Commission of the European Communities

ECLAIR Programme

Contract n° AGRE 0052

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

---

To Explore and Improve the Industrial Use of EC Wheats

Third Scientific Annual Progress Report from 1-01-1993 to 31-12-1993

Commission of the European Communities

ECLAIR Programme

Contract n° AGRE 0052

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

---

To Explore and Improve the Industrial Use of EC Wheats

Third Scientific Annual Progress Report from 1-01-1993 to 31-12-1993

Table of Contents

INTRODUCTION

***Jean-Claude AUTRAN,* Scientific Coordinator**

(IRTAC, Paris, France)

This third progress report reviews the scientific activities of the ECLAIR programme AGRE 0052 from 1-01-1993 to 31-12-1993. It is a true reflection of all our group's efforts to "Explore and Improve the Industrial Quality of EC Wheats".

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) a progress report compiled from the two-page summary of activities prepared by individual participants in the subprogramme for each task:

Partner 01	IRTAC (Coordinator), Paris	Jean-Claude Autran Monique Richard
Partner 02	Produttori Sementi, Bologna	Enzo DeAmbrogio Parivash Jenabzadeh Marilena Paolini Stefano Ravaglia Luca Bersanetti Stefano Poluzzi
Partner 03	ISC, S. Angelo Lodigiano	Basilio Borghi Norberto Pogna Rita Redaelli Anna Biancardi
Partner 04	SME Ricerche, Caserta	Giancarlo Malgarini Rita Calabria Massimo Saracino Egidio Fournier Aristide Angelillo Robert Finsterer
Partner 07C	INRA, Clermont-Ferrand	Gérard Branlard Mireille Dardevet Isabelle Felix Isabelle Gateau
Partner 07C	INRA, Clermont-Ferrand	Nathalie Robert

		Eugène Triboï Pierre Bérard Lucette Le Blevennec
Partner 07M	INRA, Montpellier	Marie-Hélène Morel Jean-Claude Autran Pierre Feillet Rita Redaelli Joëlle Bonicel Isabelle Lempereur Valérie Mélas
Partner 07N	INRA,Nantes	Yves Popineau Jacques Lefebvre Martine Le Meste Michel Cornec Jeremy Hargreaves Didier Marion
Partner 08	BSN Branche Biscuit, Paris	Aliette Verel Anne-Catherine Villain Laëtitia Kugener C. Lamiche
Partner 09	ITCF, Paris	Michel Leuillet Marie-Hélène Bernicot Christine Bar
Partner 12	IATA, Valencia	Carmen Benedito Concepción Collar Maria-Antonia Martínez-Anaya Claudia Martínez Ofelia Rouzaud Encarnacion Ibañez Elvira Seytre
Partner 13	Technical University, Berlin	Friedrich Meuser Norbert Pahne Claudia Rennau
Partner 14	FMBRA, Chorleywood	Peter E. Pritchard Brigitta Abel Sarabjit Sahi Ged Oliver Philip Greenwell Dhan Bhandari Douglas Smith
Partner 15	Gist-Brocades, Delft	Johan Plijter Mariette Uijenv
Partner 16	AFRC-IFR, Norwich	Peter Belton Ian Colquhoun Alex Grant

Partner 16	AFRC-IFR, Norwich	Mike Morgan Clare Mills Sara Holden Mary Parker Neil Rigby
Partner 17	TNO, Zeist	Robert J. Hamer Marcel Kelflkens Peter L. Weegels Roelof Orsel W.J. Lichtendonk A.M. van de Pijpekamp J.W. van Oosten H.P.M. van Laarhoven
Partner 19	AFRC-IACR, Long Ashton	Peter S. Shewry Arthur S. Tatham D.R. Hickman
Partner 22	University of Padova	Angelo D.B. Peruffo Andrea Curioni L. Furegon
Partner 23	University of Viterbo	Domenico Lafiandra Stefania Masci Mario Ciaffi Emanuele Cannarella
Partner 25	ENMP, Elvas	Francisco Bagulho Benvindo Maçãs José Cutinho Carla Moita Brites

Administrative and Financial Aspects

In the course of the report period, the administrative activities of the Coordinator were as follows:

- Preparation of the bank statements to allow payment of the 1992 funds to every partner, on the basis of the 1991 cost statements accepted by the Commission. Call letter to all partners for the 1992 cost statements (January 1993).
- The *profile sheets of participants* (consisting of updated information: name, address, phone, fax, languages spoken, involvement in ECLAIR tasks, field of expertise), as well as the *updated version of the Technical Annex* of the Programme, were finalised, printed and distributed to all participants (April 1993).
- The memorandum of the 1992 cost statements was prepared and transmitted to M. Martin Coppens in Brussels (April 14th, 1993).
- Meeting with M. Muel (UNIP) (ECLAIR Programme on Pea) on April 3rd, 1993.
- The *Second Annual Progress Report* (1992) was compiled from the three reports received from the subprogramme managers, R.J. Hamer, J.J. Plijter and N.E. Pogna, then brought in Brussels on April 14th and distributed to all participants (end April 1993).

- Meeting of the *Scientific Management Committee* on May 3rd 1993 in Brussels (M. Richard, J.C. Autran, J.J. Plijter, N.E. Pogna and S. Hardy) to restate the question of format of reports and delay of submission.
- Call letter to all partners about the new format requested for the next progress reports and newsletters, to be sent within one month after the end of each reporting period (June 30th for the Newsletter; January 31st for the Annual Report).
- Financial support of the meeting of the Programme (45 people) in Detmold, June 10th.
- The reports of the scientific results obtained in the January-June 1993 period were sent to the Commission (June 20th, 1993). A bound version of these reports, so-called *Newsletter n° 3* was distributed to all partners in October 1993.
- Meeting of the *Scientific Management Committee* on October 28th, 1993 in Paris (M. Richard, J.C. Autran, R.J. Hamer and J.J. Plijter).
- Copy of the updated financial status sent to all partners with individual information about the cumulated payments since the beginning of the programme, the cumulated justified costs, and the balance remaining to be justified (November 1993). Recall of the new deadline (January 31st, 1994) of submission of both scientific report + cost statements to the Commission, reminding that any partner in late will be paid at a next funding wave.

Scientific Aspects

The third year of the programme has been characterized by the fact that most research programmes are approaching maturity. Also, considerable attention has been given to the publication of results generated from the ECLAIR program and more than 55 publications have been already published or are accepted for publication. In addition, collaborative projects such as common set of wheat samples, book of methods, book describing agronomic and quality trials, and book of profile sheets of participants have been completed. In addition, the cohesiveness of the whole programme and the degree of communication and collaboration have been improved further as appeared in the success of the various 1993 meetings of subprogrammes in Clermont-Ferrand, Nantes and Paris (France), Bologna and Caserta (Italy), Detmold (Germany), Bristol (UK).

1) Main results obtained in 1993

In 1993, major scientific results have been obtained concerning industrial processes as well as physicochemistry or biochemical genetics.

For instance, in **milling quality**, the comprehensive model reported in 1992 has been corroborated and further extended, describing the relative influence of both chemical and morphological parameters on milling quality. An important discovery which has drawn considerable interest from millers and milling scientists is the possibility to explain 70-80 % of the variation in milling quality by endosperm ash content (especially potassium), the other factors being bran friability and kernel width.

In the **starch/gluten** project, a unique miniaturized decanter centrifuge has become available and its integration into the lab scale separation system allowed considerable reduction of residence time of gluten in the system, which has been shown to have an important effect on gluten properties. On the other hand, it was clearly demonstrated that pentosane and hemicellulose in the flour have a strong effect on gluten yield and that flour processing

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

In the **baking** studies, the main finding was the strong relation demonstrated between gel protein elastic modulus and baking performance, corroborating earlier results and extending them to wheat varieties from different countries. In addition, flour blending studies were focused on predicting dough properties from flour constituents. Again, gelproteins (or GMP - gluten macropolymer) play a key role (GMP changes from a linear polymer in flour to a three-dimensional structure in dough) and a prediction equation for the GMP content of dough was developed.

In the work on identification of flour parameters determining the quality of **semi-sweet biscuits,** a main finding is the importance of flour protein quality related parameters (amounts of gliadins and glutenins) which could also be confirmed by experiments on an industrial scale. Also, the effect of mixing conditions on the dough rheological and biochemical properties has been investigated, suggesting that the crossover between G' and G" as well as dough free water content and protein aggregation profile emerge as important parameters.

In view to determine the relation between flour properties and the quality of **sweet bakery products** on the one hand and rheological characterization of flour samples, a test bake procedure has been optimized in 1993 in terms of reproducibility and reliability. Correlations were found with flour protein content and water absorption characteristics of the flour. Dynamic rheological studies with flour slurries have succeeded to discriminate flours in terms of Ge*, which relates to structural characters of the protein network.

Investigations on **sour doughs** became very productive in terms of results. More and more it becomes apparent that due to the careful and thorough set-up of this study a valuable basis is developed for an expert system on sour dough production and related flour selection.

For studying **functional components and their interactions**, new ways of characterization of **HMW and LMW subunits of glutenin** have been improved further using still more sophisticated tools: adsorption on pore controlled glass, selective precipitation by acetone and chromatographic analyses (RP-HPLC, IE-FPLC). As a result of increased collaborations with geneticists of subprogramme C, analyses of substitution lines and null lines by a triple system (A-PAGE, SDS-PAGE, IEF) allowed to describe **the composition of the main LMW allelic types** present among European wheat cultivars. The proteins corresponding to specific alleles such as *Glu-3A* correlated to differences in dough extensibility have been purified in a reduced and alkylated form, and investigated as far as the charge distribution is concerned. The development of a simple procedure of determination of the number of cysteine residues directly from electrophoretic bands allowed to develop further the hypothesis on the relation between the number of cysteine residues of a subunit and its potential role in the determination of dough extensibility.

The studies on the effect of HMW and LMW glutenin subunits on glutenin polymer properties and on **rheological behaviour** of gluten have been extended using lines with deletion of various gliadin or glutenin loci, suggesting that the deletion of LMW loci decreased the proportion of large size polymers. Additional information was obtained from **electron spin resonance** studies of the gluten subfractions, indicating that polymerization of subunits resulted in less mobile polypeptide chains and more rigid proteins.

On the other hand, the studies on the **stability to denaturation** of a number of HMW subunits has been completed by fluorescence and circular dichroism spectroscopies while mixograph studies on incorporated gliadins or glutenin subunits to a dough have started.

In the study of **minor components associated with starch granules**, a considerable advance on the biochemical nature of friabilin was obtained. It is now clear that *in situ* friabilins have to be considered as lipoproteins. They are involved in some way with endosperm texture, but not in a way that has so far enabled to use them in a rapid diagnostic test for endosperm hardness.

New homologies between starch granule proteins and lipid binding proteins have been described through immunochemical studies, cDNA sequencing and peptide sequencing, suggesting that puroindoline a-friabilin basic 1 might be considered as "true" friabilin whereas puroindoline-b is likely to correspond to friabilin basic 2-3 and to the friabilin first isolated by P. Greenwell from starch granule of soft wheats. Moreover, puroindoline-a would be mainly located in the aleurone layer while puroindoline-b would be mainly located in the starchy endosperm. Puroindoline was also shown to interact strongly with anionic phospholipids and to exhibit an important structural flexibility which controls lipid binding specificity and foaming properties. Such a behaviour, already observed with membranotoxic proteins, might be important at the air-water interface during the gas phase expansion of bread doughs.

In the work on **interfacial behaviour of dough** during mixing it was demonstrated that the breakdown of macropolymers during mixing can be clearly seen in the surface active behaviour of dough samples, that added lipids have a strong influence on the surface behaviour, but that no difference is observed between soft and hard wheat types.

In the project on **dynamics of dough development**, 1993 has been characterized by the success in the production of monoclonal antibodies to arabinoxylans using both water-insoluble arabinoxylans from bees wing bran and arabinoxylans conjugated to BSA as a protein carrier. These antibodies have been characterized by ELISA methods and are now used for analysis of arabinoxylans in flour as well as for immunolocalisation in microscopic studies.

Simultaneously, laboratories and breeding companies involved in **biochemical-genetics and physiology (North-Western- and Southern-Europe Networks)** have made efforts to supply technologists and biochemists with wheat samples produced in highly controlled conditions and have carried out technological analyses that led to significant results in terms of 1) potential yield of the top cultivars in several European locations, 2) quality characteristics, 3) correlation between quality traits and agronomic factors, 4) effect of nitrogen fertilization, and 5) characterization of growth environments. On the other hand, the work on **genotype x environment interactions** is now focusing on the main determinants of protein content and composition.

Investigations on **gliadin and LMW glutenin subunits** provided us with a genetic approach to describe allelic composition at the *Gli* and *Glu-3* as well as mono-dimensional and two-dimensional techniques to identify the gliadin or glutenin components encoded by the different alleles at those loci. Moreover, it was decided to develop an European nomenclature of LMW glutenin subunits based on A-PAGE, SDS-PAGE and A-PAGE X SDS-PAGE fractionation of glutenins.

The work on genetic and technological aspects of **HMW glutenin subunits** and **HMW albumins** has now added new information about 1) effects of HMW subunit 2 on gluten quality, 2) DNA sequence of unexpressed subunit 2 gene in the A6 line, and 3) allelic variation for HMW albumins.

Studies on the production of lines and **near-isogenic lines** (NILS) are reaching maturity rapidly. Several NILS of cv. Alpe have been distributed to colleagues of subprogrammes A

and B for rheological studies whereas NILS from the cross Neepawa x Costantino are used for description of alleles coding for LMW glutenin subunits.

Finally, work on **sprouting resistance** has made significant progress. Progenies showing a broad variation in dormancy are in multiplication whereas germination inhibitors are currently being tested.

In conclusion, the activities of participants are now focused on completion of the various tasks and reinforcement of relationships between labs in view to retain the present network through concerted actions and to prepare future joint researches.

2) Collaborations between subprogrammes

a. Scientific Meetings in 1993

- 1st Meeting of the ECLAIR crossed group "Rheology", 16-17 March 1993 at INRA-Nantes
- 5th Meeting of Subprogramme A, 6-7 May 1993, organised by SME Ricerche, Caserta (Italy)
- 5th Meeting of Subprogramme C, 17-18 May 1993, organised by Produttori Sementi in Bologna (Italy).
- 6th Meeting of Subprogramme B, open to an international discussion on glutenins, 10 June 1993, following the 5th Gluten Workshop, 7-9 June in Detmold (Germany).
- 7th Meeting of Subprogramme B, 4-5 November 1993, organised by AFRC-IACR in Long Ashton Research Station, Bristol (UK)
- 6th Meeting of Subprogramme A, 2-3 December 1993, organised by BSN, in Paris and Athis-Mons (France)
- 6th Meeting of Subprogramme C, December 1993, organised by INRA-Clermont-Ferrand (France).

b. Updated Technical Annex of the Programme

The technical annex of the contract has been an extremely valuable document during the starting period of the programme. Because it was out of stock and also partly out of date since it was elaborated in May 1990 on the basis of the proposal of the ECLAIR project (October 1989), it was decided to update it. Keeping the general aim of the programme and of the tasks, some changes were made, including new collaborations that were not planned initially, the drop of some minor points, and the new schedule of some tasks. This new version of the technical annex was distributed in April 1993.

c. Book of Profile Sheets of Participants

To make the collaborations easier and to make clear which are the aims in the different research groups, it was decided to prepare a document corresponding to a "Who is Who in our ECLAIR Programme", consisting of a set of profile sheets.

Based on the updated content of the technical annex, it contains the address, phone, fax, languages and picture of the participants, with a short summary and field of expertise (key words), so that everybody can easily know and contact the relevant person for any problem, and can detect where other subprogrammes are the most supportive.

This document, including 80 profile sheets and an index of key words, was completed and distributed to all participants in April 1993.

3) Agenda 1994: "Important dates to recall"

- *9 June*: Plenary Meeting of the Programme in The Hague (Netherlands), following the ICC Meeting (5-8 June).

- *10 June*: 7th Meeting of subprogramme A in The Hague (Netherlands)

- *15 June*: Deadline for sending the individual contributions for the 4th Newsletter (report on January-June 1994 activities)

- 27 *September*: 8th Meeting of Subprogramme B, preceding the Meeting on "Wheat Kernel Proteins - Molecular and Functional Aspects" in Viterbo (Italy) (28-30 September)

- *15 November*: Deadline for sending the individual contributions for the Annual Report (report on 1994 activities)

5th Meeting of subprogramme A in Cetara, near Naples (Italy), 6 May 1993

5th Meeting of subprogramme C in Argelato (Bologna), Italy, 18 May 1993

6th Meeting of subprogramme B, extended to international specialists of wheat proteins, 10 June 1993, Detmold (Germany)

7th Meeting of Subprogramme B in Long Ashton (Bristol), 7 November 1993

SUBPROGRAMME A: INDUSTRIAL PROCESSES

Robert J. Hamer, **Subprogramme Manager**

(TNO, Zeist, The Netherlands)

Review of Activities

As mentioned in Newsletter no 3, the first half of 1993 was marked by the keywords 'continuation' and 'completion'. In 1993 all participants were urged to focus on completion of their tasks and to adapt their projects accordingly, if needed. This topic was the emphasis of the first 1993 meeting hosted by SME Ricerche in Caserta (6-7 May 1993). Also considerable attention was given to the publication of results generated from the ECLAIR program. The second meeting of subprogramme A was hosted by BSN and held in Paris (2-3 December 1993). In comparison to previous meetings even more results were presented, a clear demonstration that a very focused research programme is being carried out. Again much attention was given to completion of tasks and publication of results. Again a collaborative study was carried out on a common set of wheat samples. Results of that exercise are currently analysed and will be reported early 1994. Also the second edition of the methods book was completed and finalized at the Paris meeting. This edition will now be distributed to all participants. All in all the progress obtained in 1993 gives reason for satisfaction and optimism.

The work of TNO and FMBRA on milling is reaching completion. The findings at TNO have now been corroborated and further extended. The main finding is that endosperm ash content can explain 70-80 % of the variation in milling quality. Detailed analysis of the parameter led to the discovery that variation in potassium content of the complete kernel can explain 77 % of milling quality. This is really an important discovery which has drawn considerable interest from millers and milling scientists. Other factors determining milling quality are bran friability and kernel width.

The work at TUB on gluten starch extraction from wholemeal flour is making a steady progress. In 1993 a unique miniaturized decanter centrifuge has become available, which has been integrated into the lab scale separation system. This will allow a considerable reduction of residence time of gluten in the system which is considered to have an important effect on gluten properties.

The project on the improved separation of gluten and starch at TNO has enfaced substantial progress in 1993. Experiments clearly demonstrate that pentosans and hemicellulose in the flour have a strong effect on gluten yield and that flour processing properties are strongly determined by the way flour milling fractions are blended. This information is of great practical value for millers producing flour for the wheat starch industry.

At FMBRA a study was carried out to compare the baking performance of UK and German wheat varieties. The main finding of this study is the strong relation demonstrated between gel

protein elastic modulus and baking performance, corroborating earlier results and extending them to wheat varieties from different countries.

Work at TNO on flour blending was very much complementary to the work at FMBRA. In this task attention is focused on predicting dough properties from flour constituents. Again, gelproteins (or GMP) play a key role. Using statistical techniques a prediction equation for the GMP content of dough (given standard mixing conditions) was developed, showing no dependence of glutenin composition. On the contrary, the quantity of GMP in flour and dough resting time are the parameters of importance in predicting dough properties. In addition to the work of FMBRA it was demonstrated that rheological properties of GMP change drastically from flour to dough. Results indicate that GMP changes from a linear polymer in flour to a three-dimensional structure in dough.

Work on the identification of flour parameters determining the quality of semi-sweet biscuits is carried out at BSN in conjunction with INRA Montpellier (07M). BSN has studied a large series of flours to corroborate earlier findings. A main finding is the importance of flour protein quality related parameters (amounts of gliadins and glutenins) which could also be confirmed by experiments on an industrial scale. INRA has focused on the effect of mixing conditions on the dough rheological and biochemical properties. The crossover between G' and G" as well as dough free water content and protein aggregation profile emerge as important parameters from this study.

SME Ricerche focuses on the relation between flour properties and the quality of sweet bakery products on the one hand and rheological characterization of flour samples on the other. In 1993 the test bake procedure has been optimized in terms of reproducibility and reliability and a series of flours have been investigated. Correlations were found with flour protein content and water absorption characteristics of the flour. Dynamic rheological studies with flour slurries (40 %) have succeeded to discriminate flours in terms of Ge*, which relates to structural characters of the protein network.

The work at IATA on sour doughs turns out to be very productive in terms of results. More and more it becomes apparent that due to the careful and thorough setup of this study a valuable basis is developed for an expert system on sour dough production and related flour selection.

Individual Progress Reports

Task A.1.1 - Milling Quality

Partner 17 - TNO Food and Nutrition

1. Team: Dr. R.J. Hamer, Ing. W.J. Lichtendonk, Ir. M. Kelfkens, A.M. van de Pijpekamp, Ir. P.L. Weegels, J.W. van Oosten, Ir. R. Orsel

2. Progress

In 1993 investigations on the second year's set of samples and preliminary statistical analysis has been performed in the project on milling quality. Results confirm earlier findings, but also new findings have been made. In both years an important role of the endosperm ash content has been found with regard to milling quality. A large part of the variation (70 to 80 %) in milling quality is explained by the ash content of the endosperm. In the second year also 13 % of the variation can be attributed to differences in bran friability. The use of ferulic acid as a marker for bran contamination in flour has been shown to be a useful tool. When analysing the role of minerals in relation to ash content, an important effect of potassium was noted. The differences in endosperm ash content could be explained for 92 % by potassium contents in the endosperm. It also appeared that the potassium content of the kernel is a far better predictor of flour ash content than the ash content of the kernel. The potassium content of the kernel explained 77 % of the variation in milling quality, whereas the ash content of the kernel only explained 36 %. This means that the potassium content of the kernel has a high predictive value for milling quality on the basis of ash content. The analysis of morphological data revealed in independent sets of samples an important role of kernel width. Short and broad kernels are favourable with regard to flour yield and bran friability. It was also shown that below a certain kernel width the flour yield is reduced and the ash content of the flour increased.

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

Partner 17 - TNO Food and Nutrition

1. Team: see above

2. Progress

The project on the improved separation of gluten and starch has enfaced substantial progress. Spectacular differences in processing properties were observed between the different milling streams. The differences were due to differences in protein content (break roll flours) and

content (break roll flours) and probably to differences in protein quality and in fibre/hemicellulose content, which is high in the last reduction roll fraction (**Figure 1**). The reduction roll fractions were more sensitive to overmixing, which causes a decrease in gluten yield (**Figure 2**).

Since non-starch polysaccharides are known to affect gluten aggregation, pentosans (soluble hemicellulose; 2.5 %) and hemicellulose (insoluble; 5 %) were added to the flour streams and gluten yields were determined. Depending on the milling stream either hemicellulose or pentosans or both decreased gluten yield (**Figure 3**). The results indicate that the gluten yield and therefore the processing properties of wheat can be seriously affected by pentosans and hemicellulose. Furthermore, improper blending of milling fractions can affect the processing properties of flour.

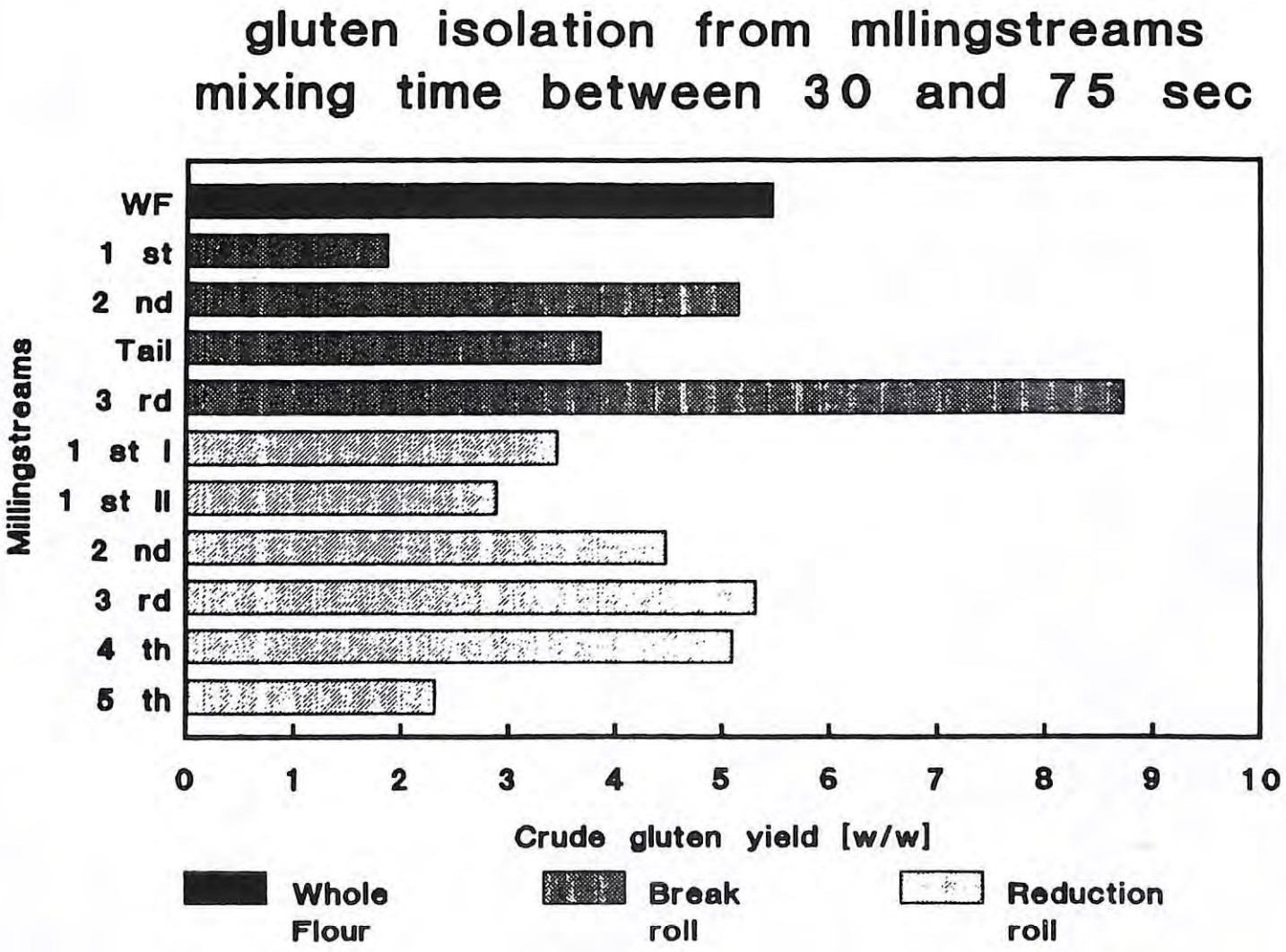


Figure 1. Gluten isolation from milling streams. Mixing time between 30 and 75 s.

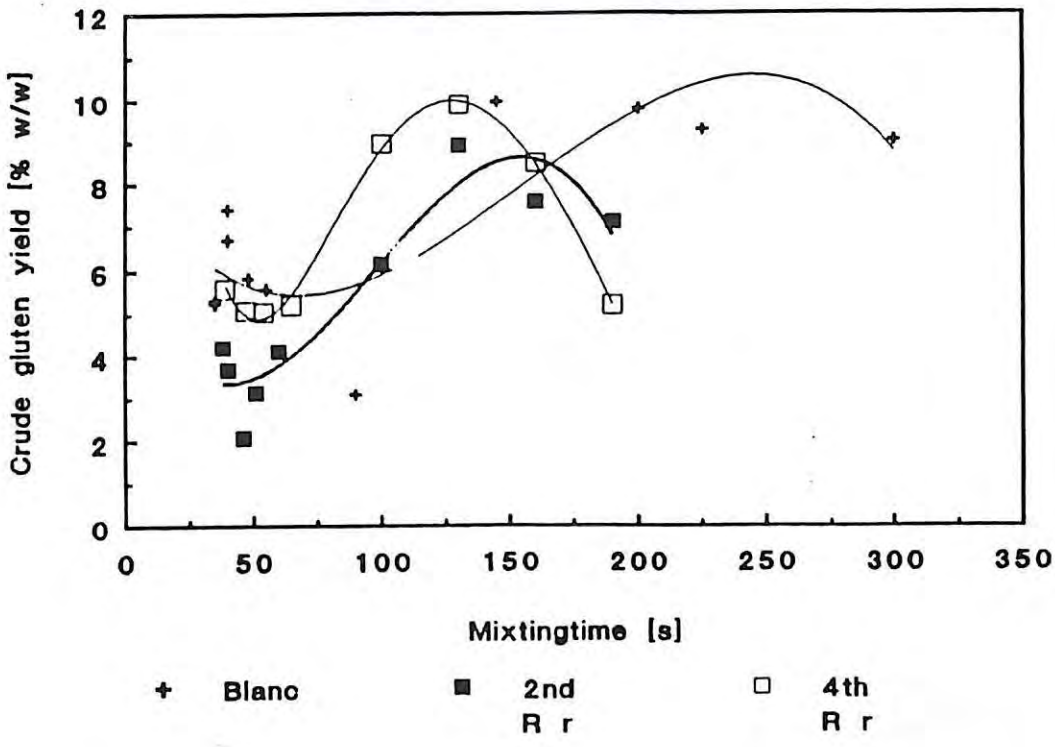


Figure 2. Gluten isolation from milling streams. Whole flour; Break roll; Reduction roll.

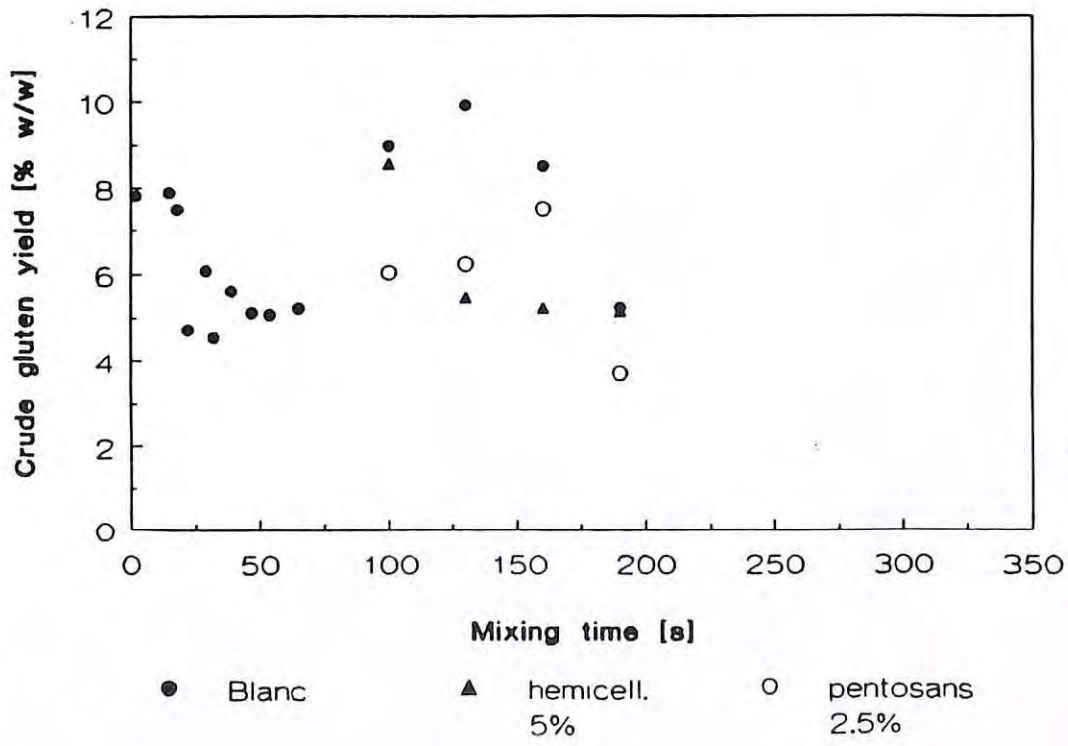


Figure 3. Effect of different Nsp addition on the gluten yield from 4th reduction roll fl.

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

Partner 13 - Technical University Berlin

1. Team: Prof. Dr. e.h. Friedrich Meuser
Dipl.-Ing. Norbert Pahne (Coworker)
Claudia Rennau (Technician)

2. Progress

Studies carried out so far have shown that wheat glutens obtained from wholemeal flours using a new separation technique for the production of starch from wheat differ in some of their characteristic properties from those which were extracted from white flours produced from identical wheat samples. The technologically most important deviations were observed in the glutens viscoelastic behaviour. Glutens obtained from wholemeal flours (G-WM) were softer and less elastic than gluten samples from white flour (G-WF). In addition to that higher contents of pentosans and hemicellulose containing substances were determined in G-WM compared to G-WF. As the same process conditions had been applied during process run the deviations observed must have been caused by the composition and the properties of the milling products used as raw materials. In this respect it is interesting to note that the milling products differed in their chemical composition as well as in their microbiological and enzymatic status.

Therefore, our recent studies were aimed to investigate the influence of microbiological and enzymatic activities occurring under the process conditions and the effect of pentosans and fibre particles on the physical properties of the gluten. For this purpose, the protease activities of the milling products, of the glutens extracted therefrom and of the process water were determined during a process run of the laboratory system over several hours. Furthermore, the change in rheological characteristics caused by the protease activity or by the addition of pentosans and fine fibre particles were studied by Glutograph measurements using a commercial gluten (C-gluten).

It could be shown that the differences in the enzymatic status of the raw materials also occurred in the glutens extracted. Wholemeal flours produced from identical wheat samples contained to some extent a higher proteolytic activity compared to white flours. Similar differences in the protease activities were observed for the glutens obtained from wholemeal and white flours. But generally the proteolytic activity of the gluten samples was lower compared to that of the flours used as raw materials. This did also apply to the other products obtained from the flours as solids (A-starch, B-starch, pentosan fraction, gluten) In summa, the largest part of the proteolytic enzymes present in the raw materials passed over to the aqueous phase, the process water. This was proven by the determination of the proteolytic activity in the recirculated process water. It was found that the proteases were concentrated in the process water during process run.

Within two hours of process run the proteolytic activity increased to a level seven to ten times higher compared to that of the flour processed. After this concentration procedure a steady-

state is achieved, which is characterised by a constant proteolytic activity. The level of this steady-state is dependent on the proteolytic activity of the raw material processed and is achieved two to three hours earlier than the equilibrium which is characterised by a constant concentration of solubles in the recirculated process water of around 6%.

The proteolytic activity present in the process water indicates that the residence time of the mass in the system must be short in order to minimize the deviations caused by enzymatic processes. Therefore, the further development of the laboratory system was mainly aimed to reduce the residence time by a modification of the separation of solids using a decanter centrifuge. This can now be reached with the newly constructed laboratory decanter centrifuge.

It is particularly interesting to note that, when processing wholemeal flours, the level of protease activity in the process water was not higher than that which occurred when processing white flours obtained from the same wheat variety. Thus it can no longer be assumed that the differences observed in the viscoelastic properties of G-WM and G-WF could be attributed to proteolytic activity during processing. Investigations carried out on reduced gluten samples using RP HPLC also showed this to be the case. The results showed no significant differences between the molecular composition of the glutens. The chromatograms of G-WM and G-WF samples taken from the same wheat variety were more or less identical.

Investigations with the aim of determining the aggregation of gluten proteins carried out on non-reduced gluten samples using SE-HPLC showed, however, that there are measurable differences between G-WM and G-WF. Fewer HMW-glutenins and more LMW-glutenins were found in all G-WM samples than in the corresponding G-WF samples. It therefore had to be investigated whether there was a causal connection between the state of aggregation of the gluten proteins or the distribution of the glutenin fractions and the physical properties of the glutens. For this purpose, the rheological properties of the glutens where also investigated using a Bohlin rheometer and a Glutograph, followed by standardised baking tests. The latter served to determine the increase in the volume during baking, 4% gluten being added to the flour used.

However, for those glutens which had been extracted using the new separation process for the production of starch, no significant correlation could be established between the molecular structure of the gluten proteins and the viscoelastic properties and the baking characteristics of the glutens.

In this connection it is interesting to note that the values obtained using the Bohlin rheometer and the Glutograph for those glutens extracted using the laboratory scale system (L-glutens) were outside the range normally expected for commercial glutens (C-glutens). The investigation of the G-WM-samples, in particular, revealed very high moduli and Glutograph times. When assessing commercial glutens, values of this kind are usually taken to indicate that the gluten structure has been damaged by lack of care during hot air drying. However, any damage of this nature to the L-glutens can be ruled out as they were dried by lyophilisation.

Further tests were therefore carried out with the aim of determining whether the differences observed could be attributed to freeze-drying. For this purpose, the viscoelastic properties of a C-gluten were investigated. The C-gluten tested had been separated industrially from wheat flour and dried both in a flash dryer and by lyophilisation. In addition to this, a gluten which

had been extracted from the same raw material using the laboratory scale system and subsequently freeze-dried was investigated. It could be shown that it was possible to expand the freeze-dried gluten to 800 BU in the Glutograph in a shorter time than the gluten, which had been dried in the flash dryer. Thus it could be proven that freeze-drying can be carried out so gently that the elastic properties of the gluten are not impaired as can occur during hot-air drying. It was therefore not possible to attribute the great differences between the Glutograph times for the L-glutens which had also been dried by lyophilisation to the drying process used. They therefore had to be caused by other factors.

The investigation into the chemical composition of the glutens showed that it was the pentosan content of the L-glutens and C-glutens extracted from the same raw material in particular which differed. The assumption that the pentosans affect the physical properties of the gluten in some way therefore appears justified. As all L-glutens with different viscoelastic properties had a high pentosan content it would appear that these properties were affected by the pentosans. In addition, the greatest differences in the rheological properties were observed in all G-WM which also had the highest pentosan contents. At the present time it is still not clear how the pentosans affect the physical properties of the glutens. This problem will therefore be the subject of further investigations.

The properties of the gluten are also affected by the process water. As has already been shown in an earlier report, the pH value of the process water decreases in all experiments regardless of the raw materials used and approached asymptotically a level of $\approx$ pH = 5. In accordance with this decrease in the pH, the acidity of the process water increases linearly. Moreover, it could be shown that the content of the total titratable acids increased at a significantly higher rate when wholemeal was processed compared to white flour. It could be shown that the increase in acidity was mainly caused by the production of lactic add. After continuously running the system for 30 hrs with wholemeal flours, the lactic acid reached a concentration of 2.5 g/l in the process water as compared to the white flour process water in which the concentration was 1.8 g/l. From this it follows that the glutens had been exposed to different acid concentrations. The effect of this exposure on the properties of the glutens had been the subject of rheological experiments.

