Commission of the European Communities

ECLAIR Programme

Contract n° AGRE 0052

Coordinator: IRTAC, 16 Rue Nicolas-Fortin 75013 Paris, France

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To Explore and Improve the Industrial Use of EC Wheats

First Scientific Annual Progress Report from 1-09-1990 to 31-12-1991

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ABSTRACT OF THE PROGRAMME

The project is aimed at Exploring and Improving the Industrial Use of EC Wheats (T. aestivum) with the objective of filling 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.

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Gist-Brocades N.V.	NL	P
TNO, Cereals, Flour and Bread Institute	NL	P
Istituto Sperimentale per la Cerealicoltura	I	A
Nuovo Crai S.p.A.	I	A
Champagne Céréales	F	A
Roquette Frères S.A.	F	A
BSN/Branche Biscuit	F	A
ITCF	F	A
"CLUB DES 5"	F	A
Istituto de Agroquimica y Tecnologia de Alimentos (IATA)	ES	A
Technische Universität Berlin	DE	A
Flour Milling and Baking Research Association	UK	A
AFRC Institute of Food Research	UK	A
AFRC Institute of Arable Crops Research	UK	A
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Estacion Experimental Rancho la Merced	ES	SC

Proposal n°: *0007/0103*
Contract n°: *AGRE.0052*

Duration: *01.01.91 - 31.12.94*

Scientific Officer: *S. HARDY*

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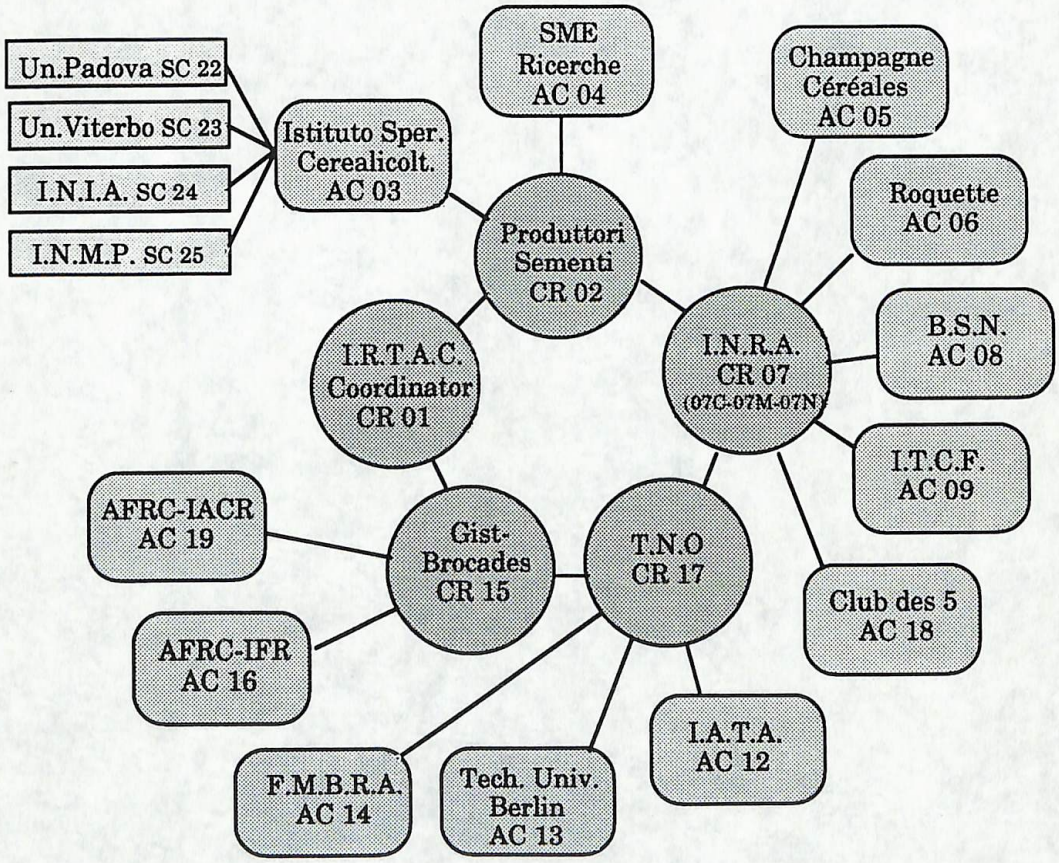
ADMINISTRATIVE STRUCTURE



INTRODUCTION

***Jean-Claude Autran*, Scientific Coordinator**
(IRTAC, Paris, France)

This first progress report reviews the scientific activities until the end of 1991, i.e. about one year after the official starting date of the ECLAIR programme "To Explore and Improve the Industrial Quality of EC Wheats.

This document has been submitted to the CEC in March 1992, at the same time as the report on the cost statements for 1991

It comprises the reports of each subprogramme A, B and C:

A - Industrial Processes, by Dr. Robert J. Hamer
B - Functional Components and their Interactions, by Dr. Johan J. Plijter
C - Biochemical-Genetics and Physiology, by Dr. Norberto E. Pogna.

Each section of the report consists of (i) a review of activities and projects, by the subprogramme manager, and (ii) the full texts of the individual progress reports prepared by each participant in the subprogramme (excepted the informations on the financial status, that belong to the report on financial accounts).

Administrative aspects

The work during this first period was greatly influenced by the delay in finalising the Contracts. During the first months of 1991, various local situations have existed due to different starting dates. Certain partners could advance funds and recruit people to work on the programme before the Contracts are signed (In particular, a warm acknowledgement must be given to TNO Organization, to Flour Milling and Baking Research Association, and to most participants in subprogramme C, for having started work earlier), while other partners could not. Mid-1991, all participants had started work and it can be said that the whole programme is now progressing in a sound way. The partners who started later and who are behind the schedule have undertaken to catch up as soon as possible and, at all events, before the end of 1992.

Scientific aspects

1. Objectives

It can be reminded that **our main objective is "To Explore and Improve the Industrial Quality of EC Wheats"** and, more specifically:

- 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).

2. Approaches

On the other hand, our programme is based on a number of **recent approaches**, that give 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. For instance:

1. The availability of isogenic, aneuploid and translocation stocks which enable to pin-point 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.

Apart from these purely scientific and technical aspects, a particularly innovative element of this project is 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. The organisation of this programme in three subprogrammes, each one of which can benefit from the results of the other two, has greatly facilitated the scientific direction, and thus the chances of success, of the programme as a whole.

3. Main results so far

Despite of the delay in contract negotiations that hampered some of the tasks, a striking progress has been made during this first year, and many promising results have already been reported. Some have been presented on a poster exhibited at the Two-Day Conference on EC Agro-Industrial and Forestry Research Programmes (March 11-12, Brussels). As an example:

a. Industrial processes

- In the **milling quality** project, it has been shown that the **image analysis** could be used to define size distributions of grains and that **ferulic acid** was a far better marker for bran friability and extraction rate than ash.

- Concerning the **starch/gluten separation**, a **laboratory scale system** was developed to investigate gluten produced from wholemeal flours and an improved separation of gluten and starch was obtained through the use of enzymes (**hemicellulases**).

- In studies on breadmaking, essential observations have been made on the relationship between **molecular structure** (glutenin depolymerisation) and **dough behaviour** during final proof. On the other hand, it became clear that the wholemeal loaf volumes could not be predicted from those in white flour breadmaking and that protein content seemed more important than gluten strength for **wholemeal bread performance**.

- In biscuit-making studies, it was found that **pentosans** contribute to explain dough stickiness and texture of 'Petit Beurre' biscuits.

- Investigating the properties of flour blends, a **low-molecular-weight protein fraction** of utmost importance for the depolymerisation of glutenin during dough processing was identified, while a prediction of the **processing properties of flour blends** proved to be possible through measurements of the 'gel-protein' fraction.

b. Functional Components and their Interactions

- New ways of **purification of gluten subfractions** (HMW and LMW subunits of glutenin) or of native aggregates were developed, based on HPLC, free-flow isoelectric focusing, or chromatography on controlled pore glass.

- Functional (viscoelastic) characteristics of **native gluten aggregates** have been determined, indicating that the rheological behaviour of gluten fractions was closely related to their glutenin polymer content, whereas gliadins contribute to rheological properties as a plasticizer.

- A new breeding test for dough extensibility was proposed, based on **allelic variation at LMW subunits** (*Gli-B1* chromosome locus).

- The investigations on **lipid-binding proteins**, especially phospholipid transfer protein from wheat flour, allowed to discover an homology with other low molecular weight sulphur-rich proteins, including **'friabilin'**, a protein associated with the surface of starch granules. The role of 'friabilin' on endosperm texture was therefore reconsidered in this new context of interactions with starch surface lipids.

c. Biochemical-Genetics and Physiology

- An essential and huge task has been the organisation of **North-Western- and Southern-Europe Networks** to supply participants with large quantities of highly controlled wheat samples and to provide information of the expression of technological quality in wheats grown in various environments.

- **Near-isogenic lines**, chromosome substitution lines, somaclonal variants, mutants lacking storage protein-encoding genes, progenies of intergeneric crosses, etc., were also produced to investigate the **genetics of endosperm proteins** and the regulation of genes expression with chromosome inter-actions.

- In addition, a bio-assay to test **sprouting resistance** was developed, and a first biochemical marker for dormancy was isolated.

In conclusion, all tasks are now well underway, and it is likely that most of the essential objectives of the programme will be reached within the required time.

However, an essential condition to amplify the success of the programme in the near fuure is the increase of the collaboration between subprogrammes:

Collaborations between subprogrammes

While the organisation of the research programme as three subprogrammes was imagined in order to make easier the preparation of the project and its management, it has become clear that the three different approaches (processes, physico-chemistry and biochemical-genetics) have to work now in close connection. A good coordination between subprogrammes and a collaboration between research groups is an essential condition to ensure the success of the programme as a whole and **to fill the gap between process development and its understanding in terms of wheat quality requirements and development of suitable breeding tests**. Any scientific problem identified in one subprogramme should benefit from the expertise of the other two, and cross actions should be developed every time it is possible.

There are some indications that this is already the case.

For instance, when facing with technological problems such as milling quality or endosperm texture (sprg A), it has been proceeded from the industrial process towards its principal components (that have been assessed using recent advances (e.g. image analysis)), then towards specific markers (e.g. purification and functional properties of friabilin) (sprg B), opening the way to molecular genetics of the marker. A similar approach has been followed in dough mixing and breadmaking studies: starting from the measurement of loaf volume and baking score (sprg A), the investigations moved to gel-protein and glutenin depolymerisation and then to the specific role of LMW- or HMW-glutenin subunits (sprg B) and to genetic studies including transfer of the relevant genes into other genotypes (sprg C) or development of new breeding tests. Another relevant example concerns the discovery of a low-molecular-weight protein that influences gluten extraction and stability of rheological properties of dough in which the approach moved from technology to physico-chemistry and perhaps to-morrow to molecular biology and genetic engineering.

Conversely, certain markers identified through simple correlations by breeders and geneticists (sprg C) have been further investigated through isolation, purification and study of functional

properties in model systems (sprg B), which should be followed by reconstitution studies at a laboratory or pilot scale (sprg A).

On the other hand, a number of actions have been encouraged to improve further the collaborations between subprogrammes:

1. Meetings of subprogrammes

All the three subprogrammes are now organising two meetings per year in alternating the meeting places. Most of these meetings were great successes, and many resolutions were passed concerning coordinative and collaborative actions, in an always excellent atmosphere.

It turns out now that the progression of many tasks would profit by going with **inter-subprogrammes discussions**. It is therefore encouraging to notice that (despite some inflation in the number of meetings to be attended !), several participants of each subprogramme now participate to the meetings of the two other subprogrammes.

2. Technical meetings

Technical meetings are aimed at encouraging in-depth inter-subprogramme discussions on selected topics and to improve cooperations.

A first technical meeting, devoted to the low molecular weight proteins was held on December 12-13 in S. Angelo Lodigiano (Milano) (see the report, pages 79-80, below). A second technical meeting on rheological methods is planned for 1992.

3. Books of methods (quality, biochemistry/physico-chemistry)

Two common books of methods are in way of completion:

- A book of quality related methods, set up by participants to subprogramme A,
- A book of biochemical procedures, set up by participants to subprogramme B.

Both books will be distributed to each partner during the first semester 1992.

4. Synopsis of the tasks

To make clear which are the aims in the different places, it was decided to prepare a document entitled "People in our ECLAIR programme: what they do actually", consisting of a set of record cards, one for each task.

Based on the updated content of the technical annex, these cards will contain the address, phone, fax, and picture of the project leader, with a short summary and key words of the task, so that everybody can easily know and contact the relevant person for any problem, and can detect where other subprogrammes are the most supportive. A project of such record cards will be prepared by the scientific coordinator, to be submitted to the next meeting of the Scientific Management Committee on April 23, 1992.

5. Availability and evaluation of wheat samples

Updated lists of samples were added to the minutes of the last meeting of subprogramme C. However, these lists are just indications of the sample reference and no sample is sent systematically to all partners: two-side agreements are necessary with the person responsible for each type of samples. On the other hand, unless mentioned, only grains are available and generally no flours.

- SEN Network: contact Dr. Pogna
- NWEN Network: contact Dr.Rousset
- Wheat collections: TNO (Dr. Kelfkens), or FMBRA (Dr. Pritchard).
- Genetic stocks: ISC, Produttori Sementi, or INRA-Clermont-Ferrand
- Special samples: contact Dr. Pogna, discuss to know the possibility of supply. If the samples are not available, put the request on a "waiting list" and send it to Dr. Pogna

An evaluation of technological quality by both laboratory tests and industrial tests was decided at a previous meeting of the subprogramme C. In addition, on a subset of samples, both conventional and future processes have to be performed.

6. General meeting of the programme: June 4th in Paris

A general meeting of the participants will be organized on June 4, 1992, during the week of the 9th Wheat and Bread Congress in Paris. Such a meeting will be an unique opportunity to bring together representatives of all partners of the Contract, to present some important results already obtained, and to make a synthesis of the overall progress.

This one-day conference will be held at "Salon Jacob, in "Méridien Montparnasse" Congress Centre, the day after the 9th International Cereal and Bread Congress. It will be restricted to the participants in this ECLAIR Programme. Non active participants that co-support some tasks will be accepted, however.

The organisation will be as follows:

- Introduction: J.C. Autran, scientific coordinator, will present an overall look on the programme as a whole.

- Three (oral + poster) sessions, each organised and chaired by one manager of subprogramme: Robert J. Hamer (A), Johan J. Plijter (B) and Norbeto E. Pogna (C), as follows:

- Each subprogramme manager will introduce his aim (10 min) and will select 2 key examples, that will be developed as oral presentations by participants (15 min + 5 min discussion each). It will be clearly expressed that these two examples are part of the whole approach. It is recommended that any other interesting result be presented as a poster (10 min discussion on posters of each subprogramme).

- General discussion stimulated by Robert J. Hamer, Johan J. Plijter and Norberto E. Pogna.

- General conclusions by J.C. Autran.

Agenda 1992: "Important dates to recall"

- *11-12 March*: Two-day conference and exhibition on EC agro-industrial and forestry research programmes ECLAIR, FLAIR and FOREST in Brussels
- *9-10 April*: 3rd Meeting of subprogramme A in Valencia (Spain)
- *23-24 April*: 4th Meeting of subprogramme B + Meeting of the Scientific Management Committee (23 April, morning) in Viterbo (Italy)
- *25-26 May*: 4th Meeting of subprogramme C in Cadiz (Spain) and Elvas (Portugal)
- *4 June*: General Meeting of the programme in Paris (the day after the 9th International Cereal and Bread Congress, Hotel Méridien Montparnasse), restricted to the ECLAIR participants
- *15 June*: Deadline for sending the individual contributions for the Newsletter n° 2 (report on January-June 1992 activities)
- *June-July* (date not appointed): Technical meeting on rheology
- *November* (date non appointed): 4th Meeting of subprogramme A in Caserta (Naples, Italy)
- *Spring 1993*: 5th Meeting of subprogramme A in Berlin.

SUBPROGRAMME A: INDUSTRIAL PROCESSES

***Robert J. Hamer*, Subprogramme Manager**
(TNO, Zeist, The Netherlands)

Review of Activities

In the first year of the programme a striking progress has been made. The first period (up to June) can be characterized by the fact that programmes had to be started at the different laboratories. At the first meeting of the subprogramme in Wageningen much emphasis was put on this respect. A series of resolutions was passed concerning setting up a common methods book, and common lists of available and wanted samples. At the second meeting in Chorleywood it became apparent that the research was on the way. The emphasis of this meeting was on the discussion of the actual progress made. Through this process numerous possibilities became apparent to intensify collaborations and make use of the expertise and knowledge of participating laboratories. Also, it became clear that next to the integration within subprogramme A also contacts and cooperation with subprogramme C was significantly improved. At the Chorleywood meeting several resolutions were accepted to improve collaboration and to benefit from the unique combination of laboratories and technologies within the programme. As an example it was decided to select a set of three wheat samples which will be analysed and technologically evaluated in all participating laboratories in 1992. Also participants were stimulated to jointly apply for mobility grants or other bursaries in order to further improve knowledge transfer and collaboration. Finally, it was decided to organize a next meeting in April 9-10, 1992 in Valencia. This meeting will be hosted by IATA.

Review of Projects.

The milling quality project is carried by **FMBRA** and **TNO**. Work has been performed using joint sets of samples. Tasks have been clearly defined. Work at FMBRA has focused for a great deal on developing suitable image analysis techniques. Their present status looks promising in terms of their use to characterize populations of wheat kernels. In terms of predicting milling quality singly on morphometric parameters optimism is fading. In this respect however the value of the work at TNO becomes apparent. Here, milling quality has been regarded as a too complex parameter from the start. Alternative parameters have been developed which as a set can describe milling quality. These parameters are measured using both biochemical and image analysis techniques. Development of these techniques has progressed satisfactorily. Linking these parameters to the image analysis results of FMBRA is possible and may yield the interesting relations looked for.

The work on gluten starch processing at **TUB** is steadily progressing. A just decision was made not to build the originally planned pilot plant, but instead improve the present laboratory scale system. At present some gluten samples have been already produced and characterized. Work is progressing well and will continue with finalizing the laboratory system and -in collaboration with other laboratories- in depth characterization of the gluten samples produced. Work at TNO focuses on the mechanism of gluten coagulation. Although some setbacks had to be overcome a micro (10-12 gram of flour) separation system was successfully developed and adapted to study the effect of hemicellulases. The action of these enzymes was studied in more detail using isolated gel-proteins.

This revealed small proteolytic side-activities the relevance of which will be clarified in the next year. Work progresses well.

Work at **FMBRA** on wholemeal and white bread showed strong parallels. For this reason both projects were merged. Extensive studies varying work input and mixing speed revealed strong differences between flours for white-bread making and hardly any differences between flours for wholemeal breads. With white bread biochemical studied revealed the importance of glutenin polymers. In wholemeal breadmaking protein quantity seems to exceed protein quality in importance. In this respect it is interesting to note that the variety FRESCO which is clearly unsuitable for white breadmaking, is suitable as a carrier wheat for wholemeal breadmaking. The present work is somewhat ahead of schedule and already had improved our understanding of technological requirements of wheat.

Work at **BSN** has started well and already yields some first preliminary results on wheat characters related to suitability for biscuit manufacture. Some of the results are confirmatory in nature others are not, but await further corroboration. The work carried out looks sound is progressing satisfactorily.

The work by **TNO** on flour blends is aimed to establish relations between biochemical and technological characters. Their finding that gel-proteins in dough relate very well with dough rheological properties is a key result. The reason for this is that this relation seems independent of variety or harvesting year. The same is not true for a relation between gel-proteins in flour and rheological parameters. A study into factors explaining this phenomena has already yielded important findings: the type and amount of HMW glutenin polymers is important and the amount of LMW proteins present. The work is original and proceeds very well.

Work at **SME Ricerche** concentrated on developing a small scale rheological test for flours. A test using flour suspensions was developed and optimized. Initial results need to be tested and compared with results obtained with doughs. Following this a decision needs to be made on the final system to be used. Work is two to three months behind schedule.

At **IATA** work has started somewhat later. At present a large and well conceived experimental plan is underway, which will be finished by March 1992. The approach and its first results have been presented. The approach looks sound and the results are promising. The project runs satisfactorily.

Main Conclusions.

In general the programme has met some delay because of administrative reasons. At this time all participants have started work and the respective projects are well underway. After some preparatory the progress report already contains some very interesting results (i.e. results with wholemeal and white bread at FMBRA and with flour blends at TNO). Other work looks promising (biscuit studies at BSN, sour doughs at IATA). The participants demonstrate a great sense of collaboration, demonstrated by the two successful meetings, the joint activities (methods book, sample sets), and the collaboration with subprogrammes B and C. In the next future collaboration will increase even further enabling even more coherence in the programme and promoting success.

Individual Progress Reports

Task A.1.1 - Milling Quality

Partner 14 - FMBRA (Flour Milling and Baking Research Association), Chorleywood, Rickmansworth, Hertfordshire, WD3 5SH, U.K.

Progress report from September 1, 1990 to November 15, 1991

1. Key measures of achievement - Objectives

Predicting milling quality of wheat samples from morphological characteristics of representative grains. Milling assessment to be completed by end of year 2. Image analysis development and evaluation to be completed by end of year 2.

