behaviour also makes them difficult to handle and makes it hard to obtain 'native' subfractions, that is, fractions retaining their functional properties. Nevertheless it proved possible, using differential solubility in increased acid concentrations, to isolate gluten subfractions that sufficiently retained their functional properties.

New methods of purification of glutenin subunits were also developed. They were carried out on both HMW subunits, which impart dough tenacity and elasticity, the essential factors of the northern European loaf, and LMW

subunits, whose genetic variation is more likely to be associated with properties of extensibility that are more critical in French and southern European breads. The methods include precipitation by acetone (which has the potential to yield large amounts of pre-purified protein groups), preparative isoelectric focussing, electroendosmotic electrophoresis, and adsorption chromatography on controlled pore glass beads.

Novel methods of characterization were also developed, including acid-PAGE for glutenin subunits, IE-FPLC, and the determination of the number of cysteines of any subunit by mixed alkylation and electrophoresis (Morel and Bonicel, 1996) (Figure 6).

### Physicochemistry and functionality of gluten subfraction

Physical studies were carried out to gain more detailed information on secondary structures. The incorporation of purified HMW subunits (such as 1Bx20) into dough using a 2 g mixograph resulted in an increase of dough strength whereas simple addition reduced it.

The rheology of various gluten subfractions was investigated by dynamic assay of shear characteristics, revealing a behaviour typical of a transient network structure and large differences in storage and loss moduli between the fractions, including a strong correlation between the plateau modulus and the proportion of the largest glutenin aggregates (excluding the SE-HPLC peak). The determination of the effect of different HMW alleles was facilitated by the availability of near-isogenic lines which differed only in HMW composition. The viscoelasticity of gluten seems therefore to be controlled by the amount of the larger glutenin aggregates, whose size can be precisely determined through dynamic light scattering. A two-level gluten structure was tentatively proposed: an aggregate level (directly involved in the observed viscoelastic behaviour) and a network level resulting from the connection of the large aggregates through non-covalent interactions. Factors affecting gluten viscoelasticity (such as temperature) have complicated effects since they operate at both levels, whereas the SS/SH process seems to act only through the aggregation state.

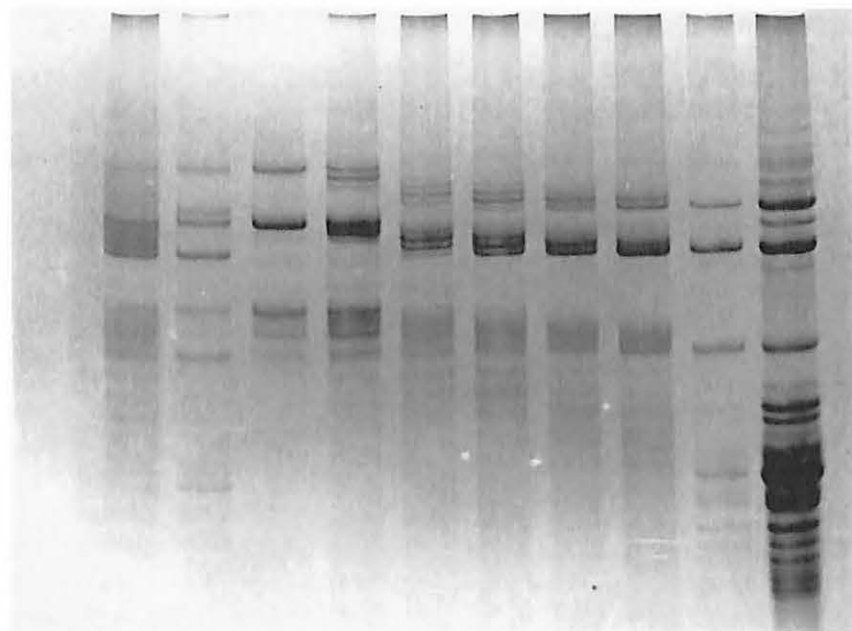


Figure 6 Determination of the number of cysteine residues in HMW subunits of wheat glutenin (variety Castan) by mixed alkylation and acid PAGE electrophoresis (from Morel, Institut National de la Recherche Agronomique, Montpellier, France).

