INDUSTRIAL USE OF EU WHEATS
Review of a Four-Year (1991-1994) European (ECLAIR) Research Programme

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Exploring and improving the industrial use of wheat *(T. aestivum)* produced in the European Union is of a key importance and has been the topic of a major project (0052) in the ECLAIR programme (1991-1994). This European project was aimed at filling the growing gap between process development and its understanding in terms of processing requirements and thus wheat quality requirements. A further objective was the stimulation of breeding in order to tailor new types of wheats, capable of satisfying the future demands of the European industries and the export market.

The rationale behind the objectives is manifold:
1. Despite the fact that wheat is an essential crop for European agriculture and for the wheat-processing industry (milling, breadmaking), European wheats are not adapted to a wide range of applications, especially to recent developments of gluten/starch separation, wholemeal breadmaking, biscuit manufacture, flour blends, sour doughs, etc.
2. The milling and baking industries require higher quality because of modern developments in technology
3. Current methods of breeding are predominantly focused on white bread-making
4. Finally, 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, whereas, in the coastal regions of Northern Europe, where the crop can be cultivated intensively, sprouting puts a severe strain on both yield and quality.

Improvement of the industrial use is likely to result from better knowledge of the various applications of wheat, each main parameter of processing and its effect being expressed in terms of functional properties of the wheat and related to specific wheat protein constituents and their interactions. On the other hand, combined functional/physico-chemical and biological advanced methodologies can be applied to quality determinants, resulting in a better understanding of their variability of composition, structure, and of their 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 of the various EU countries, the identification of improved breeding criteria (for milling quality, breadmaking or biscuit-making quality, adaptation to starch/ gluten separation, sprouting resistance, etc.) and the development of rapid tests for use in breeding programmes and trade can be obtained.

Apart from purely scientific and technical aspects, a particularly innovative element of this project was the establishment of a multidisciplinary programme (bringing together physical chemists, biochemists, immuno-chemists, 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 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.

The programme was therefore organised as three subprogrammes (Annex), each one of which benefiting from the results of the other two:
- Subprogramme A - Industrial Processes (*Dr. Robert J. Hamer*, TNO, Zeist, The Netherlands)
- Subprogramme B - Functional Components and their Interactions (*Dr. Johan J. Plijter*, Gist-Brocades, Delft, The Netherlands)
- Subprogramme C - Biochemical Genetics and Physiology (*Dr. Norberto E. Pogna*, Istituto Sperimentale per la Cerealicoltura, Milano, Italy)

In addition, the main approaches have been based on several recent advances that 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:

- The availability of isogenic, aneuploid and translocation stocks which enable to pin-point the gene products that are important in functional performance.
- The introduction of original approaches based on new concepts (*e.g.* intrinsic quality of wheat genotypes), or new protein fractions (*e.g.* friabilin, LMW subunits of glutenin, HMW-albumin, S-protein,...), that stand out clearly against the good-old Osborne's scheme.
- 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.
- 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, electron spin resonance, electro spray mass spectrometry, X-ray scattering, 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 (dough development, seed dormancy).
- The development of a range of physico-chemical techniques that determine interfacial and aggregation behaviour.

Considering the main topics of the programme (breadmaking, biscuit-making, starch/gluten separation, milling quality, protein-lipid interactions, purification and functionality of gluten subfractions, dynamics of dough development, genetics of wheat storage proteins, sprouting resistance), the major achievements can be summarised as follows:

1. Bases of breadmaking quality

This first task carried was aimed at determining the underlying physico-chemical reasons for differences in gluten strength and bread-making quality and thus providing feedback to plant breeding programmes and grain trading.

First, a test bake was developed at FMBRA (Chorleywood, UK), that proved to be suitable to demonstrate the potential of EU wheats for wholemeal and white breadmaking and reveal the "carrying" properties of strong EU wheats.

It turned out that wholemeal loaf volumes could not be predicted from those in wheat flour breadmaking and that protein content was more important than gluten strength for wholemeal bread performance.

The concept of glutenin macropolymer was defined, which makes a basis of baking quality in CBP (UK) or RMT (German). This glutenin macropolymer, whose breakdown rate during mixing depends on the baking strength, plays a key role (GMP changes from a linear polymer in flour to a three-dimensional structure in dough) was shown to be related to the composition and incorporation rate of HMW subunits of glutenin. It also revealed as a valuable tool for predicting the processing properties of flour blends.

