



# QUALITY WHEAT CRC PROJECT REPORT

Program 5

## Review of Program 5 Flour and Dough Components and their Interaction

Compiled by: Clare Johnson

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## Overview

### Dr Ferenc Békés

Dr Békés presented a detailed overview of the research program, as outlined in the accompanying slides. He then presented outcomes from project 5.1.1, *Functional properties of individual flour components*, which has been completed. Key results are the contribution of Glu1-D subunits 2+12 and 5+10 to dough strength. Analyses of mixing time, peak resistance, resistance breakdown, Rmax, extensibility, unincorporated polymeric protein, average size in SDS or acetic acid, dough development time, mixing properties in Z-arm mixer and 2g Mixograph, loaf height and puncture test force were presented. A report is to be submitted.

Dr Békés reported there was a weakness in the in vitro approach, in that the wheat background affects the response: Glu1-D will produce synergy, but Glu1-B will not. He therefore presented model dough work as it relates to noodle texture and quality, using cloned, bacterially-expressed protein, and examining protein content and gluten protein composition – again the slides are detailed. His final slides contain detailed concise outcomes from the blending project and the molecular marker program. He reported PhD student Pat Chong's work on the structure of the glutenin macropolymer in bread and noodle doughs and products was a good symbiosis with a GRDC project.

## Characterisation and introgression of novel storage protein genes

### Dr Peter Sharp

Two main *T. tauschii* proteins, T1, an  $\omega$ -gliadin that may increase dough extensibility, and T12.4, a small HMW glutenin that may reduce excessive dough mixing times, will be introgressed (Mohammad Hassani, PhD student). There have been problems introgressing T12.4 to durum and Baxter bread wheat, so the crosses will be repeated. M. Hassani will restart PCR isolation and sequencing and will also continue Cristina Gianibelli's screening of *T. tauschii*. T1 protein characterisation is on-hold at CSIRO Plant Industry. Progeny of an AUS18913/Langdon synthetic crossed with Meering will be the first for early line small scale testing, later this year.

## Dough Rheology Program

### Prof. Roger Tanner

Doughs from commercial flours (Weston) were tested for oscillatory shear, steady shear, stress relaxation, creep, viscometry and true elongation. Effects of varying composition were also reported and provide results which will fit polymers as well. Modelling must account for the 3 regions of a shear stress/strain plot, namely, strain-rate-independent, strain-softening and fracturing. Modelling of yeasted doughs has been instructive, as has analysis of elongational viscosity/strain for different HMW-GS composition – see slides. Conclusions: Gluten is different from flour, in that the linear visco-elastic limit for flour is below a strain of 0.1% (for gluten it is less than 3%), and the elongational viscosity of gluten is greater than that of flour. An increase in starch in a gluten-starch complex results in a lower linear visco-elastic limit. HMW-GS are major contributors to strength and stability of dough. Water plays an important role in dough rheology. An increase in water content of dough results in decreased elongational viscosity, storage modulus ( $G'$ ), loss modulus ( $G''$ ) and dynamic viscosity ( $\eta^*$ ) values.

**Definitions:**

The dynamic storage modulus ( $G^*$ ) is a measure of the stress when measured oscillatory applied strain. It has two components, the storage modulus ( $G'$ ) and the loss modulus ( $G''$ ). Essentially the storage modulus ( $G'$ ) can be regarded as the elastic component of the viscoelastic behaviour whilst the loss modulus ( $G''$ ) represents the viscous component.

**Diagnostics for Wheat Quality****Ms Amanda Hill**

The focus is on antibody-based methods, to create diagnostics for breeding programs and, potentially, grain receipt and processing applications. Existing tests are for 1RS translocations (ELISA kits) and alpha-amylases (rapid field test WheatRite). A late maturity amylase ELISA is in development. Only the high pI isozyme is detected. A comparative slide showing strong discrimination for single grains of Amery and Reeves was presented.

**Field diagnostics for wheat varietal identification and quality traits****Dr Kevin Gale**

A rapid antibody-based field test for varietal identification for end-use segregation, royalty payments and domestic buyers is under development. This will involve multiple antibodies and a bar-code concept in ICT card format for discrimination of results. The main 4 of the 35 major varieties grown in Australia are Janz, Spear, Frame and Stiletto, and in NSW Janz, Cunningham and Dollarbird dominate.

Michael Partridge's work produced antibodies 79115, which discriminate based on hardness. The field assay requires Yes/No discrimination rather than variations in the intensity of reaction, to avoid being confounded by environmental effects.

Other targets include granule-bound starch synthase (GBSS). There is 97% identity between these markers, for which the null phenotype gives the waxy trait, in the 3 wheat genomes, but a region which varies by 4 amino acids within a short region has been found, and antibodies to peptides based on this region give good discrimination.

Future targets will include HMW-GS alleles, puroindoline B, lipoxygenase, SBEL, SSII, amylase inhibitors, and non-conserved ESTs such as invertase. The procedure requires proteins expressed at high level in the mature grain.

It may be feasible to use microarrays of ESTs found expressed in Wyuna endosperm to screen cDNAs from different cultivars. Problems are expected due to developmental variation causing different times of expression.

**Immunodiagnosics for wheat quality and genetic screening****Dr Thomas Giersch**

Monoclonal antibodies which discriminate HMW-GS 2+12 and 5+10 have been developed and patented. As these alleles strongly influence dough properties, the antibodies will find application in rapid and high throughput quality testing in breeding programs and industrial processing.

The antibodies are being engineered by phage display and affinity maturation of single chain variable antibody region fragments (scFvs), as shown on Thomas' 9<sup>th</sup> slide, to select new specificities and improve properties of the recombinant antibody fragments. Differences in specificity for HMW-GS 7+8 and 17+18 are being detected when scFv #TG8D4 is used, enabling discrimination between progeny of a CD87 x Hartog cross. This will be extended to standard wheat varieties and incorporated in kits for breeding programs. Other specificities will be sought and purification will be optimised.

### **Molecular Markers for Hardness**

#### **Ms Kym Turnbull**

*Confidential:*

*Kym Turnbull's presentation contained data which will be written up in her PhD thesis and should not be published prior to that time.*

To determine how early hardness could be distinguished in developing seeds, endosperm texture was measured in near-isogenic Hard Heron and Soft Heron lines grown under controlled conditions. At defined intervals from 5-35 days post-anthesis, seeds were removed and wet and dry weights were taken. Samples were analysed using the Perten Single Kernel Characterisation System (SKCS4100). Grain hardness, as measured by SKCS, is determined early in development, possibly as early as 5 days, and definitely from 15 days post-anthesis. While no differences could be discerned by light microscopy, under EM, a much tighter structure between gluten and starch was observed, from 15 days. This coincides with the time at which expression of puroindoline-a is switched on.

The results showed grain hardness does not influence mass accumulation, mass loss through drying, or kernel diameter, weight or size. However hardness is mediated by the drying process.

These results suggest a re-evaluation of current hardness theories because at early stages of seed development there are very few starch granules in the cell and very little protein.

### **Polymer size and shape in cereal processing**

#### **Ms Laila Daqiq**

In an examination of size distribution of wheat biopolymers in structure/function relationships affecting the quality of the end-product, apparent polymeric protein size distribution, measured by both FFF and SE-HPLC, depends on the extraction solvent used. The native size may be estimated by charting retention time vs sonication time and extrapolating to zero. The percentages of extracted or unextracted polymeric protein do not explain the size distribution of polymeric protein. DNA from 3223 base pairs to 8188 base pairs was used to calibrate FFF, corresponding to a range from 2127180 Da to 5404080 Da. This is encouraging, but more work is required to clarify the limitations. Results correlating molecular size distribution with mixograph parameters were presented. There was strong correlation between average size and mixing time, and a negative correlation with breakdown time. Various combinations of HMW-GS were examined.

In starch analyses, different linear relationships between molecular mass and retention time were observed for each standard, indicating FFF distinguishes differences in shape. Test sample apparent molecular weights vary accordingly.

### **Micro- Z arm mixer**

#### **Dr Peter Gras**

Very consistent results are being obtained in the mixer having selected a motor size which maximises the signal to noise ratio, and the output looks like that of the farinograph and valorigraph. Linear calibration is better than 0.99. Automatic water dispensing has been incorporated, and software has been written by summer student Derek Puah, as described in the CRC's May 2000 newsletter.