2. Progress

Milling

a. Laboratory Bühler millings have been made on sample sets from the 1989 and 1990 harvests. The 1990 set comprised wheats contributed by ECLAIR partners, grown in Holland and France as well as wheats grown in the UK. Aliquots of the same wheats were exchanged for milling on laboratory Bühler mills at TNO as agreed. Ranges of extraction rates for the Chorleywood millings were:

    Harvest year 1989: 64.4-77.5 % (n = 32)
    Harvest year 1990: 65.8-79.0 % (n = 28)
    (Extraction rates expressed on the basis of % of products recovered).

b. Samples affected by the disease "take-all" to varying degrees, have been received from Rothamsted Experimental Station as forecast at the March meeting. The samples exhibit different degrees of shrivelling. They await milling and further tests.

Image analysis.

a. Image analysis measurements made on 'representative' grains from the 1989 harvest samples, presented for imaging in top-view , side-view and as sagitally bisected profiles failed to provide measurements that could be used to predict extraction rates. Even when parameters were considered together the relationships with extraction rate that emerged were not consistent with comparable analysis carried out under a previous project. Statistical analysis suggested that the number of grains taken to represent samples (<100) were inadequate, and since substantial increase in such a time consuming method could not be considered attention has been turned to presentation techniques that permit measurement on many grains simultaneously.

Some success has been achieved in reproducibly defining size distributions of grains and the value of several methods employed have been examined in relation to technologically important properties of bulk samples. Populations can be defined by as simple a measurement as the integrated projected area of a given mass of grains scattered on to a back-lit screen. Thusfar 10 g of each class of sieved grains:

Size range	Total projected area (arbitrary units)
< 2.5 mm	47.3
2.5 - 3.35 mm	40.4
3.35 - 4 mm	35.2
> 4 mm	32.5

Mixtures of grains in 25, 50 or 75 % proportions of the different classes produced linear relationships between proportions and total projected area.

b. More detailed information on population characteristics have been obtained using image processing algorithms. Beginning with samples scattered as described above the processing permits grains to be measured individually even when touching others - a condition that would normally lead to measurement of a composite of touching grains.

When 26 home grown samples were presented the mean of actual numbers of grains in each were 216 ± 17. Estimates based on image analysis data were 217 ± 17. Size distributions into 10 size categories between 0 - 30 mm^2 projected area intervals were reproducible. Further improvements are currently under development.

3. Conclusions

Developments in using image analysis to describe population characteristics are promising. If current improvements are completed they will be applied to the 1990 harvest wheats.

Grain dissection data presented earlier suggested that extraction rate was not closely related to large variations in endosperm content. It is suggested that morphometric parameters will not enable a complete prediction of the complex parameter milling quality. Integration with the work performed at TNO where the complex parameter milling quality is split into several milling related parameters (e.g. endosperm content, bran friability) and where other parameters are measured, is important at this stage.

Partner 17 - TNO (TNO-CIVO), Utrechtseweg 48, Post Office Box 360, 3700 AJ Zeist, The Netherlands.

Progress report from January 1, 1991 to November 15, 1991

1. Key measures of achievement - Objectives

First years milling assessment on 30 wheat varieties and development of image analysis system for estimates of endosperm content. (objective). Milling results and image analysis procedure. Development of markers for milling factors and evaluation of possible use (deliverables).

2. Progress

A wheat sample collection has been established including Dutch, French and British wheat varieties with a wide variation in milling quality. Samples are obtained from the partners 05 and 14. The sample set is available for both partners in the task (14 and 17). Partners have made an inventory of methods for milling and kernel quality assessments carried out at both laboratories. It was agreed that the two laboratories would perform the milling according to their own method, because of great differences in the milling objectives. This also opens the opportunity to assess the

interaction between varieties and milling method. The wheat samples have been milled and the quality in terms of ash content of flour fractions has been determined. Results show that a wide range in milling quality is available which indicates good opportunities for correlation studies. Kernel assessments at laboratory 17 have been performed. This includes the assessment of kernel length, height and breadth, thousand kernel weight, hectolitre weight, kernel hardness, protein content and falling number. Kernel volume and germ content assessments are still underway. A preliminary statistical evaluation did not reveal high linear correlations between milling quality and kernel features, which is to be expected. Better relations will be detected when milling quality is described in terms of milling factors (bran friability, endosperm content, endosperm separation).

The possibility of using ferulic acid as a marker for bran (to calculate the bran friability) has been investigated. It seems that due to differences in extractability the assessment of ferulic acid in very bran rich fractions is not possible. An arbitrary measure of bran friability can however be obtained by comparing flours at different extraction rates. **Figure 1** shows the relation of ash content and ferulic acid content to the cumulative flour yield. At high extraction rates a strong deviation between ash content and ferulic acid content is detected. This indicates that ferulic acid is a far better marker for bran friability than ash. Bran friability could be calculated from the difference in ferulic acid content of pure endosperm and flour fractions. Determinations of ferulic acid in endosperm are currently being performed.

The determination of the endosperm content is carried out by hand dissections (partner 14) and by image analysis (partner 17). A presentation technique for the estimate of endosperm content in the kernel has been developed. With this

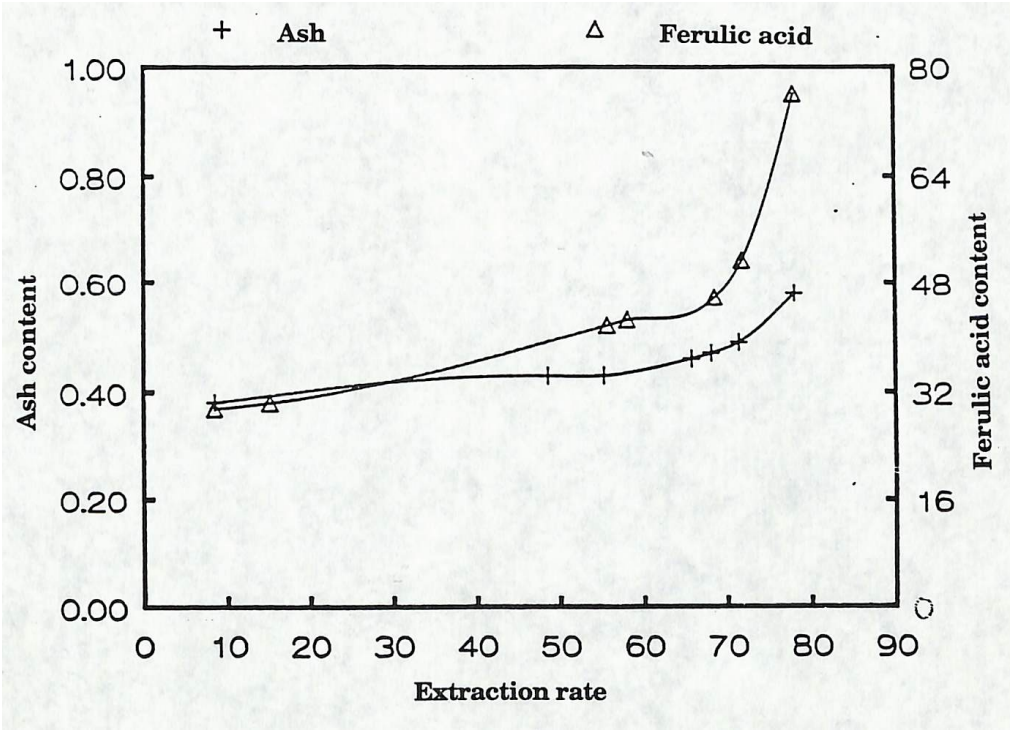


Fig. 1: Ash curve and ferulic acid curve

technique it is possible to make macroscopic images of transsections of the kernel. Kernels are embedded in synthetic resin and transsections are made by successive abrasions. After staining with iodine an image of the transsection is made and the area covered by the endosperm is calculated by image analysing. For this purpose a special program is developed. Successive transsections are made at a precise distance. The endosperm content can be calculated by integrating the endosperm areas over the transsections.

3. Conclusions

The project is proceeding according to schedule, except for the evaluation of the use of ferulic acid as marker for bran, which is a little behind schedule. This evaluation involves a large number of assessments, which are scheduled to be finished in the beginning of year 2.

Task A.1.2.1 - Improved Separation of Gluten and Starch through the Use of Enzymes

Partner 17 - TNO (TNO-CIVO), Utrechtseweg 48, Post Office Box 360, 3700 AJ Zeist, The Netherlands

Progress report from January 1, 1991 to November 15, 1991

1. Key measures of achievement - Objectives

Obtaining a better understanding of the mechanism of action of hemicellulases in starch-gluten separation and evaluate the use of enzymes as processing aids. Objective: small scale separations.

2. Progress

The work started with the development of a simplified model for gluten coagulation. The system is operated at a 10-12 gram scale. Results regarding gluten yield and starch yield correlate well with the 5 kg pilot scale system. When comparing however the action of enzymes in both systems differences were observed. The small scale protocol was successfully adapted to allow measurement of enzyme action. Characteristically, higher enzyme levels are needed in the small scale system. This is exemplified in **Figure 2** where results of enzyme addition in terms of gluten yield are compared in both systems.

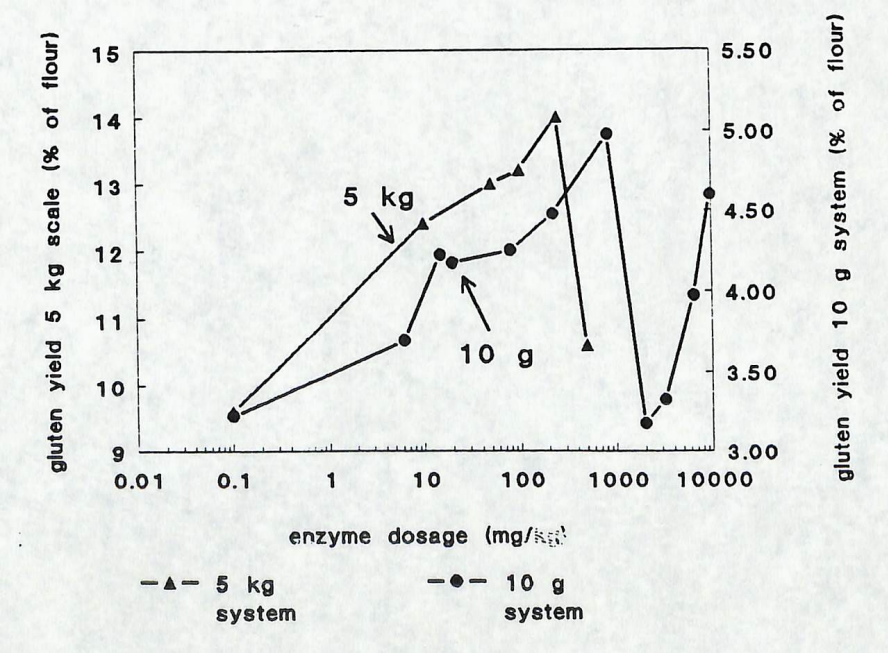


Fig. 2: Comparison of effect of hemicellulase on gluten yield in 10 g and 5 kg system

An important aspect of this work is to understand the mechanism of action of these enzymes on a biochemical level. Studies performed with the 5 kg system showed a typical decrease of pentosans associated with the high molecular weight glutenin polymers insoluble in SDS (so-called gel-protein). A similar decrease was observed with the 10 gram system although less pronounced. To enable a clear observation of this phenomena gel-protein was isolated on a larger scale and used as a substrate for a set of 5 commercial hemicellulases. Incubation led at all times to a decrease in

associated pentosans, but also to generation of free amino-groups which is indicative of proteolysis. Following this observation, hemicellulases were selected which are devoid of proteolytic activity according to the assay with gel-protein. These enzymes await testing at both 10 and 5 kg scale at present. From these results a conclusion can be made on the potential role of proteases in gluten coagulation.

3. Conclusions

The first objective: carrying out of small scale separations is nearly completed. Delays in this respect are negligible. The work with isolated gel-protein clearly has improved our ability to distinguish hemicellulolytic effects from proteolytic effects.

Task A.1.2.2 - Characterization of Wheat Gluten Produced by New Separation Processes

Partner 13 - TUB (Technische Universität Berlin), Institut für Lebensmittel-technologie Getreidetechnologie, Seest. 11, 1000 Berlin 65, Germany

Progress report from June 16, 1991 to November 14, 1991

1. Key measures of achievement - Objectives

Investigation of the causes for the differences in the main rheological characteristics of gluten extracted from whole meal flour compared to gluten washed out of white flour (Objective); Laboratory scale separations, compositional characters, qualities and physico-chemical characters of gluten fractions prepared from white and wholemeal flours (Deliverables).

2. Progress

Studies on the production of starch, gluten and by-products extracted from white and wholemeal flours have been carried out applying a new processing technique. As exemplified in the first Newsletter a laboratory system was used for these experiments instead of a pilot system, which was proposed to be built. The laboratory system has the advantage that it can be run quasi continuously on a comparatively small mass flow of about 2 kg raw material per hour. The suggested reconstruction of the laboratory system using a miniaturized decanter centrifuge, in order to reduce the residence time of the mass in the system, could not be completed. This was mainly due to problems arising from difficulties in the manufacture of the decanter's screw. But this did not hinder us to carry out first comparative investigations with the otherwise partially modified laboratory system.

The new processing technique is characterized predominantely by an initial step in which a wholemeal flour suspension is wet sieved prior to gluten agglomeration. This process step is connected with an extraction of soluble substances from the flour by recirculating parts of the process water to suspend the flour. The insoluble particles of the sieved out endosperm fraction are concentrated in the laboratory system by discontinuous centrifugation.

The sediment gained in the centrifuge's bowl was removed as a dough-like mass. In this mass the gluten proteins had already agglomerated to a certain extend. In order to keep a fully developed dough, the mass was either thinned with water in a mixer or directly kneaded by hand. The thinning of the mass is combined with a further agglomeration of the gluten proteins by mixing. By the way a pumpable dough was formed which was separated into its constituents by centrifugation. The fractions were characterized according to their chemical composition and were also used to balance the mass distribution. The kneaded doughs were used to determine their rheological properties.

It could be shown that the consistency of the doughs extracted from wholemeal flour (impact-milled grains: Ultrarotor) was far softer than that of the doughs extracted from white flour (conventionally milled: Bühler Mahlautomat; flour extraction rate 73,2%; ash content 0,61%). This could be proved by extensigramme measurements. Moreover it became obvious that the tensile strength of the doughs from wholemeal flour decreased more rapidly than that of the doughs from white flour. The changes of the dough properties were connected to the enzymic activities occurring under the process conditions. The effect of the enzymes could be proven by experiments in which their activity, present in the process water, was partially inhibited by the addition of 0,01% $HgCl_2$. Doughs treated in that way showed a lower extensibility and a far higher resistance than doughs which had been in contact with untreated process water.

Furthermore, it is of interest to note that the differences in the viscous properties of the doughs did not effect the yield of gluten. From this it can be deduced that even the white flour with an extraction rate of only 73% already contained all protein fractions which participate in gluten agglomeration. The same did not apply to the starch as only in the case of wholemeal flour almost 100% of the grains' starch ended up in the extraction process. Only 3,5% of the starch were found in the fibre-fraction separated by wet sieving, whereas in case of dry sieving the bran contained 12,1% of the grain's starch. Therefore, a far higher yield of starch was obtained when processing wholemeal flour than white flour.

The gluten samples obtained, did neither differ substantially in overall chemical nor in amino acid compositions (**Table I**). The differences observed in the contents of lipids, ash and insoluble dietary fibre were in the range of composition of commercial gluten samples. There was only one characteristic property which exceeded the variation given by the range of characteristic criteria. This was the gluten samples' colour. The gluten samples from wholemeal flour had a pronounced darker colour than that from white flour, the latter being even darker than the commercial samples.

3. Conclusions

It could be shown that the viscous properties of the doughs derived from the laboratory system from wholemeal and white flours differed remarkably. The differences were in part caused by enzymic activities which occurred during process run. The enzymic activities did not effect the gluten yield from both types of flour. Compared to the range of chemical composition of commercial gluten samples, the samples gained from the laboratory system fell well into this pattern.

Table I: Comparison between commercially available wheat gluten samples and wheat gluten samples extracted from white and from wholemeal flours using the laboratory system.

Parameter	Commercial gluten[1]		Laboratory gluten		
	Range	Average	White flour	Whole-meal flour	Wholemeal flour + $HgCl_2$
Moisture (%)	5.5 - 9.4	7.4	2.44	1.46	1.49
Ash (%)	0.59 - 1.20	0.82	0.77	1.12	1.17
Protein (%; N=6.25)	78.7 - 89.7	85.1	77.9	83.3	83.3
Starch (%)	2.7 - 15.0	10.4	15.3	12.1	12.0
Pentosan (%)	0.6 - 1.18	0.9	n.d.	n.d.	n.d.
Dietary fibre (AOAC)	n.d.	n.d.			
water insol. (%)			1.5	3.24	3.22
water sol. (%)			3.45	3.14	3.61
total (%)			4.95	6.38	6.83
Lipid					
free (%)	0.51 - 2.71	1.18	0.9	1.69	1.37
bound (%)	4.8 - 6.9	6.07	3.21	4.52	4.22
bound:free	3.6 - 9.4	6.63	3.6	2.7	3.1
pH	5.93 - 6.39	6.19	6.05	6.08	6.07
Colour (Lab-method)					
Light intensity	89.1 - 90.6	90	85.3	81.4	80.8
a-value	-0.79 - -0.87	-0.8	-0.11	0.99	0.84
b-value	13.3 - 13.7	13.5	13.32	14.62	11.87

1 Figures are partially taken from the literature (W. Bushuk and C. Wasnawan (1989); H. Boring and D. Weipert (1984)) to which own results were added

From this it follows that we have to improve and extend the methods of analytical characterization of gluten samples and doughs in order to be able to describe properly deviations in the viscous behaviour of the doughs, respectively the glutens, taking into consideration all essential processing steps of the new separation process.

Tasks A.2.1. and A.2.2 - The Characteristics and Processing Requirements of Wheat for Specific End Uses: White Bread and Wholemeal Bread

Partner 14 - FMBRA (Flour Milling and Baking Research Association), Chorleywood, Rickmansworth, Hertfordshire, WD3 5SH, U.K.

Progress report to September 30, 1991.

Introduction

The white bread project has shown that the optimum work input of single variety flours varies between 5 and 17 Wh/kg. This wide range of work inputs has made it necessary to reconsider the design of the sister wholemeal project, in which both white and wholemeal performances were being compared at a single work input of 11 Wh/kg. of dough (40 kJ/kg). At a constant work input the breadmaking of some white flours would be restricted, affecting prediction of wholemeal performance. To improve the comparison with wholemeal it was first necessary to establish the optimum work input of both types of flour, further integrating the two projects. The progress described in this report therefore combines the tests carried out in both projects.

1. Key measures of achievement - Objectives

In accordance with the principles laid down in the technical annex, the work carried out to date has addressed the following objectives:

1. The influence of single wheat variety on the bulk fermentation time requirements in traditional breadmaking process for white bread.

2. The influence of the rate of work input on optimum mixing requirement in the Chorleywood Bread Process (CBP) in white and wholemeal breadmaking, and to establish the ability of white breadmaking to predict wholemeal breadmaking performance.

3. To seek correlations between the rheological characteristics of white and wholemeal doughs with work input requirements and baking performance.

4. To establish the influence of work input and its rate on the breakdown of the glutenin fraction of wheat protein.

2. Progress

2.1 Experimental detail

Using traditional breadmaking process three single varieties, Hereward and Mercia (breadmaking) and Haven (weak) and Canadian Western Red Spring wheats (CWRS breadmaking) were compared.

Using the CBP, four varieties, Fresco ('extra strong'), Hereward, Mercia (breadmaking) and Riband (weak), were tested in white and wholemeal. Each variety was mixed to five work inputs at mixing speeds from 250 to 600 rev/min.

The work input ranges chosen were 8 to 20 Wh/kg for Fresco and Hereward, 5 to 17 for Mercia and 3 to 14 for Riband. These ranges were based on the white bread optimum found previously for these varieties.

Rheological tests were conducted on the same doughs as used for breadmaking, sampled immediately after mixing. Sampled doughs were subjected to a rapidly applied small strain which was then held constant as the decay in stress in the bulk phase of the dough, was monitored.

Glutenin (gel-protein) was determined on samples of dough after mixing, after final moulding, 20 minutes into, and at the end of final proof.

2.2 Results

a. White bread

When using traditional breadmaking processes and bulk dough fermentation, there was no clear distinction in fermentation time requirements between the varieties.

Wheat variety performance in CBP was affected by both the level and rate of work input during mixing, with distinct varietal differences.

For Fresco and Hereward loaf properties could be improved either with increased work input or its rate and most markedly with a combination of them both. Fresco gave poor breadmaking performance at low work-input levels and achieved its greatest potential at the highest work input and mixing speed tested (20 Wh/kg at 600 rev/min). Hereward had much greater tolerance to mixing conditions. It performed well at low work-input levels but achieved its best at 20 Wh/kg and 00 rev/min.

In contrast, Mercia (a successful breadmaking variety in the UK) showed that there was no advantage to be gained by raising work input beyond 11 Wh/kg or increasing mixing speed above 300 rev/min.