On the other hand, a new impetus was given to the "gel protein" fraction as a 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 the French, or South-European baking, the dough extensibility is often a more important and critical parameter. Dough extensibility was shown to be more associated with allelic variation of LMW and perhaps of gliadins than that of HMW subunits However, some LMW proteins (different from LMW subunits of glutenins) were shown to act as cysteine in preventing reassembly of GMP.

2. Bases of biscuit quality

To define an optimal sweet-biscuit flour and to improve the knowledge of the process, to increase productivity, quality and creation of new products was the aim of this task. The main results obtained by BSN (Athis-Mons, France) and INRA (Montpellier, France) include the rheological behaviour of biscuit dough, that is now better understood (viscoelastic at low strains, similar to a gel at higher strains).

An essential quality factor in biscuit-making is the stability of biscuit size. Weight, thickness and density of biscuit are related to constituents absorbing water: 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 dough free water content) and have a very negative effect on the thickness of biscuit.

On the other hand, insoluble or aggregative glutenins, *i.e.* the glutenin macropolymer impart elasticity and therefore biscuit retraction resulting in a very negative effect on biscuit width, and suggesting that biscuit-type wheats should be selected on the basis of HMW composition such as 2-7-12 or LMW allelic types '*a*' or '*m*', or perhaps simply on the basis of the gliadin/glutenin ratio.

3. Starch/gluten separation

To study the effect of processing aids (*e.g.* hemicellulases), a unique miniaturised decanter centrifuge was constructed by TUB (Berlin, Germany). Gluten and starch could be obtained from all the raw materials including wholemeal flours. Glutens from wholemeal contain more LMW and less HMW subunits of glutenin than glutens from white flours. Differences in elastic behaviour cannot be attributed to proteolytic activities but a strong effect of hemicellulose and of the process water (exposure to acid concentrations that are produced micro biologically upon continuously running of the system) was observed. Also, TNO (Zeist, The Netherlands) showed that addition of 2□% hemicellulose to flour decreased the gluten yield by 20 %, whereas addition of hemicellulase improved the gluten yield of the flour with and without extra hemicellulose.

4. Milling quality and bases of grain structure

In contrast with the considerable effort that has been devoted to the improvement of wheats in terms of bread-making quality, milling quality has had minor attention. It has been necessarily left out of breeding programmes until the last stages and physico-chemical bases of milling quality are still poorly understood. Nevertheless, taking the amount of wheat produced annually in the EU, one percent increase in milling yield represents an advantage of 40 million ECU per year for the EU millers.

Accordingly, our project was aimed at developing new ways of understanding and predicting milling quality and more especially:
- identifying the nature and relative importance of factors determining milling quality (*e.g.* endosperm hardness, bran friability, endosperm ash content, etc.).
- investigating the morphological (by image analysis) and chemical (minerals, ferulic or phytic acid, proteins associated with starch granules) bases of milling quality.
- producing a predictive (breeding) test for milling quality.

The first investigations by FMBRA on European sample sets concentrated on information gained by image analysis used to examine morphometric parameters of the kernel. However, image analysis did not prove to have a good predictive value excepted when samples contained seriously shrivelled grains. Thus, in general, endosperm content is not a factor which limits flour yield and the belief that a positive correlation exists between grain size and endosperm content is certainly unjustified. Much better relations were detected when milling quality was described in terms of milling factors (bran friability, endosperm content, 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. For instance, it was showed by TNO that:
- 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 pure endosperm and flour fractions.
- Another important discovery which has drawn considerable interest from millers and milling scientists was the possibility to explain 70-80 % of the variation in milling quality by potassium content of the kernel (that allows very good prediction of ash content of the flour), bran friability and kernel width.

On the other hand, the recent investigations of the proteins associated with the starch granules (in order to explain the physico-chemical bases of grain hardness) deserves specific development.

5. Minor protein components associated with starch granules

Previous studies by Schofield and Greenwell (FMBRA) showed an association of the 15K surface protein and 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 fact, FMBRA showed that anti-friabilin F7F antibody could not provide a predictive test on the endosperm texture in bread wheat, but a useful application for it was found in a durum wheat purity test (Durotest).

Major progress has been achieved by comparison of the basic friabilin components (through capillary electrophoresis, NEPHGE, N-terminal sequences, immunoblotting) to the lipoproteins extracted by the detergent Triton X-114 by Marion at INRA (Nantes, France).

The results obtained have considerably advanced our knowledge of the biochemical nature of friabilin, and have began to clarify the status of friabilin 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.