The instrument can be used to measure water absorption between 5 and 10%, an advantage over the mixograph. Water absorption was demonstrated to depend on the total amount of protein rather than the type, while dough development time depended on protein type, for several varieties. Only one mix was required to obtain a reliable water absorption result.

The Z-arm mixer requires less mixing time, so is a much faster test method. Dough resistance produced a linear relationship with water absorption for each variety tested.

Dr Gras is in discussions to produce the equipment. A Hungarian exchange student will spend 6 months in the CRC.

# Overview

**Dr Ferenc Békés**

Program 5 focuses on flour and dough components and their interaction, underpins much of the **research elsewhere in the CRC**. Its purpose is to **provide a basis for breeding for quality by understanding the relationship between flour composition and dough processing properties**.

In addition, the products of genes from sources outside cultivated wheat, such as from the primitive wheat, are studied for novel effects on dough function.

Much of this enhanced understanding can be used to develop breeding targets; additionally this information can be captured as **diagnostic tests using antibodies and DNA probes** (interactive with Program 1).

New methods based on **antibody engineering** are being used to manipulate the specificity and sensitivity of assays. Several assays for products of key genes are already being provided to breeding programs for routine screening.

The techniques developed in the Program provide research methods to use in studies

- on developing **molecular markers for dough quality traits (with Program 1)**,
- on the effect of **environmental growing conditions and grain storage conditions on processing quality**, on monitoring the changes in protein composition during endosperm development and on developing a mathematical model of estimating the **quality attributes of flour blends**, as well as providing **diagnostic methods for on-farm segregation (with Program 2)**.

The ability to understand the role of individual flour polypeptides and starch components comes about from the development of **small-scale equipment for dough mixing, extension and baking**. This development, together with research aimed to understand and **differentiate dough behaviour in fundamental rheological parameters** allow the results of laboratory screening tests to better predict commercial bakery performance of flours, and enable processing conditions to be more objectively manipulated to suit different end-products.



### Apparatus:

- Micro mill (\*) CRC IP (P) (U) (P) Published, commercially available  
Unique position worldwide
- 2g Mixograph (P)
- MicroZ-armed mixer (\*) CRC IP (U)
- Micro extension testers (\*) CRC IP (U)
- Micro-baking facility (U)
- Small-scale noodle-maker (U)
- RVA (P)

### Special techniques:

- Incorporation of Proteins (P)
- High-speed mixing (U)
- Extension Emulation (U)
- Blending (U)

(\*) commercializable family

Program 5 provides professional education in cereal science,

- by coordinating postgraduate programs within the CRC,
- developing mechanisms for attracting outstanding undergraduates to cereal science
- by hosting short courses for professionals already working within the industry



- 5.1. 1. Functional properties of individual flour components**
- 5.1. 4. Molecular diagnostics for wheat quality**
- 5.1. 5. Characterisation and introgression of novel storage protein genes**
- 5.1. 6. The effects of protein composition on basic and applied rheological parameters**
- 5.1. 7. Structure of glutenin macropolymer in commercial bread and low-water (noodle) doughs and products**
- 5.1. 8. Field diagnostics for wheat varietal identification**
- 5.1. 9. Polymer size and shape in cereal processing**
- 5.1.10. Benchmarking mixing and extension measurements**

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**Aims and Purpose:**

- To establish a model dough system with the minimum number of pure components, for understanding fundamental relationships between polypeptide composition and dough properties.
- To construct a mathematical model to predict the effects of specific components on dough properties.
- Through addition and dough incorporation studies, establish the effects of individual wheat- derived seed polypeptides on dough properties.

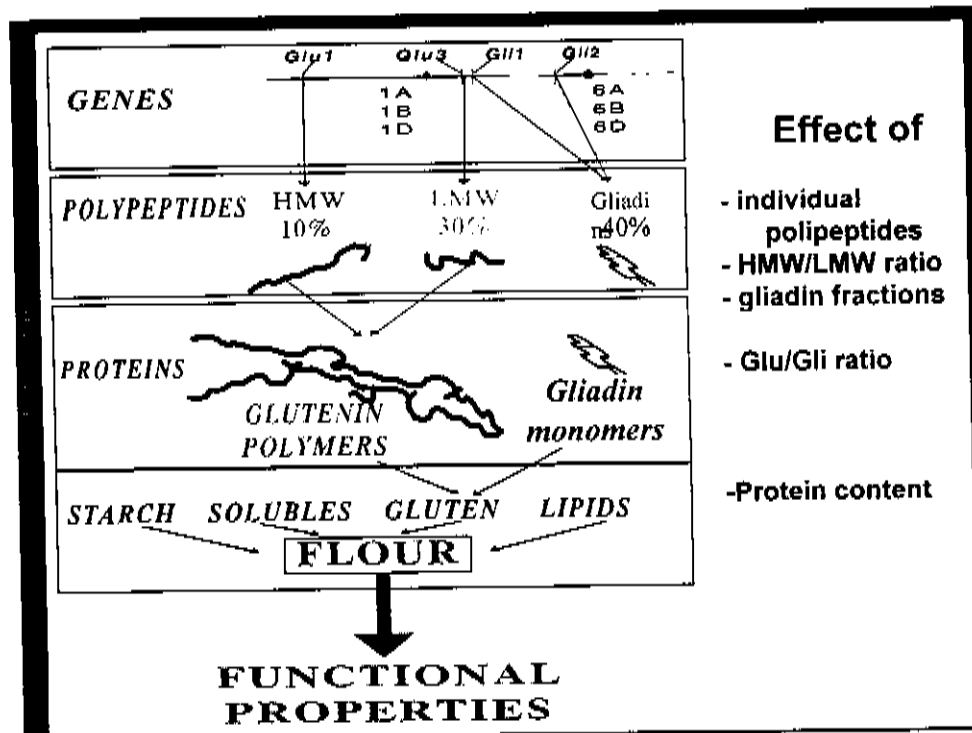
**Anticipated Outcomes:**

- Identification of new seed storage protein genes for introduction into bread wheats
- An assay system for the unequivocal determination of the effects of polypeptide sequence and structure on dough processing.
- Clear information on which genes and gene products should be targeted in breeding strategies.
- System to analyse proteins of interest prior to genetic transformation into wheat.

**What is the Commercial Application?:**

Identification of novel, useful seed storage protein variants for introduction into bread wheats or for use as ingredients/ additives.

A model dough assay system for unequivocal determination of effects of specific components on processing

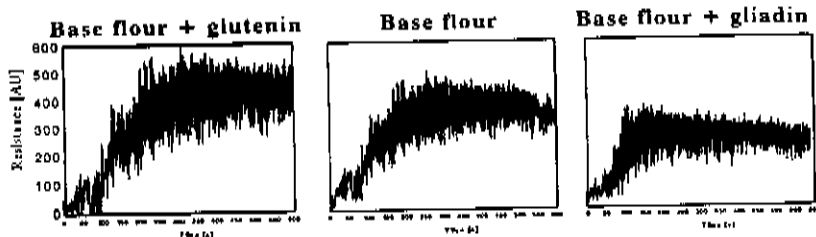




- Correlative studies on large populations
- Classical reconstitution studies
- "Base flour" method
  - *flour + component of interest*
- "Model dough" method
  - *flour is built up from known, individual components*