It became apparent that the elastic properties of the gluten were altered by the action of lactic acid, depending on the acid concentration and the exposure time. In the range investigated, the time needed to expand the gluten to 800 BU decreased to such an extent that, at a lactic acid content of 4% in the rehydration water added to the gluten, no measurements were possible after an exposure time of as short as 15 min.

As the glutens were exposed to different concentrations of lactic acid during the processing of wholemeal flour and white flour, it can be assumed that the differences in the physical properties of the glutens which were established can also be attributed to the effect of different concentrations of lactic acid, even if this does not account for them fully. The same applies to the effect of fibre particles, a higher concentration of these being found in the G-WM than in the G-WF.

In experiments to investigate this, in which different percentages of finely ground wheat bran, which contains hemicellulose, were added to the gluten, the effect on physical properties of the gluten was diametrically opposed to that which had been attributed to lactic acid. An increase in the Glutograph time could be achieved by adding 8% finely ground wheat bran. This was 2.5 to 3 times higher than the Glutograph time for rehydrated gluten without added fibre. The combined effect is currently being investigated in further experiments. In addition

to the aims already stated, the next stage of the work will be to process further raw materials with the optimised laboratory scale system and to characterise the glutens thus separated. This will enable the final analysis of results and the final discussion to be carried out with greater statistical accuracy.

Task A.2.1/2 - The Characteristics and Processing Requirements of Wheat for Specific End-uses: White Bread and Wholemeal Bread

Partner 14 - FMBRA

1. Team: Dr Peter E. Pritchard (Project Leader)
Ms Brigitta Abel (Scientist, on secondment from TUB - participant 13)
Dr S Sahi (Senior Scientist)
Mr. G. Oliver (Scientist)

2. Progress

2.1. Experimentation

During 1993, a study was conducted to compare the baking performance of UK and German (G) wheat varieties. Two breadmaking varieties; Monopol (G) and Talon (UK): two feed wheats; Jaguar (G) and Riband (UK), and samples of the breadmaking variety Urban grown in the UK and in Germany were studied. Each of the breadmaking varieties (1, 7+9, 5+10), and each of the feed wheats (null, 6+8, 2+12) shared common HMW-G subunits (subunits in brackets).

The wheat samples were laboratory milled and test baked using the Chorleywood Bread Process (CBP, UK), and by the Rapid Mix Test (RMT, G). In addition, the flours were assessed by standard quality tests such as protein content and Falling Number, and by newly developed tests such as gel-protein properties, bulk rheological properties of dough and gluten, and surface rheological properties of films of dough liquor material.

2.2. Results and discussion

Flour properties are listed in **Table I**. CBP loaf volumes and crumb scores are listed in **Table II**. In general the stronger flours performed better at high work-input levels. The German grown Urban performed better than the UK sample. The poor performance of Talon may have been due to its low protein content.

RMT loaf volume, crumb and total scores are listed in **Table III**. Increasing the proving time from 55 to 65 min improved loaf volume in all samples, but only Jaguar, Urban (UK) and Monopol further improved with 75 min.

The baking tests ranked the samples in the same order (correlation coefficient between CBP and RMT loaf volumes was r^2=0.94). See **Figure 4**.

The results of the gel-protein analysis are listed in **Table IV**. There was no link between weight and baking performance. The breakdown rate clearly differentiated the feed wheats

from the breadmaking varieties. The rheological data showed significant varietal differences. In particular the elastic modulus of the gel-protein was related to baking performance (**Figure 5**).

Loaf volume increased with increasing elastic modulus up to 36 Pa, but above that level there was a decrease in loaf volume (Monopol sample). Monopol has optimum HMW-G for breadmaking, but the high elastic modulus (57 Pa) suggests that this sample had over-strong character that prevented it achieving its full potential in standard baking tests. These results again demonstrate the value of gel-protein elastic modulus in the prediction of baking quality. Those varieties containing HMW-G subunits 7+9 and 5+10 had lower breakdown rates and higher elastic moduli than did those containing subunits 6+8 and 2+12.

The surface elasticity and viscosity of dough liquor material showed no clear relation between surface properties and baking performance (surface tension was not recorded). The quantity of dough liquor was related to the damaged starch content: the lower the damaged starch (and lower water absorption) the greater the dough liquor.

The weight and elastic modulus of gluten separated from the flours are listed in **Table V**. In general good breadmaking varieties had glutens with a high elastic modulus. However, the loaf volume achieved by the Talon sample did not reflect the elastic modulus of its gluten. This was possibly because the low protein content had a dominating influence on loaf volume.

This study confirmed that the elastic modulus of gel-protein is a useful test for baking quality for UK varieties in the CBP. In addition, it also showed that this test was valuable in the prediction of baking performance of German varieties in the RMT. Both the baking tests ranked the samples in the same order of baking quality. The German varieties were superior to those from the UK. Also, the German sample of Urban was superior to that grown in the UK. Together these results suggest that climatic or environmental effects may be important in the expression of wheat quality.

Table I. Wheat and flour analysis for all varieties

Flour	Moisture %	Protein %	Falling No. s	Damaged starch FU
Jaguar	14.2	10.4	428	35
Monopol	14.2	11.2	424	32
Riband	14.4	12.0	388	14
Talon	13.6	9.3	395	44
Urban (D)	14.1	11.6	398	30
Urban (UK)	14.0	12.1	415	29

Flour	Ash %	Zeleny ml	SDS-Sed ml	Gluten ICC 137 g
Jaguar	0.66	27	51	2.77
Monopol	0.52	60	89	2.81
Riband	0.55	29	55	3.53
Talon	0.67	46	92	2.22
Urban (D)	0.54	48	88	3.13
Urban (UK)	0.53	45	86	3.32

Table II. Baking performance in the CBP

	Loaf volume				
Wh/kg rpm	8 250	8 600	11 300	20 250	20 600
Jaguar	1421	1387	1414	1445	1369
Monopol	1539	1624	1752	1693	1713
Riband	1369	1364	1290	1468	1348
Talon	1451	1540	1539	1549	1565
Urban (G)	1729	1739	1812	1801	1841
Urban (UK)	1655	1580	1693	1748	1604
	Crumb Score				
Jaguar	4	4	3	3	6
Monopol	6	6	8	7	8
Riband	5	5	6	6	6
Talon	5	5	6	6	6
Urban (G)	7	7	8	6	6
Urban (UK)	5	5	5	6	5

Table III. Baking performance in the RMT

Loaf volume

	Proof time		
	55	65	75
Jaguar	591	609	624
Monopol	696	723	771
Riband	549	570	570
Talon	600	624	615
Urban (G)	726	771	771
Urban (UK)	678	711	729

Crumb Score

Jaguar	7	6	5
Monopol	7	7	6
Riband	5	4	4
Talon	7	6	6
Urban (G)	7	7	6
Urban (UK)	6	6	6

Total Score

Jaguar	201	188	168
Monopol	268	276	269
Riband	157	146	146
Talon	220	210	206
Urban (G)	282	302	269
Urban (UK)	236	245	252

Figure 4. Flours and loaf volumes of CBP and RMT

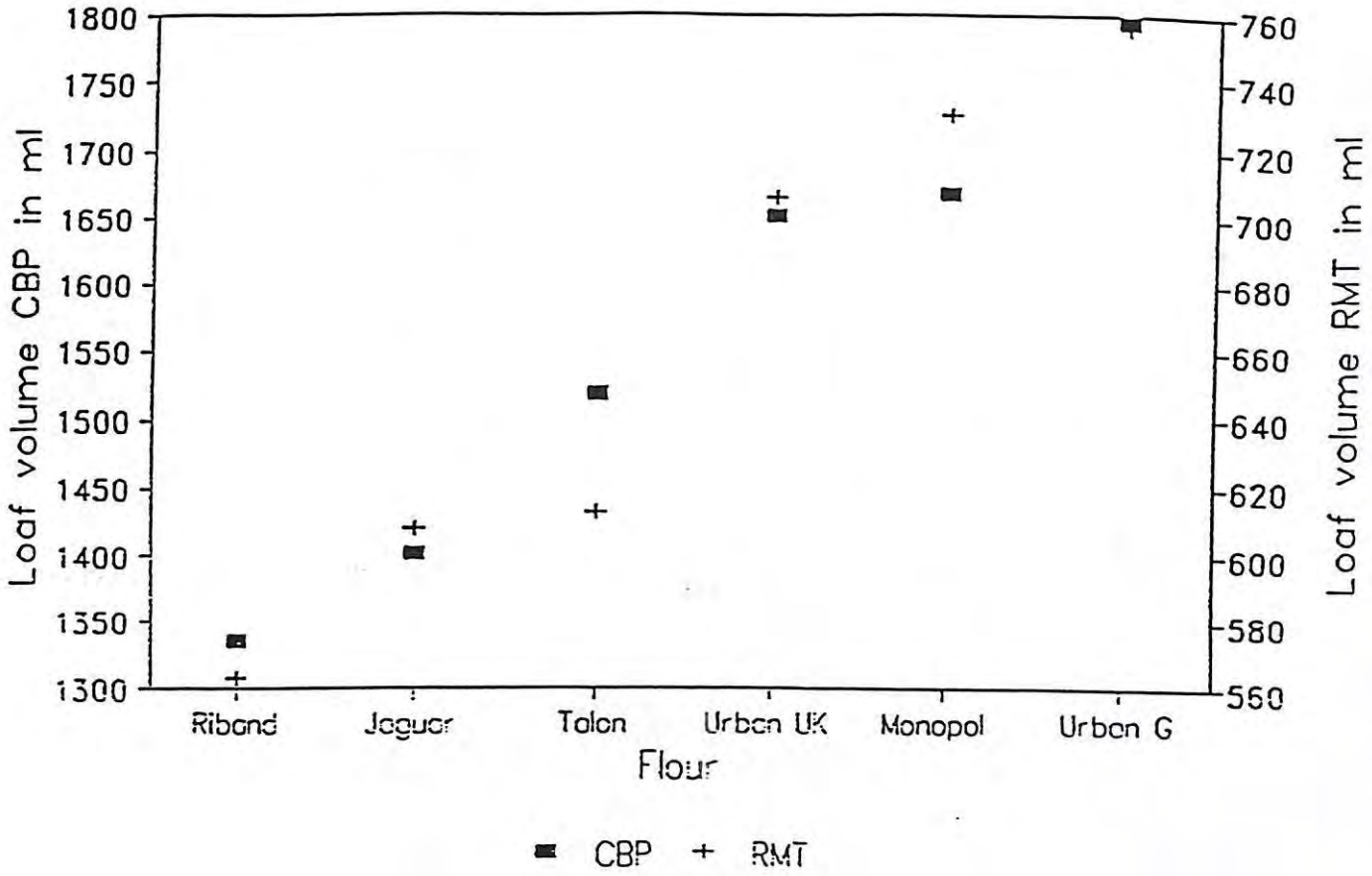


Table IV. Gel-protein characteristics

	W g/5g	Breakdown rate/min	G' Pa	δ
Monopol	10.95	0.07/0.19	57.05	36.7
Urban (G)	12.22	0.33	36.10	32.9
Talon	9.98	0.38/0.14	16.70	33.2
Urban (UK)	11.78	0.48	26.05	30.6
Jaguar	7.99	0.57	8.99	31.5
Riband	10.90	0.67	7.15	35.1

Figure 5. Estimated modulus G' of gel-proteins and loaf-volume of CBP and RMT

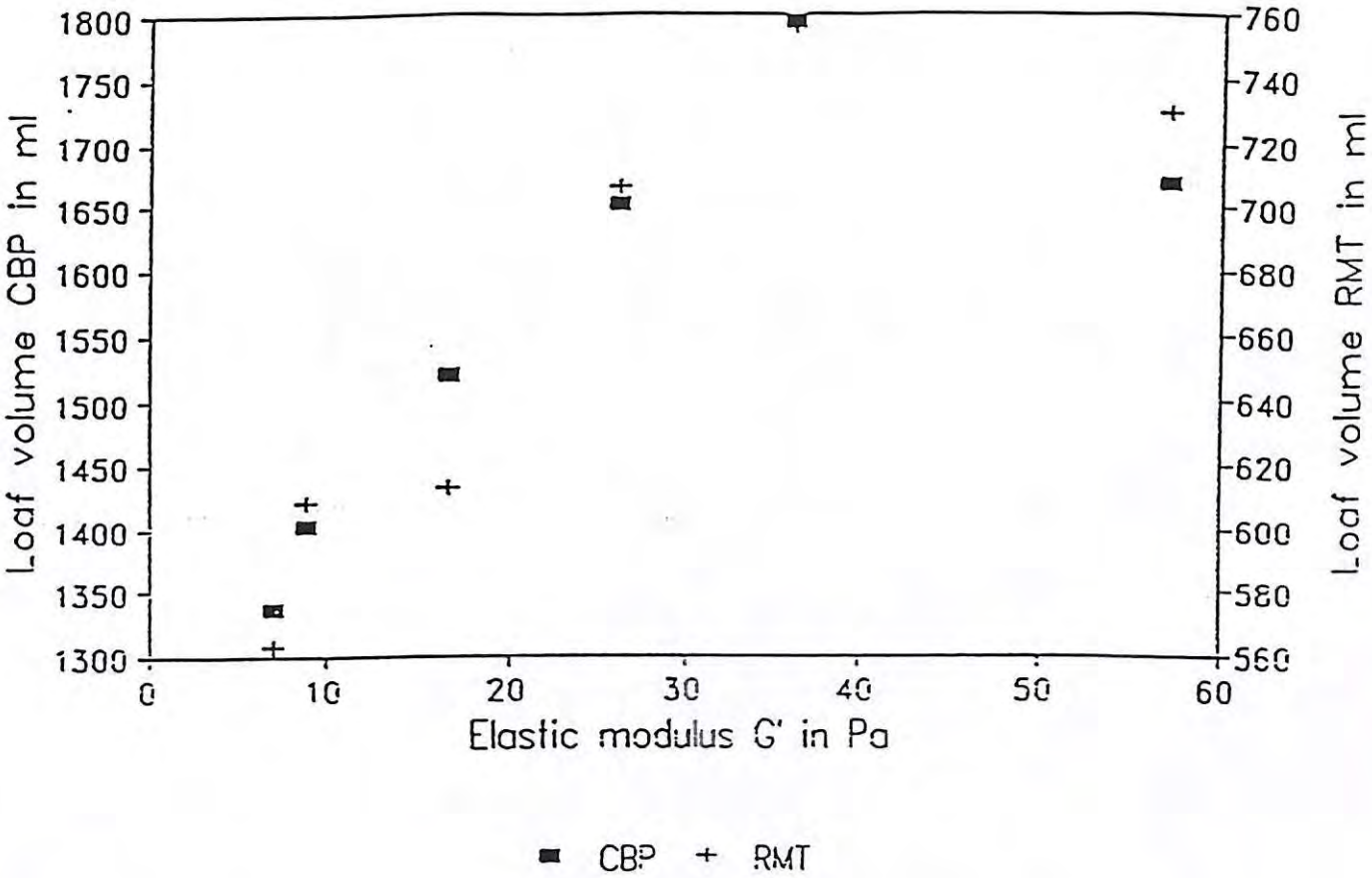


Table V. Gluten content and elastic modulus G' of the gluten at 1 Hz

	Gluten g/10g	G' Pa
Monopol	2.81	2555
Talon	2.22	1580
Urban (G)	3.13	1125
Urban (UK)	3.32	693
Jaguar	2.77	612
Riband	3.53	512

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

Partner 8 - BSN - Branche Biscuit

1. Team: Aliette Verel (Project Leader)
Anne-Catherine Villain (Researcher)
Laëtitia Kugener (Technician)
C. Lamiche (Technician)

2. Progress

Work has continued to focus on identification of flours parameters which are determining quality of semi-sweet biscuits. The set of flours samples has been extended with four flours coming from semi industrial milling of pure varieties harvested in France in 1992. The analytical characterization of flours is the same as previous years, but used the method developed by INRA (Partner 07N) for the quantification of protein fractions. The gel protein determinations have been carried out at FMBRA (Partner 14). An overview of the data obtained and the range of variation for each parameter is given in **Table VI**. Analytical data have been statistically analysed to corroborate results obtained along the previous years. The results are the following:

- We confirm that alveograph parameter P/L is increasing with components which absorb water, like damaged starch or pentosanes. So this indirect parameter, contrary to the literature, cannot really give an estimation of quality of proteins because it is based on constant hydration.

- W of alveograph presents correlations with gel protein, (correlation coefficient of 0.87) and even the breakdown rate (correlation coefficient of - 0.91). The two methods, alveograph and gel protein, which have a totally different principle, lead to obtain the same type of results.

- The hydration determined by farinograph is difficult to predict with composition parameters and a multilinear regression with damaged starch, proteins, pentosanes, median diameter lead to an explanation of only 43 % of this absorption.

- There are some relationships between pentosanes, damaged starch and ash.

The common sample set has been submitted to analyses required.

In order to improve our baking test, which was lacking of repeatability, we worked on the development of a test on a laboratory scale. New equipments have been chosen and installed. Operating conditions have been redefined for each phase: mixing, sheeting, baking. The test will be completely operational at the beginning of the year 1994. So prediction of baking quality parameters by analytical parameters would be possible next year, after the realisation of baking tests on our sample set and on the sample coming from subprogramme C (and milled at TNO (H83)).

However, measurement of variability of flours used on industrial lines confirms relationships between quality of protein (either gliadin or glutenin rate) and length and thickness of end products.

Table VI. Flours from Pure Varieties
Analytical Characteristics

Composition		Unity	Range
Moisture		%	14.0 - 16.4
Ash		% on dry matter	0.44 - 0.73
Proteins		% on dry matter	9.05 - 12.60
Albumins and globulins		% on dry matter	2.24 - 3.39
Amphiphile proteins		% on dry matter	0.84 - 1.27
Gliadins		% on dry matter	2.03 - 3.58
Glutenins		% on dry matter	3.17 - 5.37
Damaged starch		% Aud / DM	8.9 - 20.1
Pentosanes		% on dry matter	1.4 - 2.1
Soluble pentosanes		% on dry matter	0.3 - 0.7
Physical properties			
Granulometry	Median diameter	µm	19 - 64
Hagberg		s	272 - 411
Farinographe	Hydratation	%	48.6 - 56.9
	Weakening	UB	70 - 130
	Stability	min	2.0 - 12.5
Alveograph	W		71 - 206
	P/L		0.20 - 0.83
Gel protein	Weight	g / 5g	5.53 - 12.23
	Breakdown rate	per min	0.06 - 0.99
	G'		8.6 - 53.6

Another work, led in collaboration with INRA (Partner 07M), has investigated the effect of mixing conditions on the rheological properties of biscuit dough.

The rheological properties of biscuit dough (semi-sweet biscuit) were determined by oscillatory measurements on a parallel plate rheometer (Rheometrics RMS 800). A pilot scale mixer, instrumented with torque and temperature transducers in order to measure the effect of mixing conditions on the rheological properties of the dough was used. Four main factors were studied: wheat flour quality, dough water content, mixing speed and mixing time. Biscuit dough exhibits a linear viscoelastic behavior only at very low strain (below 0.25 %). At low strain (0.20 %) and whatever the mixing conditions, biscuit dough appears as a structured material, similar to a gel (G' > G", between 0.1 and 100 rad/s). However, high strains lead to a change of rheological behavior (G'/G" < 1), corresponding to a destruction of the dough's microstructure. The strain which corresponds to the crossover of G' and G" curves depends on the wheat flour variety and on mixing parameters.

3. Meetings

The 6th meeting of Subprogramme A was held in Paris, 2-3 December 1993.

Partner 07M - INRA-Montpellier

1. Team: Marie-Hélène Morel (Project Leader)
Pierre Feillet (Researcher)
Isabelle Lempereur (Research Fellow)

2. Progress

A dough undergoes various physicochemical changes during mixing that result in an improvement (followed by a decrease) of the dough handling (*e.g.* occurrence of dough stickiness), and an increase (also followed by a decrease) of the loaf volume (**Figure 6**). However, the optimum in dough handling and the maximum loaf volume do not necessarily occur for the same intensity of dough mixing. In a study aimed at monitoring a dough at different stages in the mixing and overmixing processes (MAHOT mixer) and at characterizing a physicochemical 'state of mixing', the following changes have been observed:

a) The 'free' water as expressed as the supernatant separated after centrifugation of a piece of dough, passed a minimum value at 1160 revolutions of the kneader.

b) The amount of soluble arabinoxylans gradually increased.

c) The consistency of the dough passed a maximum at 520 revolutions, then gradually decreased.

d) The amount of proteins soluble in a SDS buffer increased rapidly at first, then more slowly; it remained at a plateau value after 1160 revolutions.

e) The ratio of large-size to small-size protein aggregates, derived from the SE-HPLC elution curve, underwent a similar evolution as the SDS-soluble proteins in d).

f) The amount of gel protein rapidly dropped during the first 500 revolutions of the kneader, and then stabilized at a level four times lower than the starting value.

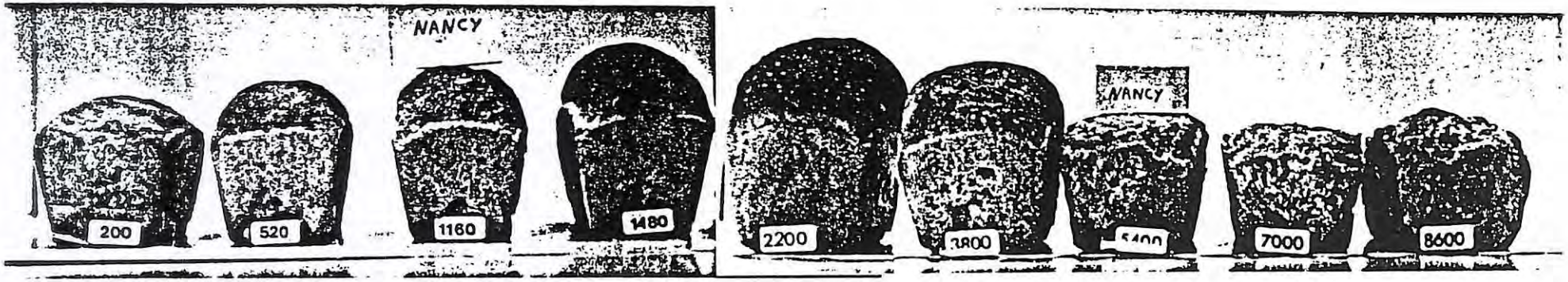
From these preliminary results, it is suggested that the best parameters for monitoring the 'state of mixing' of a dough are the 'free' water content and the aggregation profile of proteins (**Figure 7**).

3. Publications

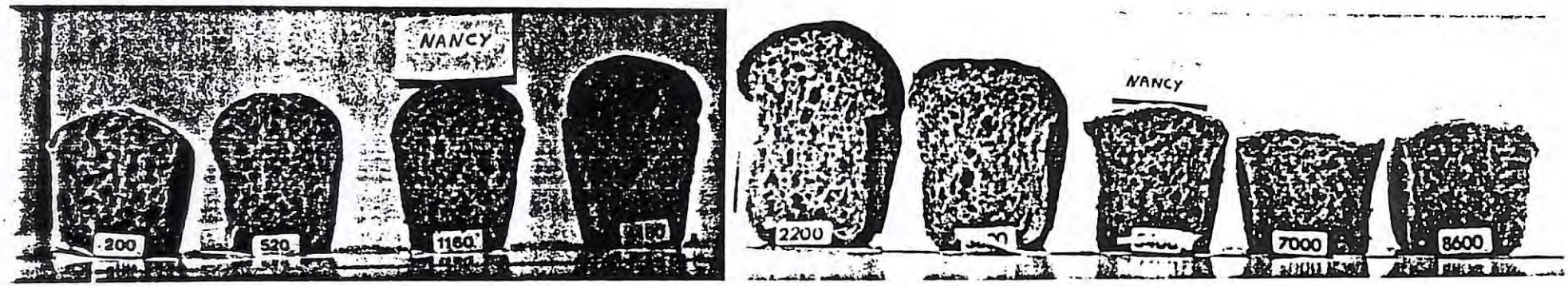
Lempereur I. 1993. Biochemical and physical characterization of the mixing state of a dough (in French). Diplôme d'Etudes Approfondies, Université des Sciences et Techniques du Languedoc, Montpellier (France), 30p.

Weegels P.L., Lullien-Pellerin V., van de Pijpekamp A.M., Autran J.C. and Hamer R.J. - 1993. Comparison of biochemical and functional properties of various cysteine-rich low-Mr wheat proteins. Lecture presented at the 78th AACC Annual Meeting, Miami, Florida, USA, 3-7 October. Abstract published: Cereal Foods World, 1993, 38, 589.

Figure 6. Micro baking test *



Loaf Volume (ml)	179	196	221	246	249	218	150	120	120

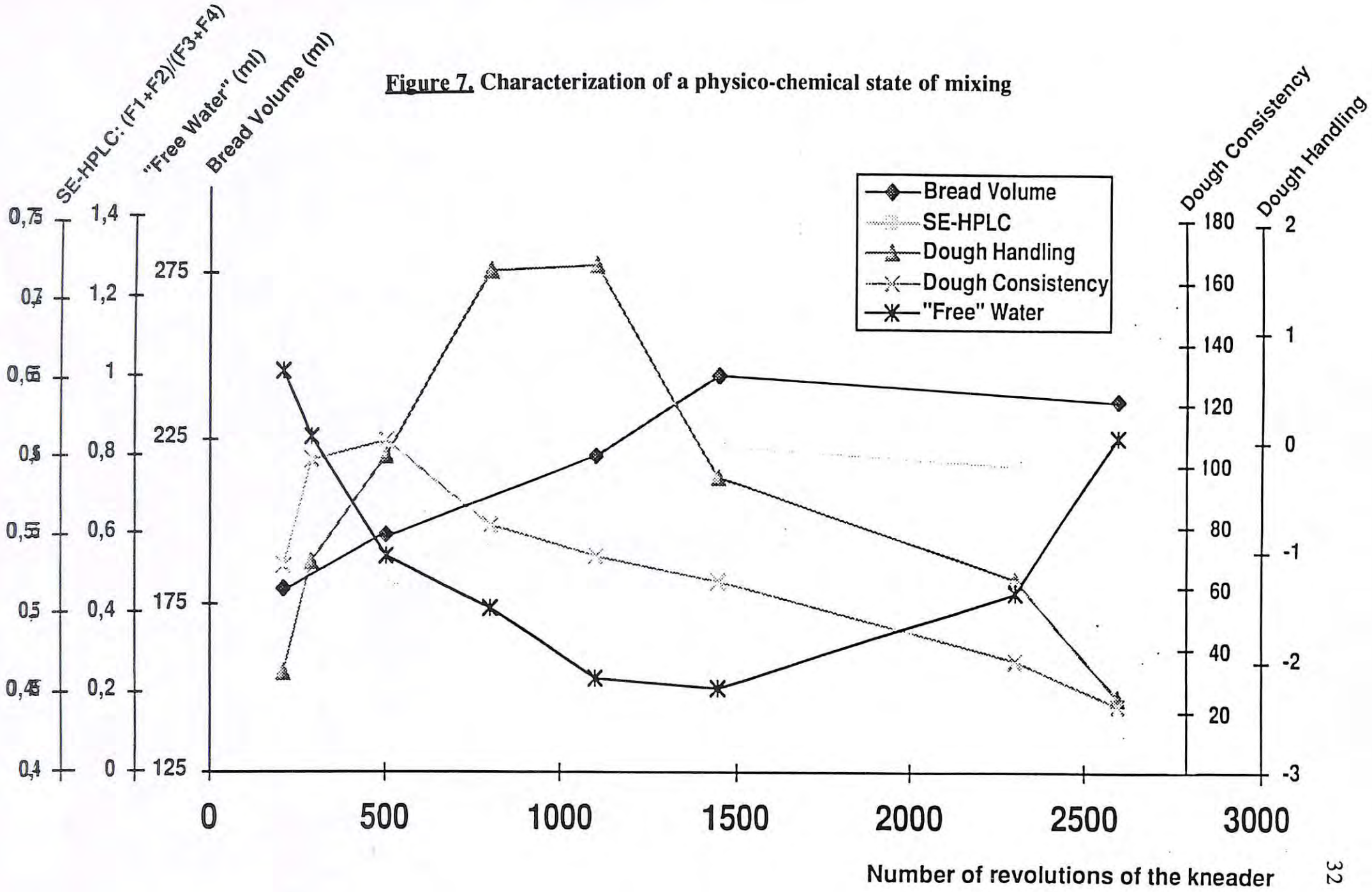


Voluminal Mass	0.27	0.24	0.21	0.19	0.19	0.23	0.32	0.42	0.42

The number on each loaf indicates the number of revolutions of the mixer

* according to Bourdet *et al.*, 1973

Figure 7. Characterization of a physico-chemical state of mixing



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

Partner 17 - TNO Food and Nutrition

1. Team: Dr. R.J. Hamer, Ing. W.J. Lichtendonk, Ir. M. Kelfkens, A.M. van de Pijpekamp, Ir. P.L. Weegels, J.W. van Oosten, Ir. R. Orsel

2. Progress

In the project on the prediction of processing properties of flour blends good progress has been made. During mixing the amount of glutenin macropolymer (GMP) decreased and during resting the amount increased again. The decrease in GMP could be predicted by an exponential decrease using a flour variable (GMP content of flour) and a process variable (mixing time; **Figure 8**). The relationship could explain 86 % of the variation in the GMP content of dough. The increase in GMP could be very well described by a function of the amount of macropolymer in flour and the resting time (87 % of the variation explained; **Figure 9**). With a similar function the quantity of the individual glutenin subunits in the polymer could be described (91 % of the variation explained). These findings indicate on the one hand that it is not so much the quality of the protein (subunit composition) which determines the reassembly of the protein during resting, but moreover the quantity. On the other hand the large amount of variation that can be explained indicates that it is possible to predict dough properties (GMP content of dough) on basis of a flour parameter (GMP content of flour) and a processing parameter (resting time). This is advantageous for the milling industry, which wants to predict dough properties prior to, i.e. without, processing, e.g. on basis of flour and processing parameters. Apart from the quantitative differences also qualitative differences occurred in the GMP. A large decrease (with one cultivar 10 times) in stiffness (G*) of the glutenin macropolymer was observed. The stiffness decreased exponentially with mixing time. Surprisingly, during resting no exponential increase in stiffness was observed, as is found normally in polymerisation reactions. Probably during mixing and resting the GMP is transformed from a linear polymer to a three dimensional gel structure. Even at mixing times where no change in amount was observed in the amount of polymer, large changes in stiffness were found. This indicates that rheological techniques are very well suited for establishing changes in the glutenin macropolymer, which cannot be detected by biochemical techniques.

3. Publications

Posters at the 5th International Gluten Workshop, June 7-9, 1993:

Orsel R., Weegels P.L., van de Pijpekamp A.M. and Hamer R.J. Relationships between the amount of glutenin macropolymer and the extensograph values.

Weegels P.L., Orsel R., Lichtendonk W.J., de Jager A.M. and Hamer R.J. Effect of LMW wheat proteins on biochemical and rheological dough properties.

Orsel R., Weegels P.L., Lichtendonk W.J. and Hamer R.J. Dynamic rheological properties of the glutenin macropolymer.

Weegels P.L., Hoffmann M.A.M., Orsel R., Hamer R.J. and Schofield J.D. Isolation, characterisation and functional properties of individual LMW wheat proteins.

Presentations at the 5th International Gluten Workshop (Proceedings):

Weegels P.L., Hamer R.J. and Schofield J.D. Depolymerisation and polymerisation of individual glutenin subunits in situ in dough - implications for the structure of gluten.

Weegels P.L., Hamer R.J. and Schofield J.D. Changes in individual glutenin subunit composition of glutenin macropolymer during mixing and resting.

Orsel R., Lichtendonk W.J., Weegels P.L. and Hamer R.J. Dynamic rheological behaviour of isolated glutenin macropolymer.

Reports: 5 (in Dutch).

Scientific publications:

Weegels P.L., Orsel R., van de Pijpekamp A.M., Lichtendonk W.J., Hamer R.J. and Schofield J.D. Functional properties of low Mr wheat proteins. II. Effects on dough properties. J. Cereal Sci. (submitted).

Weegels P.L., Flissebaalje Th. and Hamer R.J. The glutenin macropolymer depolymerises if treated improperly. Cereal Chem. (submitted).

Thesis:

Weegels P.L. Depolymerisation and repolymerisation of the glutenin macropolymer in dough and effects of low Mr wheat proteins. King's College, University of London (submitted).

Figure 8. Prediction amounts GMP dough (GD) by resting time (t) and GMP flour (GF)

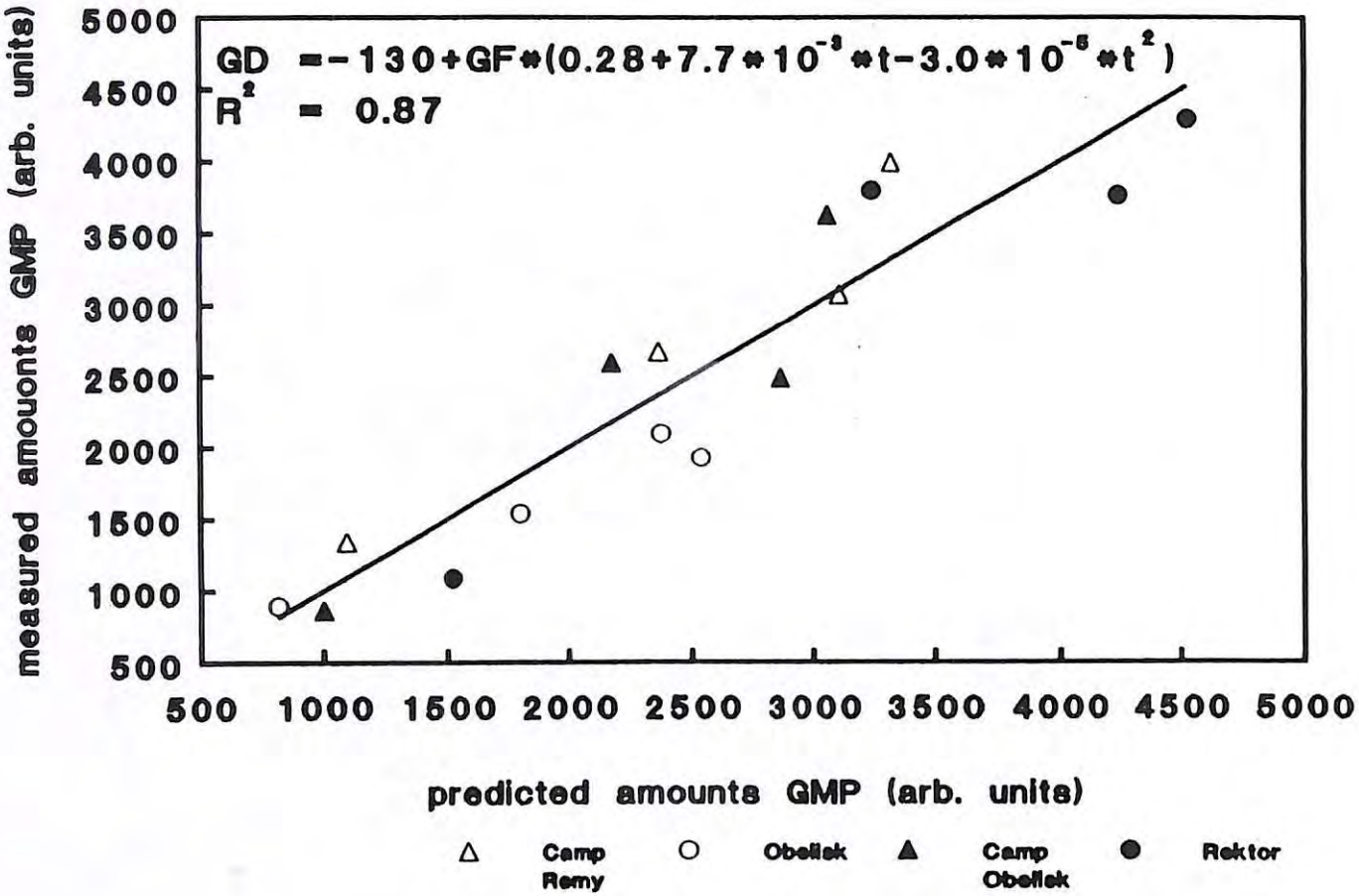
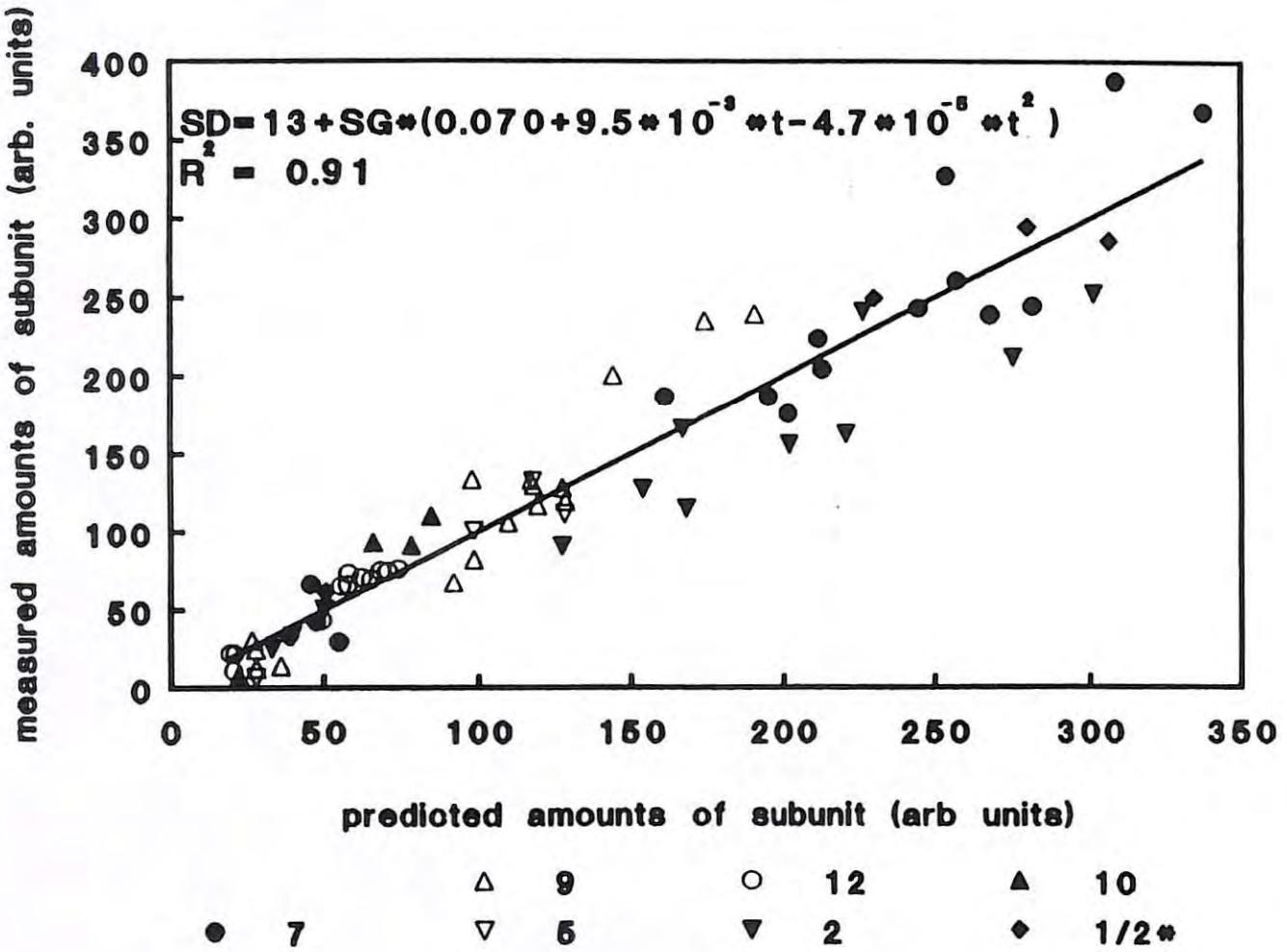


Figure 9. Prediction amounts subunit in dough (SD) by resting time (t) and subunit GMP flour (SG)



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

Partner 04 - SME Ricerche

1. Team: Giancarlo Malgarini (Research Manager)
Rita Calabria (Researcher)
Massimo Saracino (Researcher)
Egidio Fournier (Technician)
Aristide Angelillo (Technician)
Robert Finsterer (Research Fellow)

2. Key measures of achievement - Objectives

- Rheological measurement in order to obtain a better understanding of the properties of wheat; Small scale test;

- Development of a baking test to evaluate the baking quality of flour; Small scale test.