So, friabilins are involved in some way with endosperm texture, but not in a way that has so far enabled us to use them in a rapid, sensitive diagnostic test for this important quality parameter of bread wheat. Presence of friabilin on starch might occur during starch purification and further work is needed to explain the true molecular basis of friabilin starch interaction. Moreover, bases of hardness are more likely to involve a lipid-like factor binding friabilin to starch on the surface of the granules.

6. Lipid-protein interactions

In a study aim at describing the mechanisms which play a role at the interaction between lipids and other components, lipid binding proteins which were mostly recovered in the detergent rich phase after phase partitioning using Triton X-114 have been extensively characterised by INRA (Nantes).

Following the discovery of the puroindolin protein, it was showed that the protein was mainly composed of helices at pH 4, that it strongly interacted with anionic phospholipids and was stabilized by five disulfide bridges. It is therefore its important structural flexibility that controls the lipid binding specificity. Good foaming properties were also found for puroindoline, with an enhancement by the presence of lysoPC due the formation of a highly stable lipoprotein film at the air water interfaces. Such a mechanism is probably important during the gas phase expansion on proof stage and baking of bread doughs. Phospholipid-puroindoline interactions observed in model systems is similar to the behavior of different membrane invading or membranotoxic proteins.

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

In this connection, another work on interfacial behaviour of dough during mixing carried out at Gist-Brocades (Delft, The Netherlands) with the aid of an overflowing cylinder, it was demonstrated that the breakdown of macropolymers during mixing could be clearly seen in the surface active behaviour of dough samples, that added lipids have a strong influence on the surface behaviour, but that no difference is observed between soft and hard wheat types.

7. Characterisation and purification of gluten subfractions

Purification of gluten subfractions is an essential step to study their functional, rheological, physico-chemical properties, but there is a great difficulty to obtain pure subunits (especially LMW glutenins that are closely linked fractions with molecular weight similar as those of gliadins and an aggregative behaviour that make them difficult to handle) and more especially to obtain "native" subfractions, *i.e.* fractions retaining their functional properties.

Relevant results of this study include the isolation at INRA (Nantes), of gluten subfractions differing by their aggregation state with low polydispersity, based by adapting MacRitchie's procedure of differential solubility in increased acid concentrations, that sufficiently retained their functional properties to allow dynamical assay in shear to be carried out.

New methods of purification of subunits have been also developed in the course of the programme by IACR (Long Ashton, UK), INRA (Montpellier, France), Universities of Viterbo and Padova, Italy). They have been carried out on both HMW that clearly impart dough tenacity and elasticity that are essential factors of North-European loaf and LMW whose genetic variation is more likely to be associated with properties of extensibility that are more critical in French and South-European breads. For instance, precipitation by acetone (that has the potential to yield large amounts of pre purified protein groups), préparative IEF, adsorption chromatography on Controlled Pore Glass beads, electroendosmotic electrophoresis.

Novel methods of characterisation were also developed at INRA (Montpellier). They include an Acid-PAGE for Glutenin subunits, an IE-FPLC (charge differences),and a determination of the number of cysteines by mixed alkylation and electrophoresis.

8. Physicochemistry and functionality of gluten subfractions

Physical studies to gain more detailed analyses of secondary structures. Purified HMW subunits (*e.g.* 1Bx20) were incorporated in dough using a 2g mixograph, resulting in an increase of dough strength whereas simple addition resulted in a decrease (IACR, Long Ashton). The rheology of various gluten subfractions was investigated at INRA (Nantes) by dynamic assay in shear and revealed a behaviour typical of 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 (excluded SE-HPLC peak). ^{1}H and ^{2}H NMR relaxation studies at IFR (Norwich, UK) indicated that HMW subunits are <u>not</u> elastin-like in their interaction with water. Electro Spray Mass Spectrometry was used to determine molecular weights of HMW subunits for which gene sequence is available. At INRA (Nantes) and ENSBANA (Dijon, France), Electro Spin Resonance also provided information on molecular flexibility and confirmed that polymerization of subunits resulted in less mobile polypeptide chains and more rigid proteins. Also, with either TEMPO probing or labelling of cystein and lysine residues ESR suggested the presence of two liquid phases in gluten (the organised lipids and the aqueous-protein phase) which differ in polarity. X-ray scattering and scanning tunnelling microscopy studies to determine dimensions and flexibility of HMW molecules and especially indicated that subunits behave in solution as semi-rigid rods.

9. Dynamics of dough development

The aim was to study the behaviour and interactions of wheat components in doughs and baked goods with the aid of antibodies against the different component (namely polysaccharides and gluten proteins), and using microscopic techniques.