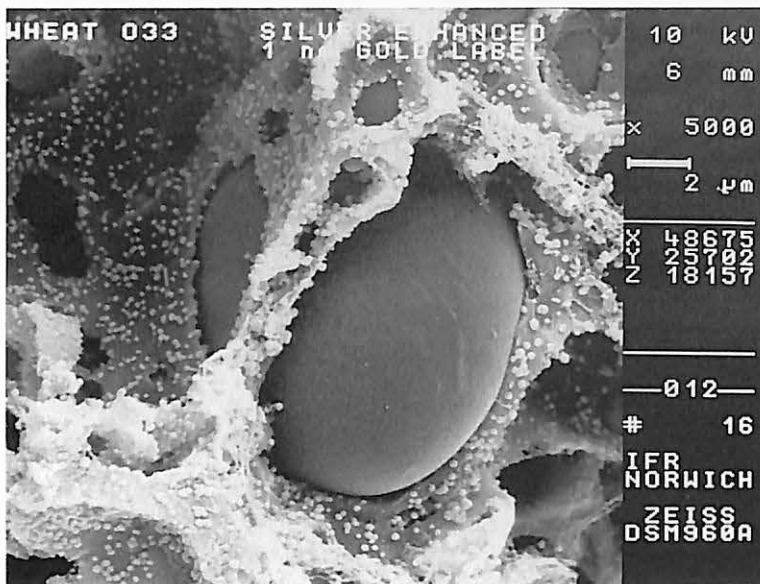


Figure 7 Silver labelling of a wheat dough sample with anti-arabinoxylan monoclonal antibodies. (From Mills, Institute of Food Research, Norwich, UK.)

NMR relaxation studies indicated that HMW subunits were not elastin-like in their interaction with water. For instance, hydration increased the mobility of the HMW subunit 20, which in turn enabled changes in favour of more extended and  $\beta$ -sheet type structure to occur. It was instead suggested that the origin of elasticity of HMW subunits was in intermolecular hydrogen bonding, which arises because of the high density of glutamine residues along the central repetitive domain (Belton *et al.*, 1994; Shewry, 1995).

Electron spray mass spectrometry was used to determine the molecular weights of HMW subunits for which gene sequences were available. Electron spin resonance (ESR) also provided information on molecular flexibility and confirmed that the polymerization of subunits resulted in less mobile polypeptide chains and more rigid proteins. ESR also suggested the presence of two liquid phases in gluten — the organized lipids and the aqueous-protein phase — which differ in polarity. X-ray scattering and scanning tunnelling microscopy studies were carried out to determine the dimensions and flexibility of HMW molecules, indicating that the subunits behave in solution as semi-rigid rods (Hargreaves *et al.*, 1994).

#### Dynamics of dough development

The behaviour and interactions of wheat components in doughs and baked goods were studied using antibodies against the different components (polysaccharides and gluten proteins) and microscopic techniques.

Polyclonal antibodies were produced in rabbits against bran arabinoxylans (AX), using lectins absorbed on micro-

titration plates to capture AX which would not be readily immobilized in the conventional way. Then, mice were injected and monoclonal antibodies to AX were obtained for the first time, a significant achievement in itself. These monoclonal antibodies were used with a new silver enhancement SEM technique (Figure 7) in order to probe the wheat and bread samples and study the structural changes that occur during baking (Mills *et al.*, 1993).

#### Genetics of wheat storage proteins

This task was mainly focused on LMW subunits of glutenins, which are the least characterized group of wheat proteins. The development of near-isogenic lines or chromosomal substitution lines made advances in this area possible.