3. Progress

3.1. Rheological measurements

Dynamic measurements carried out with the Bohlin rheometer showed that it was possible to reach the region of linear viscoelasticity to characterize flour slurries.

Measurements carried out at a concentration of 40% showed that there is a better discrimination of flours respect to the concentration of 35% because of the further development of the structure.

Strain sweep tests show that there is a linear region until a strain of 0,5%-1% and that at higher values the curves decrease.

The lowest frequency does not allow to reach the well known slopes of 1 and 2 for G' and G" respectively, typically correct for a linear viscoelastic fluid. For this reason a calculation method to characterize flours was needed.

The rheological characterization was done in terms of relaxation spectrum H(l) according to a viscoelastic analysis. As the frequencies range was limited, Ge represents not only the elastic (permanent) network but also the viscoelastic (temporary) network that has not the time to relax. For this reason we call Ge modulus, Ge*.

Only Ge* modulus allows the discrimination of flours. From this parameter we can obtain the quantity of structural unity that are formed and the kinetic of destructuration.

The obtained results indicate that flours have different values of the level of structuration (**Figure 10**) and different values of the amplitude of deformation at which begin the rupture of the system (**Figure 11**). The deformation at the strain of 2,5% is very high and all the flours were destructured (**Figure 12**). The dependence of Ge* slope from the angular velocity shows that flours have different behaviour of destructuration (**Figure 13**).

Rheological data have been correlated with the value of W (**Table VII**), the mechanical energy required until the rupture of the dough.

We suppose that a flour that is much structured and with a low rate of destructuration will need more energy than a flour that is less structured and with higher rate of destructuration.

From the rheological data we observe that strong flours have an intermedium level of structuration and an intermedium rate of destructuration; alveographic values of W were the highest.

A flour presents a lower value of W because of the higher rate of destructuration, even if the level of structuration is higher. Other samples have a level of structuration comparable to strong flours but with lower rate of destructuration; so W values were lowest. At last, some samples show low rates of destructuration and low level of structuration.

3.2. Baking test

The research activities carried out during the period were the improvement of the reproducibility of the test and, in particular, of the rapeseed method for the evaluation of the volume, the correlation with other valuation methods, in particular, with alveographic data and the evaluation of the problem of water absorption.

We obtained good reproducibility using a new oven which allowed more homogeneous temperature distribution, a standardized yeast which determined more uniform leavening. Reproducibility of the rapeseed method for volume evaluation was tested and showed accurate results.

We have correlated the data obtained by the test (volume, specific volume and weight loss during baking (**Table VIII**)) with the numbers obtained by the classification method and with the main alveographic parameters (W, P/L) (**Figure 14**).

The results show good agreement between the volume of the sample and the number of the classification method and between the volume of the sample and the protein content of the flour (**Figure 15**). The evaluation of the influence of the water absorption characteristics of flour samples has been studied and again we found a correlation between water absorption and volume after baking, particularly for high water absorption flours.

4. Conclusion

The rheological characterization allows to obtain a lot of informations about the structure of the dough. The set in gear method permits to discriminate flours by means of the Ge* modulus. We think that future work will be to elaborate these data in order to obtain a quality judgement of the flours.

The results obtained with the baking test are accurate and show good reproducibility: the volume of the sample after baking can be considered as the result of the test.

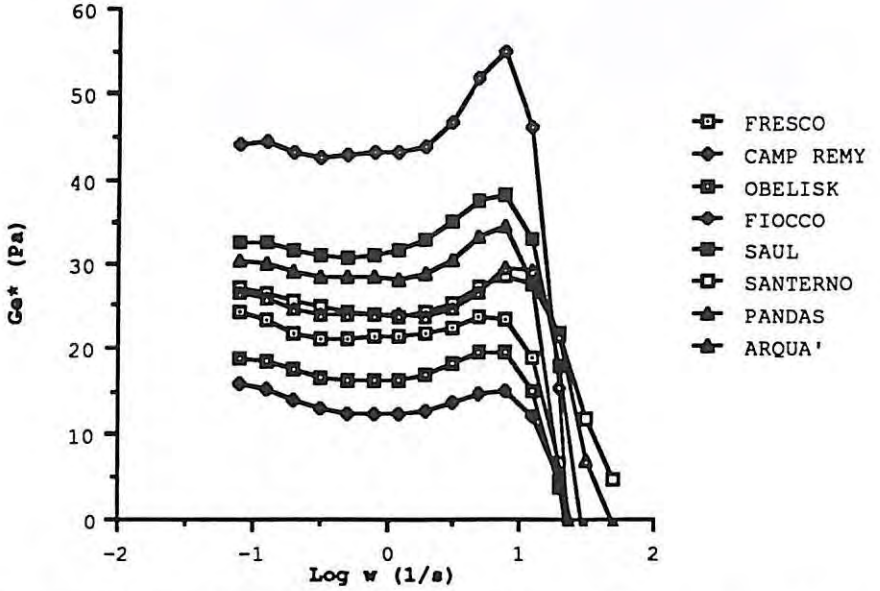


Figure 10. Equilibrium modulus comparison - A = 0.25%

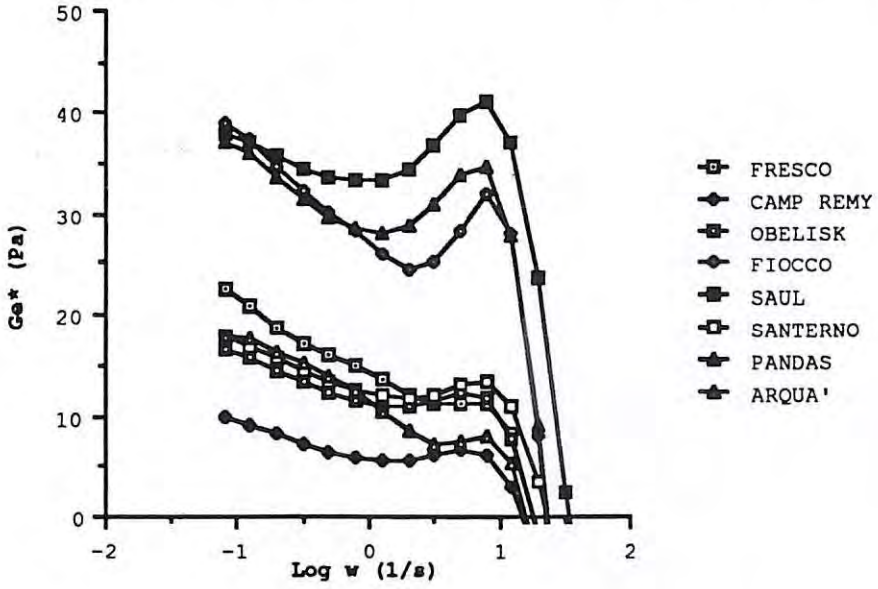


Figure 11. Equilibrium modulus comparison - A = 1%

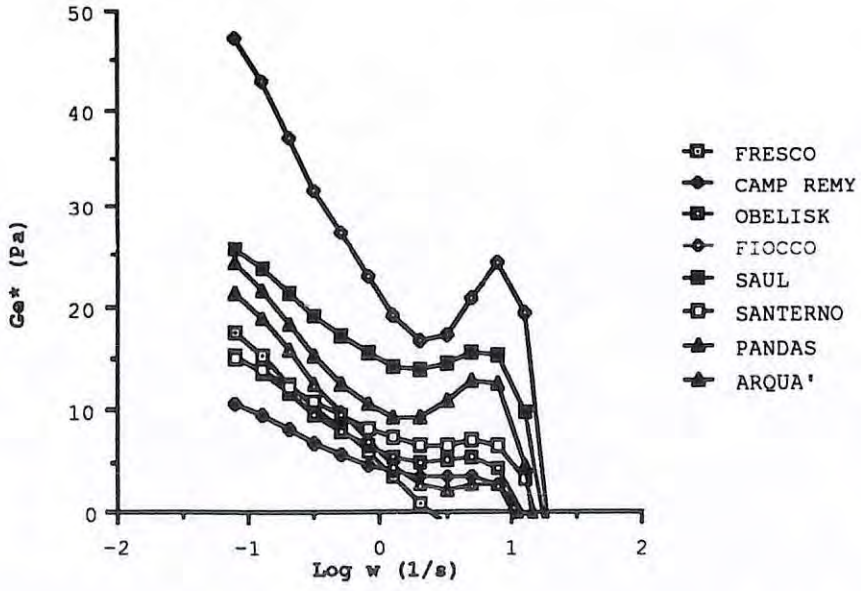


Figure 12. Equilibrium modulus comparison - A = 2.5%

Table VII. Alveographic data.

FLOUR	CLASS	G	P	L	P/L	W
FRESCO	1+	21,00	108,90	89,80	1,21	350,00
PANDAS	1	23,70	99,00	114,80	0,86	300,80
ARQUA'	1	23,60	89,80	113,50	0,79	291,00
FIOCCO	1+	21,10	97,00	80,90	1,20	281,20
CAMP REMY	2	22,30	72,80	101,30	0,72	245,00
SAUL	2	25,80	66,10	135,80	0,49	242,00
OBELISK	2	23,40	56,80	110,70	0,51	165,00
SANTERNO	3+	19,40	60,10	76,90	0,78	137,30

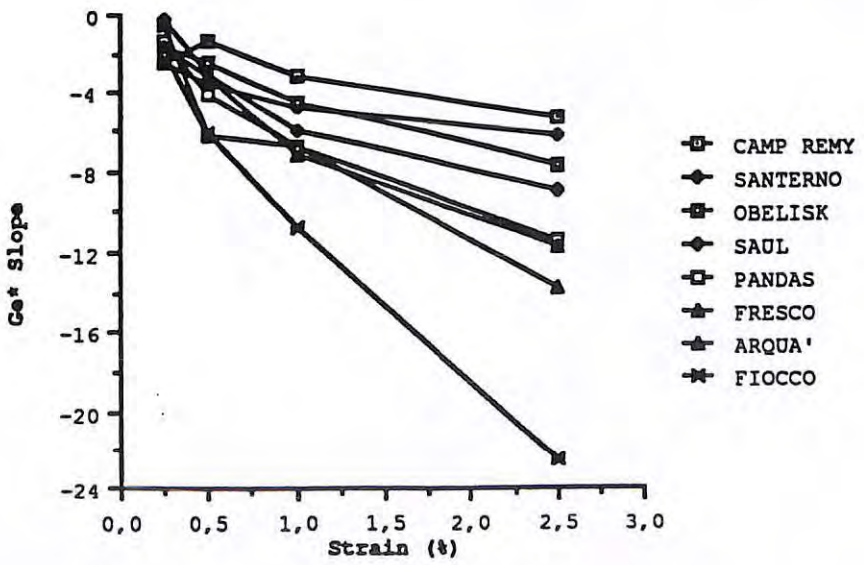
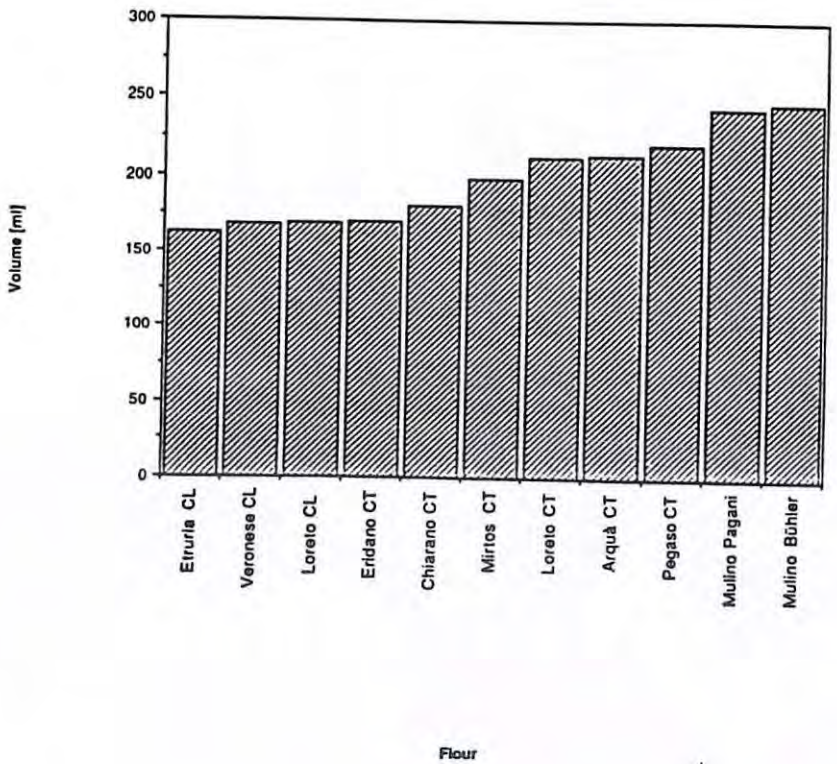


Figure 13. Equilibrium modulus slope comparison

Table VIII. Eclair Project: Results of baking test

flour	origin	volume [ml]		weight loss [g]		moisture %	
		mean	st. dev.	mean	st. dev.	mean	st. dev.
Etruria	Caltagirone	162.755	5.927	4.135	0.442	27.885	0.251
Veronese	Caltagirone	168.02	4.995	3.425	0.673	26.83	0.306
Loreto	Caltagirone	169.835	5.288	3.41	0.529	28.125	0.132
Eridano	Catania	170.47	2.398	2.85	0.401	29.13	0.131
Chiarano	Catania	180.81	6.14	4.17	0.522	28.09	0.273
Mirtos	Catania	198.185	5.241	2.9	0.607	29.435	0.336
Loreto	Catania	212.385	7.932	4.12	0.596	28.37	0.181
Arquà	Catania	213.08	7.417	3.595	0.449	28.465	0.011
Pegaso	Catania	220.3	7.891	3.79	0.74	29.77	0.161
Mulino Pagani		243.2	8.982	4.055	0.468	30.085	0.134
Mulino Buhler		246.97	7.046	4.555	0.521	29.335	0.139

Figure 14. Volume *vs.* flour.



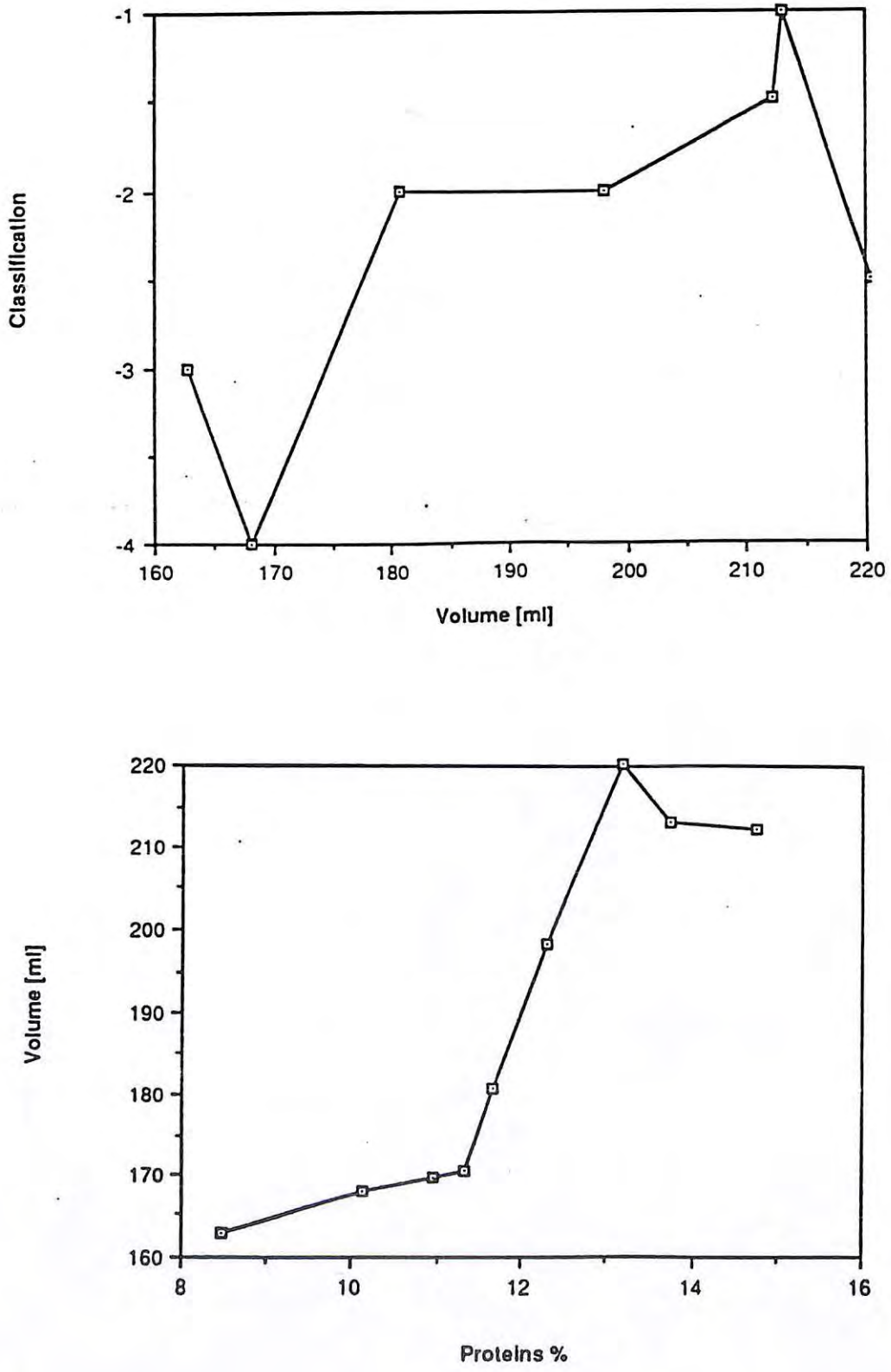


Figure 15. Correlation with other methods of flour evaluation.

Task A.2.6 - Interactions Between Selected Microorganisms and Wheat Components, and their Application to Improve Bread Making Processes

Partner 12 - IATA

1. Team: Carmen Benedito (Project Leader)
Concepción Collar (Researcher)
Maria-Antonia Martínez-Anaya (Researcher)
Claudia Martínez (Fellow)
Ofelia Rouzaud (Fellow)
Encarnacion Ibañez (Technician)
Elvira Seytre (Technician)

2. Progress

The study of biochemical aspects related with the interactions of microorganisms in flour systems have been focused on the evaluation of the effect of: 1) processing conditions, 2) processing step, 3) type of flour and starter on bread, and 4) storage time. For point I three flour samples (with varying degree of extraction) and one strain of *Lactobacillus plantarum* B-39 were used. Points 2 and 3 will include 5 different flours (MBO, GB1, B2, FR0, FR3), two microorganisms (B-39 and *L. brevis* L-62) and two kind of frozen starters (added through a sourdough (SD) and straight (ST) process). Finally point 4 was performed with FRESCO flour (70, 100 % extraction rate). The enzymatic study will consider specific enzymatic activities and changes in related nitrogen and carbohydrate fractions on model and interactive systems.

2.1. Effect of processing conditions on biochemical components of flour (**Table IX**)

A surface response design was used to study the effect of flour extraction rate, dough yield, fermentation temperature, and yeast addition. The effect of sourdough fermentation was also considered. The most influencing process variables affecting sugars and oligosaccharide levels were the presence of yeast and in a lesser extension the extraction rate of flour. Dough yield and temperature played a minor role in controlling these parameters. The ash content of flour was the main factor with positive influence on the level of nitrogen metabolites in wheat sourdoughs, and the processing condition governing the extent in the accumulation of amino acids and peptides during fermentation. Fermentation temperature governed the extent of the changes in prominent amino acids during SD fermentation and promoted the reduction of glycolipids. Dough yield increased the level of free lipid metabolites and induced variable influence on the amino acid profile of SD. The presence of yeast reduced the amount of nitrogen and lipidic components in FSD.

2.2. Effect of type of flour and starter on some biochemical characteristics of breads

2.2.1. Influence of breadmaking stage on some fermentation metabolites (**Figures 16, 17, 18**)

Breadmaking step (sour dough SD, dough fermentation UFD, FD, and baking B) and flour showed the most significant single effects on total starch, sugars and low molecular weight dextrines (LMWD). Microorganism only influenced sugar contents. Two main factors (68% variability of data) depended on LMWD, and the third factor (14%) on starch. Changes in

LMWD were primarily affected by SD and fermentation; baking did not add further differences. SD and UFD had the greatest sugar levels, and FD and B the highest of dextrines with DP > 5. Results were assessed by cluster and discriminant analysis. Content of some lipidic and nitrogen components from samples obtained along the breadmaking process characterized samples performed with flours of different extraction rate. D and B performed with white flours showed the higher levels of polar lipids and the lower of amino acids. Whole flours led to samples with the higher neutral lipid content and intermediate amounts of amino acids. SD samples were clearly separated from D and B, and properly grouped on the basis of the extraction rate of flours. Brown and whole FSD accounted for the higher amino acid values and the lower polar lipid contents; white FSD showed the higher protein and the lower level for neutral lipids. Metabolism of some individual amino acids during fermentation and their degree of participation in the baking reactions were an useful way to differentiate uninoculated from started samples, individually and binary started samples, and samples performed with white and brown/whole flours.

2.2.2. Effects of flour, microorganism and starter on some biochemical and physico-chemical characteristics of breads (**Figure 19; Tables X, XI**)

The main factor explaining variability of data was influenced by sensory attributes an physical characteristics of breads. The second factor was related with LMWD and the third one with acidity and lactic acid contents of breads. First factor separated breads in two groups on the basis of flour extraction rate, whereas LMWD allowed to differentiate white breads made with SD or straight processes. Cluster analysis included acidity and lactic acid as a separative factor grouping unstarted together with straight process breads. The best canonical correlation was established between physico-chemical characteristics of breads and carbohydrates, soluble solids and swelling power. The first pair of canonical variables showed a squared correlation coefficient of 0.986, so linear combinations of experimental variables could be determined.

2.3. Effect of storage on some biochemical characteristics of breads (**Tables XII, XIII, XIV, XV; Figures 20, 21, 22**)

This step of the working plan was performed on EC flours (FRESCO and OBELISK, with 70 and 100 % extraction rates), started with frozen B-39 and L-62. Breads were stored at 27°C until mould growth appeared. Storage variably influenced swelling power and soluble solids, depending on flour and microorganism used. Total starch and amylopectin retrogradation only underwent very slight changes. Chemical characterization of lipidic and nitrogen components of stored breads up to 4- days explained 100% of the variability of data. Factor I (68% VE) referred positively to amino acids, peptides and neutral lipids and negatively to polar lipids, and factor 2 (32% VE) positively relates to protein content. Four groups of stored breads were clearly defined after plot of scores of factor 1 *vs*. factor 2. Factor 1 separated bread samples according to the extraction rate of flours. Whole breads observed high content of neutral lipids and non protein nitrogen components; whereas white breads showed high levels of polar lipids. Along F2 axis, bread sample distribution depend on the starter they were inoculated with. Samples started with *L. brevis*, L-62 were characterized by high protein content. Within this group, the longer the period of storage, the higher the level of proteins. Breads containing *L. plantarum*, B-39 showed low levels of proteins. Protein content lowered as the period of storage increased.

3. Publications

Collar C., Martínez-Anaya M.A. and Benedito de Barber C. 1993. Interactive effects between microbial breadmaking starters and wheat flours. In: Proceedings of *Euro Food Chem Vll, Progress in Food Fermentation, Vol. 1.* Ed.: C. Benedito, C. Collar, M. A. Martinez Anaya, and J. Morell. IATA (CSIC) ISBN: 84-604-7038-5. Valencia, Spain, September 20 22. D. 75-80 (1993). Submitted for publication to *Rev. Esp. Cienc. Tecnol. Aliment.*

Collar C., and Martínez C.S. 1993. Amino acid profile of fermenting wheat sour doughs performed under different processing conditions. In: Proceedings of *Euro Food Chem VII, Progress in Food Fermentation, Vol. 1.* Ed.: C. Benedito, C. Collar, M. A. Martinez-Anaya, and R. Morell. IATA (CSIC) ISBN: 84-604-7038-5. Valencia, Spain, September 22-22, p 290-295 (1993). *J. Food Sci.,* 58 (6), 1324-1328.

Martínez-Anaya M.A., Co11ar C., and Benedito de Barber C. 1993. Comparative study on functionality of Spanish and other EC flours when used in microbiologically started breadmaking processes. In: Proceedings of *Euro Food Chem VII, Progress in Food Fermentation, Vol. I.* Ed.: C. Benedito, C. Collar, M. A. Martínez-Anaya, and J. Morell. IATA (CSIC) ISBN: 84-604-7038-5. Valencia Spain, September 20-22, p. 253-258. Submitted for publication to *J. Food Sci.*

Martínez-Anaya M.A. and Rouzaud O. 1994. Influence of flour, bacterial starter and. breadmaking stage on total starch, sugars and low molecular weight dextrines. To be presented at the 14th lCC Congress, The Hague (The Netherlands), June 5-9.

Rouzaud O., and Martínez-Anaya M.A. 1993. Effect of processing conditions on sugar and oligosaccharide profiles of wheat sour doughs. In: Proceedings of *Euro Food Chem VII, Progress in Food Fermentation, Vol. 1.* Ed.: C. Benedito, C. Collar, M. A. Martinez-Anaya and J. Morell. IATA (CSIC) ISBN: 84-604-7038-5. Valencia, Spain, September 20-22, p 308-313. Z. *Lebensm. Unters. Forsch.* 197, 434-439.

Table IXa. Sugar content[1] of unfermented sour doughs

Flour	Temp. °C	Dough Yield	Maltose		Glucose		Fructose	
			Yeast	No yeas	Yeast	No yeast	Yeast	No yeast
0.57	25	160	928	1198	751	737	520	629
		240	622	819	1028	620	603	575
	30	200	1316	592	582	325	489	327
	35	160	1013	1512	638	738	481	498
		240	1453	1470	1042	654	578	459
1.11	25	200	625	845	906	746	602	823
	30	160	904	992	850	474	680	764
		200	953	784	1254	752	737	533
		240	861	714	532	690	937	512
	35	200	880	1260	1036	1177	719	746
1.68	25	160	797	783	643	1045	515	811
		240	594	528	936	712	723	910
	30	200	792	1131	1330	1225	700	932
	35	160	792	851	1038	456	772	659
		240	849	1009	1313	843	760	707

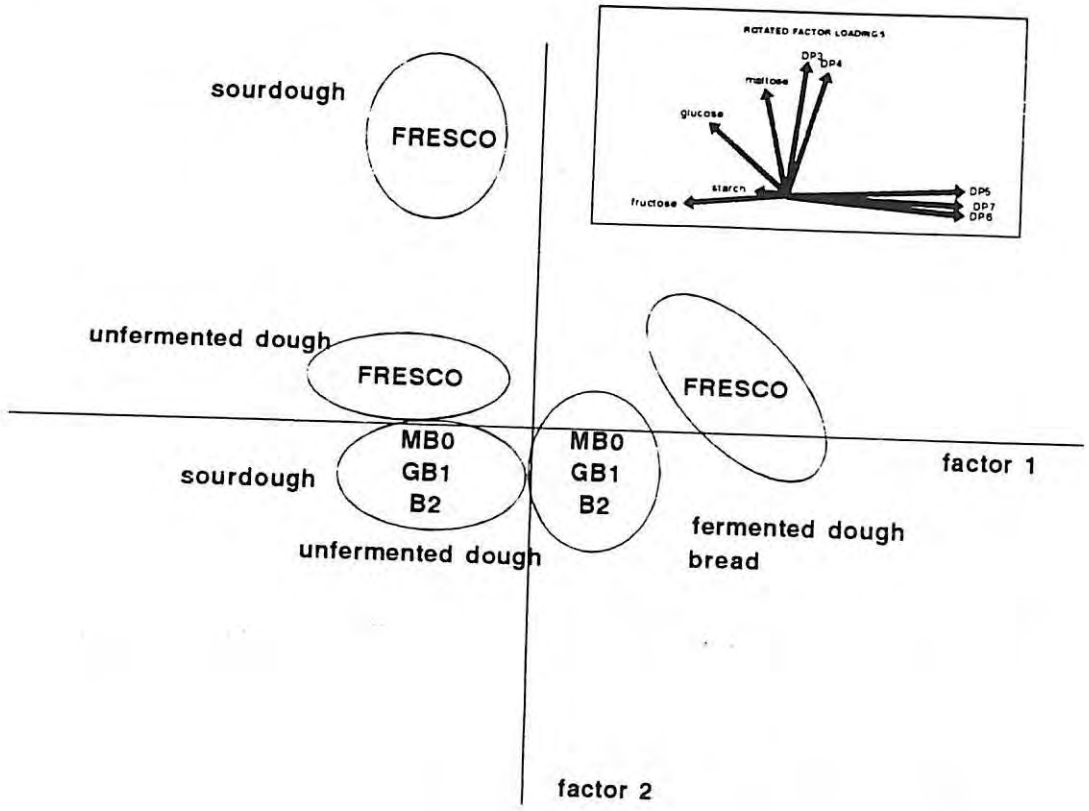
Table IXb. Sugar content[1] of fermented sour doughs

Flour	T, °C	DY	Maltose		Glucose		Fructose		DP3		DP4		DP5		DP6	
			Y	NY	Y	NY	Y	NY	Y	NY	Y	NY	Y	NY	Y	NY
0.57	25	160	2155	2249	242	498	411	488	209	198	34	28	4	3	0	0
		240	1787	2681	183	534	372	647	430	209	61	27	16	0	0	0
	30	200	1945	3154	175	387	437	867	561	423	101	53	21	4	0	0
	35	160	1900	2756	93	476	387	580	382	264	77	40	20	2	6	0
		240	88	3681	395	458	46	724	125	455	40	69	33	15	26	0
1.11	25	200	1443	1943	263	1233	606	980	507	298	78	52	19	31	0	0
	30	160	1397	2012	208	1084	589	1022	342	355	62	53	12	4	0	0
		200	210	2058	211	1206	201	982	179	356	38	52	18	9	8	0
		240	885	1939	384	1137	610	1055	396	334	60	56	30	15	11	0
	35	200	459	1805	280	1251	101	1006	223	364	43	65	11	12	5	0
1.68	25	160	1896	1448	780	1218	886	1006	629	307	104	57	23	22	0	8
		240	348	1650	226	1478	555	1201	159	309	28	54	12	18	0	0
	30	200	491	1755	129	1569	501	1002	400	349	73	50	25	10	8	0
	35	160	959	1807	953	1816	704	1152	323	469	65	104	13	28	6	0
		240	180	1396	233	1660	59	1006	135	359	21	70	9	19	8	4

[1] mg sugar/100 g flour d.w.

DY: dough yield; T: temperature; Y: yeasted sourdoughs; NY: unyeasted sourdoughs

Table IX. Effect of processing conditions on sugar and oligosaccharide profiles of sour doughs.



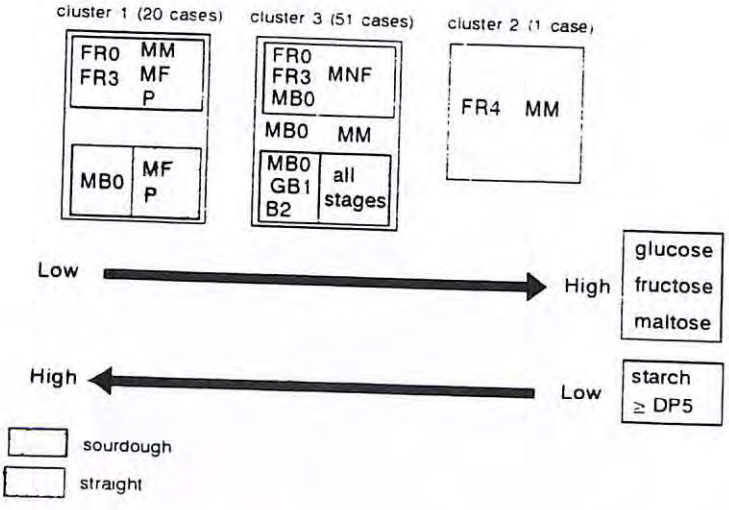
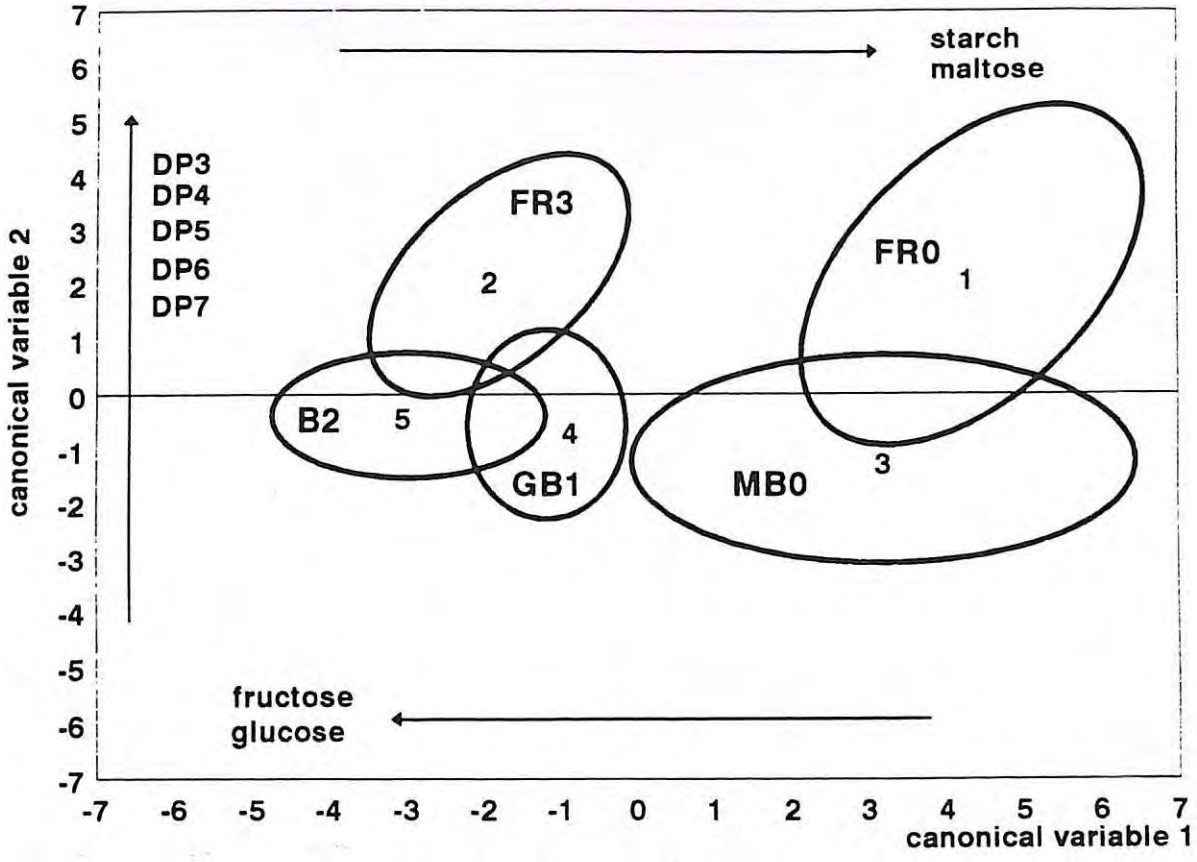


Figure 16. Influence of breadmaking process (sour dough, fermentation (unfermented and fermented doughs) and baking) on starch, sugars and low molecular weight dextrins. Up: factor analysis: characteristics of samples grouped according to the scores of the first two factors, and factor loadings. Down: K-means clustering analysis: trends of analytical variables for samples included in three preset clusters.



STEPWISE DISCRIMINANT ANALYSIS: GROUPING VARIABLE IS FLOUR.