The weak variety Riband showed a very modest response in breadmaking performance to increased work input and mixing speeds up to 11 Wh/kg at 300 rev/min. Surprisingly, there was no evidence of deterioration in performance of Riband when using the extreme mixing conditions of 14 Wh/kg and 600 rev/min, (the highest tested).

b. Wholemeal

Loaf volumes were unaffected by increases in work input and mixing speed. Only with the weak variety Riband was there any evidence that work input levels greater than 11 Wh/kg were deleterious.

c. Comparison of white and wholemeal

The relative white and wholemeal breadmaking quality of the varieties tested is indicated by the mean 400 g loaf volumes in the table below.

	Loaf volume (ml)			
	White		Wholemeal	
	Mean	Range	Mean	Range
Fresco	1573	1368 to 1734	1327	1257 to 1371
Hereward	1759	1646 to 1935	1357	1309 to 1414
Mercia	1591	1499 to 1649	1201	1129 to 1269
Riband	1426	1366 to 1477	1037	967 to 1107

It can be seen from the table, that the overall mean wholemeal loaf volumes did not always reflect those of white flour. When individual mixing conditions were compared, the prediction of wholemeal breadmaking performance from white was even less reliable.

The contrast between the performance of Fresco in white and wholemeal at the extremes of the mixing conditions is shown pictorially in **Figure 3**.

d. Rheology

For each dough (white or wholemeal) a straight line was fitted to the data corresponding to the initial rapid decrease in stress. The gradient of the line gives a measure of the viscoelasticity of the dough and its ability to store energy. The doughs that gave the lowest loaf volumes were found to have the fastest relaxation rate. This indicates that the molecular structure in these doughs broke down more rapidly than that found in doughs producing bread with high loaf volume. The breakdown of this structure may be related to the ability of a dough to undergo successful expansion during proof and baking.

This relation, of a fundamental rheological parameter with loaf volume, which is derived from both white and wholemeal doughs, suggests that there are rheological properties in doughs that can be used to predict loaf volume and which respond to changes in variety and bran contamination in the same way as loaf volume.

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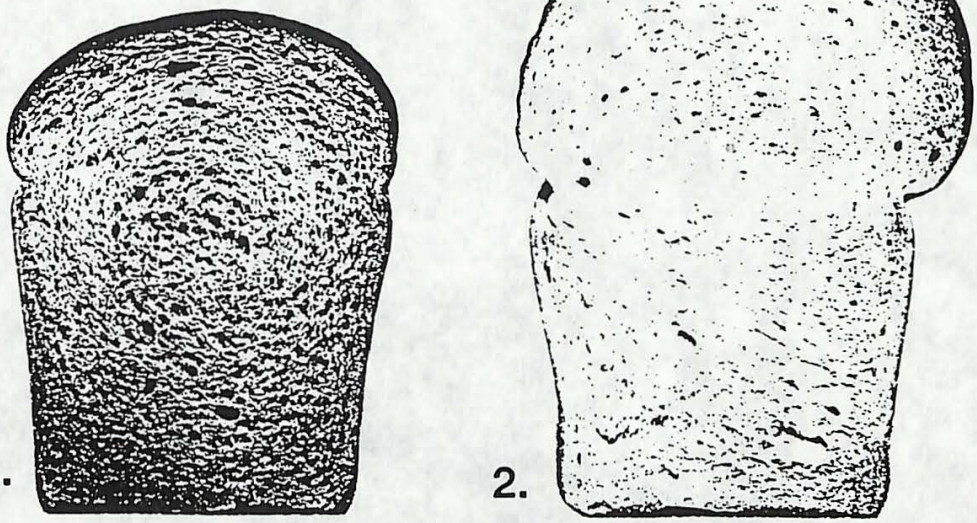
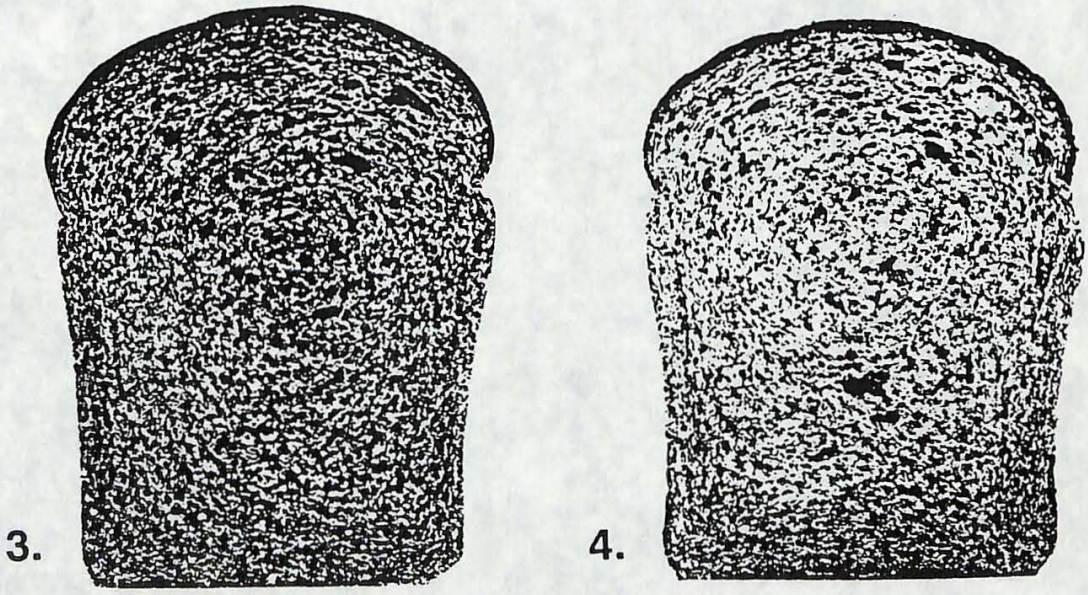
FRESCO



	RPM	Wh/kg	Volume	Score
1. White	250	8	1368	4
2. White	600	20	1723	9



	RPM	Wh/kg	Volume	Score
3. Wholemeal	250	8	1320	8
4. Wholemeal	600	20	1307	7

Fig. 3: Contrast between the performance of Fresco in white and wholemeal at the extremes of the mixing conditions.

e. Gel-protein

With the exception of Fresco white flour the gel-protein data showed that the glutenin fraction of wheat protein was depolymerised at all work inputs and mixing speed combinations and it did not reaggregate during final proof. The glutenin fraction of Fresco white flour was not totally depolymerised at 8 and 11 Wh/kg and, furthermore, did reaggregate during proof. This characteristic is typical of "extra strong" varieties and is evidence that there are factors, as yet unexplained, that allow for re-aggregation of glutenin after mixing.

3. Conclusions

The work carried out so far on wheat varieties has indicated that there are two important criteria, mixing requirements and breadmaking performance, which may be exclusive. For example, Fresco white required very high work input for best results, indicating a protein that was difficult to develop. That characteristic did not necessarily guarantee a superior performance in breadmaking, particularly in wholemeal. Wholemeal would normally be made with a high protein flour (CWRS or European, fortified with added gluten). The "extra strong" glutenin of Fresco did not automatically confer better wholemeal performance. The importance of protein content in the production of wholemeal bread will feature in future studies under this ECLAIR task. The addition of dry gluten to a number of varieties will be investigated together with the influence of endosperm texture (hard or soft varieties) on the production of wholemeal bread. The white bread aspects will include new UK and continental varieties.

Task A.2.3 - Evaluation of Technological Functionality of Wheat Flours and Protein Fractions in Baked Products.

Partner 08 - BSN Branche Biscuits, Centre International de Recherches Jean Theves, 6 rue Edouard Vaillant, 91200 Athis Mons, France.

Progress report from April 1, 1991 to November 15, 1991

1. Key measures of achievement - Objectives

To define factors that affect biscuit making quality of flours.

2. Progress

In order to establish a data base including analytical characteristics and technological results, 13 flours have been selected to cover a large range of variation. Six flours came from milling of French varieties, 5 flours , poor in protein, were prepared from these flours by air classification, 2 came from different streams of milling of blended varieties.

All 13 flours have been extensively characterized physico-chemically. The chemical analyses comprised humidity, ash, protein, damaged starch (% Audidier/ dm), total pentosans and soluble pentosans. Amylase activities were determined according to Hagberg. Flour particle size characteristics were measured using laser

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Table II: Summary of the data obtained in the definition of the factors that affect biscuit making quality of flours.

PETIT BEURRE

ANALYTICAL PARAM.	PROD. ASSESSMENT
HUMIDITY	
ASH	
PROTEINS	STICKINESS LENGHT SURFACE FRIABILITY WEIGHT
DAMAGED STARCH	
PENTOSANES	STICKINESS FRIABILITY HARDNESS
HAGBERG	
MEDIAN DIAMETER	?
WATER ABSORPTION	STICKINESS FRIABILITY SURFACE WEIGHT
STABILITY	HARDNESS ?
W	STICKINESS LENGHT SURFACE FRIABILITY WEIGHT HARDNESS
P	STICKINESS FRIABILITY SURFACE WEIGHT
G	?
P/L	

WEIGHT : NEGATIVE RELATION

WEIGHT : POSITIVE RELATION

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Table III: Preliminary results of data analysis based on 13 experimental flours (CV: coefficient of variation).

PETIT BEURRE

FLOUR TESTS		
PARAMETER	MEAN	CV
COMPOSITION		
WATER	13.5	13
ASH	0.6	11
PROTEIN	8.9	20
DAMAGED STARCH	18	10
PENTOSANES	2	15
AMYLASIC ACTIVITY		
HAGBERG	362	15
PARTICLE SIZE		
MEDIAN DIAMETER	31	28
FARINOGRAPH		
WATER ABSORPTION	53.6	6
STABILITY	3.75	56
ALVEOGRAPH		
W	104	63
P	48	40
G	19	19
P/L	0.7	39

PROD. ASSESSMENT		
BAKING TESTS	LABO 2 KG CV	PILOT 20 KG CV
DOUGH		
STICKINESS	92	72
BISCUIT		
WEIGHT	8	3
LENGHT	4.5	4
WIDTH	1.5	1
HEIGHT	6	6
HARDNESS	21	17
FRIABILITY	13	15
SURFACE ASP.	78	65

granulometry. Rheological measurements comprised water absorption and stability by Farinograph measurements. W, P, G, P/L values were determined by Alveograph testing.

All flours were technologically evaluated using the 'Petit Beurre' biscuit baking test as described in the methods book. Tests were made both on a laboratory scale (2 kg of dough) and on a pilot scale (20 kg of dough).

A summary of the data obtained is given in **Table II.** The data were analysed by principal component analysis and regression. **Table III** gives some preliminary results in highlighting tendencies. Since the number of flours (13) is still very limited these results should be viewed with caution. Nevertheless some preliminary conclusions can already be made. Analytical parameters like humidity, ash, damaged starch, Hagberg falling number, and flour particle size characteristics seem not to be correlated with any technological quality parameters. These results need to be confirmed. On the other hand, protein quantity is positively related to weight and biscuits width and negatively with dough stickiness and length, surface and friability of biscuits. The Alveograph *W* value exhibits the same correlation with these parameters. As a new finding, the quantity of total pentosans could explain dough stickiness and biscuit texture.

3. Conclusions

Work is progressing well. First results have been generated, but require corroboration by future extensions of the data set. On preliminary finding concerns the importance of pentosans. This new and intriguing result needs further corroboration.

Task A.2.4 - Processing Properties of Flour Blends, Prediction and Improvement

Partner 17 - TNO (TNO-CIVO), Utrechtseweg 48, Post Office Box 360, 3700 AJ Zeist, The Netherlands.

Progress report from January 1, 1991 to November 15, 1991.

1. Key measures of achievement - Objectives

Improvement of insight in the relation between gluten composition and dough functional characteristics in order to predict and improve the processing properties of EC flour blends and to compensate for year-to-year variation in flour quality.

2. Progress

Wheat was milled from three varieties commonly used in blending of flours. Wheat from two harvest years was used (1989 and 1990). The rheological and biochemical properties were determined both on flours and blends. With the results obtained with flours from both harvest years statistical relations were explored between dough rheological properties and biochemical properties (in particular the amount of SDS insoluble glutenin polymers, called gel-protein). **Figure 4** demonstrates a very good relation between the Extensigraphs resistance value and the gel-protein content in the flour blend. However for nearly every series of blends or even for two series of blends from two varieties and two harvesting years separate relations exist. Clearly this relation is both variety and year dependent.

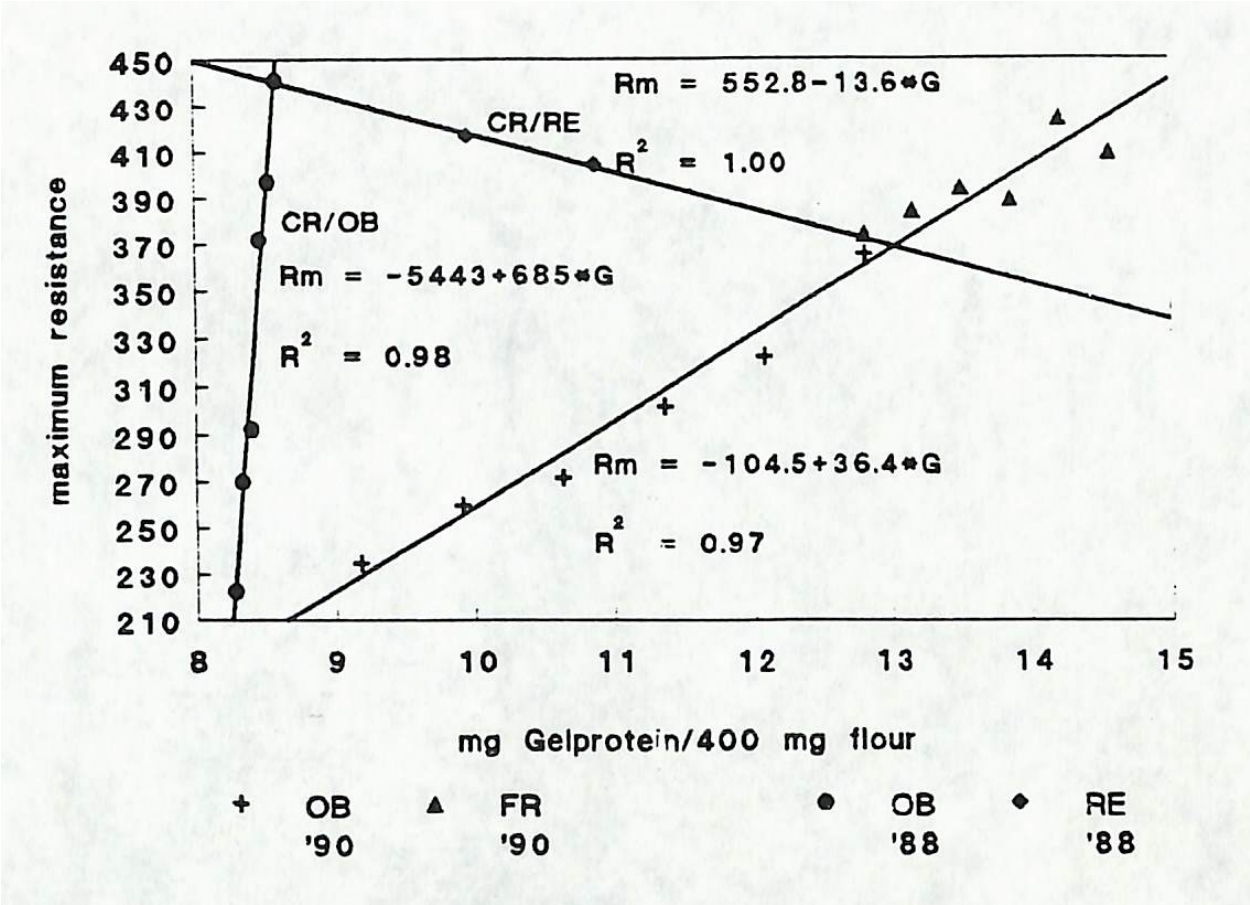

Fig. 4: Gel-protein flour *vs* Rm after blending Obelisk/Fresko/Rektor with Camp Rémy.

The next observation is in this respect very important. In **Figure 5**, the gel-protein content of the dough is graphed against the maximal resistance value. Now, a single relationship appears. This very important finding demonstrates the role of glutenin polymers in determining dough rheological properties. Also, it becomes evident that the key to understanding the effects of blending lies in the transformation of flour glutenin polymers to dough glutenin polymers.

Work therefore concentrated on other flour constituents affecting this transformation. LMW wheat proteins were isolated by sequential precipitation and added to base flours in concentration up to 0.8 %. The three base flours were mixed for different times and the gel-protein content was determined directly or after a rest period of 45 min. In all cases addition of LMW proteins led to an increased breakdown and a decreased reassembly of gel-proteins, although the extent of the effect was clearly dependent on the initial dough strength (read: amount and type of HMW glutenins). The variety containing HMW glutenin-A subunits '5+10' was more 'stable' in this respect than the two other varieties containing HMW glutenin-A subunits '2+12'. Also, lower levels of LMW protein additions were tested. Addition of 0.2 % of LMW protein did not influence the rate of gel-protein breakdown during mixing, but retarded to a large extent gel-protein reassembly during resting.

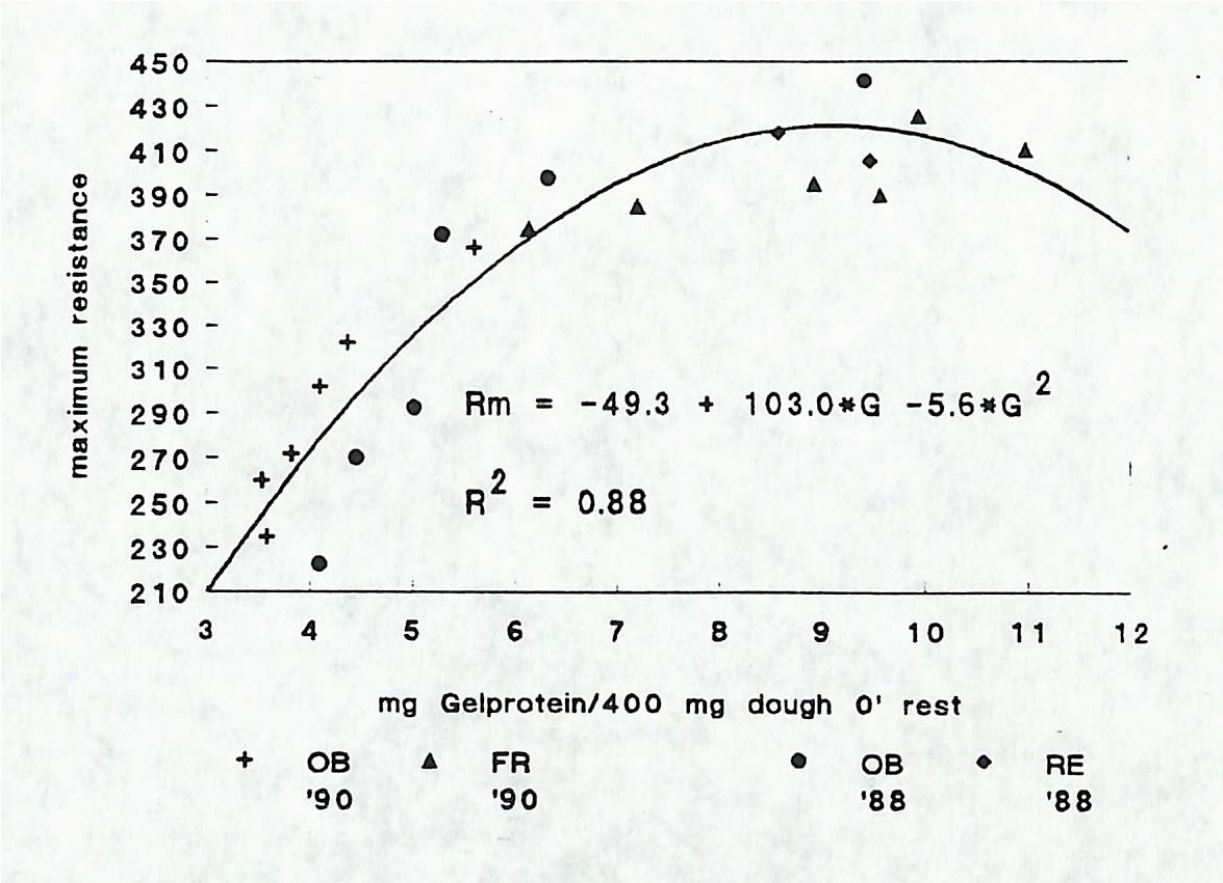

Fig. 5: Gel-protein dough 0' *vs* Rm after blending Obelisk/Fresko/Rektor with Camp Rémy.

The mechanism of action of LMW proteins was further explored using biochemical techniques. Since LMW proteins acted to a certain extent similar as cystein, the role of SH groups was studied. Blocking of the SH groups in LMW proteins eliminated their action on glutenin breakdown and reassembly. Two-dimensional electrophoresis followed by immunochemical detection of LMW proteins demonstrated that part of the LMW proteins can become covalently attached to the glutenin polymers. These results suggest that LMW proteins can prevent glutenin polymerization by interacting with glutenin SH groups generated during mixing.

3. Conclusions

A good statistical relation was found between rheological and biochemical characters of doughs. This relation appears -in contrast to a similar relation with flours- independent of flour type or harvesting year. Research therefore has to focus on factors influencing glutenin polymerization during dough preparation. In this respect next to the type and quantity of glutenins a possible role of LMW proteins is identified. The project is on schedule and proceeds very well.