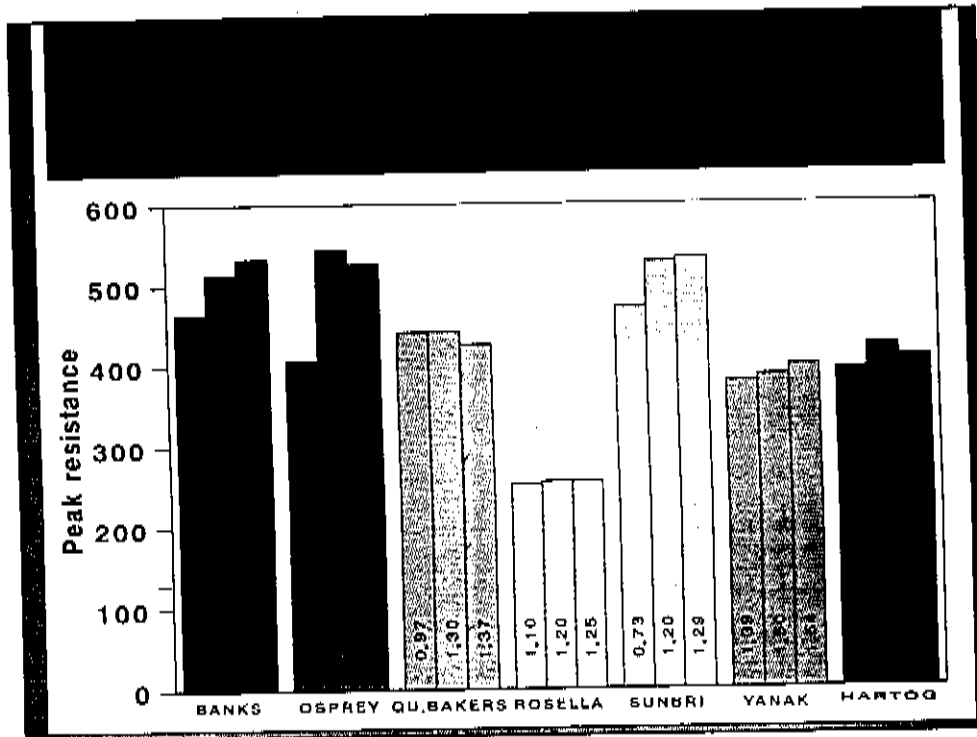
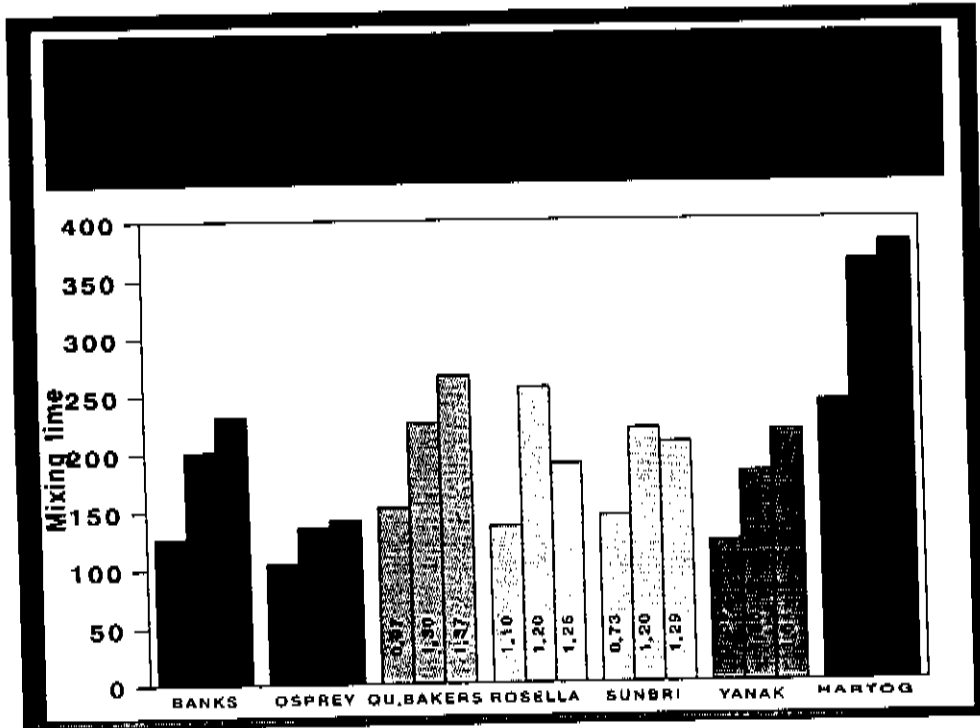


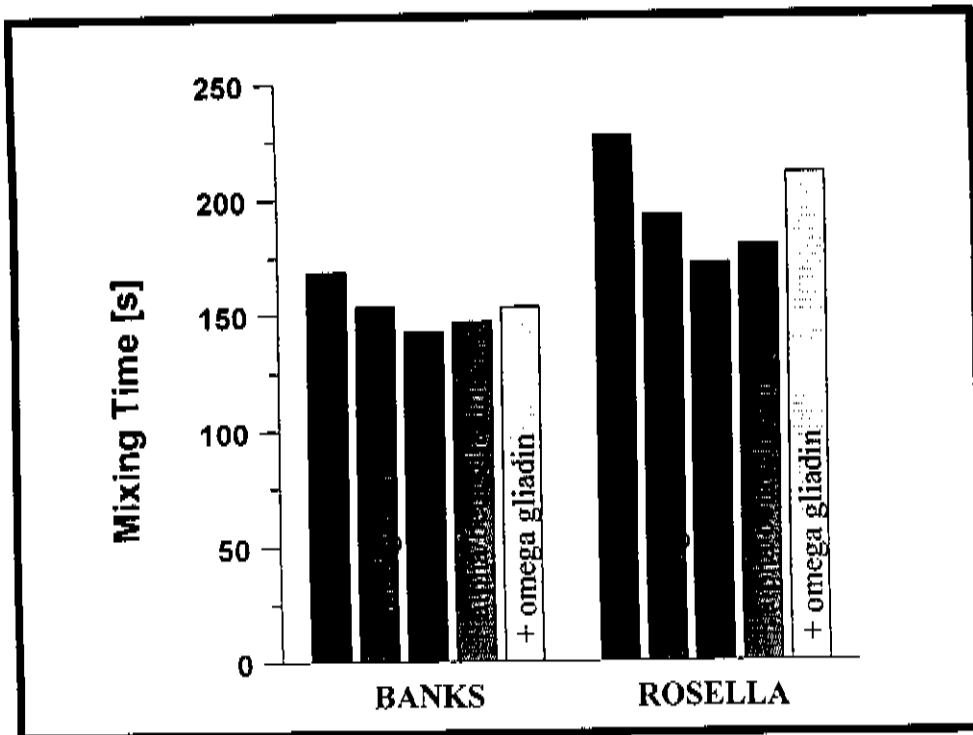
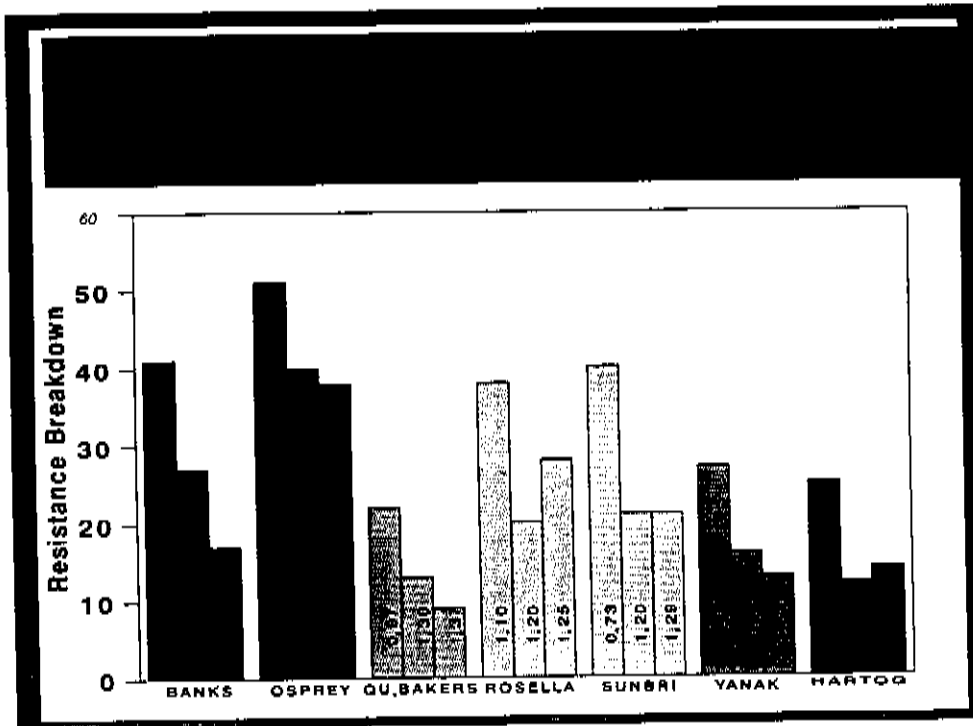
## MODIFYING THE GLUTENIN TO GLIADIN RATIO