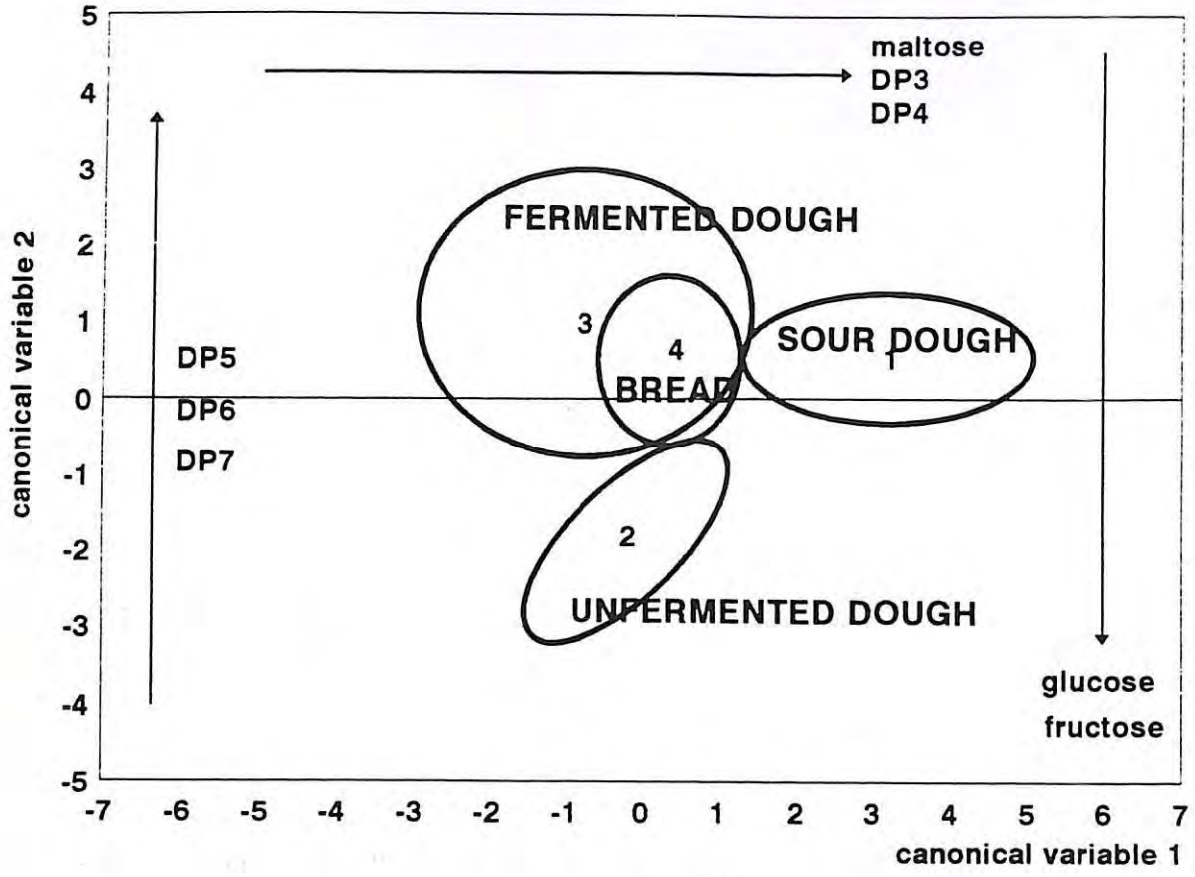
CLASSIFICATION FUNCTIONS

Variable	FR0	FR3	MB0	GB1	B2
starch	15.4000	12.6348	15.1041	13.0683	12.3651
maltose	0.0423	0.0351	0.0329	0.0294	0.0283
maltohept.	1.0327	0.8349	0.8782	0.7772	0.7226
constant	-606.4132	-408.8940	-573.1974	-430.1750	-385.4121

CLASSIFICATION MATRIX

Variable	FR0	FR3	MB0	GB1	B2	%correct
FR0	9	0	2	0	0	81.8
FR3	0	9	0	0	2	81.8
MB0	0	0	16	1	0	94.1
GB1	0	0	0	12	4	75.0
B2	0	0	0	1	16	94.1
TOTAL	9	9	18	14	22	86.1

Figure 17. Influence of breadmaking process (sour dough, fermentation (unfermented and fermented doughs) and baking) on starch, sugars and low molecular weight dextrins. Stepwise discriminant analysis with grouping variable: flour. Plotting of samples in the plane defined by the first two canonical variables, significant variables included in the classification functions, and percentage of correct classification for each group.



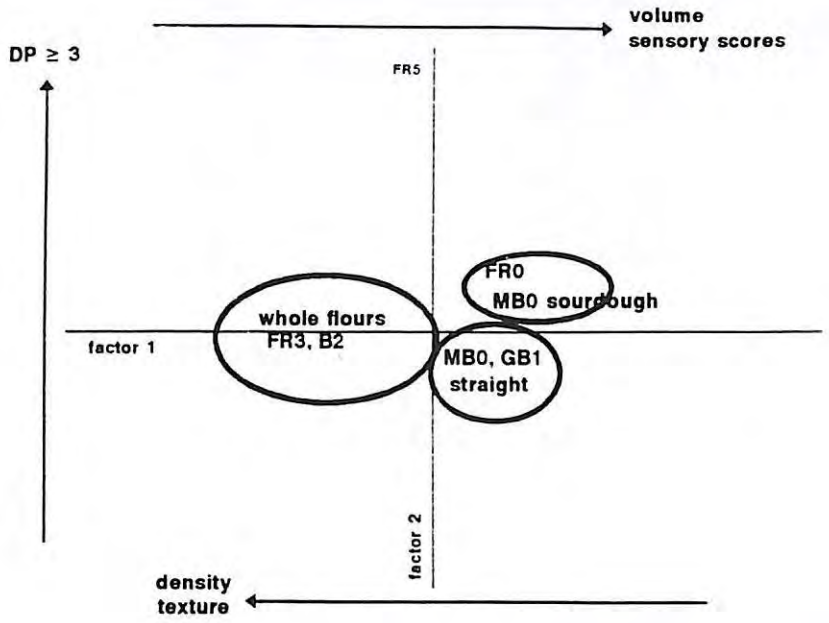
STEPWISE DISCRIMINANT ANALYSIS: GROUPING VARIABLE IS PROCESSING STAGE.

Variable	sourdough	unferm. dough	ferment. dough	bread
glucose	-0.00220	0.02540	0.01470	0.01226
maltose	0.00151	0.00765	0.00389	0.00430
maltotriose	0.02982	-0.04514	-0.02330	-0.02208
maltoheptaose	-0.07167	0.08017	0.28244	0.22414
CONSTANT	-5.71161	-7.03342	-6.10995	-4.91640

CLASSIFICATION MATRIX

STAGE	sourdough	unf. dough	ferm. dough	bread	% correct
sourdough	8	0	0	1	88.9
unf. dough	0	20	0	1	95.2
fer. dough	0	1	13	7	61.9
bread	0	1	6	14	66.7
TOTAL	8	22	19	33	76.4

Figure 18. Influence of breadmaking process (sour dough, fermentation (unfermented and fermented doughs) and baking) on starch, sugars and low molecular weight dextrins. Stepwise discriminant analysis with grouping variable: processing step. Plotting of samples in the plane defined by the first two canonical variables, significant variables included in the classification functions, and percentage of correct classification for each group.



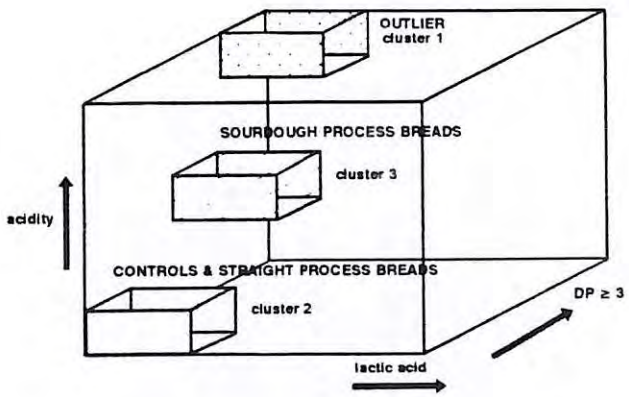


Figure 19. Effect of flour and starter addition (sourdough and straight processes) on biochemical, physico-chemical and sensory characteristics of breads. Up: factor analysis: classification of samples in the plane defined by the first two factors and trends of analytical variables related to both factors; down: K-means clustering analysis: tendencies of significant analytical variables in cases included in three preset clusters.

SQUARED MULTIPLE CORRELATIONS OF EACH VARIABLE IN ONE SET WITH ALL THE OTHER VARIABLES IN THE SAME SET

FIRST SET		SECOND SET	
Variable	R-SQUARED	Variable	R-SQUARED
volume	0.9664	swelling power	0.6576
texture	0.8948	soluble solids	0.4911
width/heigt	0.4624	total starch	0.6613
density	0.9676	glucose	0.4168
		fructose	0.5679
		maltose	0.5724
		maltotriose	0.8582
		maltotetraose	0.9286
		maltopentaose	0.9257
		maltohexaose	0.9448
		maltoheptaose	0.8995

[1] squared multiple correlations of each variable in one set with variables in the other set.

	CANONICAL VARIABLE	
FIRST SET	R-SQUARED[1]	P-value
volume	0.9686	0.0001
textura	0.9035	0.0057
width/height	0.8503	0.0260
density	0.9627	0.0001
SECOND SET	R-SQUARED[1]	P-value
swelling power	0.5527	0.0087
soluble solids	0.3408	0.1328
total starch	0.7817	0.0000
glucose	0.1586	0.5700
fructose	0.3466	0.1253
maltose	0.4453	0.0409
maltotriose	0.7773	0.0000
maltotetraose	0.7335	0.0002
maltopentaose	0.8168	0.0000
maltohexaose	0.6773	0.0008
maltoheptaose	0.6851	0.0006

CANONICAL VARIABLE LOADINGS

	CANONICAL VARIABLE			
FIRST SET	CNVRF1	CNVRF2	CNVRF3	CNVRF4
volume	-0.914	0.386	0.061	-0.112
textura	0.782	-0.406	-0.211	0.422
width/height	0.318	-0.750	0.364	0.451
density	0.942	-0.249	0.000	0.226
SECOND SET	CNVRS1	CNVRS2	CNVRS3	CNVRS4
swelling power	-0.255	0.609	0.147	0.461
soluble solids	0.535	-0.213	0.142	0.038
total starch	-0.742	0.503	0.080	-0.096
glucose	-0.026	-0.219	0.269	-0.289
fructose	0.262	-0.453	-0.204	0.302
maltose	0.119	0.545	-0.432	0.078
maltotriose	0.637	0.635	0.053	0.130
maltotetraose	0.412	0.734	0.250	0.204
maltopentaose	0.517	0.779	0.077	0.021
maltohexaose	0.345	0.746	0.228	0.145
maltoheptaose	0.115	0.826	-0.138	0.247

SQUARED CANONICAL CORRELATIONS

CANONICAL VARIABLE	SQUARED CANONICAL CORRELATION
1	0.98565
2	0.90390
3	0.85095
4	0.63439

Table X. Effect of flour and starter addition (sourdough and straight processes) on biochemical, physico-chemical and sensory characteristics of breads. Canonical correlations between selected physico-chemical (first set) and biochemical (second set) characteristics of breads: squared multiple correlations within and between set of variables, canonical variable loadings and squared canonical correlations.

Table XI. Central composite design for sampling of wheat sour doughs performed under different processing conditions.

yeast	flour	T, °C	25			30			35		
		dough yield	160	200	240	160	200	240	160	200	240
+	0.54		■		■		■		■		■
	1.11			■		■	■	■		■	
	1.68		■		■		■		■		■
−	0.54		■		■		■		■		■
	1.11			■		■	■	■		■	
	1.68		■		■		■		■		■

Table XII. Pattern of nitrogen compounds of unfermented and fermented sour doughs started with ***Lactobacillus plantarum,*** B-39.

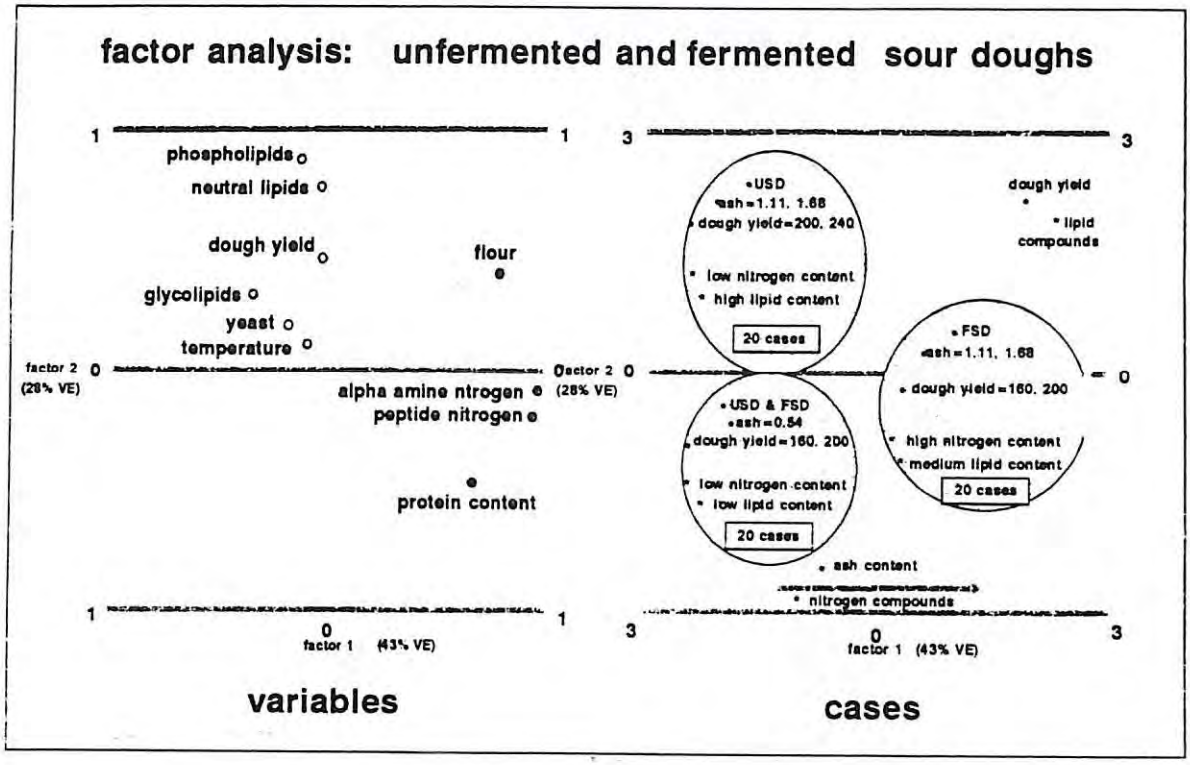
yeast	T, °C	flour	DY	amino acids (mg AAN/100 g.d.b.)		peptides (mg AN/100 g.d.b.)		proteins (mg /100 g.d.b.)	
				USD	FSD	USD	FSD	USD	FSD
+	25	0.54	160	5.511	7.411	4.595	10.288	887.53	2394.16
			240	9.540	2.901	7.694	6.064	1080.54	2360.10
		1.11	200	9.005	11.579	7.486	14.558	1021.91	1873.07
		1.68	160	6.556	22.102	3.691	16.317	1217.13	1774.09
			240	9.902	15.856	9.444	13.951	1135.06	1810.76
	30	0.54	200	5.549	3.730	3.613	5.657	1149.48	2470.20
		1.11	160	7.148	17.127	8.187	16.814	849.94	2101.90
			200	8.867	12.113	11.737	14.336	1143.38	2288.54
			240	8.927	9.625	9.155	12.472	1164.95	2298.00
		1.68	200	9.036	17.513	10.233	20.663	1168.04	1803.77
	35	0.54	160	6.401	9.801	5.747	9.548	1335.49	2637.26
			240	6.772	5.914	5.186	7.028	1154.19	2818.94
		1.11	200	8.914	21.968	7.243	26.450	1095.35	2415.04
		1.68	160	10.361	29.679	8.303	27.134	1091.73	1921.18
			240	13.686	29.011	9.491	25.191	1120.32	2194.98
-	25	0.54	160	6.644	9.106	6.148	12.295	1302.24	2810.86
			240	6.344	7.102	6.059	8.962	1146.70	2749.71
		1.11	200	8.843	16.863	8.180	15.142	1057.89	2662.54
		1.68	160	9.387	23.411	9.722	20.100	1006.14	2141.58
			240	10.295	23.202	11.665	23.621	1070.36	2269.48
	30	0.54	200	5.925	9.704	7.098	14.168	1189.40	3087.91
		1.11	160	7.113	19.746	11.224	18.009	1074.46	1973.11
			200	8.649	19.224	12.990	20.363	1027.21	2225.85
			240	8.635	19.644	10.444	19.750	1294.45	1856.11
		1.68	200	9.670	24.239	13.827	25.091	1192.88	1969.64
	35	0.54	160	6.104	12.472	9.579	13.711	1161.75	2706.96
			240	6.432	10.725	7.701	10.767	1250.86	2528.53
		1.11	200	8.242	25.884	11.015	22.451	1198.66	1944.97
		1.68	160	11.553	33.480	11.826	27.590	1084.99	1881.57
			240	10.886	33.889	9.433	29.372	1088.61	1804.39

DY: dough yield; USD: unfermented sour dough; FSD: fermented sour dough; AAN: alpha amine nitrogen; AN: primary amine nitrogen.

Table XIII. Pattern of lipid fractions of unfermented and fermented sour doughs started with ***Lactobacillus plantarum,*** B-39.

yeast	T, °C	flour	DY	total free lipids (mg /100 g.d.b.)		neutral lipids (mg /100 g.d.b.)		phospho lipids (mg /100 g.d.b.)		glycolipids (mg/100 g.d.b.)	
				USD	FSD	USD	FSD	USD	FSD	USD	FSD
+	25	0.54	160	163.00	217.92	158.92	215.48	3.74	1.81	0.34	0.63
			240	577.73	217.91	530.07	211.71	41.32	4.85	6.34	1.35
		1.11	200	255.96	167.79	246.12	161.56	9.33	4.85	0.52	1.35
		1.68	160	285.92	186.47	259.05	183.99	16.20	2.46	10.67	0.02
			240	622.96	356.03	581.02	350.56	36.38	4.83	5.56	0.64
	30	0.54	200	272.22	164.48	265.77	156.36	4.94	4.11	1.51	4.01
		1.11	160	367.00	112.72	358.62	109.91	6.61	2.36	1.76	0.45
			200	531.68	139.28	512.81	133.60	17.00	4.61	1.87	1.07
			240	551.33	204.38	520.93	197.32	27.01	5.43	3.39	1.63
		1.68	200	618.76	271.3	596.85	265.74	20.35	4.80	1.56	0.71
	35	0.54	160	186.24	244.60	180.27	243.18	3.85	1.24	2.12	0.18
			240	268.20	176.86	258.21	170.59	8.42	4.14	1.87	2.13
		1.11	200	506.52	161.66	489.93	154.89	15.46	5.46	1.13	1.31
		1.68	160	286.66	238.32	275.78	234.88	9.95	2.83	0.93	0.61
			240	672.25	241.18	640.84	230.82	30.07	8.67	1.34	1.70
-	25	0.54	160	189.52	148.46	185.36	145.71	3.31	2.30	0.85	0.45
			240	306.73	194.39	290.67	189.40	12.36	3.13	3.70	1.87
		1.11	200	580.50	151.19	552.79	146.46	25.75	3.72	1.95	1.01
		1.68	160	302.04	150.62	293.20	146.39	8.06	2.82	0.78	1.42
			240	498.00	214.56	471.62	209.50	25.41	4.44	0.97	0.62
	30	0.54	200	249.18	186.96	244.15	171.80	4.09	6.87	0.94	8.29
		1.11	160	295.93	97.92	289.40	94.67	5.75	2.87	0.78	0.38
			200	534.54	123.98	515.17	119.59	17.82	3.55	1.55	0.84
			240	450.90	135.19	426.89	131.32	22.52	3.05	1.49	0.82
		1.68	200	545.29	216.78	516.59	212.47	27.68	3.74	1.02	0.57
	35	0.54	160	203.40	134.14	199.84	131.06	2.81	2.48	0.76	0.60
			240	252.96	110.52	247.33	104.25	4.70	3.41	0.93	2.86
		1.11	200	432.54	85.24	417.62	81.05	13.89	3.67	1.03	0.52
		1.68	160	181.74	137.46	172.45	134.94	8.50	2.04	0.80	0.48
			240	475.76	190.44	457.88	182.87	16.98	7.15	0.90	0.42

DY: dough yield; USD: unfermented sour dough; FSD: fermented sour dough.



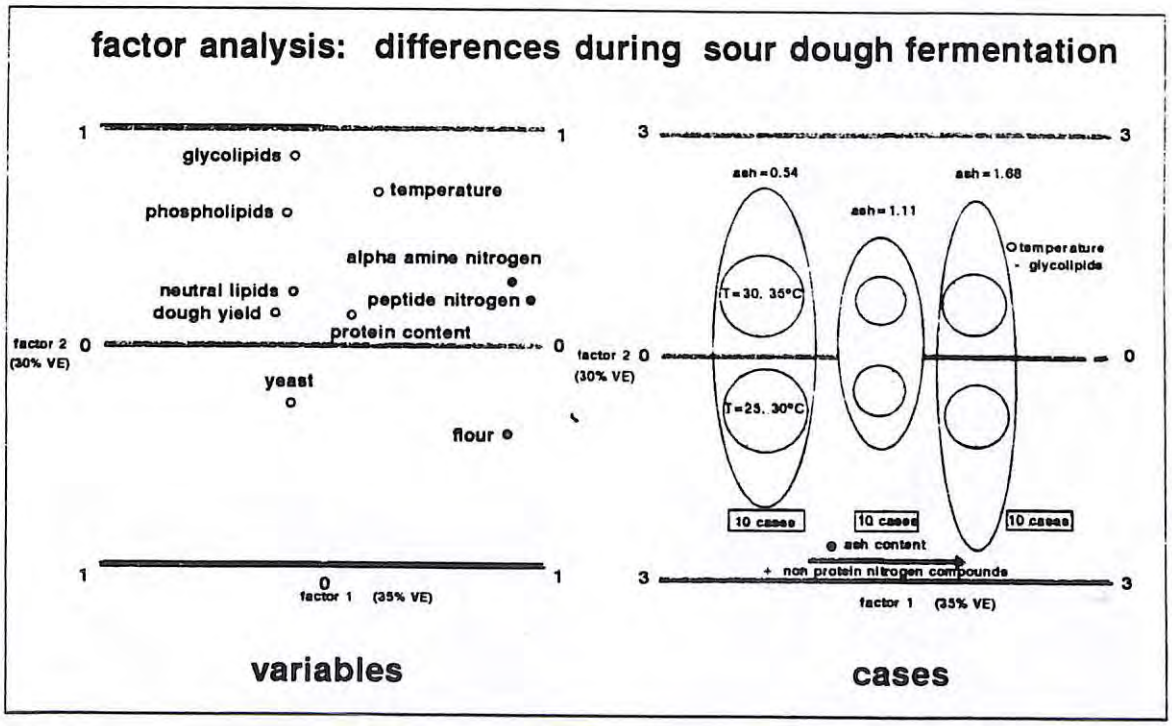


Figure 20. Distribution of variables and cases in the plane defined by factor 1 vs. factor 2 (factor analysis) when analytical and processing variables are considered for unfermented and fermented wheat sour doughs started with *Lactobacillus plantarum*.

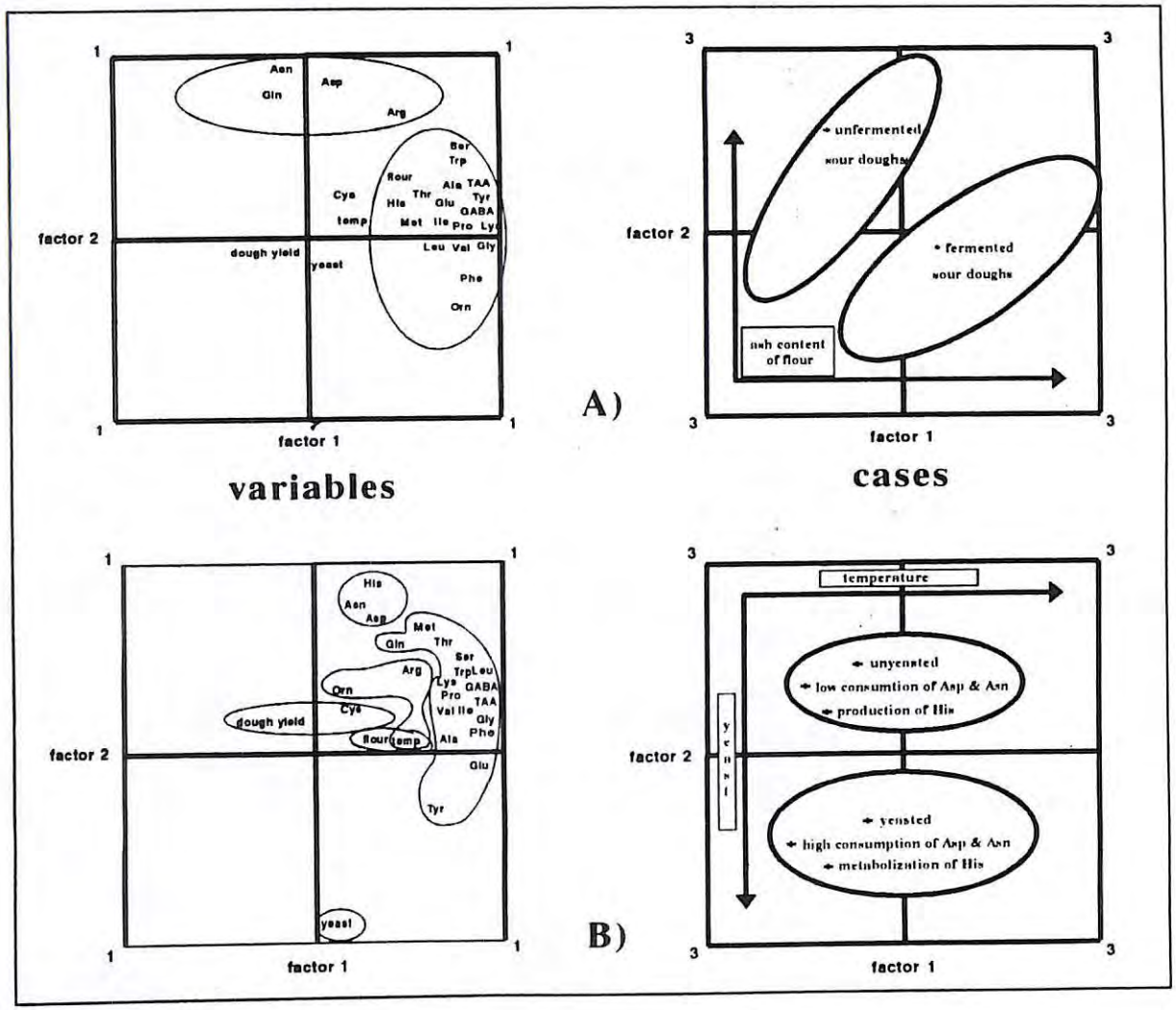
Table XIV. Free amino acid level (mg/100 g sample, d.b.) of unfermented wheat sour doughs started with *Lactobacillus plantarum.*

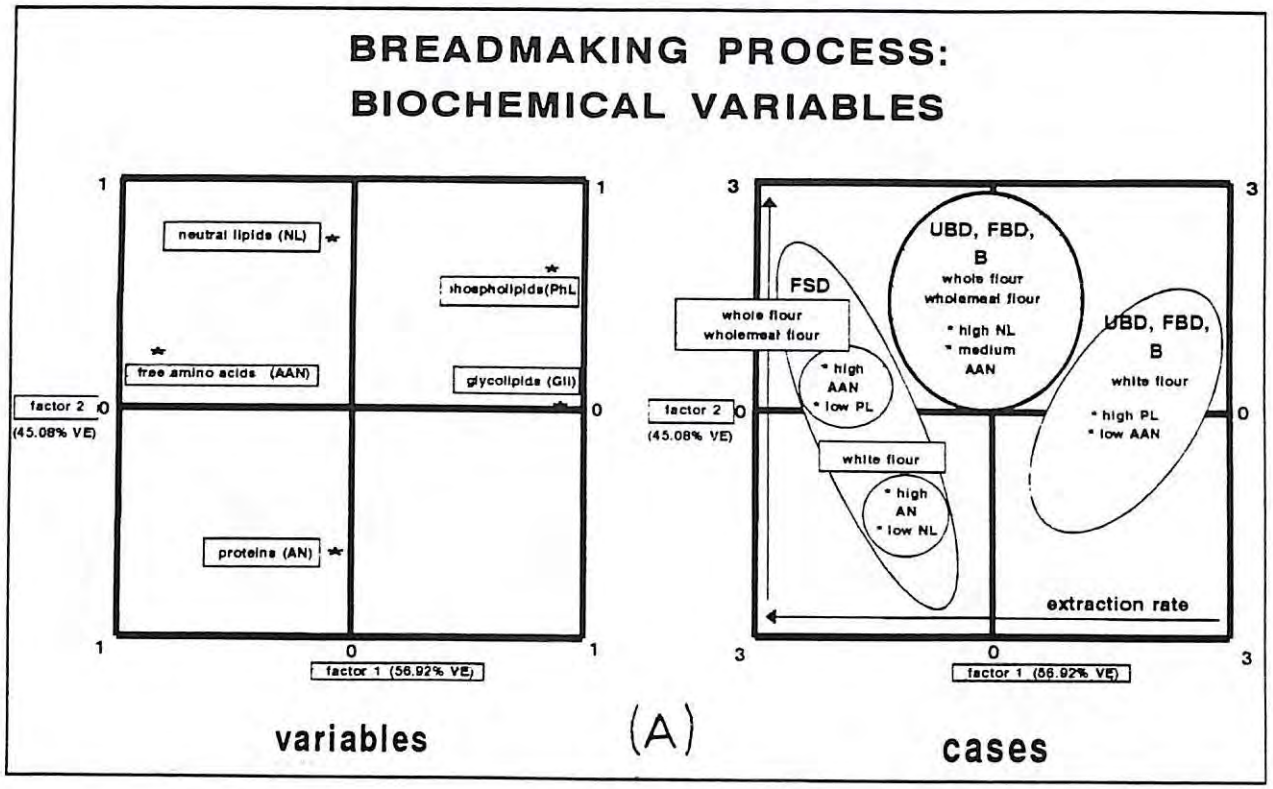
yeast	T, °C	flour	DY	Asp	Glu	Asn	Gln	Ser	Thr	Gly	Ala	GABA	Pro	Arg	Val	Met	Ile	Leu	Trp	Phe	Cys	Orn	Lys	His	Tyr	TAA
+	25	0.54	160	5.90	3.68	5050	5.55	1.22	1.27	2.64	2.58	3.66	1.01	1.29	1.23	0.15	1.05	0.85	3.37	0.66	0.35	0.16	0.51	0.15	0.77	42.55
			240	9.13	7.07	11.73	7.73	1.99	2.66	1.74	3.99	7.18	2.03	3.21	2.03	1.24	1.32	1.46	5.02	1.19	0.78	0.19	1.24	0.64	0.74	74.81
		1.11	200	8.93	5.29	15.98	7.27	1.93	1.40	1.77	4.68	10.56	2.54	4.68	2.35	0.25	1.85	3.78	7.47	1.09	0.40	0.17	1.34	0.57	0.69	84.89
		1.68	160	8.54	6.84	8.46	8.12	1.69	1.64	1.23	3.55	3.79	1.38	1.70	1.77	0.27	2.50	1.18	4.35	0.84	0.15	0.08	0.79	0.57	0.93	60.37
			240	9.24	9.30	15.13	6.84	1.30	2.11	1.72	4.06	5.63	2.12	4.32	2.11	0.16	1.34	1.46	7.53	0.96	0.22	0.15	1.17	0.73	0.96	78.56
	30	0.54	200	7.64	4.58	7.90	6.47	1.80	1.80	1.31	3.46	5.63	1.35	2.33	1.67	0.34	1.21	1.52	4.05	0.96	0.27	0.14	0.92	0.55	1.02	56.90
		1.11	160	8.63	3.77	9.31	5.54	1.55	1.18	1.37	5.19	12.58	2.66	4.42	2.97	0.16	2.08	2.84	6.29	1.23	0.16	0.15	1.32	0.14	1.08	74.64
			200	8.66	3.75	13.55	8.10	2.52	1.86	2.28	5.40	14.18	2.55	6.07	3.07	0.59	1.93	2.43	7.09	1.54	0.29	0.17	1.87	0.88	1.10	89.89
			240	6.20	3.02	7.64	4.39	1.71	1.22	1.48	3.18	8.23	1.33	1.43	1.88	0.21	1.06	2.73	4.54	1.08	0.20	0.16	1.00	0.31	0.78	52.08
		1.68	200	11.98	6.19	18.52	9.03	2.75	2.18	2.81	6.02	15.11	3.53	7.49	3.49	0.66	2.28	2.59	10.11	1.90	0.53	0.30	3.04	0.86	1.12	111.49
	35	0.54	160	5.93	2.90	9.92	4.79	1.08	1.03	0.87	2.57	4.33	1.02	1.00	1.23	tr	0.99	2.54	3.13	0.77	0.12	0.08	0.63	0.17	0.81	40.30
			240	7.43	4.38	5.73	6.39	1.28	2.11	1.12	3.21	4.31	1.29	2.00	1.57	0.23	1.17	2.91	3.80	0.89	0.17	0.08	0.72	0.37	0.81	48.17
		1.11	200	8.86	3.75	11.67	7.81	2.39	1.53	2.17	5.67	12.61	2.57	5.32	2.80	0.22	2.09	2.18	7.90	1.59	0.34	0.15	1.64	0.47	1.30	84.85
		1.68	160	7.77	4.77	14.26	6.43	2.03	1.13	1.32	3.74	7.47	2.12	3.53	2.04	tr	1.21	3.14	4.46	0.90	0.26	0.11	0.97	0.36	0.57	68.55
			240	11.38	7.04	22.71	10.32	3.54	1.54	2.56	6.48	13.78	3.62	8.87	3.61	0.45	1.99	5.83	8.96	1.81	0.59	0.17	1.94	0.93	1.08	119.24
-	25	0.54	160	8.83	5.24	9.06	7.03	2.08	0.86	1.18	3.76	5.05	1.33	1.95	1.77	0.13	1.02	1.04	3.20	0.89	0.20	0.12	0.85	0.52	1.04	57.17
			240	12.33	11.25	12.01	14.43	3.18	1.89	2.22	6.51	8.69	3.01	21.94	3.91	0.29	2.77	4.79	0.54	3.19	1.28	1.44	1.86	0.58	2.19	105.35
		1.11	200	9.31	6.09	11.23	6.31	2.22	1.36	1.48	5.47	10.09	2.58	4.70	2.84	0.14	1.91	2.17	6.17	1.42	0.11	0.19	1.53	0.68	1.74	80.73
		1.68	160	10.72	6.29	16.34	2.88	2.16	1.54	1.91	5.84	12.13	3.31	6.80	3.34	0.24	1.82	2.29	8.18	1.51	0.18	0.14	1.65	0.26	1.07	93.61
			240	7.05	4.64	12.58	4.46	1.73	1.25	1.27	4.08	6.80	1.84	3.72	2.14	tr	0.99	1.35	4.24	0.87	0.17	0.10	0.94	0.43	0.52	61.21
	30	0.54	200	5.91	3.33	6.64	4.56	1.51	0.84	0.94	2.79	3.95	1.05	1.37	1.32	0.24	0.77	0.95	2.42	0.66	0.02	0.08	0.58	0.58	0.70	40.63
		1.11	160	5.80	9.53	10.70	5.70	3.04	1.21	1.61	4.24	10.13	1.80	4.05	2.31	0.08	1.36	1.79	4.43	1.37	0.13	0.09	1.32	0.59	0.78	64.57
			200	6.35	3.30	10.56	5.06	1.78	1.53	1.94	4.74	10.98	2.29	5.04	2.89	0.31	1.78	2.16	5.25	1.42	0.22	0.20	1.67	0.63	1.16	71.27
			240	4.64	3.16	7.78	3.52	1.35	0.91	0.91	2.71	5.01	1.12	2.21	1.69	tr	0.86	1.00	2.54	0.66	0.16	0.06	0.78	0.18	0.47	41.73
		1.68	200	9.06	5.91	14.99	5.38	2.24	1.47	1.47	5.33	10.95	2.88	7.07	3.49	0.07	1.70	2.53	6.76	1.65	0.13	0.14	1.66	0.59	0.34	86.31
	35	0.54	160	6.93	3.67	10.71	8.20	4.51	1.47	1.59	4.75	7.93	2.01	4.68	2.86	0.54	1.51	2.13	4.68	1.46	0.20	0.10	1.14	0.92	1.60	71.59
			240	7.23	4.62	8.68	7.17	2.44	2.12	1.46	4.10	4.24	1.45	2.40	2.55	0.34	1.32	1.62	3.44	1.16	0.30	0.21	0.96	0.63	1.22	59.66
		1.11	200	4.61	2.54	9.13	3.63	1.72	1.31	1.37	3.23	6.79	1.55	3.18	2.33	0.38	1.03	1.30	3.06	0.87	0.14	0.12	0.94	0.56	0.59	50.41
		1.68	160	8.75	5.82	17.16	8.15	2.87	1.79	2.53	7.75	14.17	3.81	8.23	5.59	tr	2.59	3.55	10.22	2.19	0.15	0.22	3.19	1.66	1.02	111.45
			240	6.70	4.22	11.10	4.26	1.74	2.00	1.46	3.84	8.87	2.24	7.37	2.32	0.28	1.49	1.57	5.48	1.10	0.37	0.11	1.05	0.41	0.60	68.61