Task A.2.5 - Rheological Characterization of Wheat Samples and Identification of Specific Processing Requirements Related to Sweet Bakery Products and Products with Sour Starters

Partner 04 - SME Ricerche, Località "La Fagianeria", 81015 Piana di Monte Verna (Caserta), Italy.

Progress report from April 1, 1991 to November 15, 1991.

1. Key measures of achievement - Objectives

Rheological measurements in order to obtain a better understanding of the properties of wheat; (Objective). Small scale tests (Deliverable). Completion March 1992.

2. Progress

This report covers rotational and oscillation measurements of a soft flour slurry at a concentration of 45% dry weight.

A standard method for the preparation of the slurry has been developed and the influence of gap and resting time prior to testing on the viscosity have been studied. From the results, it has been decided to set the resting time at 5 min, to use two different gaps (1 and 1.5 min) and to place around each sample damp cotton. Afterwards, rotational measurements varying shear rate and oscillation measurements have been performed. The temperature of all the tests was set at 20°C.

3. Results and conclusions

The rotational data were processed according to the power law. The behaviour of shear stress in function of shear rate has been reported in **Figure 6**. No significant differences between the results of the two gaps were observed.

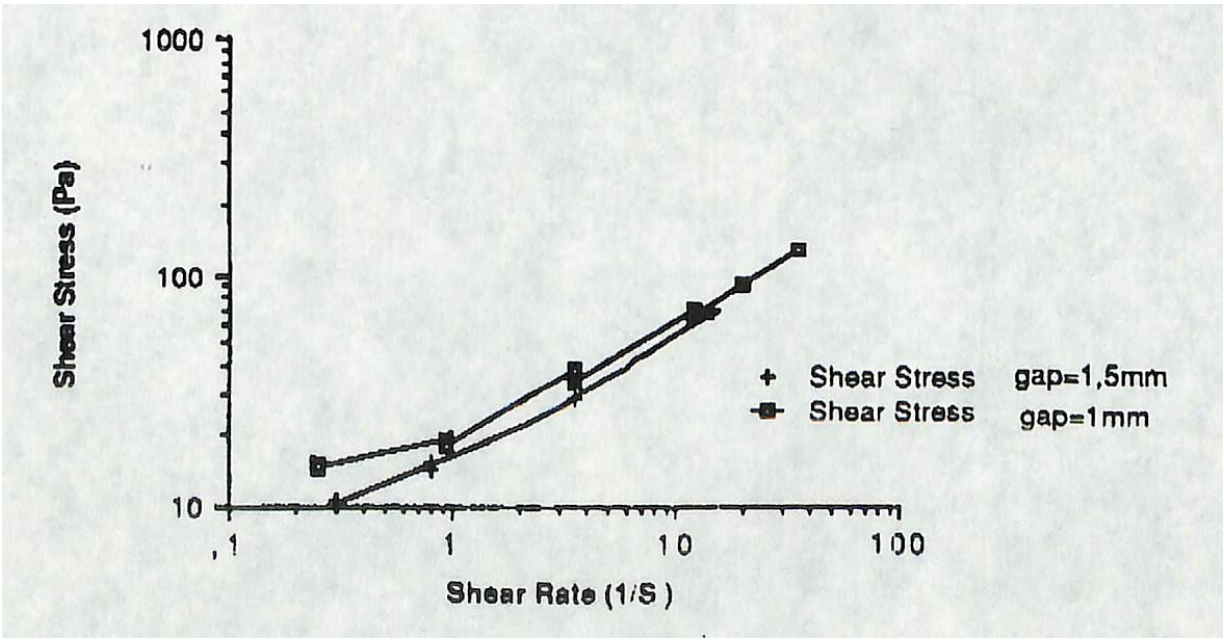

Fig. 6: Shear stress *vs* shear rate

The values of K and z for both the gaps are closed to the results presented in the previous report (15/6/91) for a 45% slurry.

Dynamic tests were carried out varying the angle at constant frequency to check the range of linear viscoelasticity. Then, a frequency sweep with a constant angle was carried out. These curves have been interpolated with a polynomial 3rd degree regression. The comparison of G' and G" for each gap (**Figs. 7 and 8**) shows a similar contribution of the elastic and viscous parts are also similar. The values of the slope of the first section of the curves for G' and G" have been obtained; the results, for the gap = 1.5, are closed to 2 and 1 respectively.

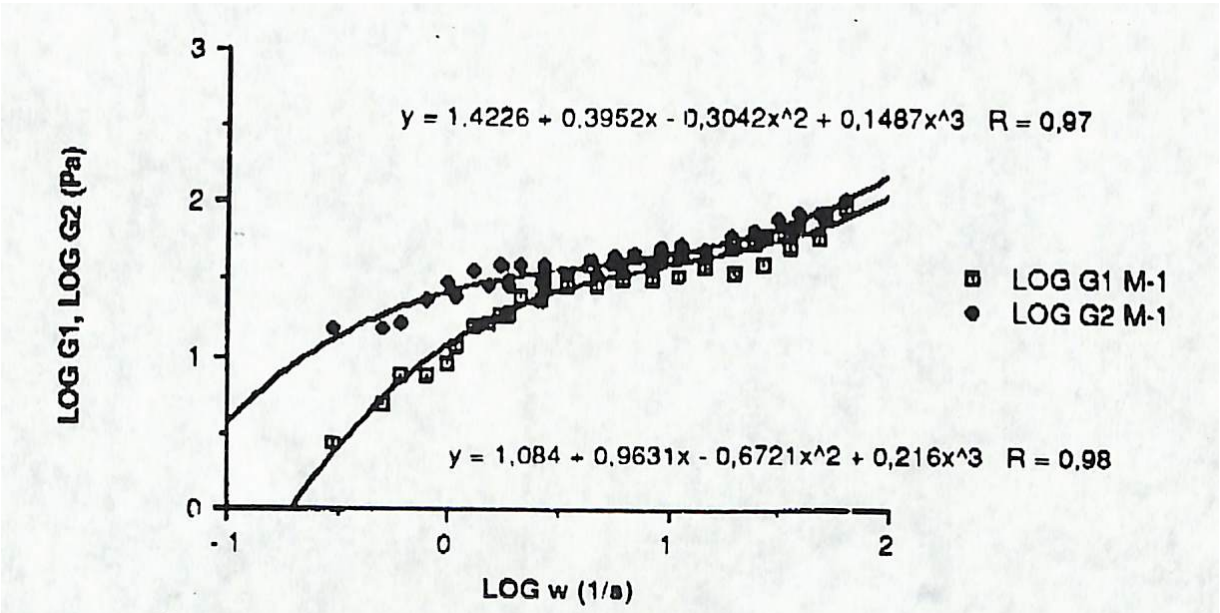

Fig. 7: Mean values of G1 and G2 for a gap of 1

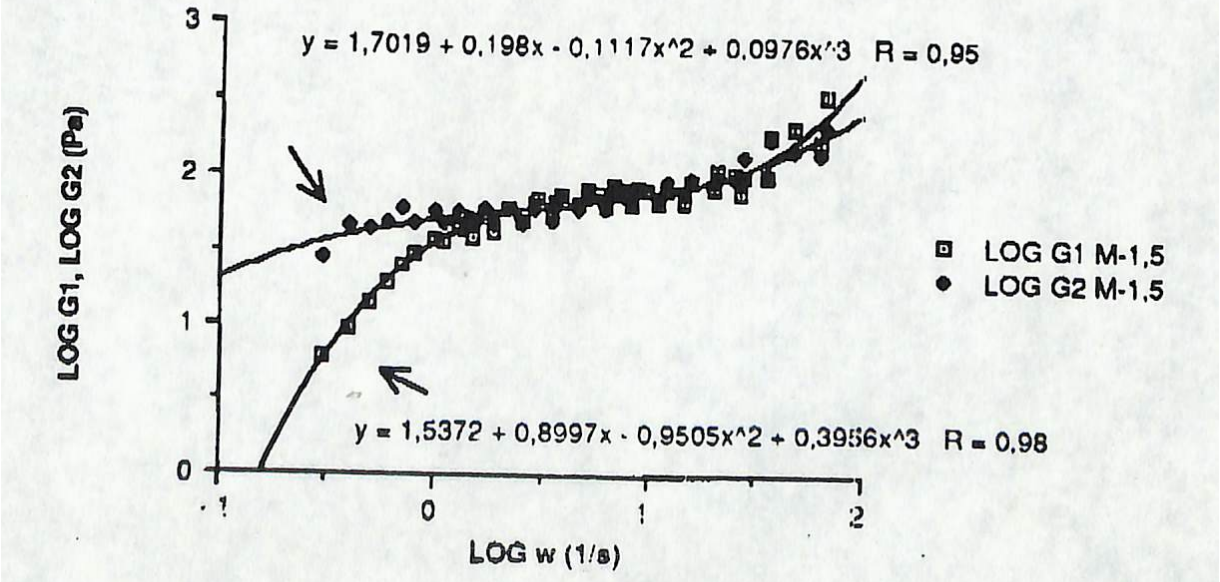

Fig. 8: Mean values of G1 and G2 for a gap of 1.5

The comparison between the behaviour of rotational viscosity and dynamic viscosity, in function of angular velocity showed a result similar to that reported in the literature, especially for the gap of 1 mm (**Figure 9**). The observed deviation may be due to the "non perfect" linear behaviour in the oscillatory measurements.

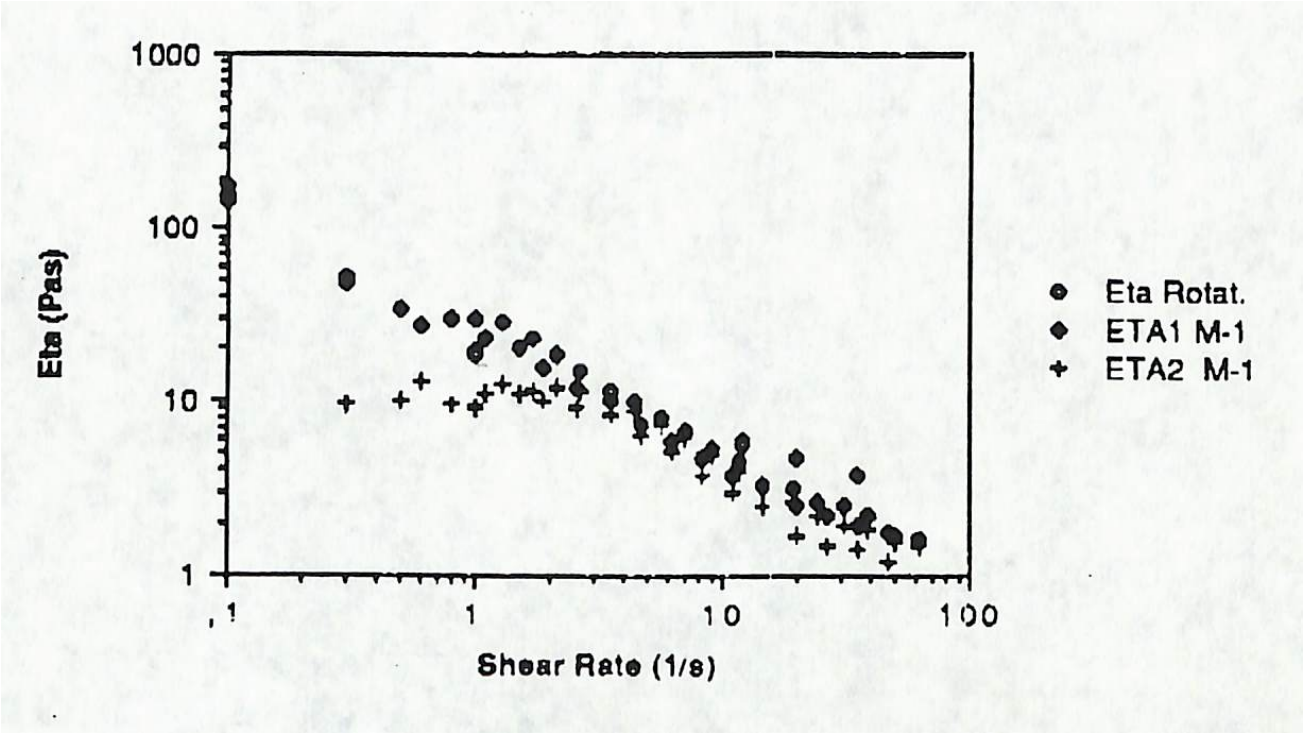

Fig. 9: Comparison of viscosity for a gap of 1

Finally the interrelation between the dynamic moduli and the relaxation modulus has been studied. The calculated G" well reproduces the experimental values with only a few iterations while the calculated G' is higher than the experimental one. Therefore, the obtained value of Ge was negative. This can be explained by the fact that it was impossible to reach a real linear viscoelastic region, hence a certain degree of non-linearity has to be expected.

3. Conclusions

Our future work will be to characterize flour slurries at lower concentrations with dynamic measurements in order to reach the region of linear viscoelasticity. Then, to compare the behaviour of different flours.

Task A.2.6 - Interactions between Selected Microorganisms and Wheat Flour Components and their Application to Improved Breadmaking Process

Partner 12 - IATA (Istituto de Agroquimica y Tecnologia de Alimentos), G.I. N4 C / Jaime Roig, 11 46010, Valencia, Spain.

Progress report from January 1, 1991 to November 15, 1991.

Progress

A selection of wheat flour samples from a pool of thirteen Spanish commercial flours located at different geographical areas, covering ranges of degree of extraction, proteolytic activity and flour quality has been performed on the basis of their physico-chemical characteristics (moisture, protein, ash and fat contents) and rheological and fermentative properties (alveogram, farinogram, extensogram, maturogram, oven-rise record, and reofermentogram).

As physico-chemical flour characteristics are concerned, the following ranges have been obtained (**Fig. 10**) : moisture : 12.75-15.31%; protein :10.85-14.56%;d.b; fat : 1.23-1.97%d.b; and ash : 0.52-2.56 %, d.b. Flour samples analysed covered a wide range of rheological and fermentative values. Alveogram parameters of white flours varied from 100 to 252 $\times$ 10^3 ergs (W), 0.32 to 1.4 (P/L) and 11.26 to 36.65 (% of degradation). Main differences in rheological characteristics corresponded to development time (farinogram : 1.5-13 min) and maximum resistance to extension (extensogram : 292-835 BU). Fermentative properties showed the widest value ranges for dough level (maturogram : 320-735 BU).

- As a general characteristic, the percent of degradation governed rheological and fermentative properties of Spanish commercial flours. This year, no flours without proteolytic activity could be found from our suppliers.

- Protein content was the chemical parameter differentiating among white flours (75 = degree of extraction). The higher protein levels results in greater flour strength (W), water absorption, resistance to extension, energy, and dough level.

- As expected increasing degree of extraction leads to higher protein, fat and ash contents. As a result, rheological and fermentative properties undergo deleterious effect as recorded in the extensigram, maturogram and impulsogram parameters. Higher water absorption and degree of softening than for white flour, have been observed.

Pure cultures of lactic acid bacteria strains (homo-and heterofermentative) have been selected according to their acidification ability. Two strains of lactic acid bacteria - *Lactobacillus plantarum* B-39 (from Cereals Laboratory collection) and *L. brevis* L-62 (Florapan, Chr Hansen) - have been selected for use in two physical conditions - frozen and freeze - dried (on a sour dough matrix)

In this step, baking tests have been performed using previously selected microorganisms as sour dough frozen bacterial starters, and selected white flours. Breadmaking performance has been evaluated by determining acidification properties (sour dough), and physico-chemical and sensory characteristics (bread). Control doughs and breads without sour dough, using commercial yeast, have been used for comparison purposes.

Concerning the performance of bacterial starters, heterofermentative L-62 when compared with homofermentative B-39 leads, in general, to greater acidification levels (TTA,pH) of sour doughs, doughs and bread, (**Table IV**) due to its higher acetic acid production during sour dough fermentation. As well, it gives higher consistency, energy, elasticity, level, and degree of softening and lower stability of doughs, (**Table V**) as well as harder texture, odder taste, more pronounced gummoness and lower overall acceptance of breads (**Table VI**). The influence of flour is mainly observed on the farinogram characteristics and on texture hardness. Weaker flour C gives the lower dough development time, stability and maximum resistance, the higher degree of softening and the harder texture of bread. Flour B yields, in general, doughs and breads with the opposite characteristics.

The effect of starter addition when compared with control doughs without added microorganisms includes higher acidification of doughs and breads, and degree of softening of doughs. Fermentation time, extensigram characteristics of dough and width/height ratio of breads show lower values for samples including bacterial sour dough starters. Other parameters such as stability, dough level, total oven rise, volume and texture depend on both flour and bacterial starter used. Control and B-39 started breads did not present significant differences in bread scoring, whereas L-62 breads received lower punctuation in sensory analysis.

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Fig. 10: Characteristics of Spanish commercial flours.

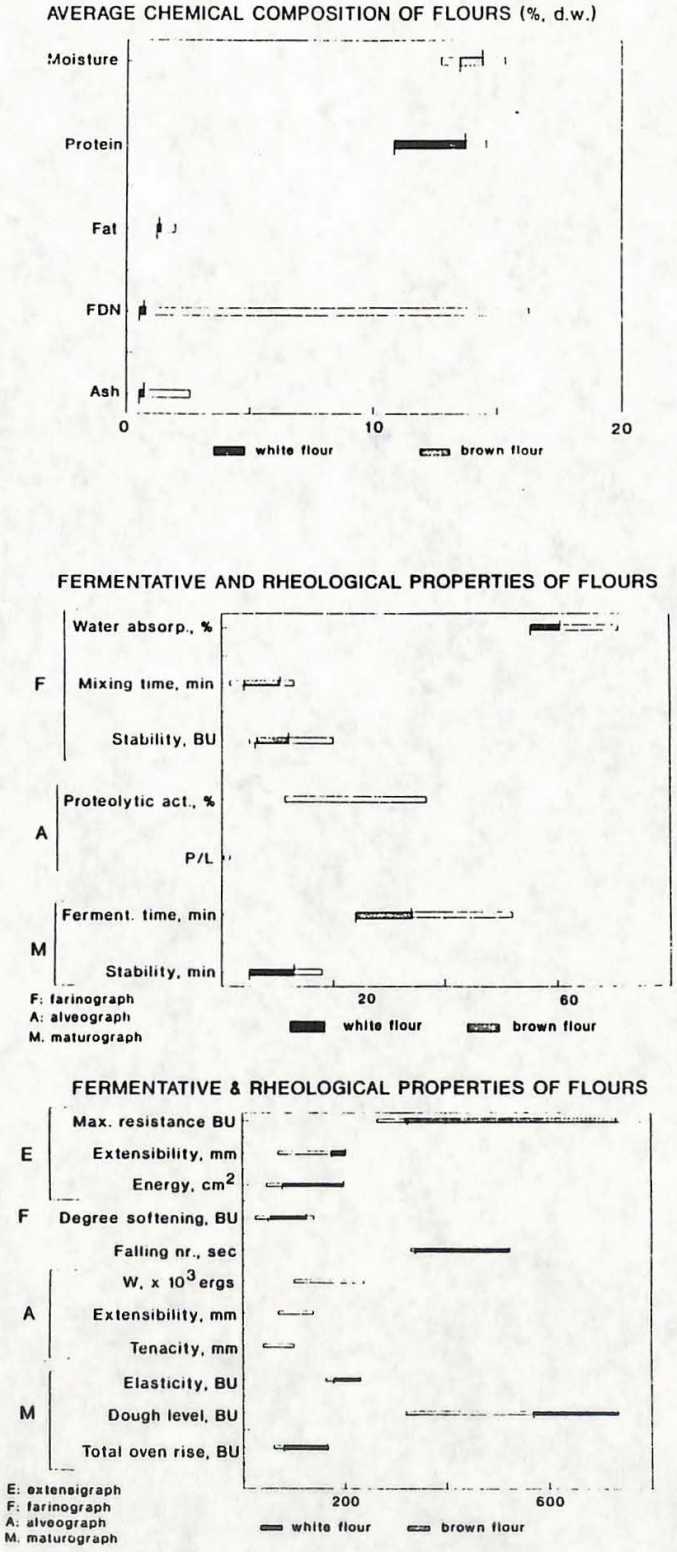

Table IV: Acidification properties.

Micro-organism	Flour	Final pH		Final TAA*		Acetic acid		Lactic acid	
		Sour dough[1]	Bread[2]	Sour dough[1]	Bread[2]	Sour dough[1]	Bread[2]	Sour dough[1]	Bread[2]
L-62	A	3.74	5.18	10.43	4.86	0.14	0.02	1.18	0.32
B-39	A	3.70	5.35	9.57	3.92	0.10	0.03	1.29	0.31
L-62	B	3.81	5.14	10.72	4.41	0.16	0.03	1.40	0.37
B-39	B	3.80	5.29	10.12	4.26	0.11	0.02	1.58	0.33
L-62	C	3.69	5.12	10.94	4.66	0.16	0.02	1.04	0.29
B-39	C	3.67	5.35	10.06	4.32	0.11	0.02	1.37	0.31
	A	-	5.92	-	2.67	-	0.02	-	< 0.01
Control	B	-	5.99	-	2.62	-	0.02	-	< 0.01
	C	-	5.92	-	2.98	-	0.02	-	< 0.01

* mL NaOH 0.1N/5 g flour, 14% m.b.

[1] g/100 g flour, as is

[2] g/100 g bread, d.b.

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Table V: Fermentative and rheological characteristics of doughs.