At IFR, polyclonal antibodies have been produced in rabbits against bran pentosan extracts, using lectins absorbed on microtitration plates to capture pentosans which would not be readily immobilised in the conventional way.

Second, mice were injected to produce Mabs to arabinoxylans (AX) and boosted with AX alone and AX-protein conjugates. Mabs against AX were thus produced, which is a great success in itself. These Mabs were are currently used with a new silver enhancement SEM technique in order to probe the wheat and bread samples and structural changes that occur during baking. An example of silver enhancement on wheat endosperm

10. Genetics of wheat storage proteins

This task was mainly focused on LMW subunits of glutenins that are the least characterised group of wheat proteins. Owing to the development by the French and Italian geneticists of near-isogenic lines or chromosomic substitution lines such as those from cv. Courtot, advances in this topic have been made possible.

Genetic variability for B, C and D groups was better described as well as genetic linkage between loci coding for gliadins and glutenins on group 1 chromosome. Because no 1D system allowed satisfactory description of LMW subunits, a multiple system was developed by INRA, Montpellier, to characterise LMW subunits, including SDS-PAGE, IEF, and a newly developed Acid-PAGE. In addition, a two-step A-PAGE/SDS-PAGE technique was developed by INRA (Clermont-Ferrand), to reveal the polymorphism of ω-gliadins. Moreover, a 2-dimensional A-PAGE x SDS-PAGE method was developed by ISC (S. Angelo Lodigiano and Rome, Italy) and INRA (Montpellier) 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.

The effects of both LMW, HMW, and gliadins on gluten properties were also much better understood. For instance, new relationships between ω-gliadin alleles and technological quality were established by ISC (S. Angelo Lodigiano), while the specific effect of allelic variation at the *Glu-3* loci was described by INRA (Montpellier), in interaction of that at *Glu-1* loci, suggesting to screen lines containing the *Glu-B3* "*o*" or "*m*" allele when aiming at breeding wheats giving extensible doughs such as biscuit-type wheats.

In addition, a new *Glu-D1* subunit (5*, different from subunit 5 normally associated with subunit 10) was also discovered by the University of Viterbo in cv. Fiorello, that was lacking the additional cysteine residue, typical of subunit 5, at the beginning of the repetitive domain. This raised doubts on previous results excluding subunit 5 as being responsible for differences in breadmaking quality observed in the pairs 5+10/2+12.

11. Sprouting resistance

Prevention of sprout damage is an objective long yearned for in the EU. 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 by TNO in this project was entirely new in both concept and methodology. Instead of detecting levels of amylase, work has focused on:
- developing a bioassay to monitor inhibitors of germination
- purifying a fraction containing a germination inhibitor (which proved to be distinct from abscisic acid) in view to a rapid detection at an early stage and perhaps prevention In addition, the determination of the broadness of the genetic basis for dormancy should allow to select for sprouting resistance in breeding programmes

Summary of results obtained

Many more results have been produced in other fields that were not detailed in this report, for instance in other applications of wheat products: sour doughs, interactions with microorganisms, sweet bakery, flour blends. Needless to say that many biochemical and technological studies have been made possible by multilocal experiments of advanced varieties and lines, and production of samples in controlled conditions by all the members of the South-Europe and North-Western Europe networks. In addition, genotype x environment interactions were studied and populations for breeding were experienced as well as effect of nitrogen fertilization, stability of quality expression, or somaclonal variation for factors affecting breadmaking quality.

Of course, a lot is still to be done to improve the industrial use of EU wheats but already now, this ECLAIR programme has largely contributed to wheat improvement at a precompetitive level, witness the 60 papers already published by the participants, by improving the linkage of agriculture and industry through research. In summary, the main results of this study consisted of:

1. A better understanding on physico-chemical bases of the industrial processing of wheat and flour (milling, white and wholemeal bread-making, starch/gluten industry, flour blends, fermented products and biscuit manufacture) which will now allows each participant to apply the newly acquired knowledge in his own industry.
2. The development of improved methods for the rapid and efficient analysis and characterization of lines in early stages of breeding and of wheat samples in trade.
3. A genetic base of strong-type lines which breeders can now utilise in view to introduce, in a longer term, and well beyond the limited framework of the four-year programme, new varieties of wheat with all the desired agronomic and technological characteristics, particularly the stability of the expression of quality in various environmental conditions of development of the plant and with the minimum use of chemical treatments.
4. A better identification of quality determinants whose genes should be identified, cloned, sequenced and possibly transferred.