Table XV. Free amino acid level (mg/1oo g sample, d.b.) of fermented wheat sour doughs started with ***Lactobacillus plantarum.***

yeast	T, °C	flour	DY	Asp	Glu	Asn	Gln	Ser	Thr	Gly	Ala	GABA	Pro	Arg	Val	Met	Ile	Leu	Trp	Phe	Cys	Orn	Lys	His	Tyr	TAA
+	25	0.54	160	3.74	4.17	2.04	0.91	0.48	1.29	1.84	2.78	9.65	2.60	1.11	3.51	0.35	1.99	6.16	3.37	3.65	0.20	2.25	2.13	0.13	1.40	56.06
			240	1.01	2.54	0.97	0.91	0.43	1.03	1.23	1.59	5.70	2.03	1.17	0.94	0.07	0.52	1.43	1.45	0.79	0.14	0.87	0.72	0.10	0.37	26.01
		1.11	200	2.83	5.08	1.89	1.28	0.75	1.23	1.81	2.59	10.79	7.03	1.45	3.43	0.22	1.34	4.00	2.67	2.81	0.98	2.84	1.82	0.13	1.59	53.74
		1.68	160	9.34	11.79	8.44	3.63	2.87	2.78	6.10	8.15	27.71	11.69	15.64	10.88	1.49	6.24	17.58	12.41	8.27	0.31	2.80	10.13	0.81	7.15	184.91
			240	1.81	5.82	2.04	1.18	0.83	1.48	2.61	2.60	12.11	3.61	1.50	3.25	0.21	1.49	4.08	3.87	3.33	0.17	4.31	2.29	0.49	1.72	61.79
	30	0.54	200	1.02	2.26	0.82	0.77	0.38	0.84	1.05	1.48	4.48	1.61	0.64	1.09	0.07	0.43	1.08	1.15	1.08	tr	1.11	0.56	0.06	0.36	22.40
		1.11	160	6.89	10.62	5.03	2.27	2.73	2.06	5.35	8.58	25.44	11.86	6.36	10.78	0.89	7.14	17.90	9.65	10.46	0.48	5.69	9.39	0.52	8.28	168.36
			200	2.00	5.97	1.41	1.46	0.91	1.27	2.69	4.00	13.27	4.07	1.97	4.01	0.35	1.98	5.32	3.50	4.47	0.17	4.08	2.64	0.21	2.51	68.27
			240	1.54	6.41	1.41	1.54	1.41	1.78	3.21	3.40	13.70	4.49	3.34	3.23	0.61	2.33	6.94	3.63	4.42	0.27	2.91	2.25	0.16	1.95	70.90
		1.68	200	0.87	1.47	0.92	0.59	0.40	0.39	0.51	1.13	1.72	0.83	0.74	1.08	0.08	0.63	1.22	0.70	0.84	0.67	0.79	0.86	0.11	0.18	16.43
	35	0.54	160	2.67	5.49	1.90	0.97	1.09	1.35	2.73	4.22	8.47	2.96	0.72	3.87	1.04	2.58	6.81	3.18	4.95	0.16	3.11	2.37	0.54	1.47	62.65
			240	2.25	8.19	2.41	1.78	1.51	2.00	3.12	5.19	5.92	3.77	3.74	4.17	0.80	3.75	7.83	1.48	5.56	0.19	1.85	2.40	0.57	1.97	70.46
		1.11	200	5.31	15.27	4.52	3.75	3.42	2.78	8.08	11.85	27.95	16.87	4.47	15.22	3.57	11.79	28.01	12.54	20.21	0.18	9.11	9.92	0.74	7.88	222.14
		1.68	160	7.50	15.81	8.94	5.61	5.66	3.77	10.13	15.58	27.81	14.58	3.37	17.71	3.99	13.23	36.39	18.54	18.53	0.41	10.01	13.23	1.08	4.23	257.19
			240	4.66	16.06	4.41	3.79	3.16	2.37	8.59	9.59	29.12	22.57	4.44	15.38	2.24	11.94	28.95	15.27	21.52	0.34	8.91	7.65	0.68	8.51	230.17
-	25	0.54	160	6.23	5.19	5.74	2.81	2.33	1.69	2.80	3.74	12.48	3.71	0.75	5.46	1.30	3.18	10.18	4.37	5.48	0.13	3.79	4.69	1.89	1.85	90.29
			240	4.15	3.59	4.80	1.72	1.93	1.89	2.48	1.82	10.14	3.04	3.43	3.76	0.99	2.04	5.51	3.53	3.43	0.08	2.75	2.15	1.28	0.80	65.29
		1.11	200	6.98	7.13	7.40	2.21	3.20	2.01	3.85	4.35	17.24	6.15	5.58	7.84	1.75	5.25	12.94	6.06	5.57	0.19	5.86	6.81	1.94	2.88	123.15
		1.68	160	3.43	5.13	4.42	2.39	1.80	1.83	2.62	5.06	9.0	5.61	2.76	5.89	0.85	4.38	10.54	4.81	4.56	0.18	4.60	5.57	1.09	0.74	87.36
			240	7.57	8.55	7.68	3.08	2.45	1.55	6.50	3.20	21.77	10.81	6.54	6.87	1.94	5.12	12.82	9.59	6.37	0.23	6.75	5.79	1.25	3.34	139.43
	30	0.54	200	7.96	4.15	6.54	2.22	21.24	1.14	3.46	3.88	16.60	4.39	1.12	5.91	1.71	4.21	12.25	5.99	7.21	0.30	4.37	3.45	1.31	1.03	102.35
		1.11	160	6.06	6.09	6.91	2.37	2.96	1.25	5.12	6.22	17.13	6.88	2.15	7.69	2.34	5.57	14.13	6.53	6.58	0.32	5.33	7.77	1.77	3.00	124.71
			200	5.53	6.10	6.73	2.07	2.17	1.74	3.80	4.81	15.01	6.43	1.70	6.64	1.21	4.96	13.09	5.49	7.09	0.36	6.18	4.70	1.38	1.70	108.87
			240	7.14	9.36	8.63	2.21	2.65	2.14	5.26	4.46	19.56	9.32	4.91	9.05	2.06	7.77	19.77	10.15	11.30	0.30	6.24	6.91	1.46	2.34	152.42
		1.68	200	10.45	9.75	10.95	3.08	3.90	3.33	6.81	8.08	26.55	20.95	11.17	12.13	7.13	9.82	25.03	18.52	12.86	0.40	8.32	9.26	2.29	8.21	228.82
	35	0.54	160	7.76	6.69	6.29	3.22	3.17	2.59	4.07	6.53	14.53	7.07	2.66	7.66	1.96	5.90	14.81	6.40	7.74	0.33	5.64	6.48	1.96	1.57	124.02
			240	5.31	5.94	5.32	1.66	1.82	1.40	3.31	3.90	12.38	4.49	2.04	5.30	1.36	4.10	11.73	4.79	7.46	0.35	4.23	3.31	0.85	1.16	92.20
		1.11	200	6.96	9.04	7.45	1.92	4.37	3.15	5.40	8.15	19.82	15.73	10.89	12.42	8.33	11.47	29.62	14.98	16.65	0.28	7.17	8.91	2.59	2.48	207.78
		1.68	160	4.90	7.33	6.31	3.42	2.57	2.62	3.74	7.23	12.87	8.01	3.94	8.41	1.21	6.25	15.06	6.87	6.65	0.26	6.57	7.96	1.55	1.06	124.80
			240	7.99	10.62	9.23	3.67	4.19	4.36	6.31	9.65	22.50	21.49	13.71	14.02	2.24	6.14	29.91	19.06	16.19	0.20	8.02	9.11	2.54	2.04	223.25

Figure 21. Distribution of variables and cases in the plane defined by factor 1 vs. factor 2 (factor analysis) when processing variables and absolute values for individual amino acids (A), as well as their changes during fermentation (B) are considered for unfermented and fermented wheat sour doughs started with *Lactobacillus plantarum*.





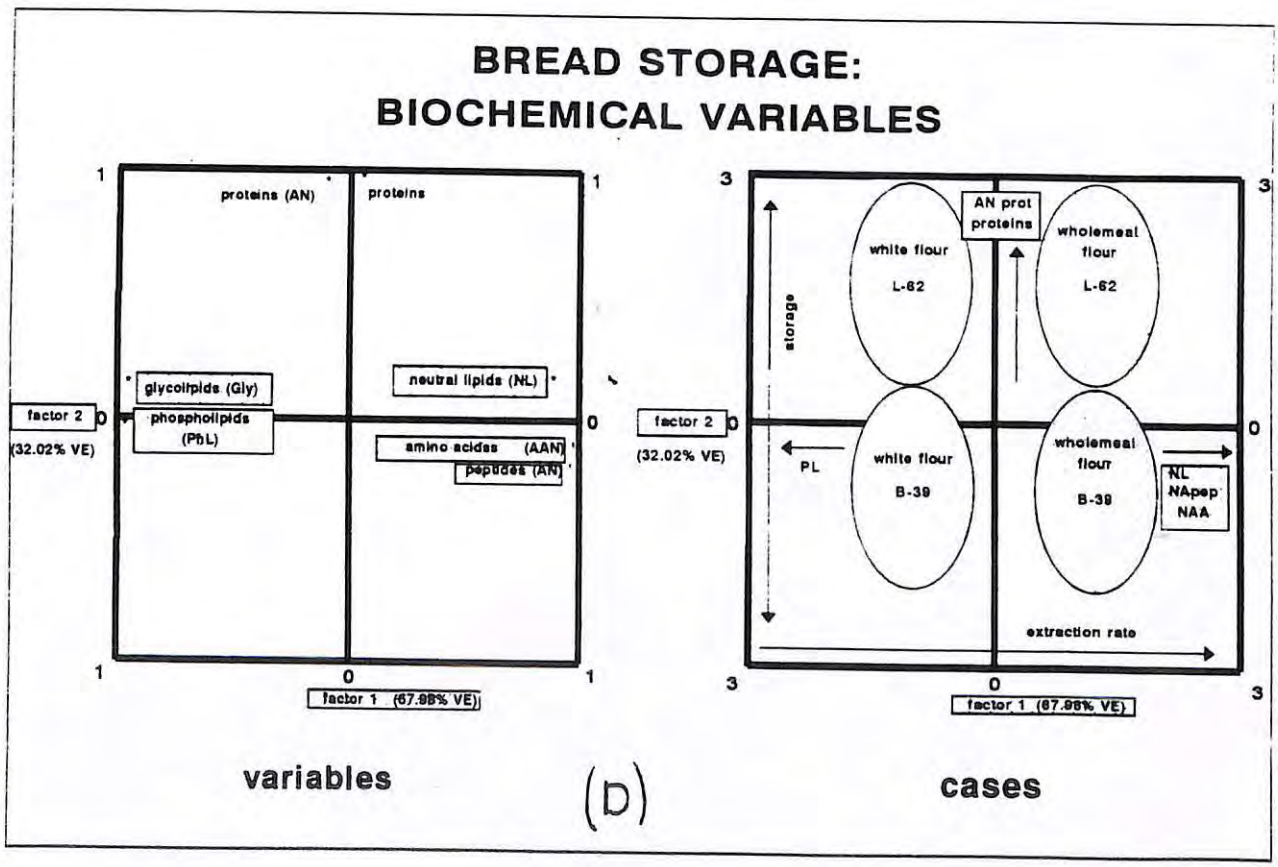
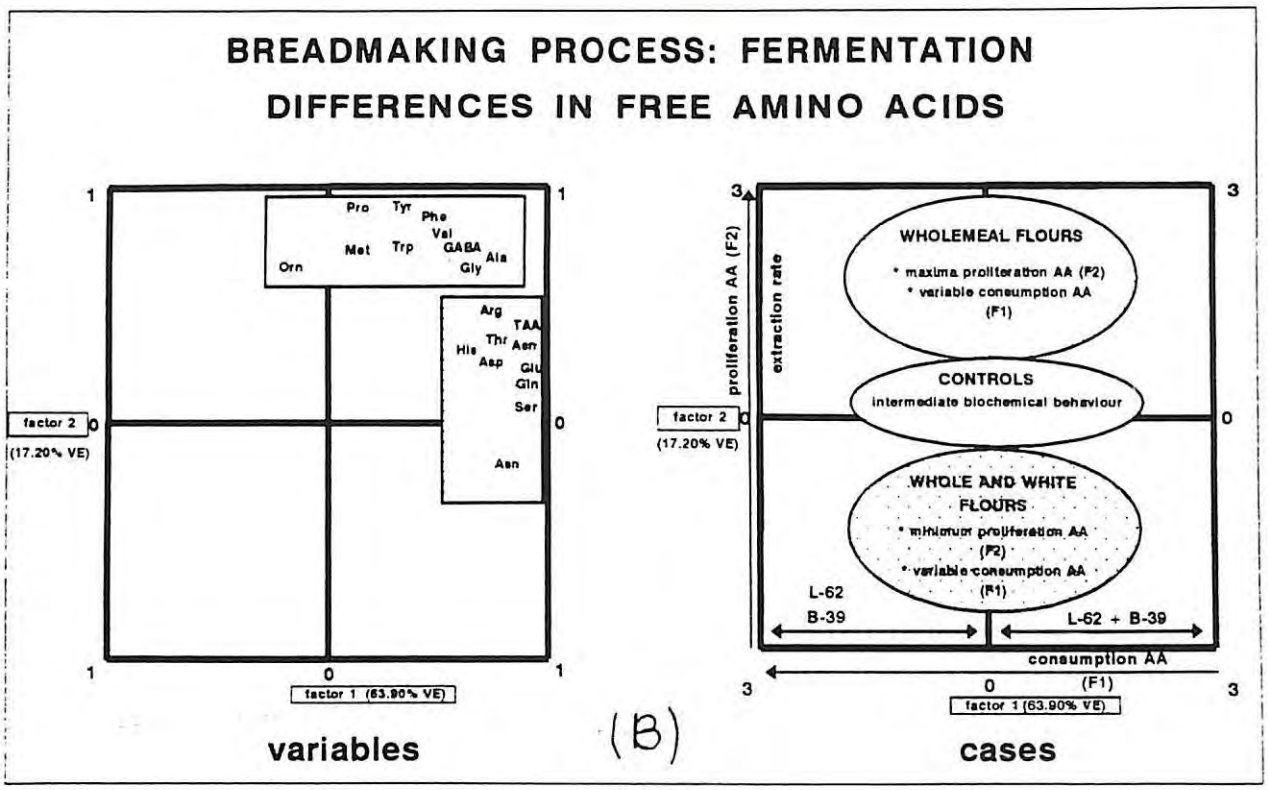


Figure 22. Distribution of variables and cases in the plane defined by factor 1 vs. factor 2 (factor analysis) when biochemical variables (lipidic and nitrogen components) characterizing breadmaking process (A), their changes during fermentation (B), baking (C), and bread storage (D) are considered for breadmaking samples (sour dough process) started with *Lactobacillus brevis*, L-62, *Lactobacillus plantarum*, B-39 and their mixtures.



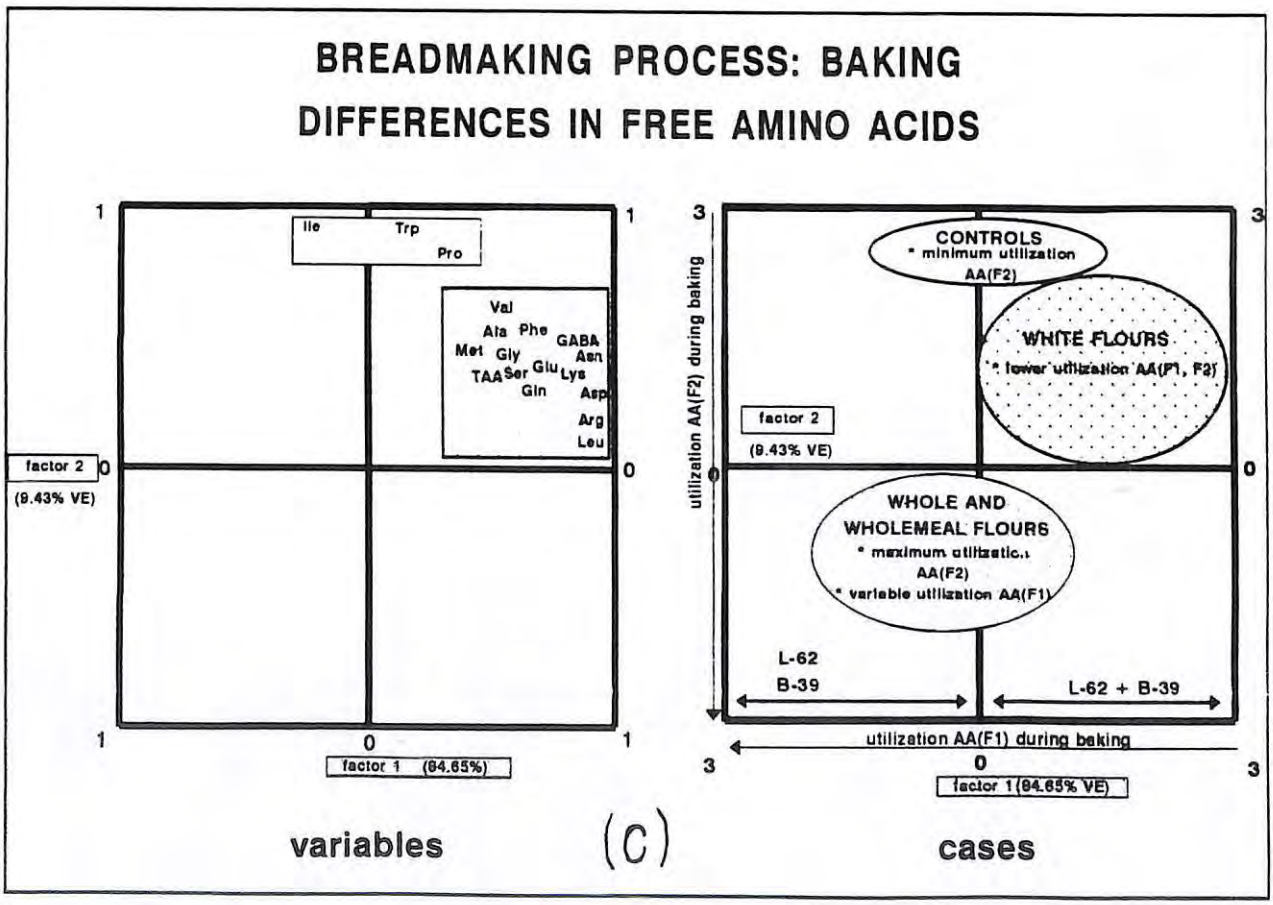


Figure 22. (contd.)

SUBPROGRAMME B: FUNCTIONAL COMPONENTS AND THEIR INTERACTIONS

Johan J. Plijter, **Subprogramme Manager**

(Gist Brocades, Delft, The Netherlands)

Review of Activities

Since the start of the programme seven meetings were held. As stated before one sees an increase in the support from the different participants for all the other research done within this subprogramme and also an increase in the cohesion and collaboration with the subprogrammes A and C. Also one sees an increase in the number of interconnections and collaborations between the different research groups, not only in this subprogramme but also with the other subprogrammes, resulting in a strong consortium. This is illustrated by the large attendance of the meeting held in Detmold in June, where a lot of people from the other subprogrammes were present at the glutenin meeting. Also the meeting in Bristol was vivid due to discussive nature. The next meeting for subprogramme B was decided to be held in Delft following the 14th ICC Congress in the Hague in June. The meetings on the interconnections between the different subprogrammes which were identified and formalized. The meetings of these subjects, pentosans in Delft, rheology in Nantes, glutenins (LMW and HMW) in Detmold, were all very successful. There will be a second meeting on glutenins in Viterbo, as a satellite to the cereal conference there.

Main Conclusions

The number of collaborations within the subprogramme has greatly extended during this year. Examples of them are given in the individual progress reports. Also the collaboration of both individual research groups of different subprogrammes has increased, besides a more closely collaboration of the different subprogrammes as can be seen from the attendance of people from one subprogramme at meetings of the other subprogrammes and the joint technical meetings (pentosans, rheology, glutenins). They resulted not only in collaborations in research, but also already in joint publications.

Individual Progress Reports

Task B.1.1. - Purification and Characterization of Gluten Subfractions

Partner 07M - INRA-Montpellier

1. Team: Marie-Hélène Morel (Researcher, Project Leader)
Jean-Claude Autran (Head of Laboratory)
Joëlle Bonicel (Technician)
Valérie Mélas (Research Fellow)

2. Progress

In the course of the reporting period, the procedure of separation of glutenin subunits according to their charge was refined on the basis of a better control of the conditions of protein precipitation by acetone. The new protocol includes the following steps: (i) extraction of glutenins according to Singh et al. (1991), with reduction and alkylation of subunits, (ii) fractionation of the latter extract on a FPLC cation exchange Mono-S column into 14 fractions, (iii) SDS-PAGE analysis and densitometric scanning of the patterns in order to determine proportions of the various LMW and HMW subunits of glutenin in each fraction and therefore to determine the charge distribution profile of any wheat genotype.

Interestingly, different cultivars give very different (and reproducible) charge distribution profiles. The study is currently extended to 50 French cultivars to assess the potential of this cation exchange FPLC method in view to better explain the physicochemical bases of baking quality and to possibly infer a quality index. Preliminary results demonstrated the presence in the most basic FPLC peaks of some LMW allelic types previously associated with a high extensibility of the dough (*e.g. o* and *n*: see report of subprogramme C, section C.4).

On the other hand, using null forms (isolated in the Italian common wheat cultivars and supplied by ISC, S. Angelo Lodigiano, Partner 03) lacking *Gli-1*-encoded gliadins and LMW subunits, as well as 1B/1R translocated cultivars, several subunits (*e.g.* some of those encoded at the *Glu-3A* locus) could be obtained in a pure state **(Figure 23)** and are currently investigated for N-terminal amino acid sequence.

Simultaneously, a method for the preparation of low-molecular-weight subunits of glutenin of bread wheat without contamination by high-molecular-weight glutenin subunits or by gliadins was investigated. Using a simple protocol based on the selective precipitation by 40 % acetone, two fractions were obtained corresponding to HMW and LMW subunits respectively. The protein fractions can be obtained either reduced or reduced and alkylated. The effects of temperature, contact time, and of successively increasing concentrations of acetone were tested, indicating that the protocol could be scaled-up to obtain large quantities of LMW subunits and thus be used as a pre-purification step.

A simple procedure of determination of the number of cysteine residues was also adapted for wheat glutenin subunits. For instance, partially purified subunits were alkylated with appropriate mixtures of anionic and cationic reagents (iodoacetic acid and 4-vinyl-pyridine) and then analysed by acid-PAGE in the presence of 2 *M* urea. For instance, from a single HMW component containing N cysteines, a pattern containing N+1 bands was generated **(Figure 24)**. The procedure has been used to check the number of cysteine residues of several already known HMW subunits from T. durum and T. aestivum cultivars and will be applied to the determination of the number of cysteine residues from unknown LMW subunits.

3. Meetings/Visits

In the course of this report period, Marie-Hélène Morel and Jean-Claude Autran visited FMBRA (partner 14) respectively on 11-12 March and 15-16 June (International Conference on Bread - from Breeding to Baking). Marie-Hélène Morel, Valérie Mélas and Jean-Claude Autran attended the 5th Gluten Workshop, 7-9 June in Detmold (Germany) and the meeting of subprogramme B, open to an international discussion on glutenins, 10 June. Marie-Hélène Morel and Jean-Claude Autran attended the meeting of subprogramme B, 4-5 November in Bristol (UK).

4. Publications

a) Refereed:

Morel, M.H. 1994. Acid-PAGE of wheat glutenins: a new tool for the separation of high and low molecular weight subunits. Cereal Chem. (in press).

Mélas V., Morel M.H., Autran J.C. and Feillet P. 1994. Simple and rapid method for purifying LMW subunits of glutenin from wheat. Cereal Chem. (in press).

(b) Other:

Morel, M.H., Mélas, V., Bonicel, J. and Autran, J.C. 1993. Multiple approach (IEF, SDS-PAGE and A-PAGE) of the composition of LMW subunits of glutenin and its effect on dough properties. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany).

Mélas V., Morel M.H. et Feillet P. 1993. Les sous-unités gluténines de faible poids moléculaire : des protéines d'avenir ? Industries des Céréales, 84, 3-14.

Figure 23. **SDS-PAGE (10 % acrylamide) of glutenin subunits of cv. Andain (1B/1R translocated). 1. Whole LMW+HMW subunits; 2. Fraction partially purified by separation on a cation exchange column (Mono-S, Pharmacia, Sweden); 3. Purified LMW subunit encoded at the *Glu-3A* locus.**

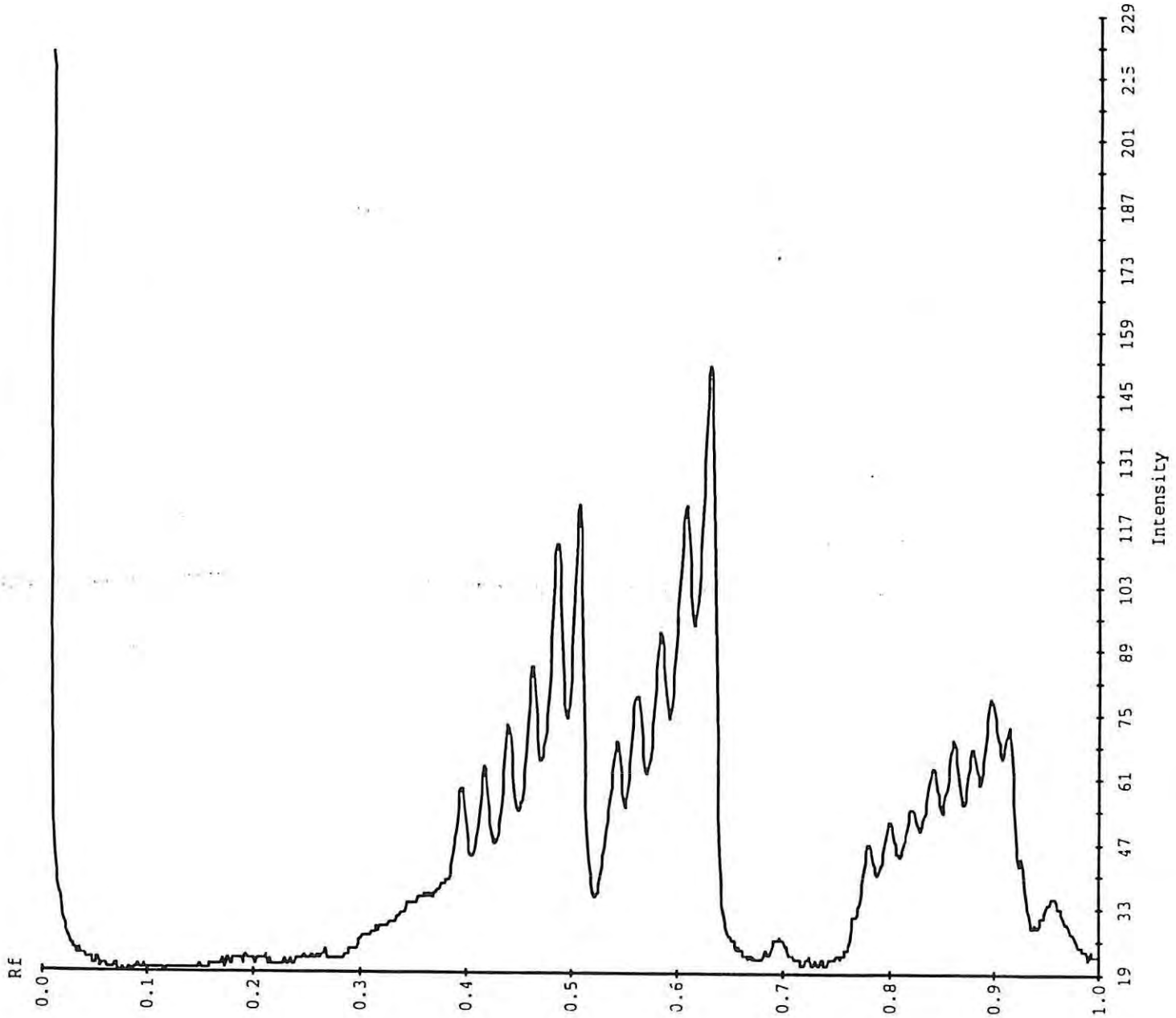


Figure 24. Determination of the number of cysteine residues by electrophoresis in three HMW subunits of glutenin from cv. Cobra (type 5-7-10): densitometric tracing showing the various mobilities of the protein after using varying molar ratios of iodoacetic acid and 4-vinyl pyridine. Rf 0 indicates the top of the gel. From left to right: subunit 5 (6 peaks → 5 cysteines), subunit 7 (5 peaks → 4 cysteines), subunit 10 (8 peaks → 7 cysteines).

Partner 07N - INRA Nantes

1. Team: Yves Popineau (Researcher, Project Leader)
Jacques Lefebvre (Researcher)
Martine Le Meste (Researcher)
Michel Cornec (Research Fellow)
Jeremy Hargreaves (Research Fellow)

2. Progress

Glutens were extracted from defatted flours of series of wheat genotypes provided by N. Pogna (Sub-programme C, Istituto Sperimentale per la Cerealicoltura, Milano, Italy) and by R. B. Gupta (CSIRO, Canberra, Australia).

- Near-isogenic lines of wheat Alpe differing by their HMW glutenin subunits encoded by *Glu-B1* and *Glu-D1* loci.

- Near- isogenic lines of wheat Alpe differing by their gliadins and LMW glutenin subunits encoded by *Gli-B1* and *Gli-D1* loci.

- Near-isogenic lines of wheat Gabo differing by their HMW subunits compositions (1A null, 1A null/ 1B null, Triple null).

- Translocation lines (1BL/1RS and 1DL/1RS) of Gabo differing by their LMW subunits encoded by *Glu-B3* and *Glu-D3* loci.

The glutens were analysed by SE-HPLC, ultracentrifugation and dynamic assay in shear.

Partner 19 - AFRC-IACR Long Ashton Research Station Bristol

1. Team: Prof. Peter S. Shewry
Dr. Arthur S. Tatham
Mr. D.R. Hickman

2. Progress

The effects of the addition of a single species purified unalkylated HMW subunit (1Bx20) was determined on medium and high protein flours using an experimental 2g mixograph (in collaboration with Dr. F. Békés, CSIRO, Australia). The simple addition of HMW subunit to a base flour resulted in similar effects to the addition of S-poor prolamins, an increase in viscosity and decrease in elasticity. By mixing less than 10 % of the added subunit was incorporated into the flour, showing that some redox reaction were occurring during mixing. However, incorporation into the doughs, using a reversible reduction-oxidation system, resulted in an increase in doughs strength and elasticity, the HMW subunit being incorporated into polymers. (Paper in press, J Cereal Sci.).

Physical studies of the chemistry of the HMW subunits have continued, using cd denaturation, fluorescence denaturation and denaturing gels. None show sigmoidal type denaturation (*i.e.* indicating a rapid change in structure), all show gradual changes, in secondary structure content (by cd), environment of the tryptophan residues (by fluorescence spectroscopy) and electrophoretic mobility (by SDS-PAGE with urea in the gels). These indicate a gradual change in conformation by denaturants. There appears to

be little difference between the physical chemistry of subunit 2 and 5. We are awaiting the results of time-resolved fluorescence, which will give information on segmental mobility, *i.e*. the overall flexibility of the molecules. Studies are continuing on subunits 10 and 12. These studies should determine whether there are differences between the subunits.

Small angle X-ray scattering studies have indicated that subunits behave in solution as semi-rigid rods, with diameters of about 2.0-2.5nm, there are no major differences in conformation between the subunits studied.

As we have found little difference between the subunits we have been using mass spectroscopy (electro-spray and matrix assisted laser desorption) to determine molecular weights of high molecular weight subunits for which gene sequences are available, basically the subunits from cvs. Chinese Spring and Cheyenne. The determined molecular weights are within experimental error similar to those of the gene sequences, suggesting that if the subunits are modified, that level of modification is very low (*i.e*. one or two molecules per HMW subunit). (Paper in preparation).

Fractions of different prolamin species (α-, γ- and ω- gliadins) have been provided to the Institute of Food Research (Partner 16) for NMR and infra-red studies of protein hydration and interaction. Different HMW subunits have also been provided for NMR studies to determine whether differences between subunits can be determined using such techniques. Papers are in preparation from these studies.

Future: We will complete the physical studies of the HMW subunits and detailed analysis of their secondary structures. We have some outstanding commitments to provide material for NMR studies, derivatised in different ways, in an attempt to perturb structure and interactions.

Partner 22 - Università di Padova

1 Team: Prof. Angelo D.B. Peruffo (Researcher)
Dr. Andrea Curioni (Researcher)
Dr. L. Furegon (Student)

2. Progress

2.1. Different glutenin fractions as obtained by CPG chromatography

A controlled Pore Glass beads (2000 Å pore size) column has been used in order to obtain acetic acid-soluble glutenin fractions differing in their behaviour at the solid/liquid interface. The proteins adsorbed onto the glass surface at acidic pH could be selectively eluted by treating the column subsequently with Na-phosphate buffer pH 7.4 containing 6 *M* urea plus 50 *mM* 2-mercaptoethanol (eluent 1) and with the same buffer made 0.5 % in sodium dodecyl sulphate (eluent 2). The eluent 1 gave rise to one protein peak (ME fraction), whose ascending part was constituted by High Molecular Weight Glutenin Subunits (HMW-GS) (**Figure 25A**, lane 1a), whereas its descending part contained some Low Molecular Weight Glutenin Subunits (LMW-GS) (**Figure 25A**, lane 1b). However, the bulk of the LMW-GS was released only by eluent 2 (SDS fraction) (**Figure 25A**, lane 2a) Moreover the descending part of the same fraction contained two proteins (**Figure**

25A, lanes 2b and 2c) that, by Acidic PAGE, could be identified as the ω−gliadins coded by chromosome 1 D. Since the adsorption on the glass surface has been shown to be dependent, at least in part, on the hydrophobic character of the gluten proteins (see our previously reported data), the described results indicated that the LMW-GS eluted by the detergent SDS are the major responsible of the hydrophobicity of the glutenin aggregates. On the contrary, the HMW-GS and the LMW-GS eluted by the eluent 1 did not show any affinity for the glass surface, indicating a much lower hydrophobicity. Moreover, the 1 D encoded ω−gliadins, which were eluted only at the end of the experiment, seem to be, among the gluten proteins, those showing the higher affinity for the glass. This may be due to the lack of intramolecular disulphide bonds, which would allow a high degree of molecular spreading onto the solid surface. The LMW-GS eluted by the eluent 1 could be further fractionated on the basis of their aggregative behaviour. In fact, when the whole ME fraction was dialysed *vs*. 0.1 *N* acetic acid containing up to 150 *mM* 2-mercaptoethanol, concentrated and chromatographed on a Sephadex G-150 column three distinct peaks were obtained: the first, eluted at the void volume, contained LMW-GS (**Figure 25B**, lane 1), while the second and the third were constituted by HMW- and LMW GS, respectively (**Figure 25B**, lanes 2 and 3). This indicated that, within the ME fraction, some LMW-GS are able to aggregate even in the presence of 2-mercaptoethanol (at acidic pH), being eluted from the Sephadex as aggregates of high molecular weight. On the contrary, the remaining part of the LMW-GS, as well as the HMW-GS did not aggregate in the described conditions. The higher aggregative tendency of the first eluted LMW-GS was confirmed by analytical SDS-PAGE of the three Sephadex peaks, electrophoresed after 24 hrs dialysis *vs*. 0.1 *N* acetic acid (**Figure 25C**). In summary, the reported results indicate that, by adsorption chromatography on CPG beads, it is possible to separate at least three different fractions of LMW-GS, which, by SDS-PAGE show the same molecular weight: one fraction, hydrophobic, eluted only by SDS, one fraction with a high tendency to aggregate through S-S bonds and, finally, one fraction which seems to be scarcely reactive.

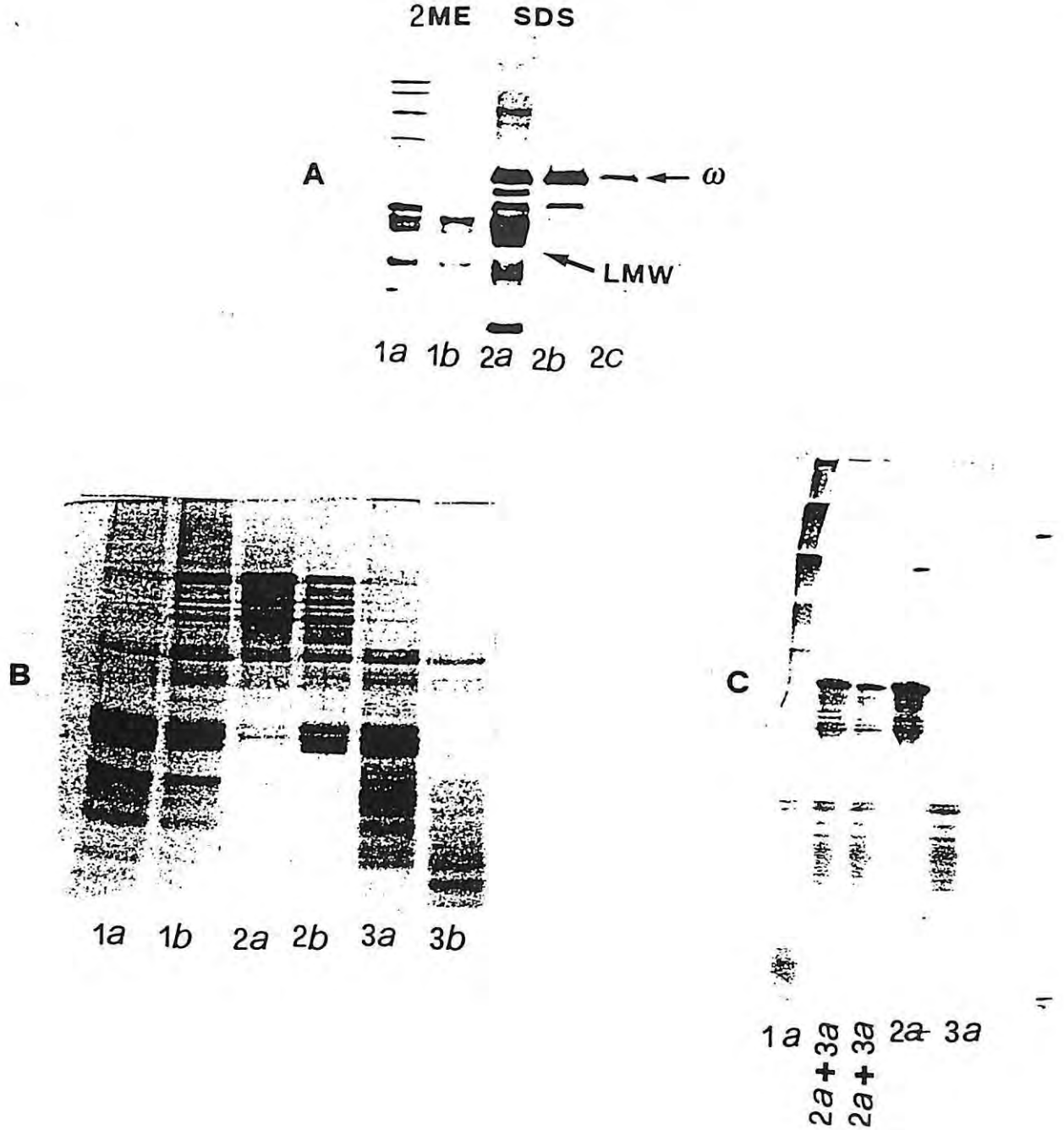


Figure 25. **A. SDS-PAGE patterns of the ME fraction (lanes 1a and 1b): ascending and descending parts of the peak, respectively) and the SDS fraction (lanes 2a and 2b: ascending and descending parts of the peak, respectively; lane 2c: tail of the peak). The 1D-encoded ω-gliadin is indicated.**

B. SDS-PAGE patterns of the three peaks eluted from the Sephadex column in reducing conditions at acidic pH. Lanes 1a and 1b: ascending and descending parts of the first peak, repectively; lanes 2a and 2b: ascending and descending parts of the second peak, respectively; lanes 3a and 3b: ascending and descending parts of the third peak, respectively.