Micro-organism	Flour	Extensigram (90 min)			Farinogram				Maturogram			Oven rise recorder	
		Maximum resistance (BU)	Extensibility (mm)	Energy (cm^2)	Development time (min)	Maximum consistance (BU)	Stability (min)	Degree os softening 20´(BU)	Fermentation time (min)	Elasticity (BU)	Doug level (BU)	Final volume (BU)	Total oven-rise (BU)
L-62	A	330	105	38.6	4.0	440	5.5	150	68	220	655	650	170
B-39	A	345	97	40.5	5.5	370	9.0	100	64	190	510	650	250
L-62	B	370	117	59.0	6.0	485	5.0	175	73	240	710	790	380
B-39	B	363	104	42.8	6.0	460	7.5	120	74	220	640	-	-
L-62	C	300	110	37.1	2.5	485	1.0	205	60	200	550	630	230
B-39	C	285	95	34.7	3.5	540	3.0	200	64	160	380	500	150
Control	A	395	115	51.8	-	-	-	-	92	210	580	555	225
(no added	B	450	118	65.5	-	-	-	-	88	210	565	630	250
starter)	C	345	119	47.3	-	-	-	-	73	190	520	500	190

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Table VI: Bread characteristics.

Micro-organism	Flour	Physical Characteristics			Bread Scores (0-10)			
		Volume (ml)	Texture* (g)	Width / Height	Aroma	Taste	Eata-bility	Overall aceptance
L-62	A	405	50	1.47	7.7	6.4	5.9	5.7
B-39	A	397	21	1.39	7.7	7.1	7.0	6.3
L-62	B	407	146	1.44	6.9	5.6	5.7	4.5
B-39	B	455	78	1.53	7.6	7.3	6.6	6.4
L-62	C	383	277	1.62	7.3	7.3	6.4	6.3
B-39	C	362	259	1.58	7.0	7.3	7.3	6.5
Control (Without added) starter	A	428	94	1.71	8.1	7.6	7.7	7.7
	B	363	118	1.75	7.6	7.7	7.4	7.5
	C	328	232	1.64	7.8	8.0	7.8	7.1

* Instron press.

Experimental conditions:

. Header strength: 500 - 5000 g

. Plunger diameter: 25 mm

. Plunger speed: 100 mm/min

. Plunger depth: 15 mm

SUBPROGRAMME B: FUNCTIONAL COMPONENTS AND THEIR INTERACTIONS

***Johan J. Plijter*, Subprogramme Manager,**
Gist Brocades, Delft, The Netherlands)

Review of Activities

Part of the activities in this period were influenced by the fact that the contracts were signed mid-1991, so that several partners involved in subprogramme B were not able to start their research in January. As a consequence, the different activities in the subprogramme B started at different times.

After a first meeting held in Paris, which was intended as a possibility for the people involved in subprogramme B to get acquainted with each other, two scientific meetings were held in 1991. The first scientific meeting was held in Montpellier, France, on July 11 and 12. The second one was held in Norwich, UK, on November 4 and 5. At both meetings, presentations were given by all participants involved in the subprogramme.

Review of Projects.

Task B.1.1 - Purification and characterization of gluten subfractions

Partner 07N INRA Nantes
Partner 07M INRA Montpellier
Partner 19 AFRC-IACR Bristol
Partner 22 Università di Padova
Partner 23 Università di Viterbo

Objectives

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

Progress

Before selecting specific LMW subunits of glutenin for purification and study of functional properties, it was necessary to determine those that impart either high or poor breadmaking quality. So, a study of the composition in LMW and HMW subunits of glutenins was carried out by partner 07M in collaboration with task C.4.

Within a set of wheats which showed a identical HMW composition it is possible to obtain a further differentiation based on their LMW partners. A relation between different LMW partners and a level of baking strength was found.

The homogeneity of the different near-isogenic and genetic stocks available at partner 19 have been checked for purity prior to multiplication under field conditions next spring; to provide defined material for the fractionation.

The work on the purification of individual HMW subunits of glutenin for the physico-chemical analysis by partner 07N has started.

Gluten fractions composed of gliadins and of glutenin polymers with various sizes must be prepared and characterized without altering their functionalities in view of structural and rheological studies.

The method of MacRitchie (1987), with some adjustments, was found to be usable for this purification. Glutens were extracted from one good French variety (Aubaine) and from three near-isogenic lines of Sicco differing in their HMW glutenin subunit compositions (1/7+9/5+10;1/7+9/2+12;-/7+9/-). Eleven fractions were obtained per gluten sample. Anew SE-HPLC procedure was developed to analyse sizes of protein polymers and monomers in the gluten subfractions. Under the conditions used in this procedure the problems occurring in other methods like non complete solubilization of proteins and a recovery which is dependent on the sample composition are avoided. A series of fractions differing 1) in their HMW subunits content and 2) in size distributions of glutenin polymers are isolated. The procedure is easy to perform and yields amounts of protein, suitable for rheological characterization, of which the functionality is preserved by the used extraction methods. Protein subfractions could be passed to partners upon request.

During 1991 several bread wheats and wild wheat progenitors related to the A and D genomes have been electrophoretically analysed in Viterbo, to identify their pattern of variation and new potentially useful variants of wheat storage proteins.

Several methods for the purification of gluten components have been tested an found useful. Analyses of LMW glutenin subunits have indicated limited variation at the *Gli-D1* and *Glu-D3* loci. These two different alleles were also found to be associated with different technological properties.

Lines with HMW glutenin subunits displaying an unusual higher molecular weight were found. These subunits are being transferred into commercial cultivars to evaluate their influence on bread making.

At the University of Padova a new purification technique is developed based on adsorption chromatography on controlled pore glass. This to purify native glutenins of a acetic acid gluten extract. In this way it was possible to purify native gluten free of monomeric protein, which is essential since in reduced conditions the LMW-glutenin subunits have a similar molecular mass to those of other wheat proteins (mainly gliadins).

In a 50% *n*-propanol gluten extract (extracted in the absence of reducing agents) small gluten aggregates are present which are formed by disulphide linked HMW and LMW glutenin subunits. They are heterogeneous in pI and show a random composition.

Conclusions

The work is proceeding according to schedule, except for partner 19 who will catch up during 1992.

Task B.1.2 - Physico-chemistry and functionality of wheat proteins

Partner 07N INRA Nantes
Partner 15 Gist-brocades

Objectives

- Study of the physico-chemical properties of gluten and subfractions of gluten from wheat of different genotypes.
- Study of protein interactions.

Progress

Viscoelastic properties of gluten and gluten subunits, isolated in task B.1.1, were studied by a dynamic assay in shear conditions insuring a linear response of the material. All results indicated that the rheological behaviour of gluten fractions are closely related to their glutenin polymer content. Gliadins are likely to contribute to gluten rheological properties as a plasticizer. Experiments showed that the subunits structure has an effect on the functionality of glutenins. Whatever was the HMW subunit compositions, a very close relationship was found between rheological properties and size characteristics of the gluten subfractions.

Conclusions

The work is proceeding according to schedule.

Task B.1.3 - Gluten hydration and interactions of gluten proteins with other proteins.

Partner 15 Gist-brocades
Partner 16 IFR-Norwich

Objectives

- Elucidation of the mechanism of gluten hydration.
- Study of the mechanism which plays a role at the interaction between gluten and other components.

Progress

The water relaxation constants were measured from doughs made of three flours which were respectively a good and weak bread making flour and a biscuit flour. The experiments showed that the NMR transverse relaxation behaviour was exponential of nature and could be represented by a three component system. The amount of each component was dependent on the water/flour ratio.

Little influence was seen of the mixing time on the relaxation properties of doughs, despite the large influence on the dough rheology. Probably the measured relaxation properties were a function of the water/starch interactions rather than that of the water/gluten interaction.

Conclusions

The work is in principle proceeding according to schedule.

Task B.1.4 - The role of minor protein components associated with starch granules.

Partner 14 FMBRA

Objectives

- To establish the role(s) of starch granule protein in relation to functional properties of wheat.
- Predictive test of the endosperm texture on basis of the starch granule protein.

Progress

Work has been devoted entirely to the starch granule surface protein "friabilin" (experimental approaches (a) and (b), as planned. Research has been done to improve the purification of "friabilin". A monoclonal antibody has been produced to assay friabilin content of whole endosperm, for comparison with isolated starches. *T.durum* and had *T.aestivum* had zero and high contents as expected, but hard *T.aestivum* had a high content in contrast to the low level on it starch. The immunoassay cannot therefore predict endosperm texture as hoped. This important objective will be pursued for a further year (with deferral of experimental approaches (c) and (d) on starch granule properties). N-terminal amino-acid sequence of friabilin shows homology with the phospholipid-binding proteins being studied under task B1.5, therefore its role on endosperm texture will be examined in this context.

Also the lipid content and surface physical-chemical properties of extracts from starch granule surfaces were examined. The starch surface lipid was extracted together with the "friabilin"; therefore the extracts were highly surface active.

Conclusions

The work id in principle proceeding according to schedule. However the unexpected homology of "friabilin" to the lipoproteins of task B.1.5 has lead to studies of starch surface lipids.

Task B.1.5 - Lipid interactions.

Partner 07N INRA Nantes
Partner 15 Gist-brocades

Objectives

- Study of the wheat lipid composition and behaviour in dough.
- Study of the mechanism which plays a role at the interaction processes between wheat lipids and other components.

Progress

Wheat lipid-binding proteins were extracted and purified by partner 07N. These proteins could be purified with the aid of Triton X114 phase partitioning, followed by size exclusion, ion exchange and reversed phase high performance chromatographies. A wheat phospholipid transfer protein was purified from wheat flour using ammonium sulphate precipitation, size exclusion and ion exchange chromatographies. A consequence of the extraction procedure with Triton X114 is the development of a new sequential fractionation of wheat proteins. In this way pure albumin-globulin, gliadin and glutenin fractions can be purified. The proteins partitioning in the Triton X114 rich phase are two γ-gliadins and a series of low molecular weight cysteine rich proteins. N-terminal amino acid analysis showed that, except for the purothionins, all proteins are unknown. They have a pI of above 8. The phospholipid transfer protein is a monomeric protein of 90 residues, MW = 9607, a pI of 10 and has eight disulphide bridges. It shows strong homology with the other known proteins of this family isolated from e.g.barley, rice, maize.Work has been done at Gist-brocades on the interfacial behaviour of dough during mixing. Dough was prepared from a commercial flour, with no other additives than salt, sugar, yeast and water. Samples were taken at different mixing times. The interfacial behaviour was studied with the aid of an overflowing cylinder. During mixing the components which determine the surface behaviour changed from a high molecular character to a low molecular weight character.

Conclusions

The work is in principle proceeding according to schedule.

Task B.2 - Dynamics of dough development

Partner 16 IFR-Norwich

Objectives

- To study the behaviour and interactions of wheat components in doughs and baked goods with the aid of antibodies against the different components and microscopic techniques.

Progress

In order to develop screening assays for putative anti-pentosan monoclonal antibodies, polyclonal antibodies have been produced in rabbits against two different wheat bran pentosan extracts. Anti-pentosan antibody capable of binding to pentosan on a solid phase was detected by addition of specific, enzyme labelled antibody. The antiserum samples observed exhibited binding at a high dilution compared to pre-immune samples. Also, mice have been immunised with a number of pentosans immunogens. Further immunization programmes are underway. Also, the existing monoclonal antibodies will be applied to the microscopic analysis of bread dough.

Conclusions

The work is little behind in schedule, but the participant will catch up with the other participants in the subprogramme in 1992.

Individual Progress Reports

Task B.1.1 - Purification and Characterization of Gluten Subfractions

Partner 07M - INRA Montpellier, Laboratoire de Technologie des Céréales, 2 Place Viala, 34060 Montpellier Cedex 1, France.

Progress report from September 1, 1990 to November 15, 1991

1. Key measures of achievement - Objectives

Determination of the composition in LMW and HMW subunits of glutenin in relation to the quality scores of the cultivars (completion December 1991) and in view to select the specific LMW subunits that will be submitted to purification (deliverable December 1992) and physico-chemical studies.

2. Progress

Before selecting specific LMW subunits of glutenin for purification and study of functional properties, it was necessary to determine those that impart either high or poor breadmaking quality. Accordingly, a preliminary study of the composition in LMW (and HMW) subunits of glutenins was carried out (see also below, the report of task C.4, page 72).

Within a set of wheats with identical HMW formulas (among which there is a large variability of baking strength) it is possible to obtain a further differentiation based on LMW patterns. Among the 70 cultivars analysed, 57 were clustered in 9 groups (see **Table XI**, in the report of task C.4, page 73). It is especially important to notice that specific LMW patterns are associated to significantly higher (e.g. LMW type 7) or lower (e.g. types LMW 1 or 9) levels of baking strength.

The variability in LMW types may contribute to explain the variability in *W* among cultivars with identical HMW subunits of glutenin. Moreover, when considering only the attribute *G* (extensibility), the reliability of the prediction is much higher than for baking strength.

In addition, the relationship between LMW and extensibility is confirmed. Whether this relationship results from a different molecular weight distribution that is induced by LMW subunits, or from their higher sulphur content (a good supply of -SH and S-S being required to allow an orderly slipping - extensibility - of molecules), needs to be addressed in future work.

Although the effect of LMW on baking quality might, at least partially, result from the relative importance of quantity of protein produced by the different alleles, it cannot be ruled out that one allele or pair of alleles might appear more effective either in strengthening a dough or in improving extensibility. As a consequence, our present work is aimed at purifying the main LMW subunits of glutenin to achieve a better understanding of their mechanism of aggregation and of differences in rheological properties of dough between wheat cultivars.

3. Conclusion

Work is progressing according to the schedule.

Future work will be aimed at (i) characterizing further the composition of groups 1, 5 and 7 of LMW patterns (see **Table XI**, below) using two-dimensional electrophoresis and (ii) purifying the main LMW components from contrasting types of bread wheat cultivars using a procedure similar to that we developed to purify durum wheat LMW subunits of glutenin, which includes the following steps:

- Extraction of 14 g of semolina with 140 ml of 500 *mM* NaCl at 4° C.
- Recovery of the pellet after centrifugation (15 min at 10,000 *g*) and removal of starch by manual lixiviation.
- Extraction overnight with 40 ml of 7.5 *M* urea containing 100 *mM* DTE and 2 % Pharmalytes, followed by centrifugation 45 min at 10,000 *g*).
- Large scale isoelectric focusing on sucrose density gradient with checking the efficiency of the separation by isoelectric focusing on ultra-thin horizontal gels (Morel and Autran, 1990).
- Dialysis and freeze-drying of the samples.
- Final separation of the LMW subunits of glutenin by ion-exchange FPLC on Mono-S column.
- Control of the purity of the fractions by IEF, SDS-PAGE and 2-dimensional electrophoresis.

Partner 07N - INRA Nantes, Laboratoire de Biochimie et de Technologie des Protéines, Centre de Recherches de Nantes, INRA, Rue de la Géraudière, B.P. 527, 44026 Nantes Cedex, France.

1. Purification and characterization of whole gluten gluten subfractions and protein subunits of different genotypes. Production of defined gluten subfractions. Completion of first step: 06/91.

2. Glutens were extracted from one good French variety (Aubaine) and from three near-isogenic lines of Sicco differing by their HMW glutenin subunit compositions (1/7+9/5+10, 1/7+9/2+12; -/7+9/-). Subfractions were prepared by a procedure derived from that described by MacRitchie (1987). It consists of a progressive extraction of gluten proteins by solubilization in dilute HCl solutions with decreasing pHs. Eleven fractions are obtained per gluten sample.

A modified SE-HPLC procedure was used to analyse sizes of protein polymers and monomers in gluten subfractions. The objectives were to solubilize completely proteins into chromatography buffer and to avoid column blocking which was unusual with known procedures. The new procedure uses home-packed SE-HPLC columns filled with Pharmacia Superose 6 Prep-Grade. Conditions of separations were as follows: 0.0125 *mM* sodium borate buffer pH 8.5, 0.1% SDS, flow rate 0.3 ml/min. Samples are sonicated under carefully controlled conditions before centrifugation, filtration and injection. Under those conditions protein solubilization is complete and recovery is constant whatever are sample compositions.

Glutens and subfractions were analysed by SDS-PAGE. Sicco lines 5+10 and 2+12 have the same HMW glutenin subunit contents whereas line double null contains about only one half. Our procedure of subfraction preparation provides a series of fractions differing 1) in their HMW subunit contents, 2) in size distributions of their glutenin polymers. Sicco lines differed in their contents of large size glutenin polymers. Size distributions of glutenin polymers are related 1) to HMW glutenin subunit contents of glutens and 2) to compositions of HMW glutenin subunits (5+10 vs 2+12).

The procedure of preparation of gluten subfractions is easy to perform. It yields amounts of proteins suitable for rheological characterisation because functionality is preserved by extraction treatments. Protein subfractions could be passed to partners upon request.

Partner 19 - AFRC-IACR (Institute of Arable Crop Research), Long Ashton Research Station, BS18 9AF, Bristol, U.K.

1. Progress

The homogeneity of the different near-isogenic and genetic stocks have been checked for purity prior to multiplication under field conditions next spring; to provide defined material for fractionation. Work has started on the purification of individual high molecular weight subunits of glutenin for physico-chemical analysis.

Studies to date indicate that the genetic stocks have remained uncontaminated during prior multiplication (by analysis of the prolamin patterns by SDS-PAGE, 20 seeds of each line analysed). Crude HMW fractions are being produced prior to separation.

2. Conclusions

The work, now started, is expected to proceed as planned. Next year, we will employ a scientist on a short term contract to 'catch-up' with the remainder of the project group.

Partner 22 - Università di Padova, Dipartamento di Biotecnologie Agrarie, Via Gradenigo 6, 35100 Padova, Italy.

1. Key measures of achievement - Objectives

Purification and characterization of gluten aggregates (objective)
Small samples of purified gluten aggregates in native form (deliverable)
Some gluten aggregates are ready for characterization.

2. Progress

2.1 Characterization of gluten aggregates by free-flow isoelectric focusing

Gluten was extracted with 50 % *n*-propanol in the absence of reducing agents. The solution was submitted to free-flow IEF in the Rotofor cell and the focused fractions analysed by SDS-PAGE. In non-reducing conditions, fractions showed to contain several low mobility bands along with monomeric proteins. Upon reduction SDS-PAGE patterns of the same fractions showed the disappearance of the low mobility bands and the appearance of HMW and LMW subunits of glutenin in all the IEF fractions. Since the position of the glutenin subunits was very different from that obtained after IEF of reduced propanol extract, it was concluded that they are part of disulphide-linked aggregates, whose composition and pI is extremely heterogeneous.

2.2 Purification of gluten aggregates in the native form

The purification of gluten aggregates was achieved by chromatography on controlled pore glass beads of the acetic acid gluten extract in non denaturing conditions. One fraction contained only aggregates made up by HMW and LMW glutenin subunits, as assessed by SDS-PAGE and A-

PAGE. Moreover, the fractions shows functional properties similar to those of the gluten, since it is able to form a viscoelastic mass after the addition of salt. Thus, the fraction can be a useful material for microscopic and biochemical studies, for reconstitution experiments and for the purification of LMW glutenin subunits.

3. Conclusions

- Relatively small propanol soluble gluten aggregates are formed by disulphide linked HMW and LMW glutenin subunits. They are heterogeneous in pH and show random composition.

- Acetic acid soluble aggregates, free from monomeric proteins are obtainable. They retain functional properties.

- Objective for the next period: further characterization of gluten aggregates; purification of LMW glutenin subunits from the aggregates.

Deliverable: purified LMW glutenin subunits.

Completion: April, 1992.

Participant 23 - Università di Viterbo, Departamento di Agrobiologia e Agrochimica, Universita degli studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy.

1. Objective

Identification of novel variants for high- and low-molecular weight glutenin subunits and their characterization in order to define their role in gluten; obtain new lines of bread wheat with different allelic variants at the *Gli-1* and *Glu-3* loci.

2. Progress

During 1991 several bread wheats (lines, cultivars and subspecies of the *aestivum* group) and wild wheat progenitors related to the A and D genomes have been electrophoretically analysed, to identify their pattern of variation and new potentially useful variants for storage proteins.

Crosses have been performed in order to evaluate the effect of some new identified variants on technological properties. Several methods for purification of gluten components (Western blotting, electroelution and chromatography at high and low pressure) have been tested.

Two-dimensional electrophoretic analyses of gliadins and low-molecular weight glutenin subunits (2-pH's, IEF or NEPHGE/SDS-PAGE, respectively) have indicated limited variation at the *Gli-D1* and *Glu-D3* loci. Two main electrophoretic patterns were in fact found at each of the above mentioned loci which resemble those found in the bread wheat cultivars Cheyenne (CNN) and Chinese Spring (CS). These two different alleles were also found associated with different technological properties, being CNN-type superior to CS-type.

Lines with high-molecular weight glutenin subunits displaying an unusual higher molecular weight were found. These subunits are being transferred into commercial cultivars to evaluate their influence on bread making.

3. Conclusions

Results are progressing in the purification of gluten components, RP-HPLC is currently being used to relate amount of different gluten fractions with flour technological properties.

Task B 1.2 - Physico-Chemistry and Functionality of Wheat Proteins

Partner 07N - INRA Nantes, Laboratoire de Biochimie et de Technologie des Protéines, Centre de Recherches de Nantes, INRA, Rue de la Géraudière, B.P. 527, 44026 Nantes Cedex, France.