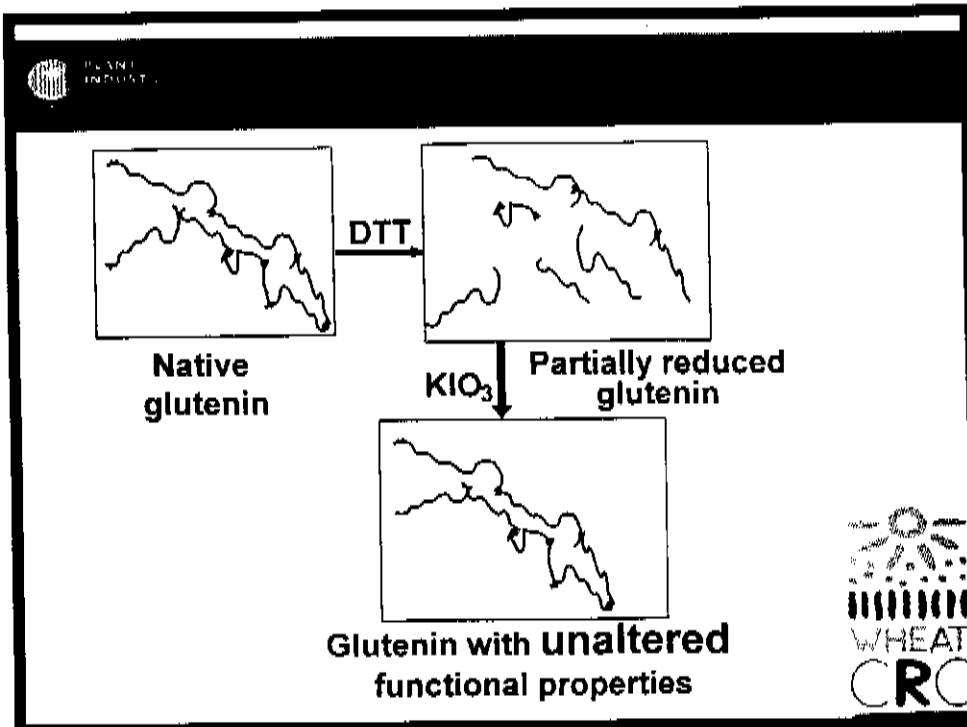
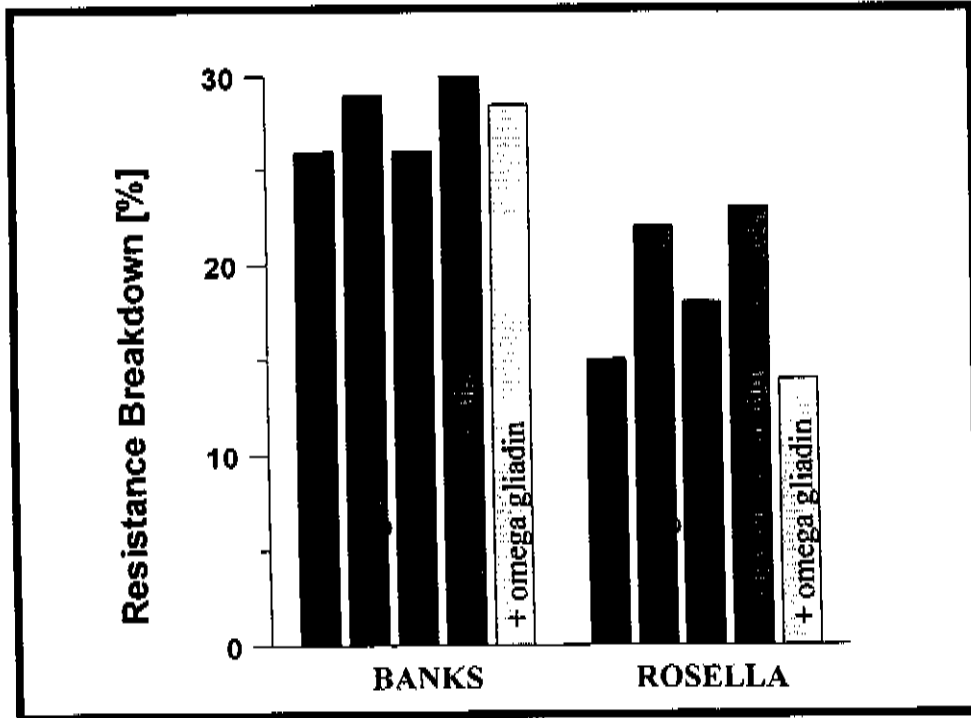


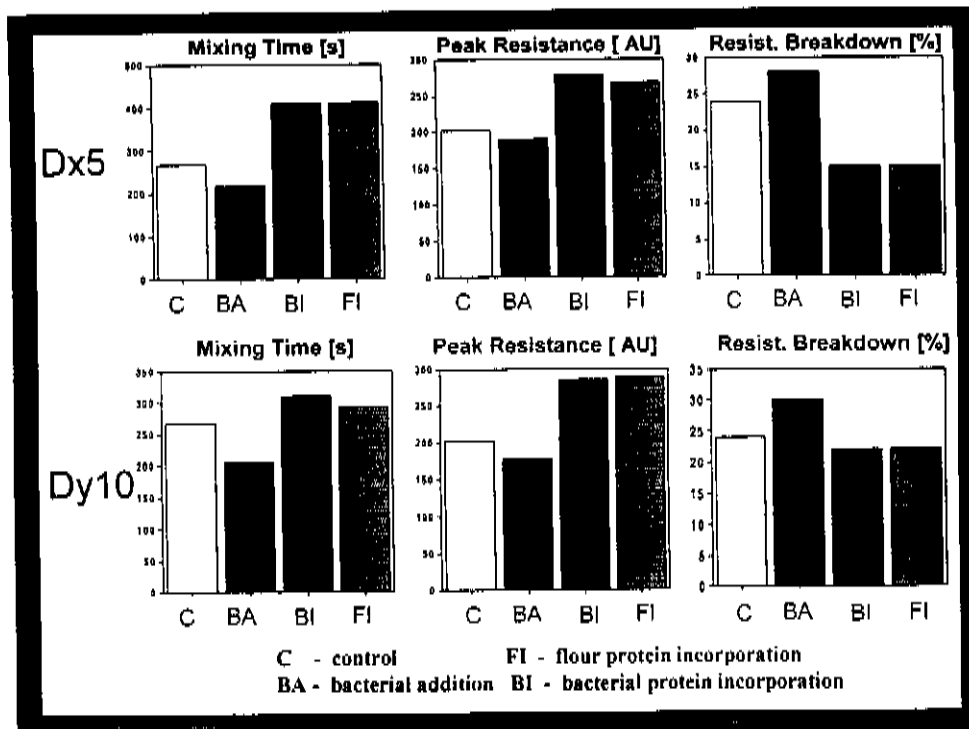
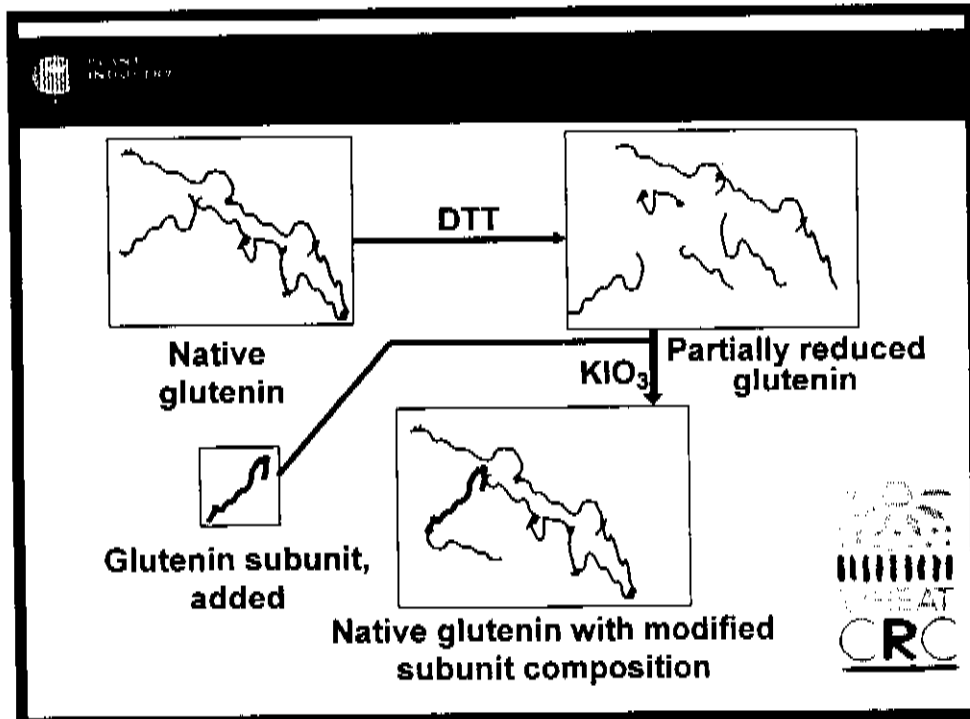
- greater mixing requirements:
  - increased mixing time
  - increased peak resistance
- increased tolerance to overmixing:
  - decrease in resistance
  - breakdown