C. SDS-PAGE patterns in unreduced conditions of the same fractions of Fig. 1B, after 24 hrs dialysis *vs.* 0.1 *N* acetic acid.

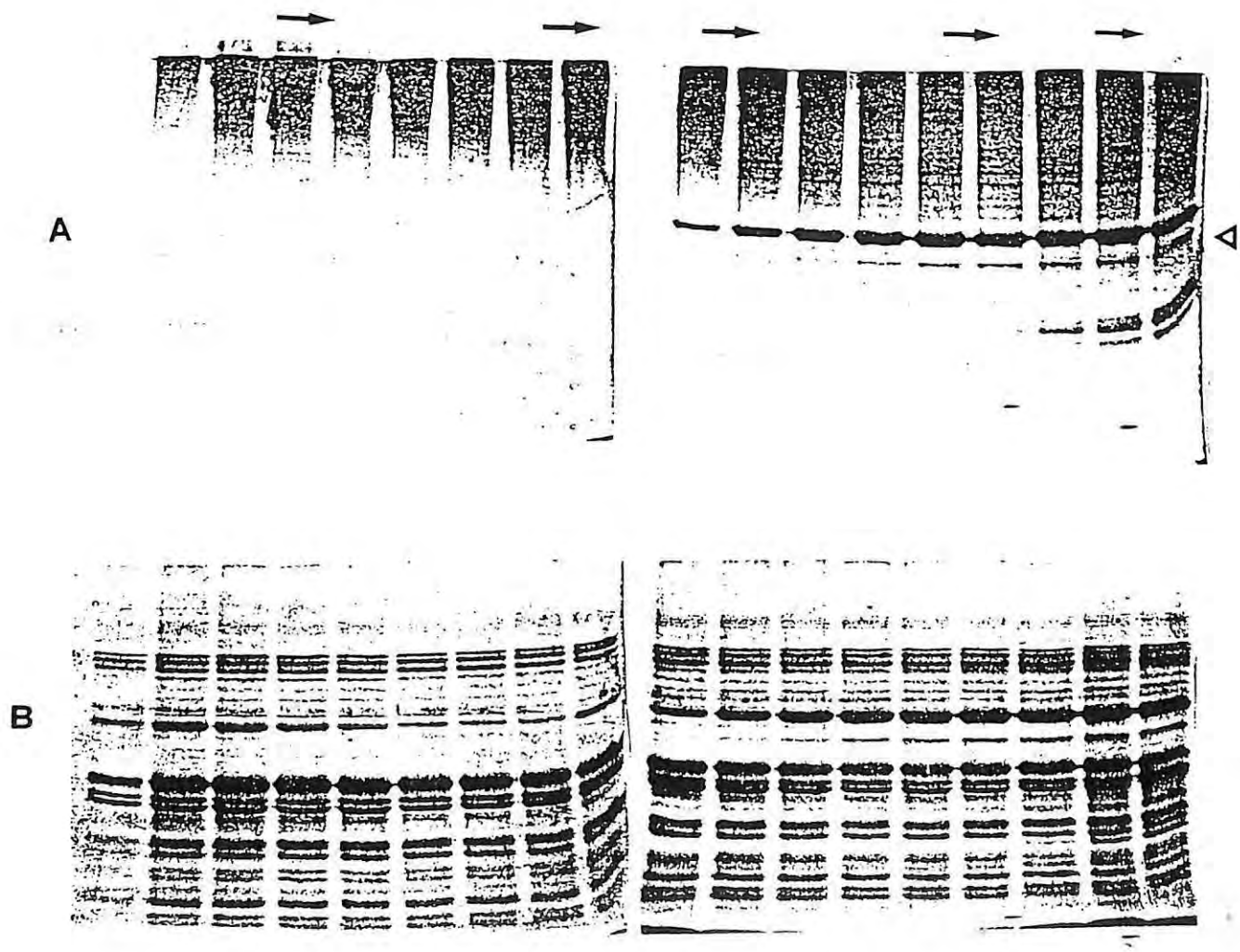


Figure 26. SDS-PAGE patterns of the fractions subsequently eluted from the Sephadex column. A and B: unreducing and reducing conditions, respectively. The arrows indicate the increasing elution volume. The arrowhead indicates the monomeric 66 kDa protein.

2.2. Separation of a Medium Molecular Weight subunit from durum wheat glutenin polymers

As previously reported, the separation of the acetic acid soluble gluten proteins by frontal analysis onto a column of CPG beads at acidic pH allowed the recovery of two distinct protein peaks. The first peak (P 1) was eluted at the void volume of the column and was constituted by glutenin polymers free from monomeric proteins, whereas the second one (P 2) contained monomeric proteins (gliadins) along with glutenin aggregates. The latter were assumed to be smaller in size in comparison to those of the P 1, since they entered the stacking gel of unreduced SDS-PAGE in a higher extent. Moreover, as far as the aggregates of P 2 are concerned, the pattern of subsequent chromatographic runs indicated that the higher the molecular weight, the lower their affinity for the glass surface. When the proteins present in P 2 were fractionated in unreduced conditions on a Sephadex G 150 column, it was possible to separate the glutenin aggregates from the contaminating gliadins (**Figure 26A**). The polymers so obtained, when reduced and analyzed by SDS-PAGE, showed to be constituted by HMW-GS, LMW-GS and a medium molecular (66 kDa) with a mobility similar to that of the D subunits of glutenin (**Figure 26B**). The occurrence of this protein is typical of the polymers present in P 2 (*i. e*. those of lower molecular weight), being almost undetectable in the reduced SDS-PAGE pattern of the polymers in P 1. Then, it is possible that the 66 kDa protein may be important in determining the degree of polymerization of the glutenin aggregates. It noteworthy that a protein with identical mobility in SDS-PAGE (**Figure 26A**, arrowhead) was found, in monomeric form, in the Sephadex fractions eluting between the glutenin aggregates and the gliadins of lower molecular weight. The characterization of the 66 kDa protein, already purified from the glutenin polymers by electroendosmotic preparative electrophoresis, is the aim of our future work

3. Meetings

In the course of this report period, A.D.B Peruffo and A. Curioni attended the 5th Gluten Workshop, 7-9 June and the meeting of subprogramme B, 10 June, in Detmold (Germany). A. Curioni attended the 2nd subprogramme B meeting in Bristol (UK), 4-5 November 1993.

4. Publications

Curioni A., Peruffo A.D.B., Pressi G., Pogna N.E. and Zamorani A. A polyclonal antibody specific for a low molecular weight glutenin subunit: preliminary results. General meeting of the ECLAIR project. Paris, 7-9 June 1993.

Peruffo A.D.B., Curioni A., Pressi G., Pogna N.E. and Zamorani A. Adsorption chromatography on controlled pore glass beads of acetic acid-soluble gluten proteins. Cereal chemistry (in press).

Peruffo A.D.B., Curioni A., Pressi G., Zamorani A. and Pogna N.E. Adsorption chromatography on controlled pore glass beads of unreduced acetic acid-soluble gluten polymers. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany) (in press).

Pogna N.E., Redaelli R., Pasquini M., Curioni A. and Peruffo A.D.B. Inheritance studies of two chromosome translocations involving chromosomes 1A, 1B and 1D in bread or durum wheat. In Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany) (in press).

Partner 23 - Università di Viterbo

1. Team: Domenico Lafiandra (Researcher)
Stefania Masci (Researcher)
Mario Ciaffi (Researcher)
Emanuele Cannarella (Technician)

2. Progress

During this year electrophoretic and chromatographic (Reversed-phase high performance liquid chromatography) characterization of high molecular weight glutenin subunits was continued; the effect on wheat technological properties of D-type low molecular weight glutenin subunits, encoded at the *Glu-D3* locus, was also investigated.

Chromatographic analyses of high molecular weight glutenin subunits were carried out on reduced and reduced and pyridylethylated subunits, using 4-vinyl pyridine. During such analyses subunits were detected which possessed similar apparent molecular weights, when separated on SDS-PAGE, but different values of surface hydrophobicities when separated by using RP-HPLC. At the *Glu-A1* locus new alleles were detected which differ from subunit 2* and 1 in surface hydrophobicities and isoelectric points. In the bread wheat cultivar Fiorello, which has been reported to possess the recombinant pair of subunits 5+12 at the *Glu-D1* locus, we demonstrated that subunit 5 is different from other subunit 5 present in other bread wheat cultivars and normally associated with subunit 10. The sequence of a cloned DNA fragment isolated from bread wheat cultivar Fiorello, corresponding to the N-terminal part of the protein, indicated the absence of the additional cysteine residue at the beginning of the repetitive domain, flanking the N-terminal region, typical of subunit 5.

SDS-sedimentation test was performed on progeny obtained from a cross between the bread wheat cultivar Darius, lacking the *Gli-D1*/*Glu-D3* components, and the bread wheat land race Nap Hal lacking high molecular weight glutenin subunits encoded at the *Glu-D1* locus. Results indicated that the absence of D-type low molecular weight glutenin subunits positively affected gluten properties. In a previous report we have described as these subunits could be the result of a minor mutation of an ω-gliadin gene(s) that give rise to a codon for a cysteine residue. If only one cysteine is present in each D subunit this can form only one intermolecular disulfide bond, with the consequence that the D subunits could determine a decrement of the size of glutenin polymers similarly to that suggested for other α- and γ-type glutenin subunits.

3. Meetings

During this year D. Lafiandra and S. Masci have participated to the 5th International Gluten Workshop where results obtained in the ECLAIR project were presented. D. Lafiandra has participated to the subprogramme B meeting in Bristol from the 4th to the 5th of November.

4. Publications

Lafiandra D., D'Ovidio R., Porceddu E., Margiotta B. and Colaprico G. 1993. New data supporting high Mr glutenin subunit 5 as the determinant of qualitative differences in the pairs 5+10 *vs*. 2+12. J. Cereal Sci. 18, 197-205

Masci S., Lafiandra D., Porceddu E., Lew J.-L., Tao, H.P. and Kasarda D.D. 1993. D-glutenin subunits are mutated ω-gliadins containing cysteine. Cereal Chem. 70, 581-585

Masci S, Lafiandra D., Porceddu E, Lew J.-L., Tao, H.P. and Kasarda D.D. 1993. 1D-coded D glutenin subunits from Chinese Spring show 1D-coded ω type N-terminal sequences. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany)

Lafiandra D., Ciaffi M., Colaprico G. and Margiotta B. 1993. Comparative effect of null lines at the *Glu-D1* and *Glu-D3* loci on wheat qualitative properties. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany)

Task B.1.2. - Physicochemistry and Functionality of Wheat Proteins

Partner 07 N - INRA Nantes

1. Team: Yves Popineau (Researcher, Project Leader)
Jacques Lefebvre (Researcher)
Martine Le Meste (Researcher)
Michel Cornec (Research Fellow)
Jeremy Hargreaves (Research Fellow)

2. Progress

2.1 Effect of HMW and LMW glutenin subunits on glutenin polymer properties and on rheological behaviour of gluten

2.l.1. HMW subunits

Gabo lines

The deletion of subunits modified the size distribution (SE-HPLC) of polymers. The lowest proportion of large size polymers (P1) was found in the triple null line. However LMW subunits were able to form by themselves a limited amount of those large size polymers. The presence of subunits 5+10 (encoded by *Glu-D1* locus) resulted in a higher proportion of P1 polymers than that of *Glu-B1* encoded 17+18 subunits. The deletion of *Glu-A1* encoded subunit did not change much glutenin content and size distribution. Ultracentrifugation showed that deletion of HMW subunits changed aggregation properties of glutenin polymers. The triple null line was very poor in strongly aggregative glutenins (insoluble and precipitated at 100 000 *g*). Glutenins were recovered mainly in the pellet of centrifugation at 435 000 *g*. Glutenin polymers containing only subunits 5+10 were more aggregative than those containing only subunits 17+18. Accordingly, the deletion of HMW subunits decreased gluten viscoelasticity, but that of double null 5+10 line was higher than that of double null 17+18 line.

Alpe lines

The analyses of Alpe lines are not yet completed but available results indicate that viscoelasticity of gluten is influenced by *Glu-B1* alleles.

2.1.2. LMW subunits

Translocated lines of Gabo

The deletion of three loci encoding LMW subunits decreased significantly the proportion of large size glutenin polymers, although the suppression of one or two loci had a much less marked effect on glutenin size distribution. The aggregative behaviour of glutenins was not altered significantly when the composition of LMW subunits was changed. However a higher gluten viscoelasticity was found for the lBL/lRS-lDL/lRS translocated line.

Alpe lines

The deletion of *Gli-B1* encoded gliadins and LMW glutenin subunits increased the storage modulus of gluten of 32 to 50% depending on the other glutenin alleles present. It shows that the rheological behaviour of glutenin polymers depends on their relative contents of LMW/HMW subunits. A large proportion of LMW subunits may be detrimental to gluten elasticity. At the opposite, the substitution of allele *m* to allele *b* at *Gli-B1* locus decreases G' of about 20% showing that properties of glutenin polymers are depending also strongly of the LMW subunit composition by itself.

Further studies of these lines are in progress to characterize glutenin polymers.

2.2. Electron spin resonance (ESR) on gluten and gluten subfractions

Spin probing and spin labelling experiments were performed on non-defatted and defatted glutens and gluten fractions. The effect of the extraction of soluble and amphiphilic proteins was also examined

2.2.1. Study of glutens

Probing with different probes indicated that gluten can be considered as a three phase system with large water pockets, lipid vesicles and an hydrated protein network having a mesh of about 0.8 nm.

Lysine and cysteine residues were labelled in hydrated glutens. Part of lysine label was bound to small non-storage proteins but cystein label is linked more specifically to gliadins and glutenins

2.2.2. Study of gluten fractions

Fractions differing by their contents in glutenin and gliadin and by their polymer compositions were analysed.

A maleimido probe was more immobilized in gliadin- than in glutenin-rich fractions. It indicates that the mesh size of the gluten protein network is modified by the gliadin/glutenin ratio. Correlation times and immobilisation of a lysine label was related to the contents of fractions in small non-storage proteins. On the other hand, the

behaviour of cysteine label appears to relate to the proportion of large glutenin polymers in the fractions: the higher their proportion, the higher the proportion of low mobility label. It suggest that polymerization of subunits results in less mobile polypeptide chains and more rigid proteins.

3. Meetings

A technical meeting on "Rheology of dough constituents" was held at INRA-Nantes (France), March 18-19, 1993. A report was annexed to the Newsletter, June 1993.

The team attended the meeting of sub-programme B held in Detmold (Germany), June 11, 1993. Y. Popineau attended the meeting of sub-programme B held in Bristol (UK), November 5-6, 1993.

4. Publications

Three papers were accepted for publication:

Hargreaves, J., Le Meste, M. and Popineau, Y. 1994. ESR studies of gluten lipid systems. J. Cereal Sci. Accepted Sept. 1993.

Cornec, M., Popineau, Y. and Lefebvre, J. 1994. Characterisation of gluten subfractions by SE - HPLC and rheological dynamic analysis in shear. J. Cereal Sci. Accepted Sept. 1993.

Popineau, Y., Cornec, M., Lefebvre, J. and Marchylo, B. 1994. Influence of HMW glutenin subunits on glutenin polymers and rheological properties of glutens and gluten subfractions of near-isogenic lines of wheat Sicco. J. Cereal Sci. Accepted Sept. 1993.

Two communications and one poster were presented at the 5th International Gluten Workshop, Detmold (Germany) June 7-9, 1993.

Hargreaves, J., Le Meste, M. and Popineau, Y. 1993. Study of the gluten proteins by ESR spectroscopy. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany).

Lefebvre J., Popineau Y. and Cornec M. 1993. Viscoelastic properties of gluten proteins: influence of prolamin composition and of temperature. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany).

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

Partner 16 - AFRC-IFR Norwich

1. Team: Peter Belton (Head of Laboratory)
Ian Colquhoun (Project Manager)
Alex Grant (Researcher)

2. Progress

2.1. Methods and Material

NMR techniques were used to investigate and compare the properties of non-polymerised and polymerised HMW subfractions of glutenin in D_2O hydration experiments (^1H) and in temperature experiments (^2H) on highly hydrated samples. These methods are described in earlier reports. The highly hydrated samples were obtained by dispersing the dry material in a large excess of D_2O for 24 hours at ambient temperature, centrifuging and loading into a NMR tube (600 % w/w D_2O). High resolution ^{13}C spectra of aqueous (H_2O) suspensions of the HMW subunits at ambient temperature were also recorded.

2.2 Results

1. The effect of hydration (0 % to 135 % w/w D_2O). Ambient temperature

^1H transverse relaxation, which was similar in both samples, could be described by a two component system *i.e.* gaussian/exponential at lower hydration and bi-exponential at higher levels. T_2 values of the dry samples were approximately 15μs (component 1, gaussian 88 %) and 60μs (components 2, exponential 12 %). Tenfold increases in T_2 (component 2) occurred on initial hydration (18 % D_2O) but remained constant at higher levels unlike component 1 T_2 which increased steadily to around 50 % D_2O. As hydration increased, the proportion of gaussian component decreased and the exponential increased. No gaussian component was visible at hydration levels of 50 % D_2O and above.

2. The effect of temperature (278 K to 363 K). 600 % w/w D_2O

^2H transverse relaxation in both samples could be described by a three component system. Changes on increasing temperature followed a similar pattern in both samples. Initially, T_2 values increased linearly with temperature but linearly ceased around 333 K and decreases in T_2 (components 2 and 3) occurred at higher temperatures. The percentage of the third component (polymerised subunit) decreased linearly with increasing temperature to approximately 20 % at 363 K, with increases in components 1 and 2. Results from the non-polymerised subunit differed only in the percentages of components found at each temperature. The trends were the same.

3. High resolution ^{13}C spectra (aqueous suspension). Ambient temperature

The spectra of non-polymerised and polymerised subunits showed sharp and well defined peaks which related to the amino-acid composition of the repetitive domain. The spectra were similar with similar with only small differences.

2.3. Conclusions

The results of hydration, temperature and high resolution work showed that the non-polymerised and polymerised subunits behaved similarly but differences, mainly in the percentages of relaxation components, did occur.

In each sample, the amount of gaussian component decreased with increasing hydration. This decrease coupled with increases in T_2 values indicated increasing protein mobility. Small increases in initial hydration levels (0 to 18 % D_2O) significantly increased protein mobility *i.e.* there was a large increase in the T_2 value of the exponential component. This may be related to a change from the glassy to the rubbery state of the subunits, the transition temperature of which is lowered to approximately 283 K at 20% moisture content. The mobility of the hydrated subunits was further demonstrated by sharp, well defined peaks in ^{13}C high resolution spectra which were obtained using solution state techniques

T_2 relaxation behaviour over the temperature range examined, could be explained in terms of chemical and diffusive exchange. As shown in previous reports, increases in temperature resulted in decreases in the proportions of the long T_2 component indicating that subunits, on heating, absorb D_2O unlike mammalian connective tissue elastin, a model of which had been proposed to explain possible elasticity in HMW subunits.

^1H relaxation measurements on C-hordein (a barley protein closely related to ω-gliadins of wheat) have shown that as water is added and the temperature raised, inter- and intra-chain H-bonding between glutamine residues is disrupted and replaced by glutamine-water interactions. However, some inter-chain interactions will still remain and a view is emerging that such interactions may be of considerable importance in determining the behaviour of these polymers.

3. Publications

Belton P.S., Gil A.M. and Tatham A.S. ^1H NMR relaxation time studies of the barley protein C-Hordein. Macromolecules (submitted).

Belton P.S., Colquhoun I.J., Field J.M., Grant A., Shewry P.R. and Tatham A.S. 1994. ^1H and ^2H NMR relaxation studies of the high M_r subunits of glutenin and comparison with elastin. J. Cereal Sci (in press).

Poster

Belton P.S., Colquhoun I.J., Grant A., Shewry P.R. and Tatham A.S. 1993. NMR relaxation studies of hydrated HMW subunits. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany)

4. Meetings

- Rheology Meeting, March 1993 at Nantes, France (IJC).

- 5th International Gluten Workshop, June 1993 at Detmold, Germany (IJC, AG).

- ECLAIR sub-programme B meeting, November 1993 at Long Ashton Research Station, Bristol, UK (PSB, IJC, AG).

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

Partner 14 - FMBRA

1. **Team:** Dr. Philip Greenwell (Researcher, Project Leader)
Dr. Dhan Bhandari (Researcher)
Mr. Douglas Smith (Technologist)

2. Progress

2.1. Experimentation

The endosperm texture (milling quality) of bread wheat, and its relationship to the 15kD friabilins (starch granule surface proteins) have continued to be the target of the ongoing work. Progress has been achieved particularly by comparison of the basic friabilin components to the lipoproteins extracted by the detergent Triton TX-114 under Task B.1.5 (Didier Marion, INRA Nantes).

(a) Water-washed starch from the soft variety Galahad was used as the source of starch granule surface proteins, which were separated into a pool of neutral friabilins and three basic friabilins by column chromatography as mentioned previously.

(b) Samples of friabilin (basic-1) and friabilin (basic-3) were subjected to N-terminal amino-acid sequence analysis (collaboration with Arthur Tatham, IACR-Long Ashton, Task B.1.1).

(c) Basic and neutral friabilins were analysed by high performance capillary electrophoresis (HPCE) in acidic buffers to complement analysis by non-equilibrium pH-gradient electrophoresis (NEPHGE).

(d) Electroblots of NEPHGE patterns of basic friabilins and TX-114 proteins were immunostained with monoclonal antibody F7F, raised against friabilin (basic-1)

(e) Attempts were made with solvents known to be effective in stripping friabilins off starch to make extracts from white flours that could be rapidly analysed by HPCE or NEPHGE. The hope was that the profile of basic friabilins might form the basis of a rapid microscale diagnostic test for endosperm texture, but in practice the patterns were too distorted by overloading by other protein types.

(f) Further work on somaclonal mutant lines of varying endosperm texture (samples from Enzo DeAmbrogio, Soc. Prod. Sementi Bologna, Task C.9), by SDS-PAGE estimation of starch friabilin on microscale CsCl-cushion starch preparations, has been done. Similar work has also been done on lines derived from crosses between biotypes of the Finnish variety Ulla (samples from Dr. T. Sontag-Strohm, Univ. of Helsinki).

2.2. Results and Discussion

(a) The essential identity of the basic friabilins with the TX-114 proteins has now been confirmed with all the techniques used for comparison. By size (SDS-PAGE), isoelectric

points (NEPHGE), immunostaining with antibody F7F and N-terminal sequences, friabilin (basic-1) is the same as the (EVGGGGG...) TX-114 protein, and friabilin (basic-3) is the same as puroindoline, the (DVAGGGG...) TX-114 protein. The antibody cross-reacted with all the basic proteins, but not with neutral components of the 15kD operational friabilin from the starch granule surface.

(b) HPCE confirmed the findings with NEPHGE that the near-neutral pool of friabilins is composed of numerous (approx. 12-15) minor components. The major components of operational friabilin from Galahad starch are friabilins (basic-1 and -2)

(c) As stated in earlier reports, the F7F-detected immuno-friabilin, *i.e*. basic friabilin, in hard and soft flours is not diagnostic of their endosperm texture, but the possibility remains that the relative amounts of the different basic friabilins might be. Attempts to demonstrate this by NEPHGE or HPCE on selective extracts of proteins with 1*M* salt/iso-propanol mixtures were unsuccessful. Possibly the TX-114 extract and RP-HPLC techniques of Task B.1.5 might be more successful.

(d) The studies of somaclonal mutant lines and Ulla biotypes are an attempt to see how the amount of granule surface friabilin responds when bread wheat shows endosperm texture differences under the influence of genes other than the Hardness gene on chromosome 5DS. The results are still being analysed.

Overall, the results obtained by this project have considerably advanced our knowledge of the biochemical nature of friabilin, and have made it clear that *in situ* they have to be considered as lipoproteins. Their nature, and their relationship to endosperm texture, have both proved to be much more complex than envisaged at the beginning of the project. Clearly, friabilins are involved in some way with endosperm texture, but not in a way that has so far enabled us to use them in a rapid, sensitive diagnostic test for this important quality parameter of bread wheat. Work on this task began early because of UK Government scheduling of its share of funding; consequently the early rundown of funds means that activity during the final year will be devoted primarily to publication of the existing results.

2. Publications

In addition to the internal project meetings, this ECLAIR-funded work was communicated as listed:

Greenwell P. 1993. Poster entitled "Flour proteins related to endosperm texture of bread wheat", presented at the 5th International Gluten Protein Workshop, Detmold, Germany, 7-9 June.

Greenwell P. 1993. Poster entitled "Flour proteins related to endosperm texture of bread wheat" presented at the International Conference on Bread- from Breeding to Baking, FMBRA, Chorleywood, U.K., 15-16 June.

Greenwell P. and Brock C.J. 1993. Lecture entitled "Identity of starch-granule-surface proteins (friabilins) of bread wheat with detergent-soluble lipid-binding proteins from flour", presented at the AACC Annual Meeting, Miami, Florida, USA, 3-7 October. Abstract published: Cereal Foods World, 1993, 38, 615-616.

Sulaiman B.D., Brennan C.S., Greenwell P. and Schofield J.D. 1993. Lecture entitled "Isolation of friabilin and the use of polyclonal antisera for immunolocation studies", presented at the AACC Annual Meeting, Miami, Florida, USA, 3-7 October. Abstract published: Cereal Foods World, 1993, 38, 616.

Task B.1.5. - Lipid Interactions

Partner 07N - INRA - Nantes

1. Team: D. Marion (Researcher, Project Leader)

2. Progress

2.1. Homologies between starch granule proteins and lipid binding proteins

N-terminal sequencing has shown that there is an homology between some lipid binding proteins and low molecular weight starch granule proteins. The main lipid binding protein was fully sequenced and named puroindoline in regard to its unique tryptophan rich domain. Now, we have isolated two isoforms named puroindoline-a and puroindoline-b. We have obtained polyclonal antibodies that react specifically with puroindoline-a. These antibodies crossreact with friabilin basic 1 isolated from the starch granule by Dr P. Greenwell. Cross reactivity does not occur with friabilin basic 2 and basic 3. Furthermore, friabilin basic 2-3 and puroindoline-b have the same retention time in reversed phase HPLC. Apparently friabilin basic 2 and basic 3 are more strongly bound to starch granules than puroindoline a-friabilin basic 1 and thus might be considered as "true" friabilin.

More recently Jolly et al (Theor. Appl. Genet., 1993, 86, 589-597) have published the N-terminal sequence of 15kD granule starch proteins GSP and of some peptides obtained from endoproteinase digest of these GSPs (**Figure 27**). It is obvious that more peptides are derived from puroindoline-b than from puroindoline-a.

These results show that the concentration of puroindoline-b is probably more important than the quantity of puroindoline-a on the surface of starch granules while in the whole seed [puroindoline-a]/[puroindoline-b] = 3 to 10. Therefore we suggest that puroindoline-b is the friabilin first isolated by Dr P. Greenwell from starch granule of soft wheats.

Preliminary results using two monoclonal antibodies specific for puroindoline-a and for puroindoline a-b indicate a spatial distribution of puroindolines. Puroindoline-a would be mainly located in the aleurone layer while puroindoline-b would be located mainly in the starchy endosperm.

2.2. Structural flexibility of puroindoline and lipid binding properties

Secondary structure of puroindoline as revealed by circular dichroism and infrared spectroscopy show that this protein is mainly composed of helices at pH 4 while the extended structure content increases when the pH increases from 4 to 7. A metastable zone is observed between pH 7 and 8 due to a slow aggregation of the protein. Whatever the secondary structure of puroindoline, it interacts strongly with anionic phospholipids. However an important decrease of its affinity for zwitterionic lipids is observed when the

helix content of the protein increases. Therefore puroindoline which is stabilized by five disulfide bridges exhibits an important structural flexibility which controls the lipid binding specificity.

2.3. Functional properties of puroindoline

In collaboration with Dr D.C. Clark from IFR Norwich the foaming properties of puroindoline and puroindoline-phospholipid complexes have been studied. Lysophosphatidylcholine has been chosen as a model phospholipid. The results obtained show that the good foaming properties of puroindoline are enhanced by the presence of lysoPC due the formation of a highly stable lipoprotein film at the air water interfaces. Such a mechanism is probably important during the gas phase expansion on proof stage and baking of bread doughs. Phospholipid-puroindoline interactions observed in model systems is similar to the behavior of different membrane invading or membranotoxic proteins. The toxicity of puroindoline against different bacteria and fungi is under investigations.

3. Publications

Blochet J.E., Chevalier C., Forest E., Pebay-Peyroula E., Gautier M.F., Joudrier P., Pezolet M. and Marion D (1993). Complete amino acid sequence of puroindoline, a new basic and cystine-rich protein with a unique tryptophan rich domain, isolated from wheat endosperm by Triton X114 phase partitioning. FEBS Lett. 329, 336-340.

Wilde P.J., Clark D.C. and Marion D (1993). The influence of competitive adsorption of lysophosphatidylcholine on the functional properties of puroindoline, a lipid binding protein isolated from wheat flour. J. Agric. Food Chem. 1570-1576.

Gautier M.F., Aleman M.E., Guirao A., Marion D. and Joudrier P (1994). Triticum aestivum puroindolines, two basic cystine-rich proteins: cDNA sequence analysis and developmental gene expression. Plant Mol. Biol. (in press)

N-terminal sequence of GSP and puroindoline-b

EVGGGGGSQEPPQERKLN (GSP)
EVGGGGGSQQCPQERKLS (puroindoline-b)

Partial sequences of major peptides isolated from lysyl-endoproteinase digests of GSP and their homology with puroindolines

VIQEAK (GSP)
VIQEAK (puroindoline-a)

GGEEHEV (GSP)
GGCEHEV (puroindoline-b)

DYVXE (GSP)
DYVME (puroindoline-b)

NFPV (GSP)
DFPV (puroindoline-a and -b)

QLQRAQS (GSP)
QLQRAQS (puroindoline-b)

EVGGGGGSQEP (GSP)
EVGGGGGSQQC (puroindoline-b)

unknown peptides A(L)AFP; ARTVQTA; SYVYEQ

Partial sequences of chymotrypsin digests

RGQVFL (GSP)
RGEVFK (puroindoline-b)

LGIR (GSP)
LGIWR (puroindoline-b)

unknown peptide: SQIAPQ

Figure 27. Sequence homologies between peptides provided by endoproteinase digests from GSP-friabilin and peptide sequences found in puroindoline-a and -b.

Partner 15 - Gist-brocades

1. Team: Johan Plijter (Researcher)
Mariette Uijen (Technician)

2. Key measurement of Achievement - Objectives

Study of the surface active behaviour in dough. The influence of lipids, mixing and wheat type on this behaviour.

3. Progress

Work has been done 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 character. The influence of lipids on the surface behaviour is an increase of it. This effect disappears during the fermentation process.

Besides this research has been done on the interfacial behaviour of doughs prepared from different flour types (hard and soft wheats), provided by Philip Greenwell (Partner 14). Doughs were prepared from with no other additives than salt, sugar, yeasts and water, just like the former studies. Also the influence of the mixing time on the interfacial behaviour was studied, with the aid of an overflowing cylinder.

The flours tested were for the soft wheats; Admiral, Hunter, the Maris Hobbit sib parent lines MHs-1-1 and MHs-3-1 and the chromosome-engineered Maris Hobbit sib lines MHs (Bez5D)-1-1 and MHs (Bez5D)-3-2. For the hard wheats the following lines were tested; Mercia, Hereward and the chromosome-engineered Maris Hobbit sib Lines MHs (Bez5D)-1-1 and MHs (Bez5D) -3-1.

With the aid of this technique no differences in surface behaviour could be observed between the flours from hard and soft wheats.

4. Conclusions

The breakdown of the gluten macropolymers during mixing can be clearly seen in the surface active behaviour of dough samples taken during this action. Also the influence of added lipids on the surface behaviour can be clearly demonstrated.

As started above one cannot observe differences in the surface active behaviour of the different soft and hard wheat types, which show clear differences in the amount of friabilin present.

5. Meetings/Visits

The rheology meeting in Nantes, March 18-19, the glutenin meeting and sub-programme B meeting in Detmold, June 11 and the sub-programme B meeting in Bristol, November 5-6, were attended.

Task B.2. - Dynamics of Dough Development

Partner 16 - AFRC-IFR Norwich

1. Team: Mike Morgan
Clare Mills
Sara Holden
Mary Parker
Neil Rigby

2. Progress

2.1. Production of monoclonal antibodies to arabinoxylans

Monoclonal antibodies (Mabs) have been produced using two types of immunogen

(i) water-insoluble arabinoxylans isolated from bees wing bran
(ii) water-soluble arabinoxylans conjugated to a protein carrier, bovine serum albumin (BSA)

Hybridomas secreting anti-arabinoxylan antibodies have been identified using two types of enzyme-linked immunosorbent assays (ELISAs, described in previous newsletters), one employing a lectin to immobilise the polysaccharide to microtitration plates, the other using feruloylated arabinoxylan fragments conjugated to BSA as the solid phase of the ELISA. Of the 100 or so antibody-positive hybridomas identified 5 have been cloned from the bees wing bran fusion (IFRN 0403, 0405, 0407, 0411, 0418) and 1 from the arabinoxylan-BSA fusion (IFRN 0410). Selection for cloning was done on the basis of titre and isotype. All Mabs produced were IgMs, except for IFRN 0410 which is an IgG. Such a predominance of IgM antibodies would be expected with such weakly immunogenic polysaccharides.

2.2 ELISA characterisation of anti-arabinoxylan antibodies

(a) Preparation of CovaLink-terminal arabinose plates

Commercially available microtitration plates that have a secondary amino-group linked by a bridge to the plate surface, have been derivatised with a bifunctional reagent to allow covalent attachment of sugar residues. Initially arabinose has been used but it is planned to couple other sugars. Using these plates it has been possible to show that two of the four Mabs tested to date, IFRN 0418 and 0410 recognize terminal arabinose; two further Mabs, IFRN 0407 and 0411 have yet to be characterised.

(b) Development of arabinoxylan inhibition ELISA

In a competitive ELISA a variable amount of soluble analyte and immobilised antigen are in competition for a small fixed amount of antibody. The concentration of the analyte in the sample is measured indirectly by the quantitation of bound antibody after it has been separated from the free antibody. The immobilised phases employed were as used in the

lectin and feruloylated fragment ELISAs. Dose-response curves have been obtained to date using both types of immobilised phases using Mabs IFRN 0418 (as culture supernatant or ammonium sulphate concentrated antibody) and 0410 (**Figure 28**). Such dose-response curves will allow the binding of these Mabs to different polysaccharides to be characterized and facilitate the development of ELISA methodology for quantification of arabinoxylans in wheat flours.