1. Key measures of achievement - Objectives

Physico-chemistry and functionality of wheat proteins. Study of rheological properties of proteins. Study of protein interactions. Completion of the first step: December 1991.

2. Progress

Viscoelastic properties of gluten and gluten subfractions were studied by dynamic assay in shear under conditions ensuring a linear response of the material. Aubaine proteins as well as of Sicco near-isogenic lines were analysed. All results indicated that rheological behaviours of gluten subfractions are closely related to their glutenin polymer content. In this respect, gliadin (i.e. monomer)-enriched fractions exhibited a much less viscoelastic character than glutenin-enriched fractions. gliadins are likely to contribute to gluten rheological properties mainly as a plasticizer.

Glutens from Sicco near-isogenic lines differed in their viscoelastic behaviours, in relationship with their HMW glutenin subunit compositions. Deleting 1A and 1D coded subunits decreased dramatically gluten viscoelasticity of line double null. Substitution of glutenin 5+10 by 2+12 modified gluten behaviour, although total protein and HMW subunits contents remained identical. It can thus be concluded that subunit structures have an effect on functionality of glutenins. Examination of gluten subfractions showed that viscoelasticity of gluten proteins is depending primarily of their contents and of the length of glutenin polymers. Whatever was HMW subunit compositions, a very close relationship was found between rheological properties (height of glutenin of MW > 5,000,000) of gluten subfractions. Low viscoelasticity of gluten from line double null is due to its low content of HMW glutenin subunits that decreases the number of large size glutenin polymers (quantitative effect). On the other hand, rheological differences between lines 5+10 and 2+12 are essentially related to abilities of their HMW subunits to build up longer or shorter polymers. Accordingly, subunits 5+10 seem to promote longer polymers than do subunits 2+12, thus gluten with higher viscoelasticity.

The study of protein interactions in gluten is just beginning because we were obliged to wait until last October to recruit a young scientist to carry out this work. Nevertheless it has now started by application of ESR to gluten proteins, and preliminary results will be available at the end of 1991.

3. Conclusions

During the first ten months the work progressed according to plan. Glutens and gluten subfractions were prepared from different genotypes and characterized. Data showed the role of large size glutenin polymers in determining viscoelastic behaviour of glutens.

During next period, fractionation procedure will be further improved to provide samples of glutenin polymers with low polydispersity. Rheological and ESR studies will be carried out on these samples also. Effects of limited S-S bond reductions and heat treatments will be examined.

Studies on rheological and surface behaviours of gliadins will start in the second half of 1992. New genotypes (substitution lines of Courtot, translocation lines) will be included in this work as soon as possible.

Task B.1.3 - Gluten Hydration and Interactions of Gluten Proteins with Other Components.

Participant 16 - AFRC-IFR (Institute of Food Research), Colney Lane, NR4 TUA Norwich, U.K.

1. Key measures of achievement - Objectives

Investigation of the water mobilities in flour doughs and hydrated glutens of wheats with varying breadmaking properties using pulsed ^1H nuclear magnetic resonance (NMR) techniques. Transverse relaxation measurements (deliverables). Completion of deliverables, December 1991.

2. Progress

^1H nuclear water transverse relaxation, characterized by time constants T2, was measured in doughs at ambient temperature using a CPMG spin echo pulse sequence. The doughs were prepared from three flours (a commercial flour with strong bread making properties; from Galahad, a variety with weak bread making properties; and from Riband, a biscuit making variety) but the bulk of the analysis was carried out on Galahad flour doughs.

Initial work showed that the transverse relaxation in wheat flour doughs was exponential in nature and could be represented by a three component system. The approximate values of component relaxation times and proportions were a) 10 ns, 75%, b) 30 ms, 23% and c) 100 ms, 2%. The variation of hydration level (0.53 to 1.4 g H_2O/g Dry Solid (DS)) in the doughs had little effect on T2 values but a significant effect on the proportions. Above 0.76 g H_2O/g DS the amount of the short component remained constant (0.6 g H_2O/g DS) and the extra water was associated with the intermediate component.

Doughs were prepared using a pin mixer but there were little effect of the length of mixing time (from 0.25 to 10 min) on the relaxation properties of the doughs. Variation of inter pulse spacing (t value) from 100 ms to 4000 ms also had little effect on the results, except for the detection of a shorter T2 component at the smallest τ values.

Methods for the measurement of ^1H NMR relaxation in wheat flour doughs have been established. Initial work, which compared doughs of three differing flours, has been carried out at one field strength i.e. 2.35 T (100 MHz). Further samples of Galahad and Riband flours have been prepared; these included defatted flour and glutens from flour, defatted flour and defatted/NaCl extracted flour. ^1H relaxation measurements, using the same techniques as above are in progress and will also be made at a lower field strength i.e. 0.47 T (20 MHz). Further NMR relaxation experiments are planned using gluten fractions to be supplied by partners within the programme (Task B.1.1). These experiments will include 2D as well as ^1H measurements.

3. Conclusions

^{1}H NMR transverse relaxation measurements have been made on doughs from a range of flour types. No difference in the relaxation properties of the dough was seen. There appeared to be little or no effect of mechanical work (despite large differences in dough rheology) or of t value, indicating that the relaxation properties were probably a function of water/starch interactions rather than water/protein. Further work is planned on relaxation properties of doughs from defatted flours and on gluten, gliadin and glutenin fractions. Efforts will be made to minimise the amount of residual starch in these fractions. The fractions should be available from January 1992.

Task B 1.4 - The Role of Minor Protein Components Associated with Starch Granules

A. Partner 14 - FMBRA (Flour Milling and Baking Research Association), Chorleywood, Rickmansworth, Hertfordshire, WD3 5SH, U.K.

1. Objectives

To devise a predictive test of endosperm texture for use in plant breeding and from mill intake. Deliverables - purified starch granule protein "friabilin", and a monoclonal antibody ELISA test to measure friabilin in whole endosperm (completion, April 1991).

2. Progress

Work has been done to improve starch isolation procedures in terms of B-granule and hence surface protein recoveries, and to optimise solubilization, recovery and chromatographic purification of the protein friabilin. Such preparations were used to establish the N-terminal amino-acid sequence and to produce a monoclonal anti-friabilin antibody. The latter was used in ELISA and immunoblotting assays to measure friabilin in whole endosperm of *T. aestivum*, *T. durum* and related diploid wheats. The lipid content and surface physical-chemical properties of extracts from starch granule surfaces were examined.

Results have included improved recovery of friabilin-rich B-granules, although the method is not satisfactory for wheats with very weak gluten. Extraction of friabilin with 1.0 *M* sodium chloride also extracted most of the starch surface lipid, which was present roughly in proportion to the friabilin; the extracts were highly surface active. Friabilin, recovered by Zn^{2+} precipitation is a very basic cystine-rich 15K protein which has N-terminal sequence homology with phospholipid-binding proteins isolated under Task B.1.5. Friabilin ELISA demonstrated that whole endosperm of hard *T. aestivum*, unlike the washed gluten, gave as high a signal as soft *T. aestivum.*

Deliverables have been achieved but with qualified success. Friabilin has been purified, but too little material for all planned purposes. An ELISA assay for friabilin has been delivered, but this did not attain the objective of being an assay for endosperm texture because the underlying hypothesis proved to be incorrect. Because of the great importance of endosperm texture to technological flour quality, it has been decided to pursue the objective for a second year (with the consent of FMBRA, UK Ministry of Agriculture and ECLAIR subprogramme-B coordinator), with experimental approaches modified in the light of developments here and elsewhere in the ECLAIR Wheat Programme. Preparation of starch will be be improved by techniques developed for Task A.1.2 (Partner 17), and purification of friabilin by analogy with the very similar lipid-binding proteins discovered in Task B.1.5 (Partner 07N). More attention will be paid to the lipid

components of the granule surface, and their surface activity (since these have been shown to differ with endosperm texture) with the objective of finding the gene product responsible for these differences. Endosperm texture mutants produced under Task C.9 (Partner 02) will be included in this study.

3. Conclusions

Good progress has been made in characterising friabilin and towards understanding the molecular mechanism of endosperm texture, but this has not produced the desired assay for endosperm texture. However, the unexpected similarity of friabilin to lipoproteins discovered under Task B.1.5 has lead to studies of starch surface lipids. The latter have been found to differ with endosperm texture, which is to remain the object of study for a further year (with deferral of studies on starch granule properties):

Objective: a predictive test of endosperm texture.
Deliverables -information on the lipids and lipoproteins of the starch surface, and on the cause of differences related to endosperm texture.

Task B.1.5 - Lipid Interactions

Partner 07N - INRA Nantes, Laboratoire de Biochimie et de Technologie des Protéines, Centre de Recherches de Nantes, INRA, Rue de la Géraudière, B.P. 527, 44026 Nantes Cedex, France.

1. Extraction and purification of wheat lipid-binding proteins

a. Extraction and purification of wheat lipid-binding proteins with Triton X114 phase partitioning.

Triton X114 as other non ionic detergents extracts specifically membrane proteins and other amphiphilic and hydrophobic proteins from wheat flour. This detergent aggregates in large micelles above 25°C (cloud point) leading to the formation of an upper detergent depleted phase and a lower detergent rich phase after centrifugation. For wheat flour, most of the membrane proteins are found in the upper detergent poor phase while the detergent rich phase is mostly composed of non membrane proteins and wheat lipids. In regard to their affinity for TX114, these proteins should exhibit an hydrophobicity and an hydrophilicity which confer them the capability to bind amphiphilic polar lipids (phospholipids and glycolipids). After detergent and lipid removal from the extract with organic solvents, these amphiphilic proteins are solubilized in 10 *mM* acetic acid and fractionated by size-exclusion, ion-exchange and reversed-phase high performance chromatographies.

b. Extraction and purification of a wheat phospholipid transfer protein.

A soluble protein involved in the transfer and exchange of phospholipids and other polar lipids between membranes was purified from wheat flour using ammonium sulphate precipitation, size-exclusion and ion-exchange chromatographies. This protein accounts for at least 3% of total wheat soluble proteins.

c. Sequential extraction of wheat proteins.

A consequence of the extraction procedure with TX 114 is the development of a new sequential fractionation of wheat proteins. The most interesting advantage of this fractionation is to give pure albumin-globulin, gliadin and glutenin fractions. First, albumins and globulins are extracted with a Tris-KCl buffer pH 7.8. Secondly, membrane and other amphiphilic proteins are extracted from the residue with the same buffer containing 2% TX114. The residue obtained was directly extracted by 70% ethanol to give pure gliadins devoid of glutenins and low molecular weight protein components. Finally, pure glutenins are obtained by extraction with a Tris buffer containing 5% SDS and 1% β-mercaptoethanol. This procedure has been successfully applied to the determination of albumin-globulin, gliadin and glutenin content of different wheat varieties. It is also possible to scale-up this procedure to purify gliadins and glutenins. In this case, the two first steps can be fused in order to extract both soluble and lipid-binding proteins.

2. Characterization of lipid-binding proteins

a. Protein partitioning in the TX114 rich phase

These proteins are mainly composed of two γ-gliadins and low molecular weight cysteine-rich proteins. The N-terminal amino acid sequence of these proteins shows that γ-gliadins and most of the LMW proteins, excepted purothionins, are unknown proteins. These proteins are basic with a pI above 8. It is noteworthy that TX114 does not extract the other γ-gliadins that are recovered with 70% ethanol from the insoluble residue. The primary structures of LMW proteins differ from this of CM proteins but they exhibit a similar distribution of cysteine residues. The amino acid sequence of the main LMW proteins was determined. It is composed of 115 residues with a MW of 12782 kDa and a pI of 10.8. The structure of this protein is stabilized by 5 disulphide bonds. A tryptophane-rich and basic amphipathic helix is found in the N-terminal part of the protein which might account for its polar lipid-binding properties.

b. Phospholipid transfer protein

This is a monomeric protein of 90 residues with a molecular weight of 9607 and a pI of 10. It is composed of eight disulphide bridges. This protein is devoid of tryptophane, methionine, and phenylalanine. Its primary sequence exhibits strong homologies with the other known proteins of this family isolated from barley,rice, maize, castor bean seeds and spinash leaves. Two amphipathic helices might account for its polar lipid binding properties.

3. Future work:

In the next year, our work will focus on the structure (primary and secondary structures) and lipid-binding properties of these proteins.

Task B.2 - Dynamics of Dough Development

Participant 16 - AFRC-IFR (Institute of Food Research), Colney Lane, NR4 TUA Norwich, U.K.

1. Key measures of achievement - Objectives

To study the behaviour and interactions of wheat components in doughs and baked goods with the aid of antibodies against the different components and microscopic techniques.

2. Progress

In order to develop screening assays for putative anti-pentosan monoclonal antibodies, polyclonal antibodies have been produced in rabbits against two different wheat bran pentosan extracts. The procedure studied involved lectin passively absorbed to the surface of microtitration plate wells. The lectins function to capture pentosans which would not be readily immobilised in the conventional way. Anti-pentosan antibody capable of binding to pentosan on the solid phase was detected by addition of species-specific, enzyme-labelled antibody.

The two pentosan fractions used as immunogens were prepared by (i) extraction at 0°C with 1*M* potassium hydroxide and collecting the highly branched 80-90% ethanol in soluble carbohydrate fraction containing arabinose (49%) and xylose (45%), or (ii) extraction at 20°C with 1*M* potassium hydroxide as before, this fraction containing arabinose (46%) and xylose (42%) with some of the polymers containing phenolic compounds.

Considerable success was observed when using a lectin from red marine algae (*Ptilota plumosa* agglutinin, specific for terminal α–D-galactosyl residues). Antiserum samples were observed to exhibit binding at high dilution compared to pre-immune samples, in spite of the early stage of the immunization programme.

Mice have been immunised with a number of pentosan immunogens. The availability of the tried and tested screening assay will aid identification and screening of monoclonal antibodies.

3. Conclusions

The immunogenicity of two pentosan fractions has been demonstrated. Further immunization programmes are underway. Identification of a suitable screening assay will aid the identification of murine anti-pentosan antibodies. Existing monoclonal anti-gliadin antibodies will be applied to the microscopical analysis of bread dough.

SUBPROGRAMME C: BIOCHEMICAL-GENETICS AND PHYSIOLOGY

***Norberto E. Pogna*, Subprogramme Manager, Istituto Sperimentale per la Cerealicoltura, S. Angelo Lodigiano, Italy)**

Foreword

This section summarizes the research activities carried out by participants in the "Subprogramme C" from October 8, 1990 to November 30, 1991. It comprises: (1) Review of activities and projects; (2) Annual report from each participant; (3) Report on the the first technical meeting held on December 12-13, 1991 at S.Angelo Lodigiano; (4) ANNEX I.

Review of Activities

The essential parts of the Subprogramme C have started and proceed well as scheduled. This subprogramme can de subdivided into two main research activities:

(I) Agronomic evaluation of EC high quality bread wheat cultivars grown in South Europe environments, and of high yielding bread wheat cultivars grown in North West Europe. This activity is providing useful information on the potential yield of the EC bread wheat germplasm as well as on the genotype × environment interaction. Moreover, it will allow us to estimate the technological characteristics of wheat cultivars grown in different environments.

The decision of wheat producers to start earlier (October 1990) proved to be quite appropriate because wheat samples are now available and partly characterized technologically to be distributed to industrial laboratories.

Seed samples of nine cultivars grown at two nitrogen levels in several locations of Southern Europe (Southern Europe Network, SEN) have been sent to Partner 07C and Partner 14 for technological analyses. A procedure for technological tests has been developed by French partners to study the quality characteristics of wheat cultivars grown in the North-Western Europe Network, NWEN). Seed samples for biochemical and rheological studies have been also supplied to Partner 04 and 23. The list of SEN and NWEN cultivars grown in 1990-91 and the quantity of seed available for distribution are reported in **Annex I**.

The 1991-92 trials of SEN and NWEN has been sown. Twenty-five SEN cultivars will be grown in 16 locations of Portugal, Spain, France and Italy and 15 NWEN cultivars will be grown in eight French locations and in one Italian location (**Table VII**).

Table VII. The SEN and NWEN trials in 1991-92.

	SEN 1991-92		NWEN 1991-92
No. of locations	16 (2P+3S+5F+6I)*		9 (8F+1I)
Name of cultivars	Centauro (I)	Castan (F)	Apollo
	Golia (I)	Courtot (F)	Armida
	Bruno (I)	MP 477 (F)	Artaban
	Maestra (I)	Soissons (F)	Baroudeur
	Manital (I)	Avital (F)	Beauer
	Mec (I)	Sidéral (F)	Camp Rémy
	Pandas (I)	Prinqual (F)	Génial
	Pegaso (I)	Almasor (P)	Récital
	Salmone (I)	Amazonas (P)	Renan
	Veda (I)	Mira (P)	Rossini
	Cajeme (S)	Mondego (P)	Sidéral
	Jecora (S)	Tua (P)	Soissons
	Rinconada (S)		Talent
			Thésée
			Viking
Treatment	2 levels of nitrogen		± fungicide
Replications	3		3

* P, Portugal; S,Spain; F,France; I, Italy.

In order to study the physiological and environmental factors affecting breadmaking properties and the stability of technological parameters, a research programme has been activated to determine kinetics of dry matter accumulation in both vegetative organs of plant and grains.

(II) Biochemical and genetic studies have been set up to gain information on albumins, HMW- and LMW-glutenin subunits, gliadins and factors involved in sprout resistance. Moreover, near-isogenic lines, intervarietal substitution lines, somaclonal variants, synthetic populations, mutants lacking storage protein-encoding genes and progenies from intergeneric crosses are currently being produced in several laboratories. These projects are proceeding according to schedule and preliminary results have been already presented and discussed during the third meeting of subprogramme C held in Paris last September 17-18, as well as during the first technical meeting organized in S.Angelo Lodigiano last December (3).

Review of Projects

Task C.1 - Multilocal Experiment of Advanced Lines and Varieties, and Production of Samples in Controlled Conditions

Sub Task C.1.1 - Sub Network 1: Southern Europe (SEN)

Sub Task C.1.2 - Sub Network 2: North Western Europe Network (NWEN)

Partner 02 Società Produttori Sementi
Partner 03 Istituto Sperimentale Cerealicoltura I.S.C.
Partner 07C INRA, Clermont Ferrand
Partner 18 Club des 5
Partner 06 Roquette Frères, S.A.
Partner 05 Champagne Céréales
Partner 08 BSN
Partner 09 ITCF
Subcontractor 24 INIA
Subcontractor 25 INMP

Partner 02 - Società Produttori Sementi S.p.A., Galleria del Reno n. 3, 40122 Bologna, Italy

1. Key measures of achievement - Objectives

Wheat lines and varieties grown in different environments in Southern Europe and North Western Europe are used to study the effect of genotype × environment interaction on breadmaking quality, to evaluate new genetic resources, and to provide raw material for biochemical and technological resources. (Derivable).

2. Progress

A SEN trial was sown in November 1991 and carried out according to the instructions. The late sowing caused an irregular emergence and the crop density varied from plot to plot.

All the data obtained with the requested observations were sent to Partner 03 (ISC) for elaboration.

The weather favoured some of the varieties with longer growing cycle more than locally adapted varieties. The shorter varieties had the disadvantage of competing with much taller ones, which sometimes lodged over them.

SDS sedimentation microtest and N.I.R.A. were performed just after harvest (single plots) and in November when we compared the plot mixtures which received the 2 different nitrogen fertilizations (See **Table VIII**). From the Table it is obvious that, unexpectedly, we cannot draw any clear cut conclusion on the effect of the supplementary late application of nitrogen.

Seed samples were sent to Partners 07C (INRA) and 14 (FMBRA). The rest of the seed was stored in case of other requests from Cooperators.

The 1991-92 trial was sown in November 8, 1991.

3. Conclusions

The data obtained are the first contribution of this project towards a better understanding of the effect of genotype and environment on wheat quality.

The next trials should make more complete our knowledge of this subject.

Table VIII: SEN Trial 1991. Argelato (Bologna, Italy)
SDS sedimentation value (microtest) and Protein content of wheat (N.I.R.A.).
N1 = 132 Units/ha of nitrogen
N2 = N1+42 Units/ha of nitrogen at heading time

Variety	SDS sedimentation value		Protein content %	
	N1	N2	N1	N2
Almansor	81	85	12,60	12,70
Amazonas	102	100	13,60	13,30
Castan	108	109	13,80	14,30
Centauro	84	79	11,80	12,80
Cajeme	98	100	14,50	15,10
Courtot	109	110	14,20	14,50
Florence Aurore	101	101	14,60	14,80
Golia	100	96	14,80	15,60
Libra	106	105	15,60	16,30
Loreto	95	99	14,70	14,30
Maestra	84	87	15,00	15,60
Mec	91	89	13,50	13,40
Mondego	93	90	14,30	13,90
Manital	90	95	15,50	15,40
Mira	89	88	12,10	12,70
MP 477	100	99	12,80	12,20
MP 906	84	91	15,00	15,00
Pandas	96	92	13,10	14,20
Pegaso	97	104	12,30	13,70
Prinqual	104	104	14,30	15,50
Rinconada	105	101	14,40	14,00
Salmone	110	110	13,70	14,70
Tua	94	95	13,30	13,50
Veda	104	103	13,90	14,40
Jecora	110	106	14,60	14,90

Partner 03 - Istituto Sperimentale per la Cerealicoltura, Sezione Operativa di S. Angelo Lodigiano, Via Mulino 3, 20079 S. Angelo Lodigiano (Milano), Italy.