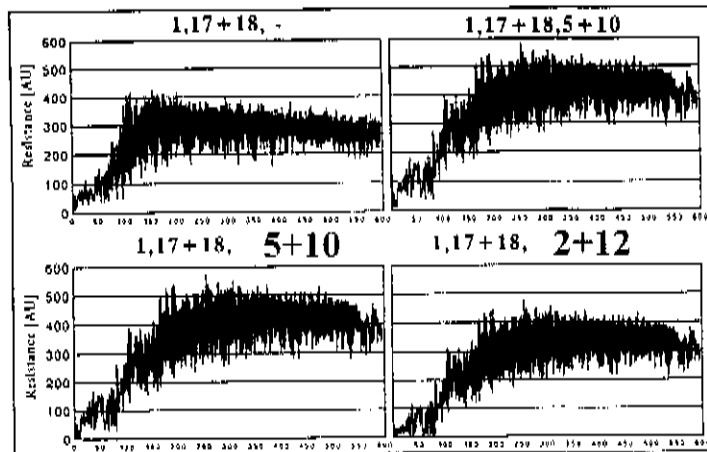
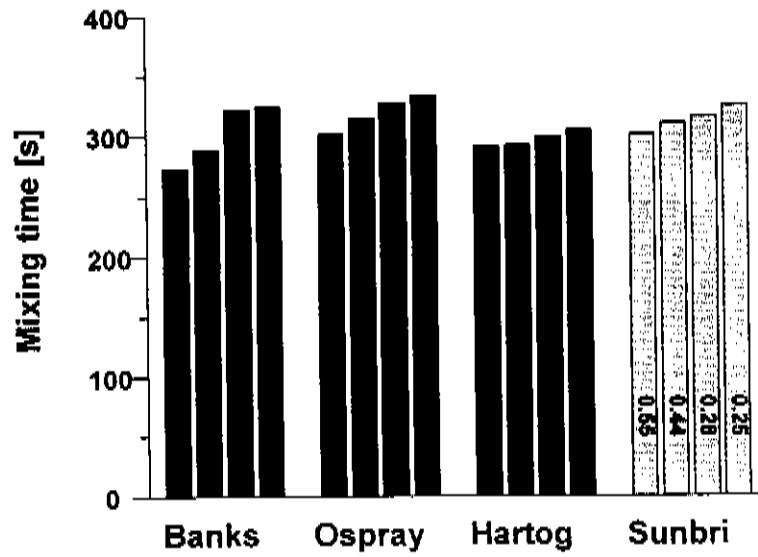
- shorter mixing requirements:
  - decreased mixing time
  - decreased peak resistance
- more rapid breakdown







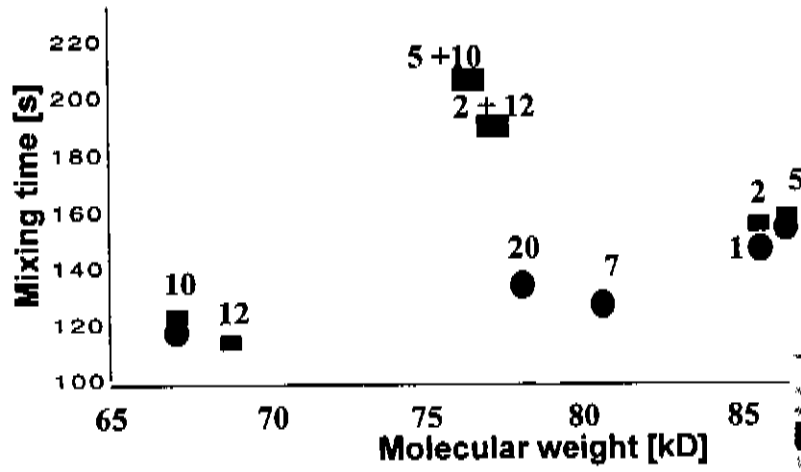








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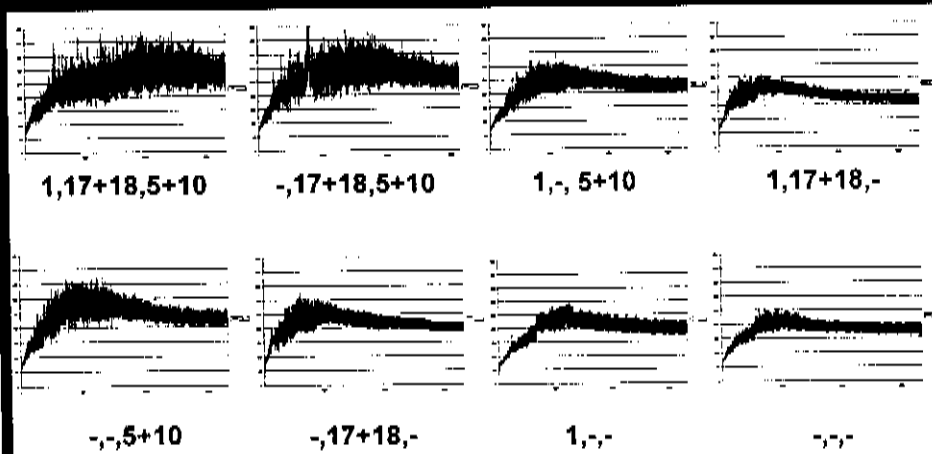


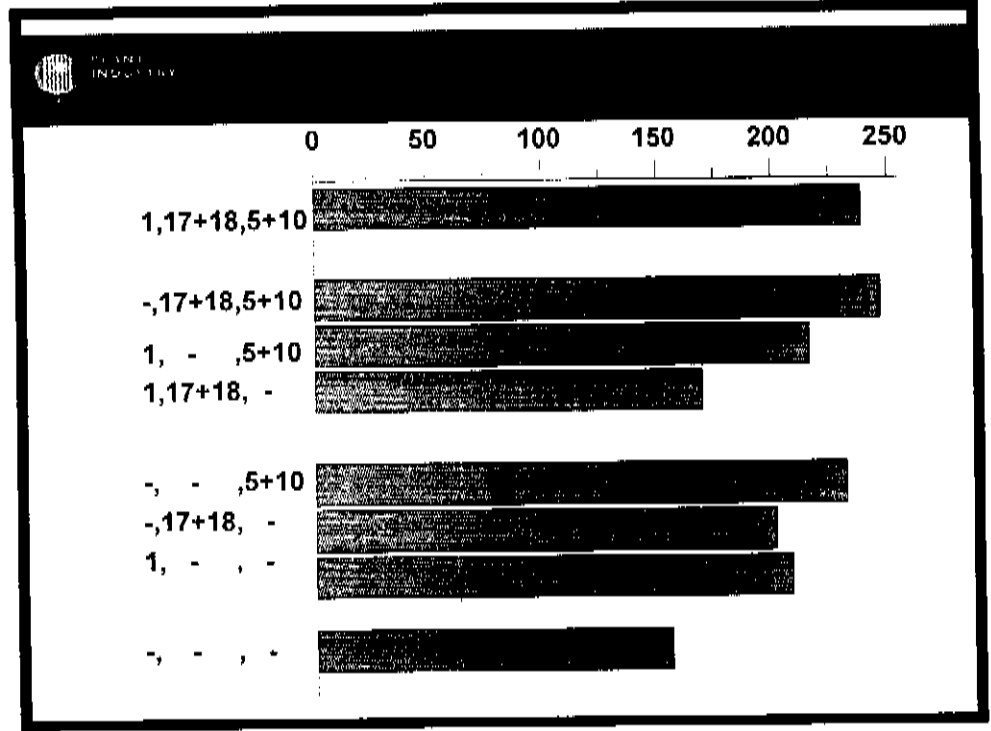
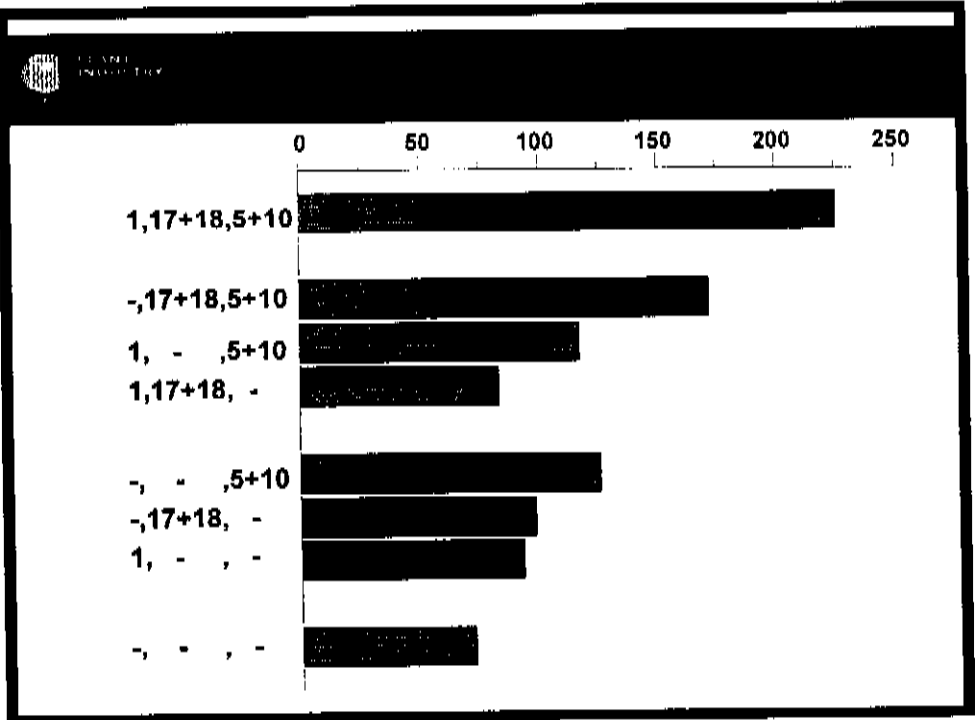
In collaboration with  
Anderson, O., USDA, WRRC, Albany