Beside scientific results, another aspect of success of the programme is in a social sense. A few years ago, there was a lot of competition between our research groups. Today, a large and united family of scientists has been built. It is a kind of consortium having a huge scientific power and in which basic research is really open. Moreover, working four years together and meeting welcoming each other frequently in our various places, led us to realise that we, European people, have a wonderful potential due to our old and rich civilisations. The new and straightforward atmosphere that has emerged between ECLAIR participants and our close friendship are perhaps the most essential elements for setting up future collaborative research programmes, aimed at improving the quality of EU wheats but also aimed at the achievement of our common European project.

ECLAIR PROGRAMME AGRE 0052

To Explore and Improve the Industrial Use of EC Wheats

WORK CHART

Participants	IRTAC, Paris, FR	Prod. Sementi, Bologna, IT	I. S. Cerealicoltura, Milano, IT	SME Ricerche, Caserta, IT	Champagne Céréales, FR	Roquette, Lestrem, FR	INRA - Clermont, FR	INRA - Montpellier, FR	INRA - Nantes, FR	BSN - Biscuit, Athis-Mons, FR	ITCF, Paris, FR	IATA, Valencia, ES	TUB, Berlin, DE	FMBRA, Chorleywood, GB	Gist Brocades, Delft, NL	AFRC - ICR, Norwich, GB	TNO, Zeist, NL	Club des 5, Paris, FR	AFRC - IACR, Bristol, GB	Univ. Padova, IT	Univ. Viterbo, IT	EERM, Cadiz, ES	ENMP, Elvas, PT
	Co.	C	C	C	AC	AC		C		AC	AC	AC	AC	AC	C	AC	C	AC	AC	SC	SC	SC	SC
ECLAIR Code Number	1	2	3	4	5	6	07C	07M	07N	8	9	12	13	14	15	16	17	18	19	22	23	24	25
A - INDUSTRIAL PROCESSES				□				□		□		□	□	□			□						
I - Processing Properties of Wheat																							
1.1 Milling quality														X			X						
1.2 Gluten/Starch Separation																							
. Use of Enzymes																	X						
. New Separation Processes													X										
II -Applications of Wheat Products																							
2.1 White Bread														X									
2.2 Wholemeal Bread														X									
2.3 Evaluation in Baked Products										X													
2.4 Flour Blends																	X						
2.5 Sweet Bakery Products																							
2.6 Interaction with Microorganisms																							
B - FUNCTIONAL COMPONENTS AND THEIR INTERACTIONS								□	□					□	□	□			□	□	□		
I - Component Interactions																							
1.1 Purification and Characterization of Gluten Subfractions								X	X										X	X	X		
1.2 Physico-chemistry and Functionality of Wheat Proteins								X	X						X								
1.3 Gluten Hydration and Interactions with Other Components															X	X							
1.4 Minor Protein Components Associated with Starch Granules														X									
1.5 Lipid Interactions									X						X								
II - Dynamics of Dough Development																							
2.1 Development of Microscopic Techniques																X							
2.2 Polyclonal and Monoclonal Antibodies to Pentosans																X							
2.3 Role of Pentosans																X							
2.4 Interactions between Proteins, Pentosans and Lipids																X							
2.5 Effects of Heat and Mechanical Work																X							
C - BIOCHEMICAL-GENETICS AND PHYSIOLOGY		□	□	□	□	□	□	□		□	□						□	□				□	□
I - Multilocal Experiment and Production of Samples in Controlled Conditions																							
1.1 Southern Europe		X	X	X	X	X	X				X							X				X	X
1.2 North-Western Europe				X		X	X			X	X							X					
II - Genotype x Environment Interaction																							
2.1 Ecophysiological Approach											X							X					
2.2 Experimentation in Controlled Environment											X							X					
III - Experimentation on Populations for Breeding																							
IV - Genetics of LMW Glutenin Subunits																							
V - Genetic and Technological Aspectsof LMW Glutenins and Other Proteins			X				X																
VI - Production of Lines and Near Isogenic Lines			X																				X
VII - Chromos. Location of Storage Protein Genes							X																
VIII - Biochemical Markers							X																
IX - Somaclonal Variation for Factors Affecting Breadmaking Quality		X																					
X - Sprouting Resistance																	X						

PROCEEDINGS
OF THE INTERNATIONAL MEETING

WHEAT KERNEL PROTEINS
molecular and functional aspects

S. Martino al Cimino, Viterbo (Italy)
September 28-30, 1994