1. Key measures of achievement - Objectives

As above

2. Progress

The SEN trials were sown in S.Angelo Lodigiano (North Italy), Tolentino (Central Italy) and Foggia (South Italy). At S.Angelo Lodigiano emergence took place late in December. Previous crop was maize. Nitrogen was applied twice during winter for a total of 90 kg/ha. Late nitrogen application on three replications was given as an urea foliar application at heading (25 kg/ha of nitrogen).

Weather conditions were unfavourable during autumn and winter, with heavy rains in October, early frost in December and -15°C in February without snow cover. Late frost (-1°C) occurred in April 20th.
In spite of this unfavourable conditions, the crop appeared in good conditions during spring. Plant height and biomass appeared reduced. Diseases were not severe with a slight mildew attack. No chemical control against foliar diseases was applied.

The results of the variety trial organized at S.Angelo Lodigiano are represented in **Table IX**. The most productive cultivars gave plot yields above 8 t/ha. Florence Aurore, Prinqual, MP 906 and Mira appeared to be too tall for our conditions. A preliminary evaluation of bread making quality made with the Chopin Alveograph and with the SDS Sedimentation test indicates that most of the cultivar included in the trial fall in the first Italian quality class (**Table X**).

We have coordinated the exchange of seed samples for the next SEN trials and prepared field books for all the cooperators.

The 1991-92 SEN trials cover 16 locations and include 25 varieties grown at two nitrogen levels with three replications for a total of 150 plots.

A report on the agronomical and qualitative characteristics of wheat cultivars grown in 1990-91 in Southern Europe has been distributed to SEN cooperators in December 1991.

3. Conclusions

The SEN trials were organized in September 1990 during a first meeting held at Montpellier and seed were sown in November - December 1990 in eleven locations in Southern Europe. This is the first example of an EC network for agronomic and quality evaluation of the European wheat germplasm of good breadmaking characteristics.

Table IX: Results of the variety trial organized at S.Angelo Lodigiano

S. ANGELO LODIGIANO (MI) ITALY Year 1990-91

Varieties	Grain yield (t/ha)	Grain moisture (%)	Test weight (kg/hl)	Heading (days from April 1st)	Plant height (cm)	SDS-Sedimentation test (mm)
MP906	8.50	12.6	83.5	51	99	69
Avital	8.37	11.9	81.6	51	93	66
Courtot	8.03	13.9	80.0	54	66	76
MP477	8.00	11.4	83.7	50	88	64
Mira	7.90	11.7	84.0	51	99	69
Maestra	7.88	12.3	84.7	50	82	51
Prinqual	7.77	11.4	84.7	50	103	83
Amazonas	7.73	11.2	82.6	50	97	78
Golia	7.45	10.8	83.9	46	62	69
Pandas	7.36	11.4	83.0	44	84	85
Castan	7.28	11.2	80.2	52	93	85
Centauro	7.20	11.1	83.4	46	73	68
Almansor	7.19	11.6	84.1	50	95	62
Mondego	6.99	11.4	83.6	49	87	72
Loreto	6.95	11.9	86.3	51	92	73
Tua	6.86	12.0	84.9	50	93	86
Manital	6.76	11.1	83.6	46	68	75
Cajeme	6.75	11.1	84.1	45	76	84
Veda	6.56	11.1	82.9	45	69	74
Pegaso	6.50	10.9	80.8	50	74	73
MEC	6.39	11.4	84.4	50	74	73
Florence Aurore	6.32	11.8	84.3	48	122	78
Jecora	6.17	11.3	84.4	45	68	80
Salmone	5.92	11.4	83.4	52	74	87
Rinconada	5.28	11.6	84.2	45	86	84
Mean	7.13	11.6	83.5	49	85	75
m.s.d 5%	0.60	0.7	1	1	4	
C.V.	7.32	4.89	1.03	1.52	4.36	

Table X: Breadmaking quality as measured by the SDS-sedimentation volume and Alveograph parameters in 25 bread wheat cultivars grown in two locations

Varieties	Jesi (AN)	S.Angelo Lodigiano (MI)						
	SDS volume	SDS vol.	SDS(1) vol.	Chopin Alveograph[1] *G*	*P*	*P/L*	*W*	Quality class
France								
Castan	86	79	90	19.8	99.0	1.24	266.2	2*
Courtot	71	68	83	27.9	53.9	0.34	198.2	2+
Fl. Aurore	87	70	86	26.9	72.9	0.50	373.0	1+
Prinqual	82	80	85	24.6	110.4	0.90	459.1	1+
MP 477	63	55	72	21.5	113.7	1.20	385.0	1
MP 906	64	69	69	20.5	122.1	1.43	413.0	1*
Italy								
Centauro	61	65	70	21.7	73.7	0.77	243.9	2
Golia	73	61	76	23.5	103.4	0.92	400.0	1+
Libra	87	55	77	24.7	72.6	0.59	239.4	2+
Loreto	72	73	72	26.3	61.6	0.44	191.0	2
Maestra	62	46	56	24.7	76.8	0.62	327.7	1+
Manital	84	69	81	24.6	95.3	0.78	408.1	1+
Mec	64	64	82	23.4	60.5	0.54	196.2	2
Pandas	77	79	90	26.6	76.2	0.53	279.9	1
Pegaso	73	61	84	24.5	67.1	0.55	249.8	2+
Salmone	87	86	88	27.3	74.7	0.49	360.0	1+
Veda	81	69	79	25.4	77.0	0.59	321.1	1
Portugal								
Almanson	61	53	70	27.2	65.5	0.44	239.4	2+
Amazonas	68	71	85	25.3	57.2	0.44	261.6	1
Mira	64	60	78	22.8	99.7	0.93	297.6	1
Mondego	67	68	75	24.3	78.5	0.66	262.9	1
Tua	78	86	85	25.9	66.2	0.48	259.6	1
Spain								
Cajeme	85	79	88	28.4	75.3	0.46	372.8	1+
Jecora	79	77	82	25.1	74.4	0.58	328.3	1+
Rinconada	75	81	87	22.2	84.7	0.84	360.0	1+

[1] 30 kg/ha of Nitrogen at heading time

Partner 07C - INRA-Clermont-Ferrand, Station d'Amélioration des Plantes, Domaine de Crouelle, 63039 Clermont-Ferrand Cedex, France

Participants : M. Rousset and N. Robert (07C), F. Kaan (07M), M.H. Bernicot (09)

1. Key measures of achievement - Objectives

As above

2. Progress

In 1990-91 SEN and NWEN trials were carried out to study the effects of genotype × environment interaction on quality. The SEN trial involved 24 high quality wheat cultivars grown in two locations by Partners 07C and 07M, and in other two locations by Partner 18. Two levels of nitrogen with two replications each were applied. The NWEN trial involved 12 cultivars grown by Partner 07C (4 locations), Partner 18 (3 locations) and Partner 09 (2 locations).

Seeds of SEN and NWEN trials have been sown in November 1991 with three replications in order to provide raw material for technological analysis.

A procedure for technological tests has been planned between the French partners implicated in the subprogramme C.1.2 (NWEN). NWEN samples have been dispatched to Partner 09 (ITCF), for cleaning and measure of protein content.

We are waiting some SEN samples for performing small-scale technological tests (Pelshenke, SDS sedimentation and PSI tests) and observations on grains.

3. Conclusions

Work is well underway.

Subcontractor 24 - INIA, Estacion Experimental, Rancho de la Merced, Apartado 589, Jerez de la Frontera, Cadiz, Spain.

Participant : J. De Juan-Aracil

1. Key measures of achievement - Objectives

As above.

2.Progress

A SEN trial with 25 European wheat cultivars and three replications has been carried out in three locations in Spain (Jerez de la Frontera, Carmona and Granada).

Relatively high yields were obtained in some cultivars. The wheat samples are currently being submitted to technological analysis.

The second SEN trial including 25 wheat cultivars has been recently sown.

3. Conclusions

Taking into account the agronomic problems encountered at Granada in 1990-91, the SEN trial has been transferred to Albacete. The project is proceeding according to schedule.

Subcontractor 25 - INMP, Estaçao de Melheramento de Plantes, 7351 Elvas Codex, Portugal.

Participants: F. Bagulho and B. Macas.

1. Key measures of achievement - Objectives

As above

2. Progress

Twenty-five varieties have been sown in two locations (Elvas and Beja). According with instructions of the SEN-group, two nitrogen manure conditions were used with three replications for each condition.

Spring in South Portugal was very dry specially in April and May and heavy water stress occurred in Beja during flowering and grain filling. For this reason the Beja trial was lost.

At Elvas, due to different type of soil and the occurrence of some rain and dew, high yields were obtained.

With regard to technological analysis, a very good experience has been achieved during this first year of studies.

3. Conclusions

Good experience was achieved during this first year and we are planning to continue in the same way according to the instructions from the meeting held in Paris in September 1991.

Partner 05 - Champagne Céréales, 3 Rue Gabriel Voisin, B.P. 2736, 51059 Reims Cedex.

Participants: F. Plénier, M. Lemeur-Plunet and S. Variéras

1. Key measures of achievement - Objectives

We are involved in the evaluation of baking quality of NWEN wheat cultivars. For this purpose, Alveograph tests are currently being applied to 12 wheat varieties (Thésée, Apollo, Récital, Soissons, Talent, Camp-Rémy, Renan, Génial, Baroudeur, Artaban, Viking, Rossini) grown in 1990-91 in six locations. Moreover, four cultivars (Talent, Apollo, Soissons, Camp-Rémy) grown in these six locations with or without fungicide treatment are currently being analysed to evaluate the effects of the treatment on wheat quality.

2. Conclusions

The starting date of our research work is January 1992 after receipt of wheat samples grown in 1990-91.

Task C.2 - Genotype × Environment Interaction

Subtask C.2.1 - Ecophysiological Approach to the Genotype Expression

Partner 07C - INRA-Clermont-Ferrand, Station d'Amélioration des Plantes, Domaine de Crouelle, 63039 Clermont-Ferrand Cedex, France

Participant: N. Robert

1. Key measures of achievement - Objectives

Objective of the project is to study the accumulation of dry matter and storage proteins during the grain filling period. As a first approach for understanding the stability of breadmaking quality. Variations in accumulation kinetics, quantity and ratio of storage proteins will be studied in relation with stability of technological properties. Variations of storage proteins (mainly glutenins and gliadins) in relation to quality will be also analysed on seed samples from SEN and NWEN trails.

2. Progress

Kinetics of dry matter accumulation in vegetative organs determined. Dry matter and protein accumulation is currently being analysed in seeds obtained from freeze dried spikes. Measurements of nitrogen content in all organs (stems, leaves, grains and husks), sugar in stems as well as storage proteins are in course.

No significant differences between dressing treatments were obtained for yield, Alveograph parameters and baking tests. This may be accounted for by the dry conditions which occurred at Clermont Ferrand during the growing season.

3. Conclusions

Work is well underway.

Subtask C.2.2 - Experimentation in a Controlled Environment

Partner 09 - ITCF
Partner 18 - Club des 5
Partner 07C - Clermont Ferrand
Partner 05 - Champagne Céréales
Partner 06 - Roquette Frères S.A.
Partner 08 - BSN Branch Biscuits

No progress has been made in this project. Therefore, these is a slippage on the planned programme of one growing season.

Task C.3 - Experimentation on Populations for Breeding

Partner 02 - Società Produttori Sementi S.p.A.
Partner 03 - Istituto Sperimentale Cerealicoltura

Progress report from January 1, 1991 to November 15, 1991

Partner 02 - Società Produttori Sementi S.p.A., Galleria del Reno n. 3, 40122 Bologna, Italy

1. Key measures of achievement - Objectives

Production and evaluation of synthetic populations having good bread-making quality. Use and assessment of new biochemical analyses and technological microtests devised by cooperators from other subprogrammes.

Small samples of selection synthetic populations. Information on these populations. Evaluation of new analytical methods (Deliverable).

2. Progress

We started working at this task on October 1991 by sowing a trial including the synthetic populations and the selection rows including the same material we received from Partner 03 (ISC)

The trial will give us information on the performance of the populations whereas the selection rows will allow us to select single plants for agronomic characters and mainly for disease resistance, taking advantage of the artificial inoculations of leaf rust and powdery mildew that we apply to our selection fields.

Seed from selected plants will be used for many purposes: sowing in the following season, crosses, SDS sedimentation microtest N.I.R.A., and storage protein electrophoresis.

3. Conclusions

The project started in October 1991, that is the first sowing period following the official starting date of the EC Contract.

The aim of this work is to increase the frequency of alleles having a positive effect on bread-making quality in synthetic populations. At the same time we are trying to improve the agronomic performance of these populations using the selection for agronomic characters and the information obtained with the production trials.

Partner 03 - Istituto Sperimentale per la Cerealicoltura, Sezione Operativa di S. Angelo Lodigiano, Via Mulino 3, 20079 S. Angelo Lodigiano (Milano), Italy.

1. Key measures of achievement - Objectives

Isolation of superior wheat lines by conventional breeding, SDS or anther culture from synthetic wheat populations characterized by a high concentration of alleles affecting agronomic and quality traits.

Small samples of seed from synthetic populations selected for quality (Deliverable).

2. Progress

A total of 100 S3 lines were grown in 1.5 m^2 plots with two replications. Forty lines were selected on the basis of their morpho-physiological characteristics and harvested. The best 20 lines as evaluated by the SDS sedimentation test were retained and sown in October 1991 in replicated plot trials at two locations.

We have grown the SINT population mentioned in the programme for a total of 100 S3 lines on 1.5 m^2 plots and two replications. By visual selection we reduced to 40 the number of lines to be harvested. After a qualitative evaluation by means of SDS sedimentation test the best 20 lines have been retained and sown in replicated plot trials at two locations.

3. Conclusions

Some wheat lines with high SDS-sedimentation volumes were selected and will be used for a new cycles of intermating selection. Work proceeds well as planned.

Task C.4 Genetics of LMW Glutenin Subunits

Partner 07M - INRA, Montpellier
Partner 07C - INRA, Clermont Ferrand

Progress report from January 1, 1991 to November 30, 1991

**Partner 07M - INRA Montpellier, Laboratoire de Technologie des Céréales,
2 Place Viala, 34060 Montpellier Cedex 1, France.**

Participants: Marie-Hélène Morel and Valérie Gazanhes (07M)

1. Key measures of achievement - Objectives

To assess the possible relationship of LMW subunits with dough properties of flour from bread wheat and its consequences in the stimulation of breeding and developments of French and South Western Europe baking industries.

2. Progress

To obtain a clear-cut separation between reduced glutenin polypeptides and gliadin monomers and enable a routine characterization of LMW and HMW by 1-D SDS-PAGE, albumins, globulins and gliadins were selectively extracted. We used Triton X 114 extraction procedure similar to that used in membrane protein studies, followed by a more conventional extraction with 70% ethanol. The

last pellet containing glutenins was then extracted with an SDS-ß-mercaptoethanol solvent. After SDS-PAGE, the HMW subunit composition was determined according to the nomenclature of Payne and Lawrence. The components with relative mobilities between 750 and 850 were assumed to consist of LMW type B subunits and the different patterns where clustered taking into account homologous patterns.

Among the 70 cultivars analysed, 57 were clustered in 9 groups whose characteristics are presented in **Table XI**. Seventeen cultivars gave fuzzy patterns or were present in less than 3 genotypes according to LMW or HMW glutenin composition and were discarded from the subsequent segmentation analyses which were used to evaluate the contribution of LMW and HMW composition to the value of technological parameters from Chopin Alveograph.

Our results led to the following conclusions:

a) Within a set of wheats with identical HMW formulas (among which there is a large variability of baking strength) it is possible to obtain a further differentiation based on LMW patterns. It is especially important to notice that specific LMW patterns are associated to significantly higher (e.g. LMW type 7) or lower (e.g. types LMW 1 or 9) levels of baking strength. The variability in LMW types may contribute to explain the variability in *W* among cultivars with identical HMW subunits of glutenin.

b) Among French bread wheats (because of the specificity of French or South-Western European bread-making and possibly of environmental conditions), the variation of LMW subunits seems to be of almost equivalent importance as that of HMW subunits in determining baking quality. Accordingly, it can be recommended to breeders to take into account both types of subunits. At present, the association of HMW "7+8" or "7+9" with LMW types "7" or "4" seems to correspond to the highest potential of baking strength.

c) When considering only the attribute *G* (dough extensibility), the reliability of the prediction is much higher than for baking strength (**Table XII**). This emphasizes the interest of LMW subunits in breeding wheats for French type of bread-making. Because dough extensibility is presently the most difficult attribute to control in France, new wheat genotypes should be selected first on the basis of their LMW patterns (a new routine tool is now available), instead of on that of HMW, the latter being used as additional markers (cumulative effect of allelic variation at both loci). At present, the association of HMW "7+8" with LMW types "4", "5" or "7" seems to determine the highest potential of dough extensibility.

d) The effect of LMW on baking quality might, at least partially, result from the relative importance of quantity of protein produced by the different alleles (e.g. LMW class # 1, the only one that contains one single B band, is a grouping of extremely poor cultivars).

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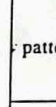
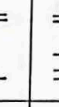
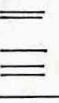
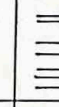
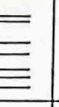
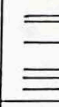
LMW	1	2	3	4	5	6	7	8	9
n of varieties	3	10	3	12	8	7	3	3	8
pattern									
W mean	80	100	105	114	102	94	135	96	79
sd	11	34	47	37	40	24	51	56	42
G mean	77	86	81	91	96	83	101	82	75
sd	13	7	5	9	21	13	0	28	7
P/L mean	266	178	301	196	171	226	181	386	274
sd	128	56	53	110	156	157	124	485	155

Table XI: Scheme of the 9 main types of LMW-B subunits of glutenin observed among a set of 57 French bread wheat cultivars.

Mean values and standard deviation of each class for attributes of dough quality: *W, P/L, G* (Alveograph) (expressed as % of the score of the standard cv. Capitole)

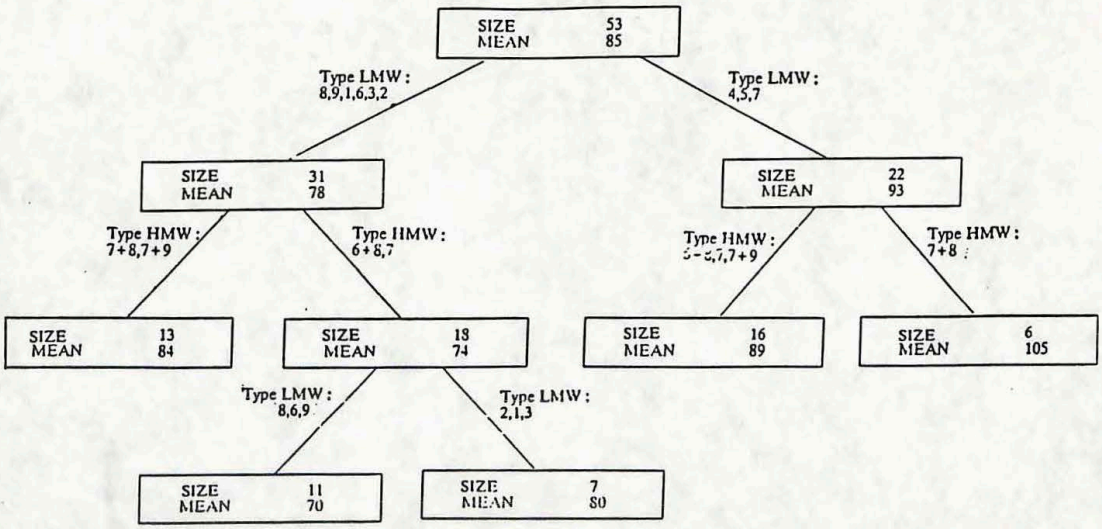


Table XII: Segmentation analysis taking into account both LMW and HMW subunits of glutenin (explained variable: *G* from Alveograph).

3. Conclusions

Work is progressing according to the schedule.

The present work is aimed at characterizing further the composition of groups 1,5 and 7 of LMW patterns using two-dimensional electrophoresis and at improving the classification of the LMW patterns and their statistical analysis in connection with technological data.

Partner 07C - INRA-Clermont-Ferrand, Station d'Amélioration des Plantes, Domaine de Crouelle, 63039 Clermont-Ferrand Cedex, France

Participants: G. Branlard, D. Khelifi and M.T. Nieto-Taladriz

1. Key measures of achievement - Objectives

The allelic diversity of the D zone proteins was established for a very large set of bread wheat cultivars and the effect of the D zone proteins on some technological parameters was tested. Some D zone alleles seem to play a role in the extensibility of dough, therefore the use of the D zone proteins in addition to the HMW glutenin subunits for quality prediction will be tested.

2. Progress

The D zone proteins encountered in durum wheat cultivars were analysed. Their allelic diversity was achieved from a set of more than 240 international collection of durum wheats. The LMW glutenin type B are under investigation. A very diverse set of wheats will be analysed. The relations between the protein polymorphism of the LMW-B glutenin and the technological quality will be assessed. Our results were discussed during the first technical meeting held at S.Angelo Lodigiano.

3. Conclusions

The schedule of the programme follows the proposed plan. The results obtained seem to be very helpful for the success of the project.