3. Future Aims

1. Finish characterisation of monoclonal antibodies and refine ELISAs for analysis of arabinoxylans in flour.

2. Examine the location of binding of anti-arabinoxylans Mabs to wheat using electron microscopy.

3. To investigate the effect of baking on the binding of anti-prolamin Mabs to gluten in doughs and bread.

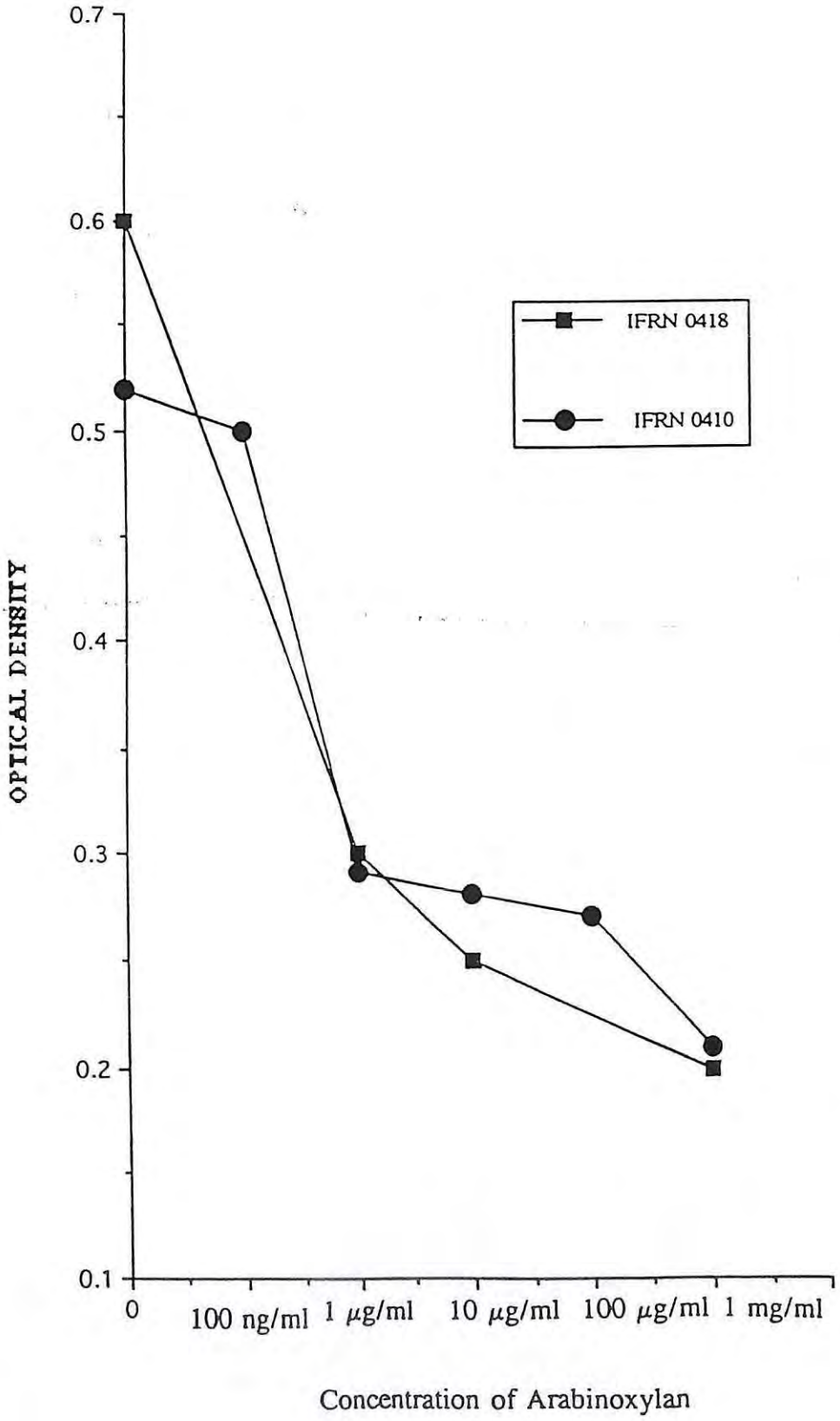


Figure 28. Inhibition ELISA curves for Arabinoxylan

SUBPROGRAMME C: BIOCHEMICAL-GENETICS AND PHYSIOLOGY

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

Review of Activities

The second period (up to December) of the third year of activity has been characterized by the fact that most research programmes are approaching maturity and providing publications.

At the last meeting in Clermont-Ferrand (December 13-14, 1993) it became apparent that the agronomic trials of SEN and NWEN and the technological measurements carried out on the SEN & NWEN samples during four year of testing (including the 1993 - 94 growing season) represented a valid and unique experience on top wheat varieties at the European level.

Therefore it was decided to put this experience in concrete form in terms of a four-chapter book including (1) morphological and physiological description of cultivars (2) agronomic trials (3) quality testing and (4) protein composition (allele composition at *Gli*, *Glu-1* and *Glu-3* loci).

Moreover the work of INRA (Montpellier), INRA (Clermont Ferrand) and ISC on gliadin and LMW glutenin subunits provided us with a genetical approach to describe allelic composition at the *Gli* and *Glu-3* as well as mono-dimensional and two-dimensional techniques to identify the gliadin or glutenin components encoded by the different alleles at those loci.

Therefore, in collaboration with E. Jackson from PBI (Cambridge) it was decided to develop an European nomenclature of LMW glutenin subunits based on A-PAGE, SDS-PAGE and A-PAGE X SDS-PAGE fractionation of glutenins.

The work at INRA (Clermont Ferrand) on genotype x environment interactions is now focusing on the main determinants of protein content and composition TNO Food and Nutrition has made significant progress with its task. Progenies showing a broad variation in dormancy are in multiplication whereas germination inhibitors are currently being tested.

The work at ISC on genetic and technological aspects of HMW glutenin subunits and HMW albumins has now added new information about (1) effects of HMW subunit 2 on gluten quality (2) DNA sequence of unexpressed subunit 2 gene in the A6 line, (3) allelic variation for HMW albumins.

The work at ISC on production of lines and near-isogenic lines (NILS) is reaching maturity rapidly. Several NILS of cv. Alpe have been distributed to colleagues of

subprogrammes A and B for rheological studies whereas NILS from the cross Neepawa x Costantino are used for description of alleles coding for LMW glutenin subunits at INRA (Montpellier).

In conclusion the activities of participants in subprogramme C are now focused on completion of the different tasks and reinforcement of relationships between labs in view of future joint researches.

Individual Progress Reports

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

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

Partner 02 - Produttori Sementi

1. Team: Enzo DeAmbrogio (Research Manager)
Parivash Jenabzadeh (Researcher)
Marilena Paolini (Researcher)
Stefano Ravaglia (Researcher)
Luca Bersanetti (Technician)
Stefano Poluzzi (Technician)

2. Progress

The SEN Trial was sown in October. Field emergence was very good due to the nearly optimal sowing date and to the moisture present in the soil.

Partner 03 - Istituto Sperimentale per la Cerealicoltura

1. Team: Basilio Borghi (Researcher)
Norberto Pogna (Researcher)
Rita Redaelli (Researcher; from January to September 1993)
Anna Biancardi (Technician).

2. Progress

SEN Trial includes the following 25 varieties grown in replicated plot trials (3 replications, plots of 10 m2):

ALMANSOR, AMAZONAS, AVITAL, BEAVER, BRASILIA, COURTOT, ERIDANO, GOLIA, JECORA, MAESTRA, MANITAL, MEC, MIRA, MONDEGO, PANDAS, PEGASO, PRINQUAL, RIBAND, SALMONE, SIDERAL, SOISSONS, TAYLOR, TUA, VEDA.

SEN Trial of the year 1993/94 was sown in Italy in October, November in five locations: SEN-l: S. Angelo Lodigiano (Milano), SEN-2: Lonigo (Vicenza), SEN-3: Argelato (Bologna), SEN-4: Voghera (Pavia), SEN-5: Ancona.

Partner 25 - Estação Nacional de Melheramento de Plantas - Elvas

1. Team: Francisco Bagulho (Researcher)
Benvindo Maçãs (Researcher)
José Cutinho (Researcher)
Carla Moita Brites (Researcher).

2. Progress

2.1. Experimental

In order to contribute to the definition of yield potential and quality characteristics for food uses, of 25 European top bread wheat cultivars the SEN Trial was grown, during 3 years, at Elvas.

With the different origins, the genotypes have distinct growth habits from very early (spring types) to late types to our conditions (alternative or winter types) **(Table XVI)**

The trial was performed according to the instructions of the established protocol.

So, 2 Nitrogen treatments were applied (N1-120 kg/ha; N2- 170 kg/ha) in 3 reps.

This 92/93 and previous (91/92) season were characterized by drought that occurred during tillering and flowering (**Figure 29**). On the other hand, heavy frosts occurred in March, affecting early genotypes, when they were at the booting stage leading to death of tillers and plants.

Protein content and SDS test have been determined on whole grain samples. Alveograph test was also completed.

2.2. Results and discussion

Due to the above mentioned climatic constraints (amount and distribution of rainfall and frosts) yields were low (3642kg/ha trial mean) **(Table XVII)**, and poor grain were obtained **(Table XVIII)**.

No significant influence was observed between Nitrogen treatments. Looking back for the last three years one can conclude that the most stable genotypes, for yield, are the ones with medium to late cycles.

Concerning quality, 1992/93 was the most favourable season with genotypes showing high protein, and good values in the alveograph test **(Table XIX)**. With quality, seems that the best hard wheats are in the early group of studied germplasm.

Meetings/Visits:

- Francisco Bagulho attended meeting of sub-programme C, 17-18 May in Bologna

- Carla Moita Brites attended the 5th international gluten Workshop, Detmold, June 7-9, 1993 and meeting of sub-programme B open to an international discussion on gluten proteins, 10 June 1993.

3. Publication

A publication concerning studies on yield and quality stability is being prepared

Table XVI. Growth habits of the cultivars of the Southern European Network trials at Elvas (Portugal).

No	Variety	Growth cicle
1	Golia	M-E
2	Salmone	L
3	Mec	M-L
4	Manital	M-E
5	Courtot	L
6	Centauro	M-L
7	Pegaso	L
8	Pandas	E-M
9	Veda	E
10	Yecora	E
11	Festa	M-E
12	Maestra	M-E
13	Cajeme	E
14	Castan	M-L
15	Mondego	E
16	MP 477	M-E
17	Tua	M
18	Sideral	L
19	Soisson	L
20	Avital	M-L
21	Primqual	E
22	Mira	M
23	Almansor	M
24	Amazonas	M-L
25	Rinconada	E

E - Early
M - Medium
L - Late

Figure 29. Rainfall distribution and number of rainy days (at Elvas, Portugal).

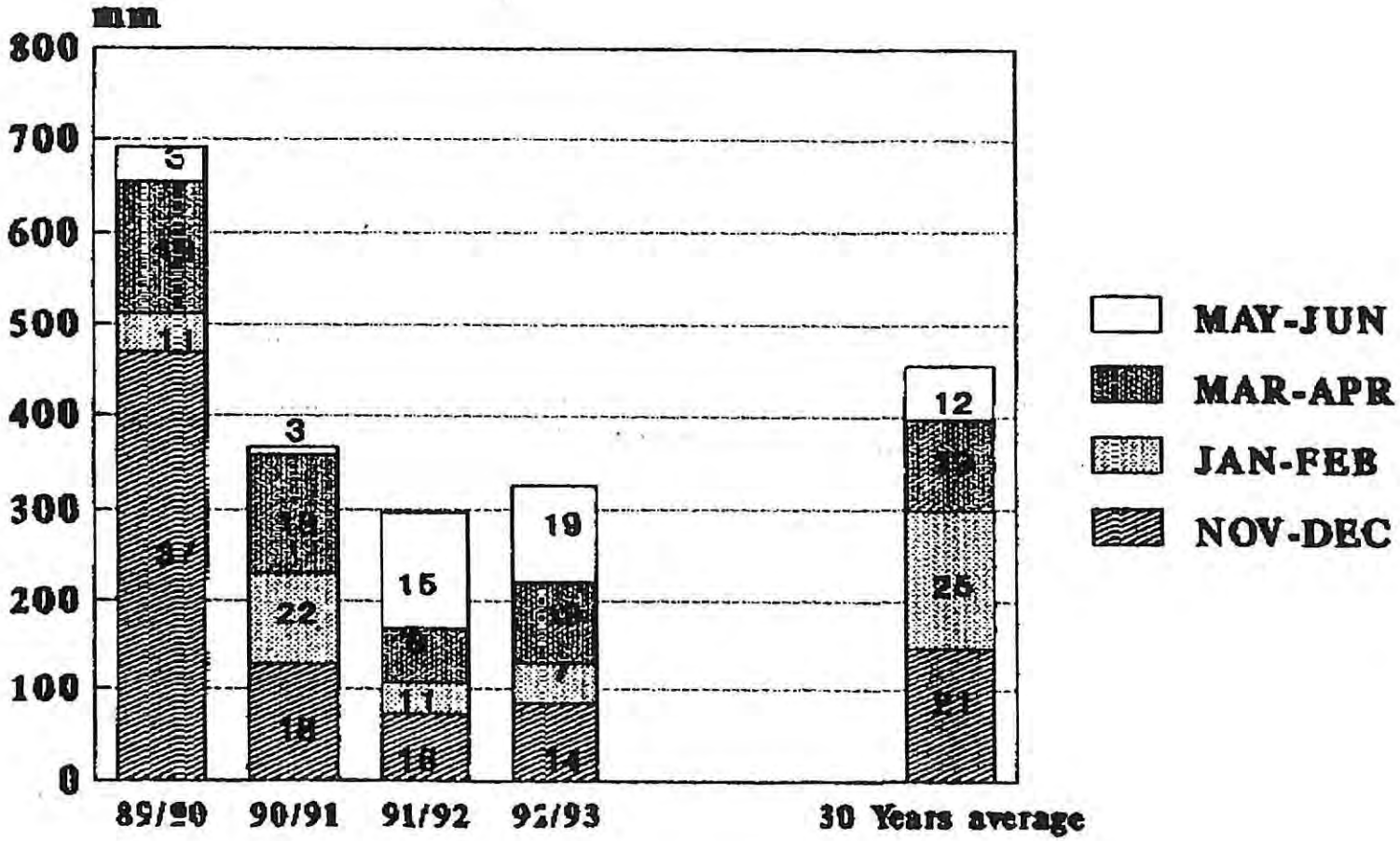


Table XVII. Southern European Network, Elvas (Portugal), 1992/93.

Grain yield of the varieties.

Latitude 38° 54' Longitude 7° 09'
Date of sowing: 17.Nov.92
Nitrogen: 1-120Kg/ha; 2-170Kg/ha

VARIETY Nº	VARIETIES	GRAIN YIELD (Kg/ha)	
11	Festa	4786	A
18	Sideral	4777	A
20	Avital	4591	AB
23	Almansor	4519	ABC
22	Mira	4404	ABC
17	Tua	4377	ABC
9	Veda	4368	ABC
16	MP477	4366	ABC
24	Amazonas	4132	ABCD
15	Mondego	3988	ABCDE
12	Maestra	3987	ABCDE
19	Soisson	3953	ABCDE
14	Castan	3704	ABCDEF
6	Centauro	3591	BCDEF
8	Pandas	3565	BCDEF
3	Mec	3535	BCDEF
25	Rinconada	3453	BCDEF
4	Manital	3385	CDEF
21	Prinqual	3187	DEFG
7	Pegaso	2876	EFGH
1	Golia	2823	EFGH
13	Cajeme	2559	FGH
10	Jecora	2180	GH
5	Courtot	2100	GH
2	Salmone	1852	H
MEAN		3642	
L.S.D. (1%)		1177	

Table XVIII. Southern European Network, Elvas (Portugal), 1992/93. Test weight of the varieties.

Latitude 38° 54' Longitude 7° 09'
Date of sowing: 17.Nov.92
Nitrogen: 1 120Kg/ha; 2 170Kg/ha

VARIETY Nº	VARIETIES	TEST WEIGHT (Kg/hl)	
24	Amazonas	75.40	A
17	Tua	75.05	AB
19	Soisson	74.55	AB
11	Festa	74.37	AB
1	Golia	74.13	ABC
22	Mira	73.60	ABCD
12	Maestra	73.33	ABCD
13	Cajeme	73.28	ABCD
21	Prinqual	73.15	ABCD
23	Almansor	73.13	ABCD
25	Rinconada	72.95	ABCD
18	Sideral	71.78	ABCDE
20	Avital	71.68	ABCDF
4	Manital	71.65	ABCDE
16	MP477	70.92	ABCDEF
15	Mondego	70.57	ABCDEF
8	Pandas	70.17	ABCDEFG
6	Centauro	70.15	ABCDEFG
10	Jecora	69.72	ABCDEFG
14	Castan	69.57	BCDEFG
9	Veda	68.35	CDEFG
2	Salmone	68.02	DEFG
5	Courtot	66.52	EFG
3	Mec	65.30	FG
7	Pegaso	64.72	G
MEAN		71.28	
L.S.D. (1%)		5,805	

Table XIX. Southern European Network, Elvas (Portugal): Protein content and SDS-sedimentation value of whole meal from N1 and N2 treatments; alveograph tests of samples from the N1 treatment.

	N1 TREAT.						N2 TREAT	
Variety	% PROT (Nx5.7)	SDS (*mm)	W (10-4J)	P (mm)	L (mm)	P/L	%PROT (Nx5.7)	SDS (*mm)
Golia	15.0	65.0	320.0	193.0	41.0	4.8	13.5	57.0
Salmone	15.1	95.0	319.0	108.0	89.0	1.2	15.5	78.0
Mec	15.6	77.0	147.0	97.0	39.0	2.6	15.9	57.0
Manital	15.5	80.0	358.0	164.0	56.0	3.0	15.7	68.0
Courtot	14.8	67.0	206.0	110.0	61.0	1.9	15.2	68.0
Centauro	12.7	75.0	228.0	109.0	56.0	2.0	13.5	57.0
Pegaso	13.4	80.0	321.0	115.0	85.0	1.4	14.4	65.0
Pandas	13.2	80.0	337.0	200.0	43.0	4.7	13.4	70.0
Veda	14.5	87.0	420.0	159.0	78.0	2.0	15.1	90.0
Yecora	15.9	80.0	418.0	125.0	99.0	1.3	16.9	80.0
Festa	13.9	90.0	283.0	121.0	73.0	1.7	13.7	72.0
Maestra	14.1	65.0	309.0	146.0	59.0	2.5	13.7	55.0
Cajeme	15.7	82.0	352.0	124.0	68.0	1.8	15.5	82.0
Castan	13.4	97.0	280.0	189.0	39.0	5.0	12.9	60.0
Mondego	13.4	70.0	390.0	197.0	56.0	3.6	13.5	67.0
MP477	13.6	85.0	389.0	198.0	48.0	4.2	13.5	65.0
Tua	13.5	75.0	346.0	138.0	79.0	1.8	14.1	73.0
Cideral	11.8	65.0	157.0	97.0	50.0	2.0	12.8	45.0
Soisson	12.3	68.0	253.0	81.0	101.0	0.8	12.9	65.0
Avital	10.8	47.0	184.0	143.0	36.0	4.0	12.0	50.0
Prinqual	14.4	83.0	457.0	188.0	68.0	2.8	14.7	92.0
Mira	12.7	83.0	314.0	186.0	56.0	3.3	12.7	65.0
Almansor	14.0	60.0	316.0	133.0	89.0	1.5	13.2	60.0
Amazonas	13.3	85.0	223.0	97.0	73.0	1.4	14.4	75.0
Rinconada	14.5	92.0	451.0	103.0	138.0	0.8	15.2	73.0
Mean	13.9	77.3	311.1	140.8	68.0	2.5	14.2	67.6

*-Dick & Quick,1983

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

Partner 07N - INRA - Clermont-Ferrand

1. Team: Nathalie Robert (Researcher)
Pierre Bérard (Technician)

2. Progress

Samples were analysed for % protein, sds sedimentation volume, Pelshenke (NWEN and SEN) and alveograph (NWEN). For both networks there was sprouting thus we have to consider carefully results obtained for 1992.

Stability for technological traits was defined by a low variance and was analysed by a regression approach (value of one genotype in one location regressed on the mean value of the locations). The slope of the regression line (b) represents the response of the genotype to environment (predictable part of the genotype X environment interactions,) the residues of the regression represent the non predictable part of the interactions. A stable genotype should have a low variance due to a low response to environment and a good confidence of this response.

Data for the two years were analysed according to the additive model: genotype + environment + year + genotype x environment + genotype x year + year x environment + error. The different interactions were significant. Causes of the interactions were graphically investigated: scale effects and rank order modifications were present.

Stability analysis was performed each year. Stability parameters were compared between years to understand if the response to environment of one genotype was largely influenced by the year conditions.

Heterogeneity of slopes was tested to distinguish between varieties with a response lower to that of the mean ($b<1$), and varieties with a higher response ($b>1$).

Partner 09 - ITCF - Paris

1. Team: Michel Leuillet (Project Manager)
Marie-Hélène Bernicot (multilocal experiment)
Christine Bar (quality evaluation)

2. Progress

Agronomical following of the 3 trials sown in October 1992 (2 for the NWEN and 1 for the SEN). Crops notations have been performed.

2.1. Agronomical results of 1993 harvest

a. NWEN (North Western Europe Network)

2 trials have been performed, one in Boigneville (Beauce) and one in St Hilaire (Champagne). Results are summarized with the trials coming from Club des 5 (Partner AC 18) an INRA (07) in **Table XX**.

They are in accordance with the results of French 1993 harvest;

-excellent performance of late varieties as APOLLO and BEAVER;

-diseases caused a lot of damages: yield decrease between 2,3 and 3 tons/ha depending on the location and the variety. Yields of foreign varieties are less important than the French ones. RENAN is the most notable variety because it has confirmed its good level of diseases tolerance and has maintained an interesting yield even in untreated replications.

CAMP-REMY, SOISSONS and REKTOR have the most regular yields in different locations. The interaction genotype x environment is lower for these varieties.

b. SEN (South Europe Network) (**Table XXI)**

One trial has been performed in Montans (Tarn). Sed have met difficulties to come up. Salcome cv. has not come up.

Yields are in the average of the year, between 4,5 and 7,8 tons/ha for SIDERAL.

2.2. Quality evaluation (**Tables XXII and XXIII)**

Protein contents have been analysed on each sample. On this basis, INRA Clermont-Ferrand (07) and BSN (08) have chosen samples in order to continue the technological evaluation of the harvest.

Locations Cappelle (NWEN) and Gramond (SEN) have sprouted.

Table XX, North-Western European Network.
Agronomical results of the 1993 harvest at the ITCF growing locations.

Location	Boigneville	St Hilaire au temple		Orgerus		Cappelle		Verneuil l'Etang		MERN	MERN
Country	91	51		78		59		77		q\ha	q\ha
Sowing period	14.10.92	12.10.92				06.11.92		30.10.92			
Previous plant	peas	peas				sugar beet		peas		all samples	treated
T\UT fungicides	T	T	UT	T	UT	T	UT	T	UT		samples
APOLLO (C)	108,0	82,4	70,8	87,5	54,3	82,2	78,7	76,4	45,8	76,1	87,3
ARMINDA	89,3	84,2	70,7	74,2	50,4	78,2	78,1	60,4	41,0	69,6	77,3
BAROUDEUR	94,3	84,4	65,5	81,1	67,1	75,7	79,6	76,6	56,7	75,7	82,4
BEAVER	105,7	88,6	71,4	84,8	56,7	-	-	77,7	42,4	-	-
CAMP REMY	94,0	78,3	68,9	71,5	52,7	80,3	74,6	66,5	46,5	70,4	78,1
MERCIA	92,3	80,7	68,7	64,1	48,8	79,4	81,4	54,5	35,8	67,3	74,2
RECITAL (C)	93,6	78,1	57,5	77,1	53,1	72,1	69,1	71,7	46,6	68,8	78,5
RECKTOR	89,4	75,9	66,3	62,8	47,1	69,8	64,6	61,9	40,2	64,2	72,0
RENAN	90,5	76,2	73,7	79,9	72,1	64,7	62,7	72,4	61,8	72,7	76,7
ROSSINI	99,8	-	-	76,2	58,1	79,2	77,7	70,4	48,8	-	-
SIDERAL	100,4	84,6	73,2	87,9	69,4	79,0	79,2	78,7	46,6	77,7	86,1
SOISSONS (C)	97,5	81,6	73,9	82,7	63,1	79,5	78,5	77,1	52,6	76,3	83,7
TALENT	90,8	75,8	61,1	65,0	50,5	65,0	73,0	57,6	43,6	64,7	70,8
THESEE (C)	94,0	86,7	71,0	64,1	45,1	79,1	70,7	75,4	45,5	70,2	79,9
VICKING		-	-	74,7	49,9	91,6	80,8	69,8	31,8	-	-
Controls (q)	98,3	82,2	68,3	77,9	53,9	78,2	74,3	75,2	47,6	72,9	82,3
Standard deviation	3,5	3,7		2,7	3,9	4,7		4,6	4,7		

Table XXI. South European Network.
Agronomical results of the 1993 harvest at the ITCF growing locations.

CULTIVARS	DISEASE NOTES			HEIGHT	FLATTENED WHEAT		EARS /m2		YIELD	
	rusty	*septorium*	*oidium*		*Nitrogen X*	*Nitrogen X + 50*	*Nitrogen X*	*Nitrogen X + 50*	*Nitrogen X*	*Nitrogen X + 50*
CASTAN	1	3	1	94	2,00	1,33	365,00	414,00	54,23	51,72
COURTOT	1	5	0	63	0,00	0,00	419,50	588,50	50,03	60,30
MT 47 77	1	2	4	84	1,67	1,67	348,50	452,50	62,79	63,96
SOISSONS	3	5	0	83	4,00	1,00	646,00	389,00	57,13	55,46
SIDERAL	1	3	4	85	3,00	0,33	645,50	594,00	78,36	72,58
AVITAL	0	2	1	84	0,67	0,00	523,50	556,00	71,98	72,93
PRINQUAL	1	3	0	80	5,67	5,33	392,00	436,00	50,17	49,29
CENTAURO	2	4	0	61	2,67	1,00	338,00	321,50	48,37	57,78
GOLIA	2	4	0	61	0,00	0,00	470,50	357,00	54,76	47,88
BRUNO	0	2	0	78	0,00	0,00	335,00	444,50	46,84	60,41
MAESTRIA	1	1	2	77	0,00	0,00	369,00	317,00	59,98	56,11
MANITAL	1	4	0	69	0,00	0,00	567,50	547,50	50,38	46,61
MEC	1	5	1	77	1,00	1,67	532,00	469,00	58,51	58,94
PANDAS	0	2	0	84	1,67	1,67	269,50	383,50	56,93	62,06
PEGASO	1	2	0	71	0,33	0,67	315,00	269,50	54,75	56,16
VEDA	2	2	2	71	2,00	0,00	636,50	571,50	53,14	49,11
CAJEME	1	3	4	73	2,33	2,33	388,00	585,50	44,88	50,24
YECORA	0	4	4	67	2,67	3,33	475,00	546,00	44,92	45,52
RINCONADA	0	2	4	84	2,33	2,67	264,50	297,50	50,39	54,67
ALMANSOR	2	6	0	99	2,00	0,00	469,00	351,00	48,28	44,87
AMAZONAS	0	1	4	95	1,67	3,33	509,00	517,00	67,70	58,10
MIRA	1	4	0	93	2,33	2,33	333,00	509,00	52,01	53,95
MONDEGO	0	3	3	74	1,67	2,33	359,00	433,50	52,88	55,78
TUA	0	2	2	101	0,00	1,33	428,50	404,50	63,72	63,73

Table XXII. North-Western European Network.
Protein content (N x 5.7 DM) of the 1993 harvest at the ITCF growing locations.

	CLUB DES 5						INRA						ITCF	
	Verneuil (77)		Orgerus (78)		Cappelle (59)		CLERMONT		LE RHEU		DIJON		BOIGNEVILLE	ST HILAI
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Treated	Treatec
APOLLO	13,7	12,5	13,0	13,2	10,6	10,7	12,8	13,1	11,2	10,7	13,2	13,5	11,9	12,1
ARMINDA	14,5	14,3	13,7	13,5	11,3	11,0	13,4	13,7	10,9	10,9	14,1	-	12,1	11,8
BAROUDEUR	13,6	13,1	14,0	13,9	11,3	11,2	13,5	13,9	11,1	10,8	13,7	14,1	12,2	11,8
BEAVER	14,8	12,6	14,4	12,8	-	-	13,6	13,7	11,2	10,9	14,0	13,5	11,4	12,1
CAMP-REMY	14,1	14,2	14,3	15,1	11,3	11,4	13,7	14,1	10,6	11,2	14,6	15,0	11,8	12,2
TALENT	14,6	14,1	14,5	14,8	11,7	11,4	13,8	14,4	11,7	11,6	14,2	14,5	-	12,9
EUREKA	-	-	-	-	-	-	-	-	-	-	-	-	12,3	13,4
MERCIA	15,7	14,3	15,3	14,6	11,4	11,0	13,7	13,6	11,4	11,1	12,7	13,1	11,6	11,9
RECITAL	13,5	12,3	12,9	12,7	11,0	11,0	11,7	12,2	10,4	10,3	14,4	14,8	12,3	11,5
REKTOR	13,3	13,5	14,7	13,6	10,7	11,5	13,8	14,3	11,2	11,9	14,3	14,4	12,2	12,8
RENAN	13,5	13,7	14,8	14,4	12,2	12,1	14,7	14,8	11,9	12,3	13,1	13,1	12,0	13,2
ROSSINI	13,8	13,5	13,9	14,9	11,6	11,4	12,5	13,0	11,0	11,3	13,6	13,8	11,5	-
SIDERAL	14,1	12,8	14,3	13,5	11,1	11,6	12,8	13,4	10,4	10,5	12,8	13,6	11,4	11,9
SOISSONS	12,4	13,2	12,5	13,6	10,9	11,5	11,5	13,2	10,9	10,9	14,5	14,3	11,6	12,8
THESEE	12,4	13,1	13,2	13,0	11,2	11,2	12,1	12,7	11,3	11,1	12,0	13,1	11,5	11,5
VICKING	14,4	12,4	14,4	13,3	10,0	10,1	12,0	12,6	10,4	10,2	13,6	12,9	-	

Table XXIII. South European Network.
Protein content (N x 5.7 DM) of the 1993 harvest at the ITCF growing locations.

	ITCF GRAMONT (81)	
	Nitrogen level X	Nitrogen level X + 50
ALMANDOR	14,7	14,7
AMAZONAS	13,4	16,6
AVITAL	13,5	14,4
BRUNO	17,0	17,3
CAJEME	17,2	17,7
CASTAN	15,8	16,4
CENTAURO	15,1	15,0
COURTOT	17,3	17,7
GOLIA	16,2	16,2
MAESTRO	14,9	15,3
MANITAL	17,3	17,4
MEC	16,1	16,6
MIRA	14,1	15,1
MONDEGO	15,5	15,5
PANDAS	15,8	16,7
PEGASO	16,5	17,5
PRINQUAL	16,4	-
RINCONADA	17,0	18,6
SALMONE	-	-
SIDERAL	14,6	14,7
SOISSONS	13,9	13,9
TUA	15,9	16,3
VEDA	15,1	15,6
YECORA	16,5	17,8
MT 47.7	-	15,3

Task C.2- Genotype x Environment Interaction

Partner 07N - INRA - Clermont-Ferrand

1. Team: Nathalie Robert (Researcher)
Lucette Le Blevennec (Technician)
Eugène Triboï (Researcher)

2. Progress

The kinetics of accumulation of protein and dry matter were studied for four bread wheat varieties. For each sample, protein fractions were extracted by a sequential extraction scheme. Rate was calculated for accumulation of dry matter, total nitrogen, albumin and globulin, gliadin, glutenin and insoluble fractions. They were differences between varieties for the different rates. Accumulation of each protein fraction was split up into accumulation of total nitrogen relative to growth of one grain and into allocation of total nitrogen to the fraction considered. There was no significant difference for accumulation of total nitrogen relative to growth, but some significant differences existed for allocation to various fractions. The final weight of on grain seemed to be a major determinant of protein quantities. The cultivars were compared for final protein composition defined as absolute quantities, proportions of total nitrogen or percentages of dry matter, Varieties classified differently according to the definition chosen for protein composition.

The same approach will be undertaken on SE HPLC data obtained in J.C. Autran's laboratory. Thus the four varieties will be compared for their protein aggregation pattern during grain filling.

3**. Publications**

Robert N., Le Blevennec L. and Triboï E. 1993. Accumulation of protein fraction during grain filling: comparison of four bread wheat varieties. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany).

Robert N., Le Blevennec L. and Triboï E. 1993. Protein composition during grain filling: comparison of four bread varieties. Cereal Chem. (submitted).

Task C.3- Experimentation on Populations for Breeding

Partner 02 - Produttori Sementi

1. Team: Enzo DeAmbrogio (Research Manager)
Parivash Jenabzadeh (Researcher)
Marilena Paolini (Researcher)
Stefano Ravaglia (Researcher)
Luca Bersanetti (Technician)
Stefano Poluzzi (Technician)

2. Progress

The trial including the synthetic populations provided by I.S.C. (S. Angelo) was harvested and **Table XXIV** shows the yield results. Quality analysis will be performed during the winter.

The 1993/94 trial was sown in October.

Partner 03 - Istituto Sperimentale per la Cerealicoltura

1. Team: Basilio Borghi (Researcher)
Norberto Pogna (Researcher)
Rita Redaelli (Researcher; from January to September 1993)
Anna Biancardi (Technician).

2. Progress

The best 12 lines derived from a synthetic population and 3 Italian quality varieties were inter crossed during 1993 according to the proposed recurrent selection scheme. During 1994 the seed will be increase and it will be released to the breeders. The agronomic and qualitative results of the best lines are reported in **Table XXV.**

Table XXIV. Experimentation on populations for breeding. Yield trial results at Argelato (1993).

N°	Variety or Population	PROD t/ha	
21	ERIDANO	11.17	A
11	PROD 4	10.08	AB
14	PROD 32	9.54	BC
2	QUAL 4	9.39	BD
16	PROD 54	9.37	BD
13	PROD 16	9.23	BD
20	CHIARANO	9.14	BD
9	QUAL 87	9.11	BD
22	GOLIA	9.10	BD
1	QUAL 3	9.08	BD
12	PROD 9	9.03	BD
15	PROD 49	9.02	BD
7	QUAL 69	9.01	BD
17	PROD 60	8.99	BD
3	QUAL 7	8.75	BD
24	ODERZO	8.75	BD
19	CENTAURO	8.71	BD
18	PROD 64	8.70	BD
25	PANDAS	8.69	BD
10	PROD 3	8.66	BD
5	QUAL 23	8.58	BD
23	MAESTRA	8.57	BD
8	QUAL 76	8.43	CD
6	QUAL 44	8.41	CD
4	QUAL 8	7.82	D

Table XXV. Experimentation on populations for breeding. Agronomical and qualitative scores of the best lines at I.S.C. (1993).

Name	Grain yield (t/ha)	Test weight (kg/hl)	Plant height (cm)	SDS Sedim. volume (ml)
23	5.95	74.8	103	74
44	5.82	72.8	88	82
4	5.80	70.8	98	90
7	5.67	71.9	92	84
87	5.66	74.0	108	88
3	5.65	73.2	98	88
76	5.61	70.6	82	86
8	5.45	71.7	102	87
69	5.09	72.5	93	84
ERIDANO	7.30	75.6	100	87
GOLIA	6.64	72.3	75	87
MAESTRA	5.79	75.7	97	89
CENTAURO	5.63	70.6	83	85
SALMONE	4.35	75.5	78	87

Task C.4- Genetics of LMW Glutenin Subunits

Partner 07N - INRA - Clermont-Ferrand

1. Team: Gérard Branlard (Project Leader)
Mireille Dardevet (Assistant Engineer)
Isabelle Felix (Research Fellow)
Isabelle Gateau (Technician)

2. Progress

2.1. Methods

The diversity of the HMW and LMW glutenin subunits was analysed using the one step method proposed by SINGH et al., 1991. The ω−gliadins were separated by SDS PAGE and the nomenclature of the allele found in the material studied was that proposed by KHELIFI et al., 1992. The identification of the different LMW GS patterns was achieved: 1) by pooling the sample of similar mobility phenotype on the second gel run out for that purpose, and 2) by using the cultivars Cappelle Desprez, Courtot and Chinese Spring as checks.

In each population studied the quality of the cultivars was assessed through the use of the following technological tests: grain protein content, Chopin alveograph and Pelshenke swelling time.

2.3. Material

Two main populations were used to study which was the part of the quality attributed to HMW GS, LMW GS and ω-gliadins. Population I which was constituted of 132 French bread wheat cultivars released between 1949 and 1984. These cultivars were grown at the INRA plant breeding station of Clermont-Ferrand. Population II, represented by a set of 61 cultivars originated from more than 15 countries, was also cultivated at Clermont-Ferrand for several years. These two populations were very different for their genetic origin. Population I represented the diversity of the French wheat cultivars and the main genetic source of the actual varieties grown in France. Whereas population II gathered numerous strains of good to very good quality from very diverse geographical origins. In addition wheats from bad to medium quality were also present in population II.

2.3. Results

Both population I and II exhibited numerous allelic variants at *Glu-A1*, *Glu-B1* and *Glu-D1* loci. The *Gli-1* alleles encountered in these two populations as revealed by SDS-PAGE also were numerous **(Table XXVI)**.

Few cultivars of identical protein composition were encountered in each population rending the variance analyses more difficult when the quality means attributed to each pattern were compared. The effects attributed to the *Glu-1*, *Glu-3* and *Gli-1* pattern diversity on the quality parameters were particularly studied **(Table XXVII)**.

Both populations I and II raised similar conclusion about the limit of variation explained by the three groups of chromosomes I encoded proteins.

The best estimated alveograph parameter was the W, whereas both P and G were the worst. For all the tested parameters tested the part attributed to the D zone ω-gliadin was higher than that attributed to LMW GS. For P and G these two protein families encoded at Glu3 and Gli1 loci had total effects almost equivalent to those attributed to Glu1 major loci.

Other analyses were carried out to assess the part of the LMW GS and the D zone ω–gliadins on quality. Several progeny were analysed, particularly from crosses including the French cultivar Darius. Preliminary conclusions evidences that some very good alleles for rheological dough quality may be nevertheless found in the Glu3/Gli1 loci.

Table XXVI. Number of different patterns found in the two populations for the *Glu-1* (HMW GS), *Glu-3* (LMW GS) and *Gli-1* (D zone ω–gliadins).

	HMW GS	LMW GS	D zone ω gliadins
Population I N = 132	32	16	20
Population II N = 92	29	39	22

Table XXVII. Part encountered of the alveograph quality parameters explained by the genetic diversity encountered at *Glu-1*, *Glu-3* and *Gli-1* loci coding for HMW GS, LMW GS and D zone ω–gliadin respectively (132 French wheat cultivars).

	HMW GS	LMW GS	D zone ω gliadins
Strength W	0.59	0.07	0.10
Tenacity P	0.31	0.08	0.16
Swelling G	0.30	0.10	0.17
Extensibility L	0.37	0.10	0.17

Partner 07M - INRA - Montpellier

1. Team: Marie-Hélène Morel (Researcher, Project Leader)
Rita Redaelli (Researcher, on secondment from Istituto Sperimentale per la Cerealicoltura, Italy, Partner 03, from October 1993)
Valérie Mélas (Research Fellow)
Jean-Claude Autran (Head of Laboratory)

2. Progress

Because of the difficulty to describe exhaustively LMW alleles with one single system *e.g.* SDS-PAGE, the native polyacrylamide gel electrophoresis system buffered by acetic acid (A-PAGE), described in the previous report, was carried out on reduced and alkylated subunits of glutenin in combination with SDS-PAGE and IEF separations. These techniques were applied to a set of 10 intervarietal chromosome substitution lines (supplied by INRA-Clermont-Ferrand, Partner 07C) and to 22 Italian cultivars (supplied by ISC, S. Angelo Lodigiano, Partner 03) in which the allelic variation at the *Gli-1* locus (ω-gliadins) was previously determined by N.E. Pogna on the basis of Metakovsky's nomenclature. In a set of forty-two French bread wheat cultivars, respectively 4, 5 and 4 allelic variants were observed at the loci *Glu-A3*, *Glu-B3* and *Glu-D3* **(Figure 30)** The specific effect of LMW subunits (whose variation respectively explains 35 % and 25 % of the variations in baking strength and dough extensibility among the same set of French bread wheats), as well as the interactions between glutenin components encoded at the *Glu-1* and *Glu-3* loci might explain some major discrepancies observed in the relation between HMW composition and dough properties. When aiming at breeding, for instance, breeding-type wheats, it is recommended to screen for genotypes containing the *Glu-B3 III* (≈ *Gli-B1 e,f*) allele associated to the *Glu-D1* allele encoding subunits 2+12. On the other hand, the amount of protein expressed by the various *Glu-B3* alleles might be related to their effect on dough extensibility.