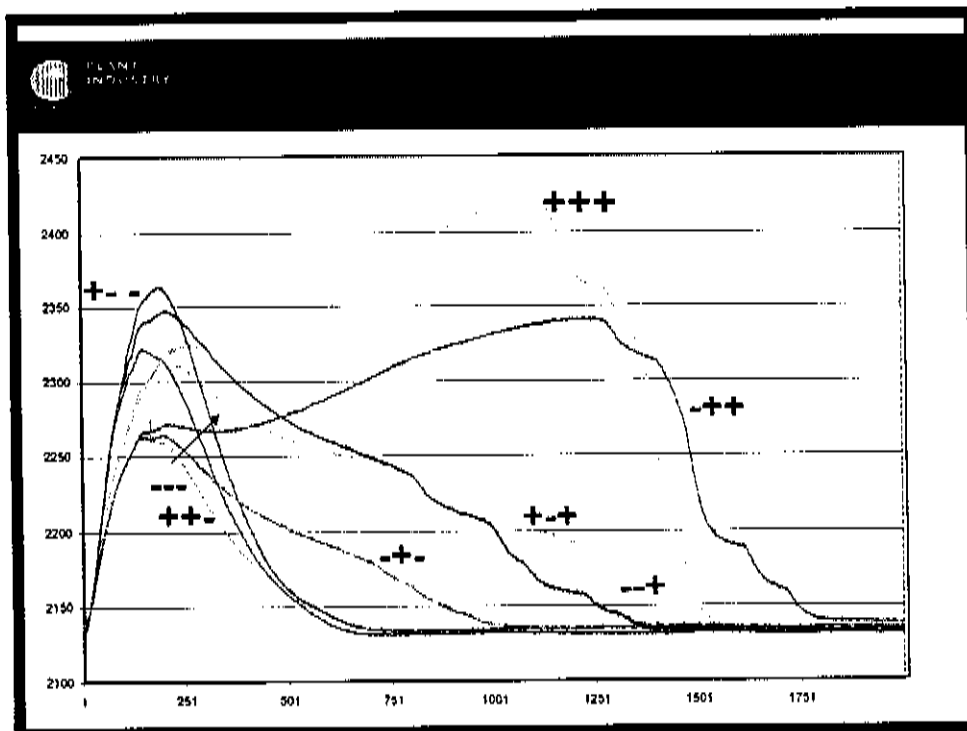
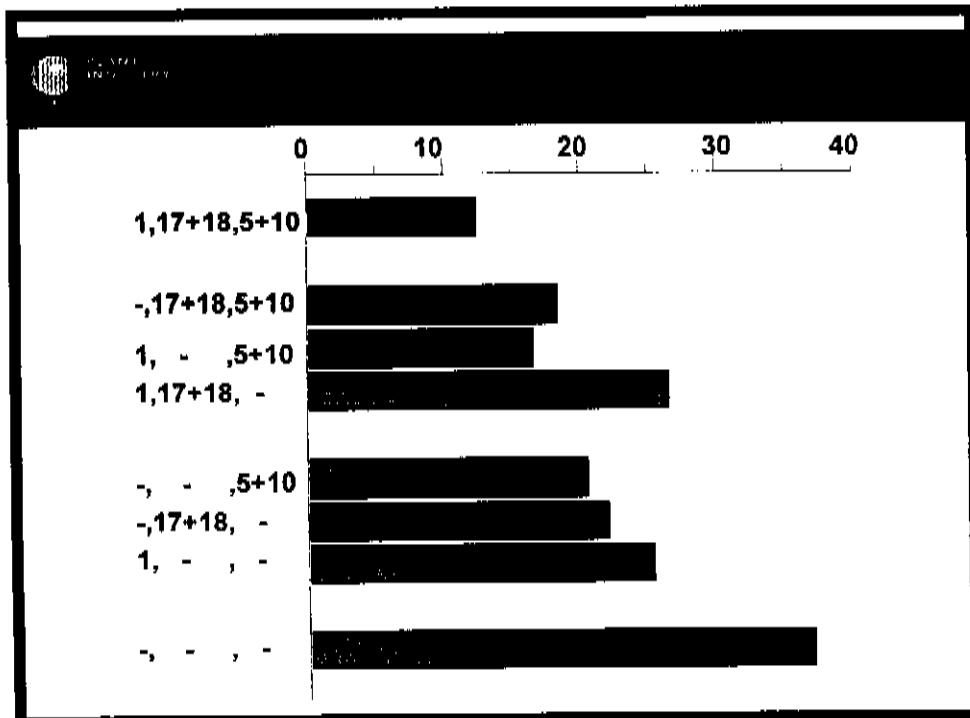
● purified from flour  
■ produced by bacterial expression