Genetic variability for B, C and D groups of LMW subunits was described, as well as genetic linkage between loci coding for gliadins and glutenins on group 1 chromosomes. Because no one-dimensional system allowed satisfactory description of LMW subunits, three new electrophoretic methods were developed: a multiple system (SDS-PAGE/IEF/A-PAGE) to characterize LMW subunits, a two-step technique (A-PAGE/SDS-PAGE) to reveal the polymorphism of



Figure 8 New two-dimensional A-PAGE  $\times$  SDS-PAGE electrophoresis for detailed description of LMW subunits in wheat cultivars (from Redaelli, Istituto Sperimentale per la Cerealicoltura, Italy).

$\omega$ -gliadins and a 2-dimensional method (A-PAGE  $\times$  SDS-PAGE) to allow detailed description of LMW subunits in various cultivars, following a genetic approach based on the correspondence between the alleles at the *Gli-1* and *Glu-3* loci (Figure 8).

The effects of LMW and HMW glutenins and gliadins on gluten properties are now much better understood (Pogna *et al.*, 1993; Branlard and Dardevet, 1994). For instance, new relationships between  $\gamma$ - or  $\omega$ -gliadin alleles and technological quality were established; a specific effect on breadmaking quality of D-type LMW subunits of glutenin and of fractions controlled by genes on *Gli-D1/Glu-D3* loci was shown; a null allele with positive effect on quality was identified; and allelic variation at the *Glu-3* loci was described in terms of interaction with the *Glu-1* loci, suggesting that screening lines containing the *Glu-B3* 'o' or 'm' allele would be useful when aiming to breed wheats for extensible doughs.

In addition, a new *Glu-D1*-encoded subunit (5\*, different from the subunit 5 that is normally associated with subunit 10) was discovered in cv. Fiorello. It lacked the additional cysteine residue, typical of subunit 5, at the beginning of the repetitive domain. This raised doubts about previous results excluding subunit 5 as being responsible for differences in breadmaking quality observed in the pairs 5 + 10 and 2 + 12 (Lafiandra *et al.*, 1993).

#### Sprouting resistance

Prevention of sprouting damage is an objective long sought in the EU. The average annual cost of sprout damage occurring once in every five years (leading to a 10% loss in yield and a reduction in the amount of flour of bread-making quality by 50%) is 50–60 million ECU. The approach taken in this project was entirely novel in terms of both concept and methodology. Instead of detecting levels of amylase, work has focused on developing a bioassay to monitor inhibitors of germination, and purifying a fraction containing a germination inhibitor (which proved to be distinct from abscisic acid) with a view to a rapid detection of germination at an early

stage and perhaps its prevention. In addition, the determination of the broadness of the genetic basis for dormancy should allow selection for sprouting resistance in breeding programmes.

#### Conclusion

Taken as a whole, this truly integrated precompetitive programme has clearly contributed in the first place to filling the gap between the development of new processes and understanding of processing requirements and wheat quality requirements, and secondly to stimulating the breeding and development of wheats capable of satisfying the present and future demands of European industry. The main results of this study were as follows:

1. A better understanding of the physicochemical basis of the industrial processing of wheat and flour (milling, white and wholemeal bread-making, starch/gluten extraction, flour blends, fermented products and biscuit manufacture) which will benefit each of these industries.
2. The development of improved methods for the rapid and efficient analysis and characterization of lines in the early stages of breeding and of wheat samples in trade.
3. A genetic base of strong-type lines which breeders can utilize now, in the medium term, and well beyond the limited framework of the four-year programme. The new varieties of wheat carry all the desired agronomic and technological characteristics, particularly those conferring stability of the expression of quality in various environmental conditions in which plants develop.
4. A better identification of the quality determinants whose genes could be identified, cloned, sequenced and possibly transferred.

Moreover, because of these fruitful exchanges of knowledge between participants and the great success of the programme from a social point of view, a European network with considerable scientific power and excellent levels of communication has been developed over the last few years, enabling us to be optimistic about future research and development programmes in wheat science and technology.

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