On the other hand, several F_7 recombinant lines, obtained from the cross between the two bread wheat spring cultivars Neepawa and Costantino, and characterised by different alleles at the three *Gli-1* loci, were analysed by two-dimensional electrophoresis to determine allelic composition at *Glu-3* loci. Reduced and alkylated glutenin subunits were fractionated in acid PAGE as described by Morel (1994) in the first dimension and by 15%, pH 8.4 SDS-PAGE (Dachkevitch et al. 1993) for the second dimension.

By comparison between the two-dimensional maps of parental cultivars and recombinant lines, the main polypeptides coded at each *Glu-3* locus were identified. In Costantino, 1, 5 and 5 subunits are coded, respectively, by *Glu-A3*, *Glu-B3* and *Glu-D3* whereas, in Neepawa, the three loci code for 1, 6 and 4 subunits, respectively **(Figure 31)** (Redaelli et al., 1994).

3. Publications

Morel, M.H., Mélas, V., Bonicel, J. and Autran, J.C. 1993. Multiple approach (IEF, SDS-PAGE and A-PAGE) of the composition of LMW subunits of glutenin and its effect on dough properties. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany).

Redaelli R., Morel M.H., Pogna N.E. and Autran J.C. 1994. Fractionation of low-molecular-weight glutenin subunits of wheat by two-dimensional electrophoresis A-PAGE x SDS-PAGE. J. Cereal Sci. (submitted).

Poster

Redaelli R., Morel M.H., Pogna N.E. and Autran J.C. 1993. Genetical analysis of low-molecular-weight glutenin subunits of wheat by two-dimensional electrophoresis. Poster presented at "Alpes-Phorèse", 11th Symposium of the French Society of Electrophoresis, December 1-3, Chambéry (France).

Partner 23 - Università di Viterbo

(see report at Task B.1.1 above)

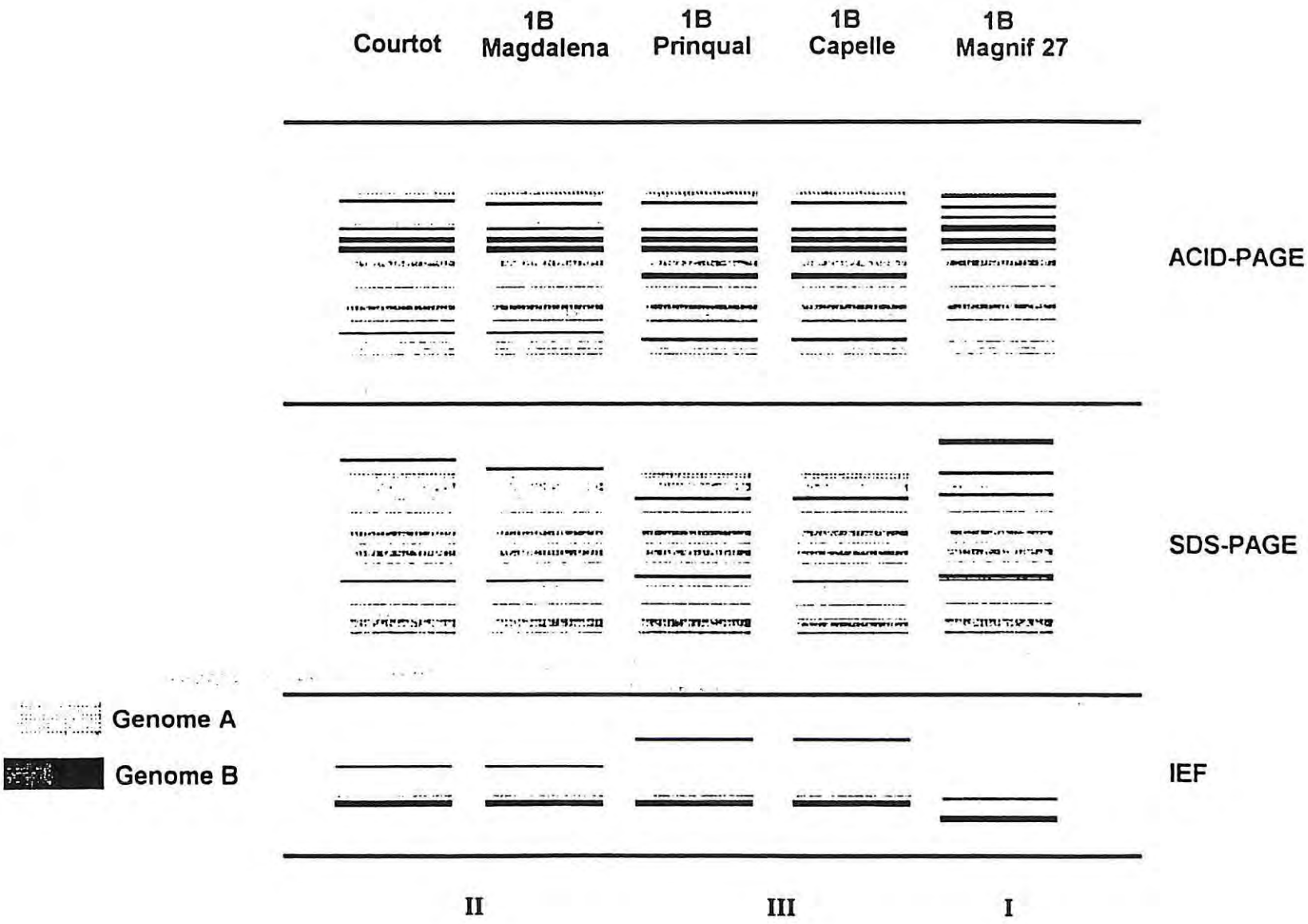


Figure 30. Schematic summary of the allelic variation observed at the Glu-B3 locus among chromosome-1B substitution lines of cv. Courtot on the basis of A-PAGE, SDS-PAGE, and IEF patterns.

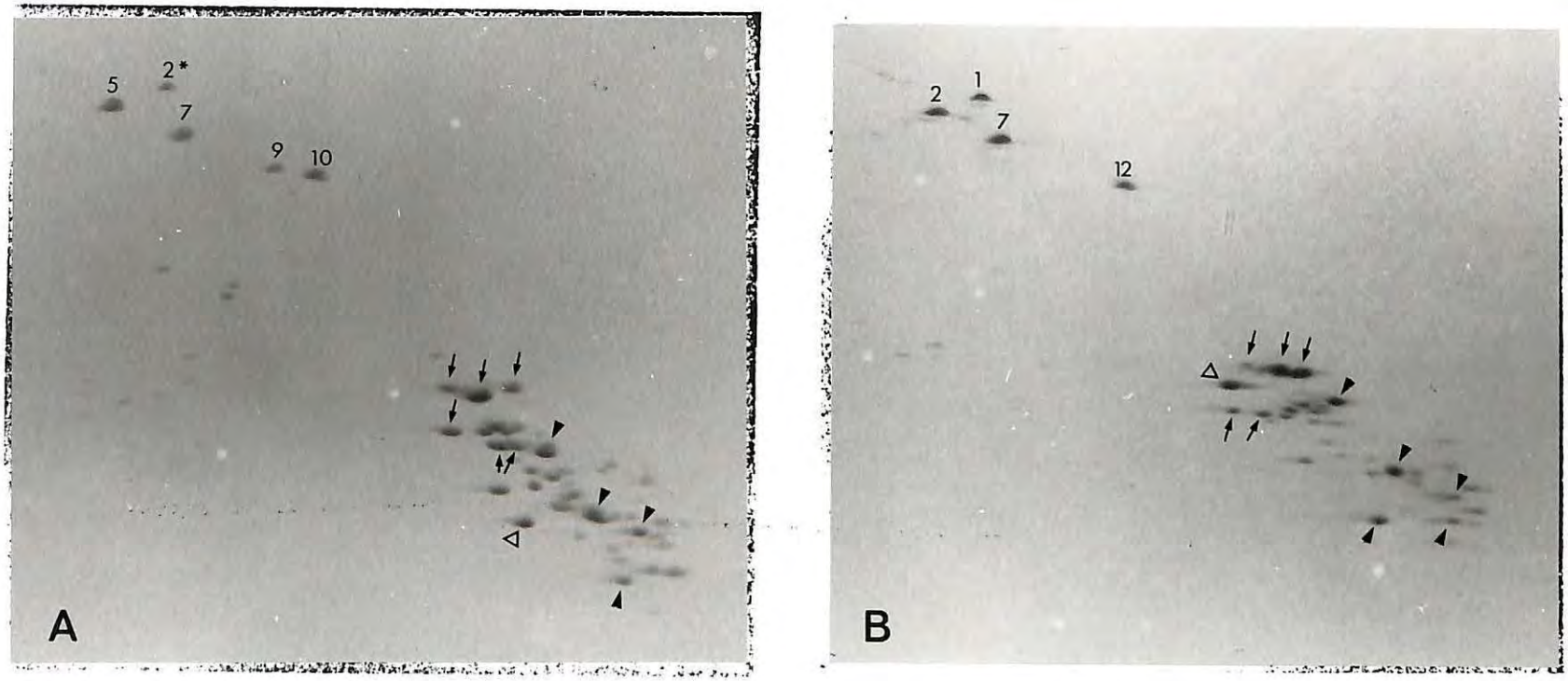


Figure 31. Two-dimensional (A-PAGE x SDS-PAGE) fractionation of glutenin subunits from cultivars Neepawa (A) and Costantino (B). On the left (top) HMW-GS are numbered. Fractionation of LMW-GS coded at the *Glu-A3* (open triangles), *Glu-B3* (arrows) and *Glu-D3* (arrowheads) is shown on the right (bottom).

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

Partner 03 - Istituto Sperimentale per la Cerealicoltura

1. Team: Basilio Borghi (Researcher)
Norberto Pogna (Researcher)
Rita Redaelli (Researcher; from January to September 1993)
Anna Biancardi (Technician)

2. Progress

Biotype 1 (*Glu-D1* encoded HMW subunits 2+12) and biotype 2 (only HMW subunit 12) have been characterized at the molecular level (PCR analysis), confirming that the *Glu-D1*-1 gene coding for HMW subunit 2 is present but it is not expressed in biotype 2. The DNA sequence of this gene is currently being determined. The SDS-sedimentation test carried out on flour from both biotypes grown in replicated plots showed that biotype 1 has superior bread-making quality compared to biotype 2, suggesting a positive effect of HMW subunit 2 on gluten quality. Both biotypes have been sown in replicated plots at S. Angelo Lodigiano. Several allelic variants of HMW albumins encoded by the group 4 chromosomes have been identified in the F2 segregating progenies from seven crosses between Italian and French cultivars. The F3 progenies have been sown in head-rows at S. Angelo Lodigiano.

Task C.6 - Production of Lines and Near-Isogenic Lines with Different Glutenin Subunit Composition and Null-Forms

Partner 03 - Istituto Sperimentale per la Cerealicoltura

1. Team: Basilio Borghi (Researcher)
Norberto Pogna (Researcher)
Rita Redaelli (Researcher; from January to September 1993)
Anna Biancardi (Technician)

2. Progress

Fourteen Near-isogenic lines (NILs) of cv. Alpe have been characterized using monodimensional and 2D - electrophoresis of storage proteins. These lines were grown in replicated plots in two locations and submitted to rheological analyses (alveograph and farinograph). Gluten form seven NILs lacking the *Glu-D1*/*Glu-D3* locus showed high elasticity and low extensibility compared to NILs possessing that locus. A strong interaction for gluten viscoelastic properties occurred between *Glu- B1*, *Glu-D1*, *Glu-B3* and *Glu-D3* loci. About 20 NILs isolated from the cross Neepawa x Costantino have been

sown in replicated plots at S. Angelo Lodigiano these lines are currently being analysed using the SDS-sedimentation test.

3. Meetings/Visits

Norberto Pogna attended the meetings of subprogramme C in Bologna (17-18 May) and Clermont-Ferrand (12-13 December); he also participated in the 5th Gluten Workshop in Detmold (Germany, 7-9 June). Basilio Borghi and Rita Redaelli attended the meeting of subprogramme C in Bologna.

4. Publications

Ng P.K.W., Redaelli R., Vaccino P., Accerbi M., Pogna N.E. and Bushuk W. 1993. Biochemical and genetical characterization of novel HMW glutenin subunits and their effects on breadmaking quality. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany) (in press).

Pogna N.E., Redaelli R. and Biancardi A., 1993. Production and genetic analysis of 14 Near-Isogenic Lines in the bread wheat variety Alpe. J. Genet. & Breed, 47 (in press).

Task C.8. - Prediction of Quality from Protein Diversity

Partner 07N - INRA - Clermont-Ferrand

(see report at Task C.4 above)

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

Partner 02 - Produttori Sementi

1. Team: Enzo DeAmbrogio (Research Manager)
Parivash Jenabzadeh (Researcher)
Marilena Paolini (Researcher)
Stefano Ravaglia (Researcher)
Luca Bersanetti (Technician)
Stefano Poluzzi (Technician)

2. Progress

The vernalization requirement of the Salmone and Oderzo somaclonal variants was assessed and no difference was found between variants and original varieties.

A set of monoclonal variants was included in trials sown in October.

Task C.10. - Sprouting Resistance

Partner 17 - TNO Food and Nutrition

1. Team: Dr. R.J. Hamer
Ir. M. Kelfkens
Dr. H.P.M. van Laarhoven

2. Progress

2.1 Development of germplasm

Material from parent varieties has been characterised and indicate that there is a broad variation in dormancy, although the level of dormancy was rather low, due to the weather conditions before harvest. Progenies have been selected and are multiplied by the breeders according to plan. Harvest of the final material took place in August 1993. This material was highly variable in dormancy. Although a complete statistical analysis still has to be performed, a first analysis showed that the variation in dormancy exceeds the variation between parents. The statistical analysis could indicate how broad the genetic basis for dormancy is, and the degree of progress that can be made by selection.

2.2 Isolation of markers

Several promising purified fractions from six varieties are available and ready to test for inhibitory action in the bioassay. However problems have been risen in the aleurone test used as a testing system for endogenous germination inhibitors. Several possible causes have been evaluated, but non of them has been found as a final cause. This part of the project was therefore temporarily stopped.

A biochemical approach was chosen by purifying possible candidate inhibitors. The fractions that were already purified and known from the bioassay as containing inhibitors were tested for abscisic acid and methyl esters of abscisic acid. From earlier experiments (gel filtration) was known that ABA would possibly not be the inhibitor. The molecule was expected to be larger (1000 - 2000 Mw).

Several monoclonal antibodies raised against pure (+)-ABA isotope and ABA methyl esters were used as a detection system.

2.3 Gel filtration

Figure 32 shows briefly the purification methods that were used. In the gel filtration fractions that were purified and still contain inhibitors, ABA esters were found (not shown).

Reversed phase and ion chromatography

The next steps in purification, ion exchange and reversed phase chromatography showed a further concentration and purification **(Figure 33a)**. The collected fractions were tested for ABA and methyl-ABA **(Figure 33b)**. The peak concentration of methyl-ABA has been found at the peak level in **Figure 34a**. In **Figure 34** the chromatogram after reversed phase fractionation is shown. There is a

clear peak in fraction 34 that contains a possible ABA methyl ester. ABA elutes under the same conditions a few fractions later (**Figure 34b**). A known ABA methyl ester was also used as a reference (**Figure 34c**). The concentration of different methyl esters will be measured in six varieties. Preparative columns are used to obtain sufficient material for identification of the ABA derivative. Further purification is in progress in order to elucidate the structure with NMR and Mass Spectrometry.

3. Publications

van Laarhoven H.P.M., Kelfkens M. and Hamer R.J. - 1993. Comparison of two methods to test the level of endogenous wheat germination inhibitors. In: Pre-harvest sprouting in Cereals 1992 Ed. K.K. Walker-Simmons and R.L. Ried 1993.

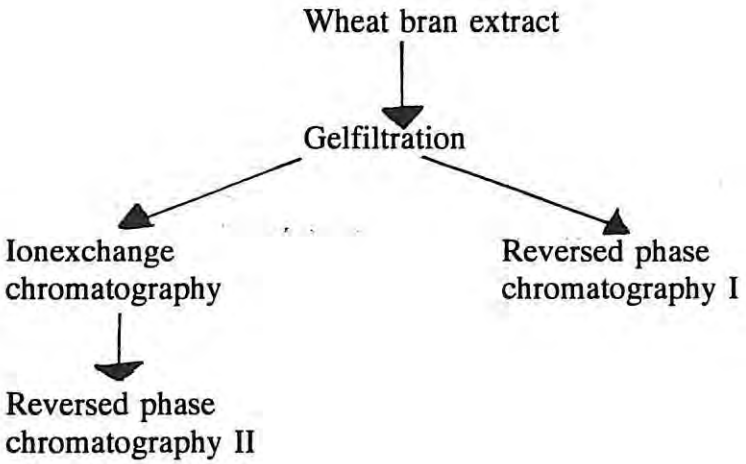


Figure 32. Purification schedule wheat bran extract

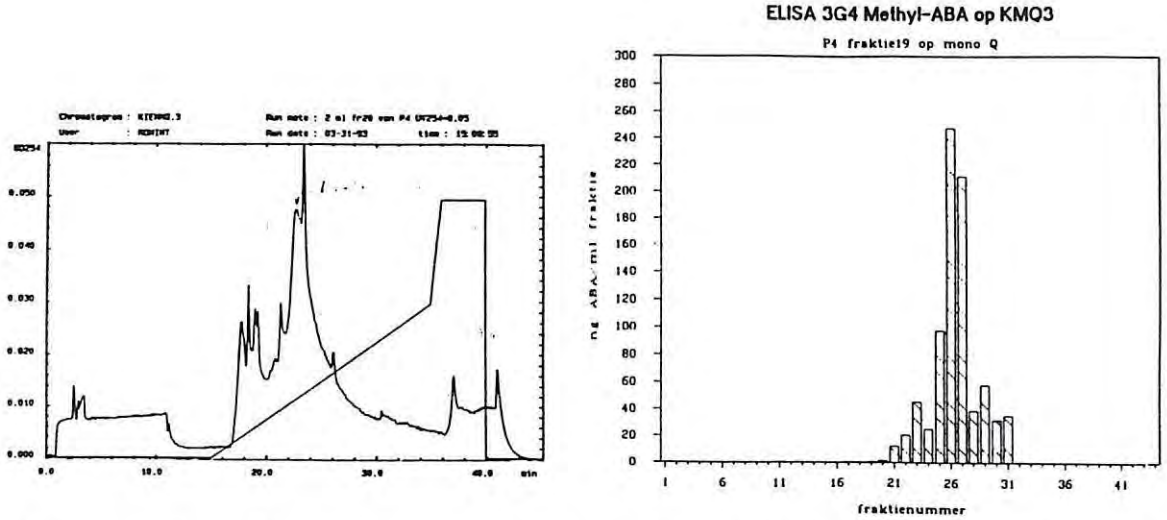


Figure 33. Anionexchange chromatography (Mono Q), 2.5 mM Tris-HCl pH=7,2 of gelfiltration fraction 20 (see figure 1)

figure 33a: Gradientelution with 0.0 to 1.0 M AMCO pH = 7.6 HCl. Detection: 254 nm

figure 33b: Elisa with monoclonal antibodies specific for methyl-ABA and ABA of ionexchange column fractions

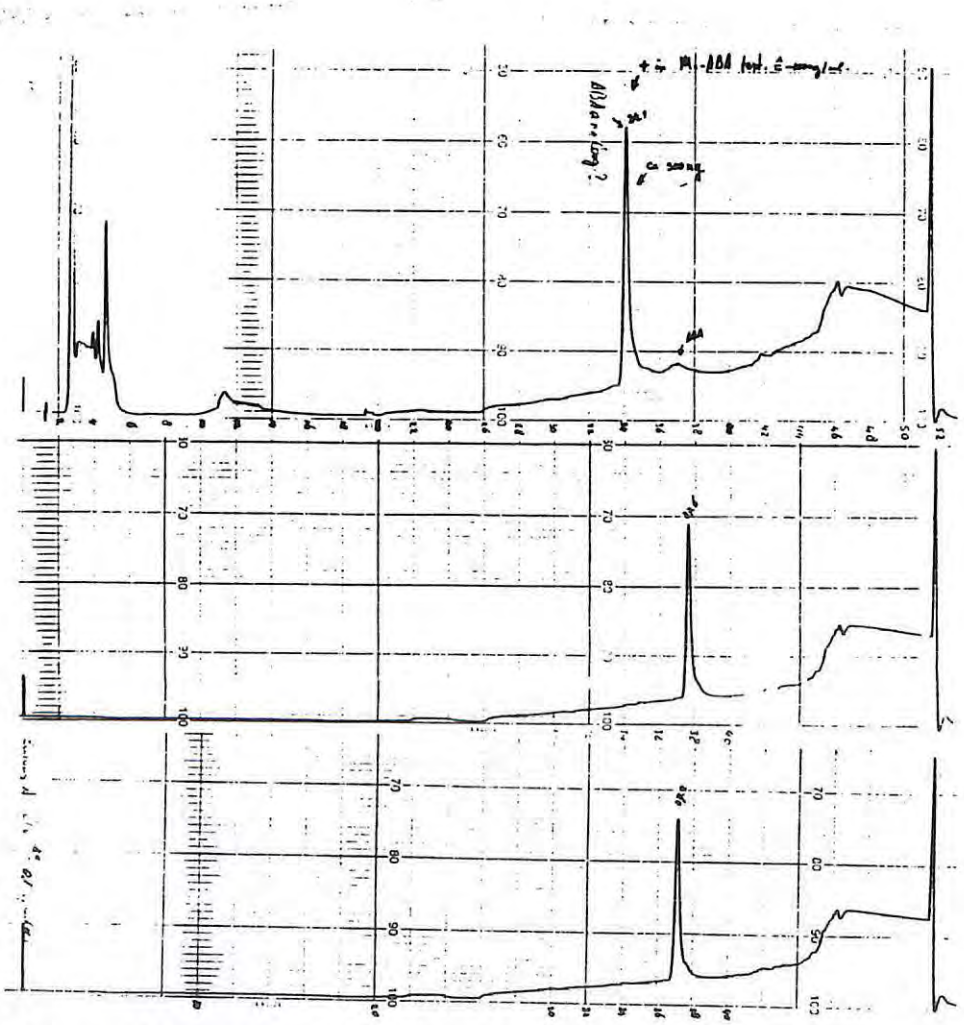


Figure 34. Reversed phase fractionation (RP18). Polair phase: Milli-Q water, apolair phase: methanol

figure 34a: Material from fraction 26/27 after ionexchange chromatography.

figure 34b: Abscisic acid

figure 34c: Synthetic methyl-ester

Annex

Publications by participants in the ECLAIR Programme AGRE 0052 (under date of April 1st, 1994)

BELTON P.S. - 1994. Does the elasticity of the high molecular weight subunits of glutenin originate in intermolecular interactions ? J. Cereal Sci. (submitted).

BELTON P.S., COLQUHOUN, I.J., FIELD J.M., GRANT A., SHEWRY P.R. and TATHAM A.S. - 1994. ^{1}H and ^{2}H NMR relaxation studies of the high M_r subunits of glutenin and comparison with elastin. J. Cereal Sci (in press).

BELTON P.S., GIL A.M. and TATHAM A.S. - 1994. ^{1}H NMR relaxation time studies of the hydration of the barley protein C-hordein. Macromolecules (submitted).

BENEDETTELLI S., MARGIOTTA B., PORCEDDU E., CIAFFI M. and LAFIANDRA D. - 1992. Effects of the lack of proteins controlled by genes at the *Gli-D1/Glu-D3* loci on the breadmaking quality of wheat. J. Cereal Sci., 16 (1), 69-79.

BLOCHET J.E., CHEVALIER C., FOREST E., PEBAY-PEROULA E., GAUTIER M.F., JOUDRIER P., PEZOLET M. and MARION D. - 1993. Complete amino acid sequence of puroindoline, a new basic protein with a unique tryptophan-rich domain, isolated from wheat endosperm by Triton X-114 phase partitioning. FEBS Letters, 329 (3), 336-340.

BRANLARD G., DARDEVET M., NIETO-TALADRIZ M.T. and KHELIFI D. - 1993. Allelic diversity of the ω-gliadins as revealed by SDS-PAGE: their possible implication in quality variation. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany).

COLLAR C., and MARTÍNEZC.S. - 1993. Amino acid profile of fermenting wheat sour doughs performed under different processing conditions. In: Proceedings of Euro Food Chem VII, Progress in Food Fermentation, Vol. 1. Ed.: C. Benedito, C. Collar, M. A. Martinez-Anaya, and R. Morell. IATA (CSIC) ISBN: 84-604-7038-5. Valencia, Spain, September 20-22, p 290-295 (1993). J. Food Sci., 58 (6), 1324-1328.

COLLAR C., MARTÍNEZ-ANAYA M.A. and BENEDITO DE BARBER C. - 1993. Interactive effects between microbial breadmaking starters and wheat flours. In: Proceedings of Euro Food Chem VII, Progress in Food Fermentation, Vol. 1. Ed.: C. Benedito, C. Collar, M. A. Martinez Anaya, and J. Morell. IATA (CSIC) ISBN: 84-604-7038-5. Valencia, Spain, September 20-22. D. 75-80 (1993). Submitted for publication to Rev. Esp. Cienc. Tecnol. Aliment.

CORNEC M., POPINEAU Y. and LEFEBVRE J. - 1994. Characterisation of gluten subfractions by SE-HPLC and rheological dynamic analysis in shear. J. Cereal Sci. (accepted)

DACHKEVITCH T., REDAELLI R., BIANCARDI A.M., METAKOVSKY E.V. and POGNA N.E. - 1993. Genetics of gliadins coded by the group 1 chromosomes in the high-quality bread wheat cultivar Neepawa. Theor Appl. Genet., 86 (2-3), 389-399.

DeAMBROGIO E., Di FONZO N., ROCCHETTI G. and BORGHI B. - 1992. I grani di forza in Europa. L'informatore Agrario, 48, 33-37.

GAUTIER M.F., ALEMAN M.E., GUIRAO A., MARION D. and JOUDRIER P. - 1994. *Triticum aestivum* puroindolines, two basic cystine-rich proteins: cDNA sequence analysis and developmental gene expression. Plant Mol. Biol. (in press)

GREENWELL P. and BROCK C.J. - 1993. Identity of starch-granule-surface proteins (friabilins) of bread wheat with detergent-soluble lipid-binding proteins from flour. Presented at the AACC Annual Meeting, Miami, Florida, USA, 3-7 October. Abstract published: Cereal Foods World, 1993, 38, 615-616.

HAMER R.J., WEEGELS P.L., FLISSEBAALJE Th.D. and SKERRIT J.H. - 1993. The depolymerisation of gluten studied by 2-D electrophoresis and immunoblotting. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany).

HARGREAVES J., Le MESTE M. and POPINEAU Y. - 1993. Study of the gluten proteins by ESR spectroscopy. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany).

HARGREAVES J., Le MESTE M. and POPINEAU Y. - 1994. ESR studies of gluten lipid systems. J. Cereal Sci. (accepted)

KHELIFI D. and BRANLARD G. - 1992. The effects of HMW and LMW subunits of glutenin and of gliadin on the technological quality of progeny from four crosses between poor breadmaking quality and strong wheat cultivars. J. Cereal Sci., 16 (3), 195-209.

KHELIFI D., BRANLARD G. and BOURGOIN-GRENECHE M. - 1992. Diversity of some D-zone w-gliadins of bread wheat as revealed by 2 step A-PAGE/SDS-PAGE technique. J. Genet. Breed., 46 (4), 351-357.

LAFIANDRA D., CIAFFI M., COLAPRICO G. and MARGIOTTA B. - 1993. Comparative effect of null lines at the *Glu-D1* and *Glu-D3* loci on wheat qualitative properties. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany).

LAFIANDRA D., D'OVIDIO R., PORCEDDU E., MARGIOTTA B. and COLAPRICO G. - 1993. New data supporting high Mr glutenin subunit 5 as the determinant of qualitative differences in the pairs 5+10 *vs*. 5+12. J. Cereal Sci., 18 (2), 197-205.

LEFEBVRE J., POPINEAU Y. and CORNEC M. - 1993. Viscoelastic properties of gluten proteins: influence of prolamin composition and of temperature. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany).

MARTÍNEZ-ANAYA M.A. and ROUZAUD O. - 1994. Influence of flour, bacterial starter and. breadmaking stage on total starch, sugars and low molecular weight dextrins. To be presented at the 14th lCC Congress, The Hague (The Netherlands), June 5-9.

MARTÍNEZ-ANAYA M.A., COLLAR C. and BENEDITO DE BARBER C. - 1993. Comparative study on functionality on Spanish and other EC flours when used in microbiologically started breadmaking processes. J. Food Sci. (submitted).

MARTÍNEZ-ANAYA M.A., COLLAR C., and BENEDITO de BARBER C. - 1993. Comparative study on functionality of Spanish and other EC flours when used in microbiologically started breadmaking processes. In: Proceedings of Euro Food Chem Vll, Progress in Food Fermentation, Vol. I. Ed.: C. Benedito, C. Collar, M. A. Martínez-Anaya, and J. Morell. IATA (CSIC) ISBN: 84-604-7038-5. Valencia Spain, September 20-22, p. 253-258.

MARTÍNEZ-ANAYA M.A., PITARCH B. and BENEDITO de BARBER C. - 1993. Biochemical characteristics and breadmaking performance of freeze-dried wheat sour dough starters. Z. Lebensm. -Unters. Forsch., 196 (4), 360-365.

MARTÍNEZ-ANAYA M.A., PITARCH B. and BENEDITO de BARBER C. - 1993. Chemometrics quality of breadmaking starters. J. Food Sci., 58 (2), 419-425.

MASCI S., LAFIANDRA D., PORCEDDU E., LEW E. J.-L., TAO P. and KASARDA D.D. - 1993. 1D-coded D glutenin subunits from Chinese Spring show 1D-coded ω type N-terminal sequences. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany)

MASCI S., LAFIANDRA D., PORCEDDU E., LEW E. J.-L., TAO P. and KASARDA D.D. - 1993. D-glutenin subunits: N-terminal sequences and evidence for the presence of cysteine. Cereal Chem., 70 (5), 581-585.

MÉLAS V., MOREL M.H., AUTRAN J.C. and FEILLET P. - 1994. Simple and rapid method for purifying low-molecular-weight subunits of glutenin from wheat. Cereal Chem. 71, (in press)

MOREL M.H. - 1994. Acid-PAGE of wheat glutenins: a new tool for the separation of high and low molecular weight subunits. Cereal Chem. 71, (in press)

MOREL, M.H., MÉLAS, V., BONICEL, J. and AUTRAN, J.C. - 1993. Multiple approach (IEF, SDS-PAGE and A-PAGE) of the composition of LMW subunits of glutenin and its effect on dough properties. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany).

NG P.K.W., REDAELLI R., VACCINO P., ACCERBI M., POGNA N.E. and BUSHUK W. - 1993. Biochemical and genetical characterization of novel HMW glutenin subunits and their effects on breadmaking quality. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany).

OLIVER G. and PRITCHARD P.E. - 1993. Rheology of the gel protein fraction of wheat flour. Spec. Publ. - R. Soc. Chem., 113 (Food Colloids and Polymers: Stability and Mechanical Properties), 225-229.

ORSEL R., LICHTENDONK W.J., WEEGELS P.L. and HAMER R.J. - 1993. Dynamic rheological behaviour of isolated glutenin macropolymer. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany).

PERUFFO A.D.B., CURIONI A., PRESSI G., POGNA N.E. and ZAMORANI A. - 1994. Adsorption chromatography on controlled pore glass beads of acetic acid-soluble wheat gluten proteins. Cereal Chem. 71 (in press).

PERUFFO A.D.B., CURIONI A., PRESSI G., ZAMORANI A. and POGNA N.E. - 1993. Adsorption chromatography on controlled pore glass beads of unreduced acetic acid-soluble gluten polymers. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany).

POGNA N.E., METAKOVSKY E.V., REDAELLI R., RAINERI F. and DACHKEVITCH T. - 1993. Recombination mapping of *Gli-5*, a new gliadin-coding locus on chromosomes 1A and 1B in common wheat. Theor Appl. Genet., 87, 113-121.

POGNA N.E., REDAELLI R. and BIANCARDI A., - 1994. Production and genetic analysis of 14 Near-Isogenic Lines in the bread wheat variety Alpe. J. Genet. & Breed, 47 (in press).

POGNA N.E., REDAELLI R., DACHKEVITCH T., CURIONI A. and PERUFFO A.D.B. - 1992. Benefits from genetics and molecular biology to improve the end use properties of cereals. Presented at the 9th International Cereal and Bread Congress "Cereal Chemistry and Technology: a Long Past and a Bright Future", Paris, 1-5 June 1992, pp. 83-93.

POGNA N.E., REDAELLI R., PASQUINI M., CURIONI A. and PERUFFO A.D.B. - 1993. Inheritance studies of two chromosome translocations involving chromosomes 1A, 1B and 1D in bread or durum wheat. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany) (in press).

POPINEAU Y., CORNEC M., LEFEBVRE J. and MARCHYLO B. - 1994. Influence of HMW glutenin subunits on glutenin polymers and rheological properties of glutens and gluten subfractions of near-isogenic lines of wheat Sicco. J. Cereal Sci. (accepted)

PRIETO J.A., WEEGELS P.L. and HAMER R.J. - 1993. Functional properties of low Mr wheat proteins. I. Isolation, characterisation and comparison with other reported low Mr wheat proteins. J. Cereal Sci., 17 (3), 203-220.

REDAELLI R., MOREL M.H., POGNA N.E. and AUTRAN J.C. - 1994. Fractionation of low-molecular-weight glutenin subunits of wheat by two-dimensional electrophoresis A-PAGE x SDS-PAGE. J. Cereal Sci. (submitted).

REDAELLI R., POGNA N.E., DACHKEVITCH T., CACCIATORI P., BIANCARDI A.M. and METAKOVSKY E.V. - 1992. Inheritance studies of the 1AS/1DS chromosome translocation in the bread wheat variety Perzivan-1. J. Genet. & Breed., 46, 253-262.

ROBERT N., Le BLEVENNEC L. and TRIBOÏ E. - 1993. Accumulation of protein fraction during grain filling: comparison of four bread wheat varieties. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany).

ROBERT N., Le BLEVENNEC L. and TRIBOÏ E. - 1994. Protein composition during grain filling: comparison of four bread varieties. Cereal Chem. (submitted).

ROUZAUD O. and MARTINEZ-ANAYA M.A. - 1993. Effect of processing conditions on sugar and oligosaccharide profile of wheat sourdoughs. Z. Lebensm. Unters. Forsch., 197, 434-439.

ROUZAUD O., and MARTÍNEZ-ANAYA M.A. - 1993. Effect of processing conditions on sugar and oligosaccharide profiles of wheat sour doughs. In: Proceedings of Euro Food Chem Vll, Progress in Food Fermentation, Vol. 1. Ed.: C. Benedito, C. Collar, M. A. Martinez-Anaya and J. Morell. IATA (CSIC) ISBN: 84-604-7038-5. Valencia, Spain, September 20-22, p 308-313.

SULAIMAN B.D., BRENNAN C.S., GREENWELL P. and SCHOFIELD J.D. - 1993. Isolation of friabilin and the use of polyclonal antisera for immunolocation studies, Presented at the AACC Annual Meeting, Miami, Florida, USA, 3-7 October. Abstract published: Cereal Foods World, 1993, 38, 616.

van LAARHOVEN H. - 1993. Purification and characterisation of an endogenous biochemical germination inhibitor from wheat bran (in Dutch). TNO-report B 93.205.

van LAARHOVEN H.P.M., KELFKENS M. and HAMER R.J. - 1993. Comparison of two methods to test the level of endogenous wheat germination inhibitors. In: Pre-harvest sprouting in Cereals 1992 (K.K. Walker-Simmons and R.L. Ried, eds.), pp. 280-287.

WEEGELS P.L. - 1994. Depolymerisation and repolymerisation of the glutenin macropolymer in dough and effects of low Mr wheat proteins. King's College, University of London (submitted).

WEEGELS P.L., FLISSEBAALJE Th. and HAMER R.J. - 1994. The glutenin macropolymer depolymerises if treated improperly. Cereal Chem. 71 (in press).

WEEGELS P.L., HAMER R.J. and SCHOFIELD J.D. - 1993. Changes in individual glutenin subunit composition of glutenin macropolymer during mixing and resting. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany).

WEEGELS P.L., LULLIEN-PELLERIN V., van de PIJPEKAMP A.M., AUTRAN J.C. and HAMER R.J. - 1993. Comparison of biochemical and functional properties of various cysteine-rich low-Mr wheat proteins. Presented at the 78th Annual AACC Meeting, 3-7 October, Miami, USA. Abstract published in Cereal Foods World, 38, 589.

WEEGELS P.L., ORSEL R., van de PIJPEKAMP A.M., LICHTENDONK W.J., HAMER R.J. and SCHOFIELD J.D. - 1994. Functional properties of low Mr wheat proteins. II. Effects on dough properties. J. Cereal Sci. (Submitted)

WEEGELS P.L., van de PIJPEKAMP A.M., HAMER R.J. and SCHOFIELD J.D. - 1993. Depolymerisation and polymerisation of individual gluten subunits in situ in dough. Implications for the structure of gluten. In: Proc. 5th Int. Gluten Workshop, June 7-9, Detmold (Germany).

WILDE P.J., CLARK D.C. and MARION D. - 1993. The influence of competitive adsorption of Iysophosphatidylcholine on the functional properties of puroindoline, a lipid binding protein isolated from wheat flour. J. Agr. Food Chem. 1570-1576.