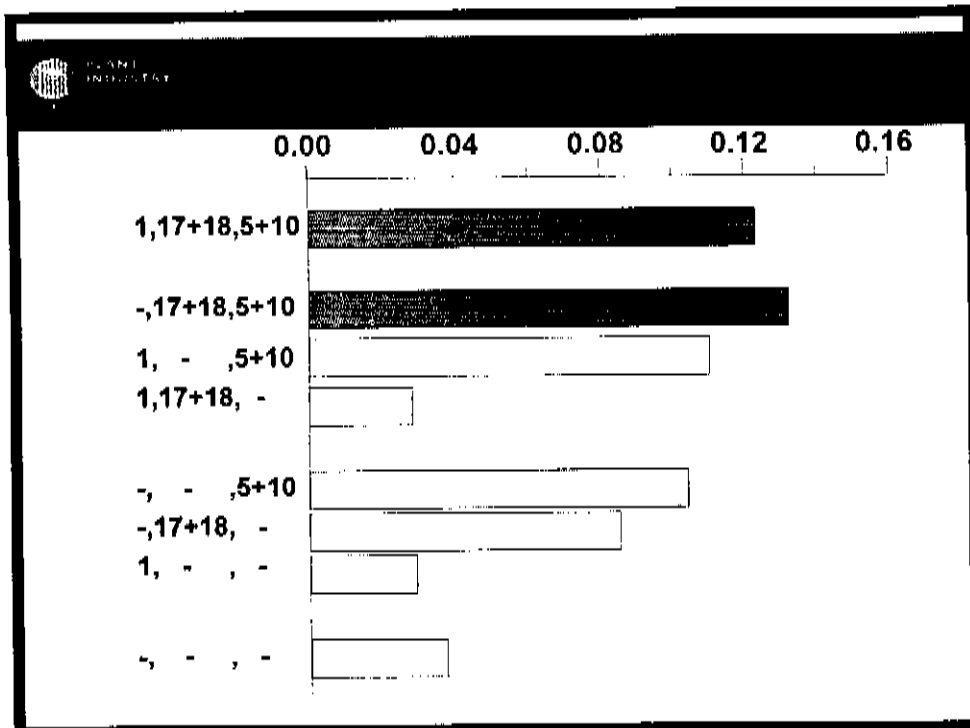
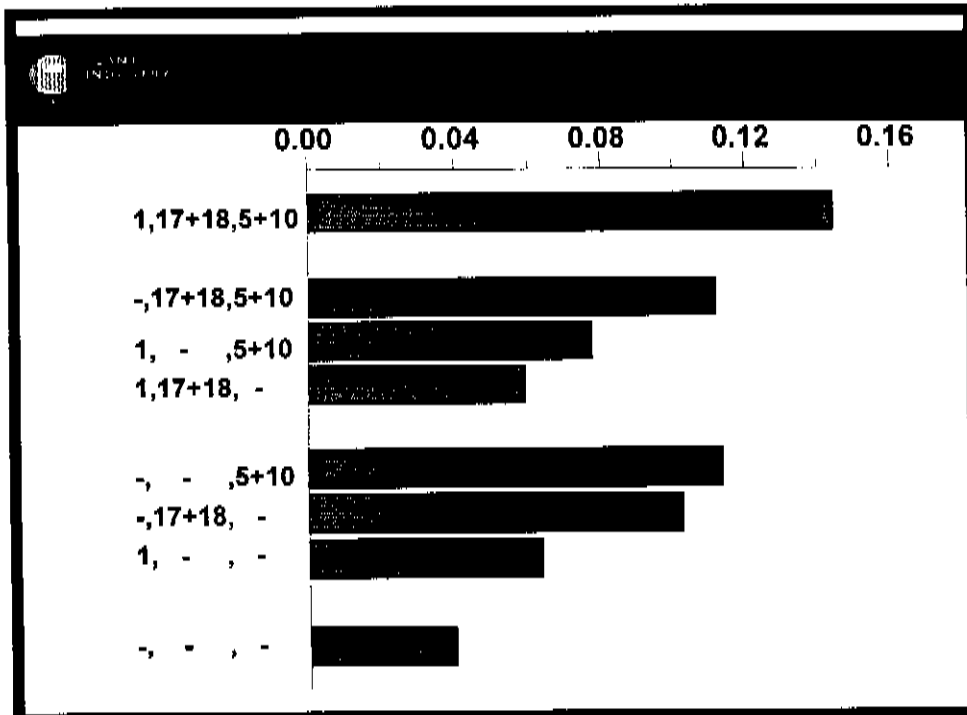


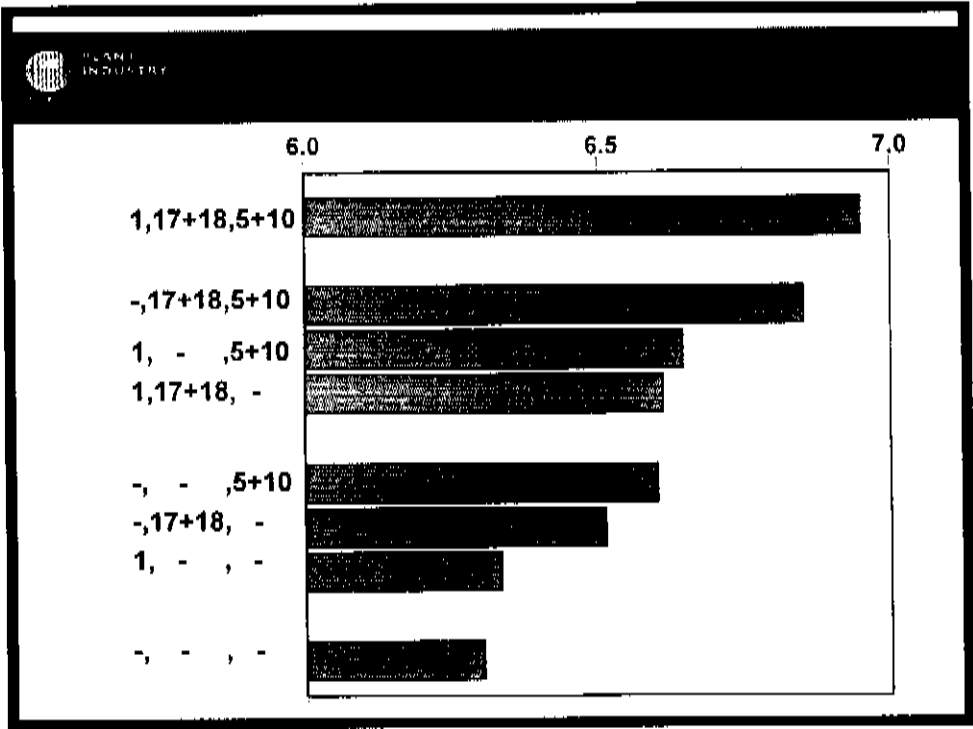
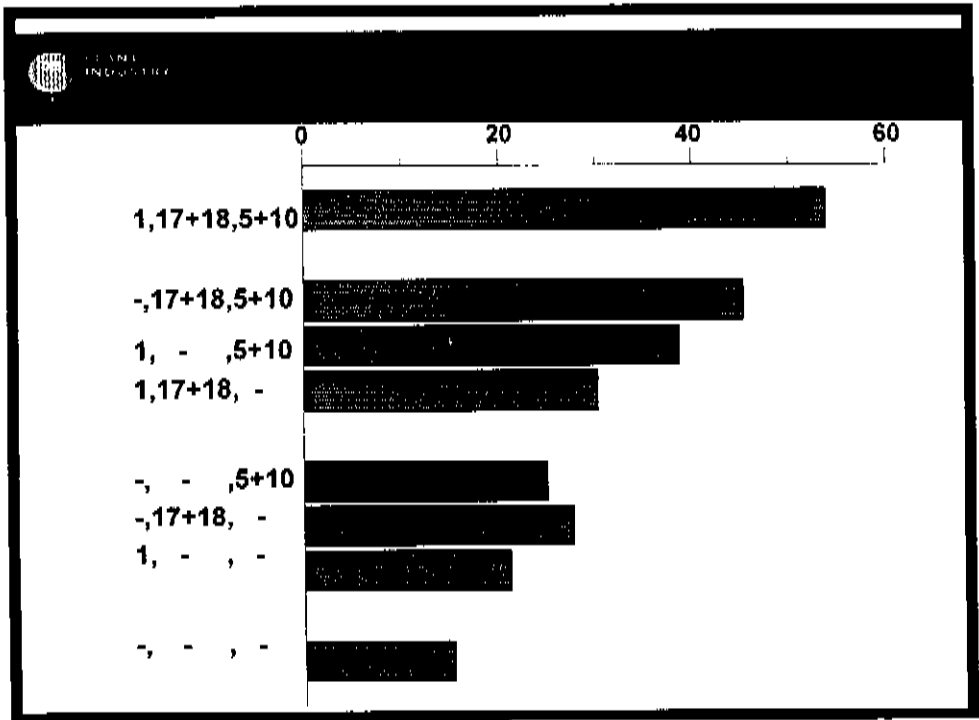
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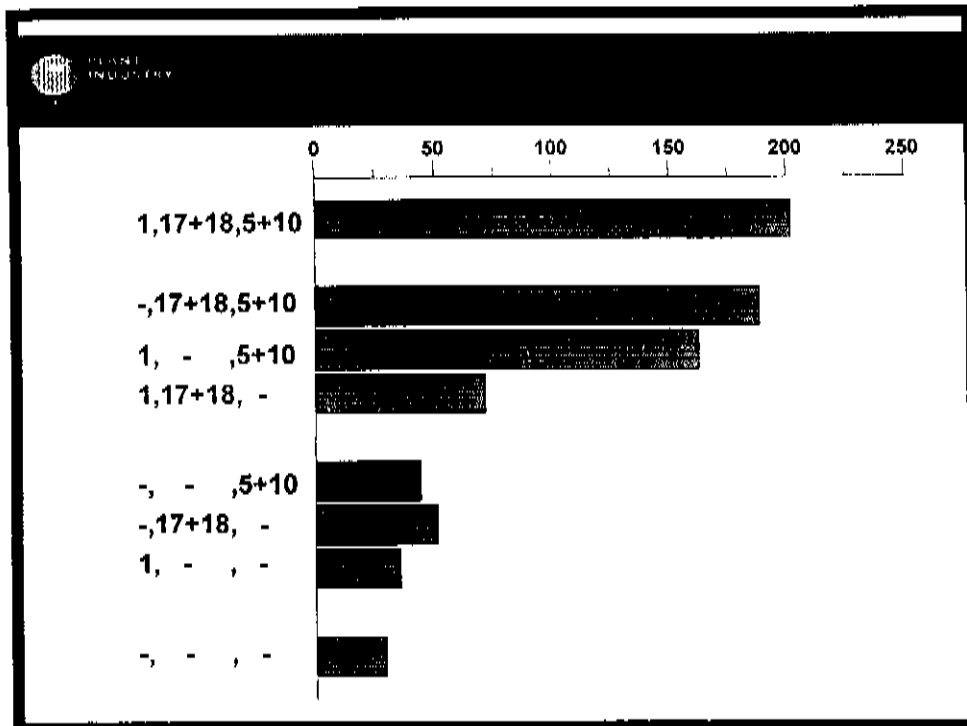
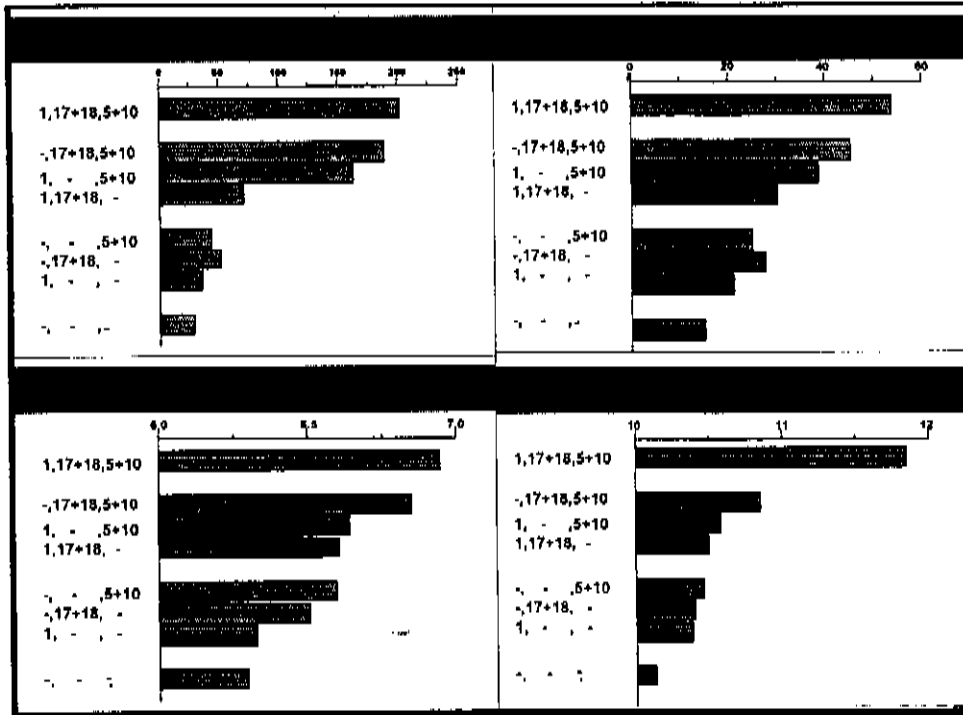