Task C.5 - Genetic and Technological Aspects of HMW Glutenin Subunits, HMW-Albumins and S-proteins

Partner 03 - Istituto Sperimentale per la Cerealicoltura, Sezione Operativa di S. Angelo Lodigiano, Via Mulino 3, 20079 S. Angelo Lodigiano (Milano), Italy.

Progress report from January 1, 1991 to December 14, 1991

Participants: R. Redaelli, N.E. Pogna, T. Lafranchis

1. Key measures of achievement - Objectives

Obtaining information on genetic control of HMW-albumins and S-protein, and on genetic variability for HMW glutenin subunits. To study the effects of albumins and HMW glutenin subunits on viscoelastic properties of gluten.

2. Progress

Water-soluble proteins from the endosperm of aneuploid lines of Chinese Spring were fractionated by SDS-PAGE. Some HMW components (about 60 kDa) were found to be coded by genes on chromosomes 4AL and 4DL, while some LMW polypeptides (about 14 kDa) were found to be controlled by chromosome 3DS.

LMW albumins were purified by EPE (electroendosmotic preparative electrophoresis) at pH 8.4 (B-PAGE). Proteins eluted from EPE apparatus were monitored at 280 μm wavelength. This method resulted to be suitable for the purification of different albumin components, and it could be employed also to separate and characterize other classes of proteins.

Fractions collected by EPE were also used to raise polyclonal antibodies against a major LMW albumin component.

Genetic variation was found for HMW albumins and LMW albumins in 50 Italian bread wheat cultivars analysed by SDS-PAGE at pH 8.4. F2 progeny from several crosses are currently being analysed to study allelic variants for HMW albumins.

A novel pair of HMW glutenin subunits was found in the French wheat line Ben 84290. Analysis of the F2 progeny from the crosses Ben × Centauro and Ben × Thésée showed that these new subunits are coded at the *Glu-D1* locus. The 1D-
encoded y-type band of Ben is the fastest moving HMW subunit described in bread wheat cultivar.

Work is in progress to determine the amino acid composition of this band after purification by electroendosmotic preparative electrophoresis.

The F2 seeds from the two crosses involving line Ben were sown in the field. The SDS sedimentation test will be performed on the F3 progeny in order to obtain preliminary information on the effects of the novel HMW subunit on gluten quality.

3. Conclusions

Preliminary results were obtained on the chromosomal control of HMW- and LMW albumins. A novel HMW glutenin subunit was isolated in the bread wheat line Ben 84290. Work is well underway.

Task C.6 - Production of Lines and Near-Isogenic Lines with Different HMW-Glutenin Subunits Compositions and of Null Forms

Partner 03 - Istituto Sperimentale per la Cerealicoltura, Sezione Operativa di S. Angelo Lodigiano, Via Mulino 3, 20079 S. Angelo Lodigiano (Milano), Italy.

Progress report from January 1, 1991 to December 14, 1991

Participants: N.E. Pogna, R. Redaelli, T. Lafranchis, A. Biancardi

1. Key measures of achievements - Objectives

Production of near-isogenic lines and null-forms lacking storage protein genes to be used for studying gluten structure and functionality. Transfer of genes coding for LMW-2 subunits from durum to bread wheat.
Seed samples for biochemical/technological analyses (Derivable).

2. Progress

By crossing four Alpe biotypes having different gliadins and HMW glutenin subunits, 15 near-isogenic lines have been selected by electrophoresis and sown in replicated plots in October 1991 (**Table XIII**). Small quantity of seed from each biotype are available for distribution.

Null forms lacking *Gli-A1*, *Gli-B1* and *Gli-D1* encoded gliadins and LMW glutenin subunits have been isolated in different bread wheat cultivars (**Table XIV**). Some of these lines have been used for studying gluten structure, genetic control and purification of LMW glutenin subunits, cœliac disease and baking quality.

The Canadian cv Neepawa was crossed with the Italian cv Costantino. These parents have contrasting alleles for gliadins and LMW glutenin subunits coded by the *Gli-1* and *Glu-3* loci, respectively. At each generation from F2 to F6, seeds from six spikes segregating at *Gli-1* and *3* were grown in head-rows. F7 head-rows are currently being grown in the field. Genetic analysis of F2 progeny allowed us to isolate one mutant line lacking 1B-encoded gliadins and LMW glutenin subunits. Moreover, cv Neepawa was found to possess a "selfish" gene coding for two ω-gliadin polypeptides. This gene is located on chromosome 1B, 18 cM from the *Gli-B1* locus. The effects of this gene on viscoelastic properties of gluten will be investigated.

The durum wheat cultivars Rodeo and E. Avanzi were crossed with the bread wheat cultivars Neepawa and Gladio; the resulting F1 and F2 seed were submitted to A-PAGE and SDS-PAGE electrophoresis. Seeds containing gliadin γ–45 and 1D-encoded gliadins were backcrossed to the bread wheat parents. Some F1 or F2 seeds were found to contain a new ω-gliadin band which was

absent in both parents. The nature of this new band will be investigated by two-dimensional electrophoresis techniques.

3. Conclusions

The project is proceeding according to the schedule. Seed samples of near-isogenic lines and null forms were produced and provided to some collaborators. Good progress was made on the occurrence of selfish genes in cv Neepawa. Seed will be provided to partner 07M for joint research.

Table XIII: List of near-isogenic lines having different gliadin/HMW glutenin subunit compositions, obtained from cultivar Alpe.

Line			HMW glutenin subunit			Gliadin		
			Glu-A1	*Glu-B1*	*Glu-D1*	*Gli-A1*	*Gli-B1*	*Gli-D1*[a]
1	Alpe	1 I	2*	7+8	5+10	Allele *b*	43.5	+
2		1 II		7+9	5+10		43.5	+
3		2 I		7+8	2+12		40	+
4		2 II		7+9	2+12		40	+
5		3 I		7+8	2+12		43.5	+
6		3 II		7+9	2+12		43.5	+
7		4 I		7+8	5+10		40	+
8		4 II[b]		7+9	5+10		40	+
9		1 I		7+8	5+10		43.5	–
10		1 II		7+9	5+10		43.5	–
11		2 I		7+8	2+12		40	–
12		2 II		7+9	2+12		40	–
13		3 I[c]		7+8	2+12		43.5	–
14		3 II		7+9	2+12		43.5	–
15		4 I		7+8	5+10		40	–
16		4 II		7+9	5+10		40	–

a) *Gli-D1* encoded gliadins present (+); *Gli-D1* encoded gliadins absent (–)
b) Few seeds available
c) No seeds available

Table XIV: List of null forms lacking gliadin proteins isolated in bread wheat cultivars.

Origin	Gliadins encoded by			Denomination
	Gli-A1	*Gli-B1*	*Gli-D1*	
cv Salmone	+	–	+	Salmone 3
cv Alpe	+	+	–	Alpe 3
cv S.Pastore	+	–	+	S.Pastore 4A
cv Sprint	+	–	–	Sprint 3
Alpe × S.Pastore	+	–	+	Several lines
	+	+	–	Several lines
	+	–	–	D.M.(sev. lines)
D.M. × cv Raeder	–	–	–	T.M.[a]
Neepawa × Costantino	+	–	+	Three lines

+ Present; - absent
a Few seeds available by May, 1992

Task C.7 Chromosomal Location of Storage Protein Genes, Chromosome Interaction, Protein Synthesis and Development of New Germplasm

Partner 07C - INRA-Clermont-Ferrand, Station d'Amélioration des Plantes, Domaine de Crouelle, 63039 Clermont-Ferrand Cedex, France

Progress report from January 1, 1991 to November 30, 1991

Participants: A. Bouguennee, M. Bernard and M. Rousset

1. Key measures of achievement - Objectives

Obtaining information on variability and expression of genes coding for storage protein in wheat, and on their effects on gluten quality.

2. Progress

Thirty six intervarietal substitution lines (with duplications) have been obtained in the recipient variety Courtot using six varieties with different breadmaking characteristics as donors of the chromosomes belonging to the homeologous groups 1 and 6. Two trials have been harvested. Technological analysis and statistical analysis of the results are currently being realized.

3. Conclusions

Work proceeds well as planned.

Task C.8 - Biochemical Markers for Screening Early Generations

Partner 07C - INRA-Clermont-Ferrand, Station d'Amélioration des Plantes, Domaine de Crouelle, 63039 Clermont-Ferrand Cedex, France

Progress report from January 1, 1991 to November 30, 1991

Participants: G. Branlard, D. Khelifi and M.T. Nieto-Taladria

1. Key measures of achievement - Objectives

A theoretical study was carried out on the efficiency of electrophoresis of biochemical markers in breeding. The effects of (1) methods of sampling, (2) number of implicated loci, (3) frequency of the alleles to be screened and (4) generation of the grain analysed on the efficiency of electrophoresis of biochemical markers in breeding were particularly studied. A publication has been submitted to the International Journal "Theoretical and Applied Genetics".

2. Progress

The quality score based on the relationship between individual LMW subunits of glutenin and quality is being established on the basis of allelic diversity of the LMW-B zone proteins. Another quality score including HMW glutenin subunits and D zone proteins is currently studied. This second index will be achieved during 1992.

3. Conclusions

These indices regarding six loci will be of a great help for breeders. We have to confirm the effect of some alleles by analysing several offsprings before using these biochemical markers.

Task C.9 - Somaclonal Variations for Factors Affecting Breadmaking Quality

Partner 02 - Società Produttori Sementi S.p.A., Galleria del Reno n. 3, 40122 Bologna, Italy

Progress report from January 1, 1991 to November 15, 1991

1. Key measures of achievement - Objectives

To evaluate the modifications induced by somaclonal variation in characters affecting quality. Seed samples of somaclonal variants and information on these variants (Deliverable).

2. Progress

In 1990, about 600 lines (from R3 to R5) belonging to the genotypes Adria, Eridano, Genini, Granarolo, Leopardo, Oderzo, Pandas, Salmone were sown in a selection field.

These lines were carefully examined in order to find deviations from the original genotypes. When deviations occurred, the plants were harvested and part of their seed was analysed in order to find out if they differed from the original genotype also in SDS sedimentation value and in N.I.R.A. data. If that was the case the remaining seed was sown in 1991 in order to check if the deviations we observed are stably inherited.

Some of the somaclonal variants which stably inherited the deviations from the original genotype are included in a trial sown in 1991.

Table XV gives information about the variants we studied in more detail in cooperation with Partner 23 (Viterbo University). We believe all of them lack the satellite of the 1B chromosome and therefore also the *Gli-B1* and *Glu-B3* loci.

Table XV: Somaclonal variants (R5 generation) showing deviations from the original cultivar (check).

Genotype	SDS sedimentation value (microtest)	Grain Hardness (N.I.R.A.)	Protein content % (N.I.R.A.)	NOTES
Salmone (check)	88	257	14,6	Red glumes. Semi-erect leaves
Salmone variants				
10435	76	251	15,6	White glumes. Recurved leaves
10441	76	250	16,6	" " "
10456	77	252	15,3	" " "
10523	86	244	15,9	" " "
10524	78	247	15,3	" " "
10529	80	247	15,3	" " "
10514	95	235	16,7	White glumes. Semi erect leaves
10516	89	237	17,1	" " "
Oderzo (check)	67	222	11,6	
Oderzo variants				
5053/5	73	213	14,2	
5059/5	84	202	15,0	

We found also other variations, but we do not know yet if they are fixed; these include absence of α-gliadins in Oderzo and lack of *Gli-A1* and *Gli-B1* gliadins in Salmone. A Gemini variant showing a change from hard to soft grain texture did not confirm this modification but was sown again for a second check; in any case we found the change in grain texture in a naturally occurring mutant of the same variety.

In 1991 we sowed in the selection field also F3 plants derived from crosses between somaclones of Oderzo and Pandas and R2 lines of Eridano and Pandas. Seed was sent to Partner 14 (FMBRA).

3. Conclusions

Somaclonal variation offers a unique possibility to compare the effect of the lack with the effect of presence of genes coding for storage protein in the same genetic background. Our aim is to obtain stable variants differing from the original varieties for the lack of these genes.

The production trial gives us the possibility to know the performance of these variants and to produce seed for technological tests that should show us how single loci affect technological quality.

Task C.10 - Sprouting Resistance

B. Partner 17 - TNO (TNO-CIVO), Utrechtseweg 48, Post Office Box 360, 3700 AJ Zeist, The Netherlands.

Progress report from January 1, 1991 to November 26, 1991

1. Key measures of achievement - Objectives

First year's deliverables: start of germplasm production, selection of the basis of an assay, evaluation of the assay.

2. Progress

A breeding program has been developed in cooperation with four wheat breeders, consisting of crosses between material varying in dormancy as well as α–amylase content in the absence of sprout damage. Material from the parents of harvest 1991 is characterized for dormancy and α–amylase levels. The results indicated that the progenies of the crosses will be useful. Progenies are multiplied by the breeders and will be available in 1993.

Varieties with a dormant or non-dormant character have been selected using a germination test. The character of the varieties has been confirmed by ABA-sensitivity testing, which showed that dormant varieties are more sensitive to ABA-inhibition. These results confirm that the germination test reveals a biochemically based form of dormancy.

A bio-assay has been developed to test the inhibitory effects of ABA or wheat extracts on germinating embryos. Results show that the test is able to discriminate between sensitive and non-sensitive varieties. Also, inhibitory effects of extracts can be measured with this bio-assay. disadvantage of this test is the relative insensitivity and therefore the amount of extracts necessary.

An other assay has been developed based on aleurone layer mediated induction and secretion of α–amylase by added gibberellic acid. This bio-assay is more sensitive and therefore less material is necessary. With this method several varieties have been tested for gibberellic acid sensitivity. Addition of extract to the test-medium shows inhibitory effects on measured α–amylase activity. The inhibitor does not directly affect the α–amylase activity, indicating that the factor(s) is not an

inhibitor of the class of α–amylase inhibitors but is possibly regulated at the induction and/or secretion level. This test will be used as the bio-assay for isolation of the inhibitor.

Work has started on the isolation of the markers for dormancy in wheat bran. The results of bio-assay testing show that inhibitory action is present in water-soluble, heat-stable and ethanol-soluble extracts. This indicates that markers for dormancy are present in the extracts. Preparative fractionation of the extracts with molecular sieve HPLC shows inhibitory action in certain fractions. The inhibitory compounds are not as selective in the sense of working as an osmoticum. The fractions will be further characterized.

From these active compound containing fractions new analytical purifications have been started with RP-HPLC.

Results so far, show that no modifications in the program are necessary. The isolation of the markers can be continued according to plan.

3. Conclusions

The project is proceeding according to schedule.

Report of the Technical Meeting "Workshop on Protein Purification", held at S. Angelo Lodigiano, December 12-13, 1991

Following the decision taken during the Meeting of the Scientific Management Committee (Paris, September 24, 1991), a workshop on protein purification has been organized by the Istituto Sperimentale Cerealicoltura (I.S.C.) at S.Angelo Lodigiano in December 12-13, 1991. The workshop has been attended by the Scientific Coordinator J-C. Autran and by colleagues from Partner 07M (M.H. Morel and V. Gazanhes), 07C (G. Branlard), 04 (G. Malgarini), 02 (S. Ravaglia), 06 (P. Wong Fat), 22 (A. Peruffo and A. Curioni and G. Pressi), 23 (D. Lafiandra and M. Ciaffi), 25 (C.M. Brites) and 03 (R. Redaelli, T. Lafranchis, N.E. Pogna). During the two-days workshop the following topics have been discussed.

1. Characterization and purification of B-group LMW-glutenin subunits. Dr. M.H. Morel has presented the progress achieved in identification of LMW glutenin subunits in several bread wheat cultivars and on the procedure for characterization and purification of those polypeptides.

2. Subunit composition of polymers extracted from gluten by 50% propanol. Dr. A. Curioni has presented the results obtained in the application of free-flow isoelectric focusing for characterization of subunit composition of gluten polymers. A paper has been submitted for publication "Cereal Chemistry".

3. Fractionation of gluten proteins and purification of HMW and LMW glutenin subunits by glass chromatography. Dr. A. Peruffo has described the application of chromatography on glass beads for fractionation of native proteins extracted from gluten by acetic acid solutions, and for isolation of a protein fraction containing HMW- and LMW-glutenin subunits free from gliadins and monomeric proteins.

4. Two-step one dimensional electrophoresis for characterization of storage proteins. Dr. G. Branlard has illustrated the results obtained in the characterization of proteins extracted from flour by propanol + acetic acid solutions and fractionated by two-step one dimensional electrophoresis (A-PAGE × SDS-PAGE). A paper regarding allelic variation at loci coding for D-zone protein has been submitted for publication to "Theoretical and Applied Genetics".

5. HPLC and two-dimensional electrophoresis for characterization of glutenin subunits and gliadins. Dr. D. Lafiandra has described the results obtained from HPLC analysis of HMW and LMW gluten subunits. A correlation has been found between retention time and number of cystein residues in the HMW gluten subunits as well as between dough extensibility and LMW glutenin subunits alleles. Two dimensional electrophoresis (Acidic-PAGE × BASIC-PAGE) has been also used to study γ- and ω-gliadins encoded by the *Gli-1* loci.

6. Characterization and purification of HMW and LMW albumins, HMW glutenin subunits and "selfish" gliadins. Dr. R. Redaelli has illustrated the preliminary results regarding genetic polymorphism of HMW and LMW albumins, and purification of a LMW albumin (14 kDa) by electroendosmotic preparative electrophoresis. Dr. T. Lafranchis has shown the occurrence of two "selfish" gliadins encoded by genes removed from the *Gli-B1* locus in cv Neepawa. Dr. N.E. Pogna has described a new HMW glutenin subunit, its genetic control and purification by electroendosmotic preparative electrophoresis.

The Workshop stimulated collaborative studies and exchange of samples for joint researches.

CONCLUSIONS

Participant in Subprogramme C faced several problems in the first year of activity: definition of a general methodology of recording agronomical, physiological and technological data, inter-laboratory evaluation of the effects of genotype × environment interaction on technological properties of wheat cultivars, coordination of collaborative studies with participants in subprogrammes A and B. All these problems have been discussed during the meetings of the Subprogramme Managers and have been largely solved. Research projects having limited interconnections with others (that is projects involving 1 to 3 participants such as Tasks C.3 to C.10) are on schedule with very few changes and produced the first significant results. Tasks C.1 and C.2 are expected to provide reliable results by the end of 1992 - Spring 1993. However, a preliminary report of the agronomical and qualitative evaluation of 1990-91 SEN trials has been already prepared and distributed to cooperators. The final report including data of rheological tests carried out in Spain, France and Italy is scheduled to be distributed in spring 1992. Participants in three 1991 meetings of Subprogramme C showed a strong sense of collaboration as also demonstrated by the short term exchanges of scientists which occurred (or has been planned for 1992) between laboratories involved in joint projects.

Annex I

List of Southern Europe Network (SEN) wheat cultivars grown in 11 locations in 1990-91

	Cultivar		Location
1	Amansor	1	ELVAS (P)
2	Amazonas	2	GRANADA (S)
3	Avital[a]	3	JEREZ DE LA FRONTERA (S)
4	Cajeme	4	TOMEJL (S)
5	Castan	5	CLERMONT-FERRAND (F)
6	Centauro	6	MONTELIMAR (F)
7	Courtot	7	TOULOUSE (F)
8	Florence Aurore	8	ANCONA (I)
9	Golia	9	BOLOGNA (I)
10	Jecora	10	FOGGIA (I)
11	Libra	11	S.ANGELO LODIGIANO (I)
12	Loreto		
13	Maestra		
14	Manital		
15	Mec		
16	Mira		
17	Mondego		
18	MP 477		
19	MP 906		
20	Pandas		
21	Pegaso		
22	Prinqual		
23	Rinconada		
24	Salmone		
25	Tua		
26	Veda		

[a]Grown only at S.Angelo Lodigiano, Toulouse, Montélimar, Clermont Ferrand, Elvas.

Amount of seed from SEN and NWEN available for distribution

Participant in charge for distribution	Seed sample[a]		Rheological analyses performed
	Location	Amount	
AC 03 test (S.Angelo Lod.)	S.Angelo Lod.	0.5 kg for each plot plus 5 kg of each cultivar at N1	SDS-Sedimentation Alveograph at N2
	Ancona and Foggia	0.1 kg for each plot	--
AC 02 (Soc. Produttori Sem.) test	Bologna	10 kg for each plot cultivar at N1 and N2	SDS Sedimentation (protein content)
AC 07C (INRA Clermont F.	Clermont F.	4 to 6 kg for each plot[b]	Several tests are in course
AC 18 (Club des 5)	Montelimar	10 kg of each cultivar at N1 and N2[b]	"
	Toulouse	20 kg of each cultivar at N1[b]	"
SC 25 (ENMP-Elvas) test	Elvas	5 kg for each plot	SDS-Sedimentation
SC 24 (EER de la M. Spain) (Club des 5)	Granada Jerez Tomejl	5 to 10 kg for each cultivar at N1 and N2	Alveograph

[a] Each cultivar has been grown at two levels of nitrogen (N1 and N2) with 2-3 replications each (25 cultivars × 3 replications × 2 levels of N = 150 plots)

[b] Seed from NWEN is also available (4 to 5 kg for each plot at Clermont Ferrand, 5 to 12 kg for each cultivar at Montélimar and Toulouse). The NWEN cultivars are: Apollo, Artaban, Baroudeur, Camp Rémy, Génial, Récital, Renan, Rossini, Soissons, Talent, Thésée and Viking.