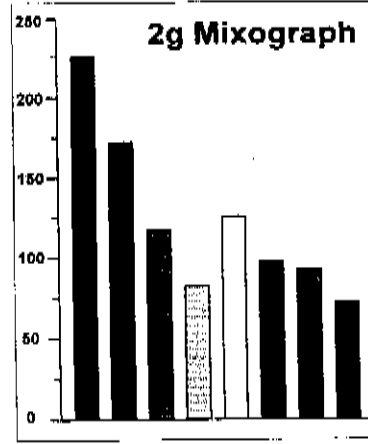
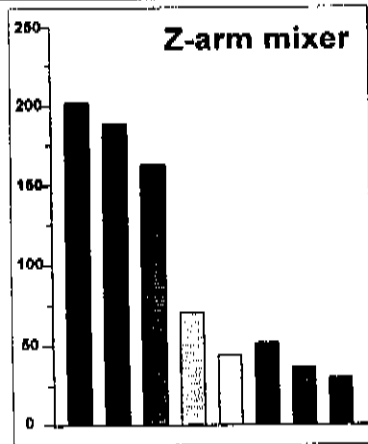








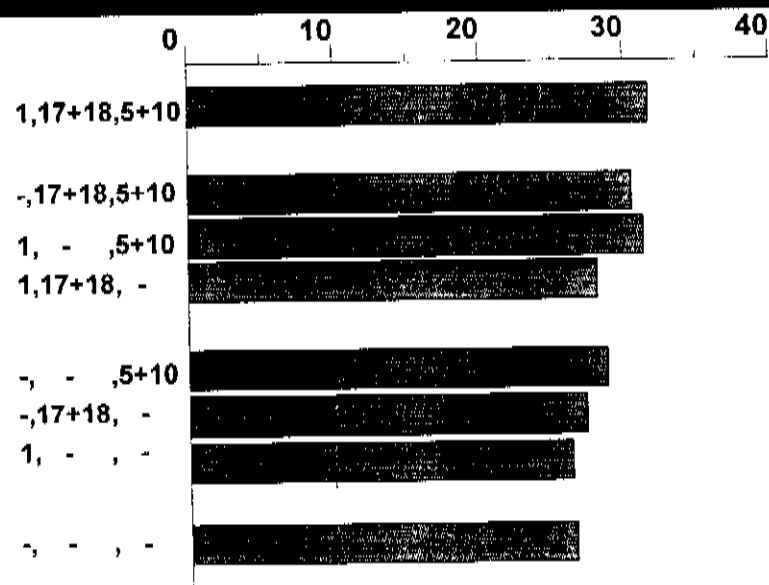
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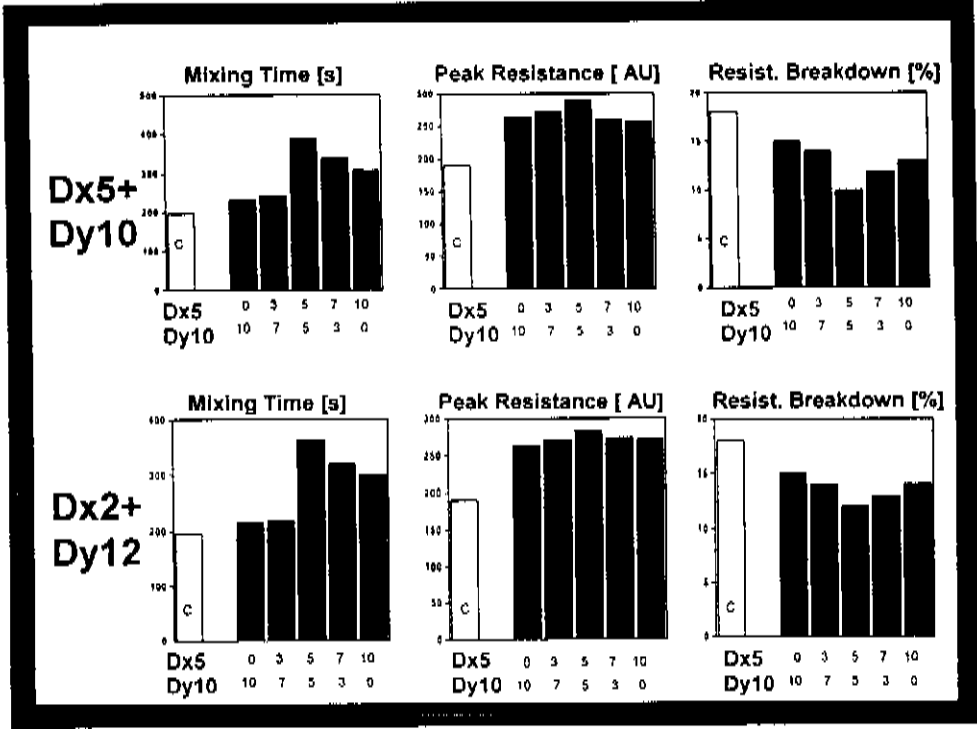
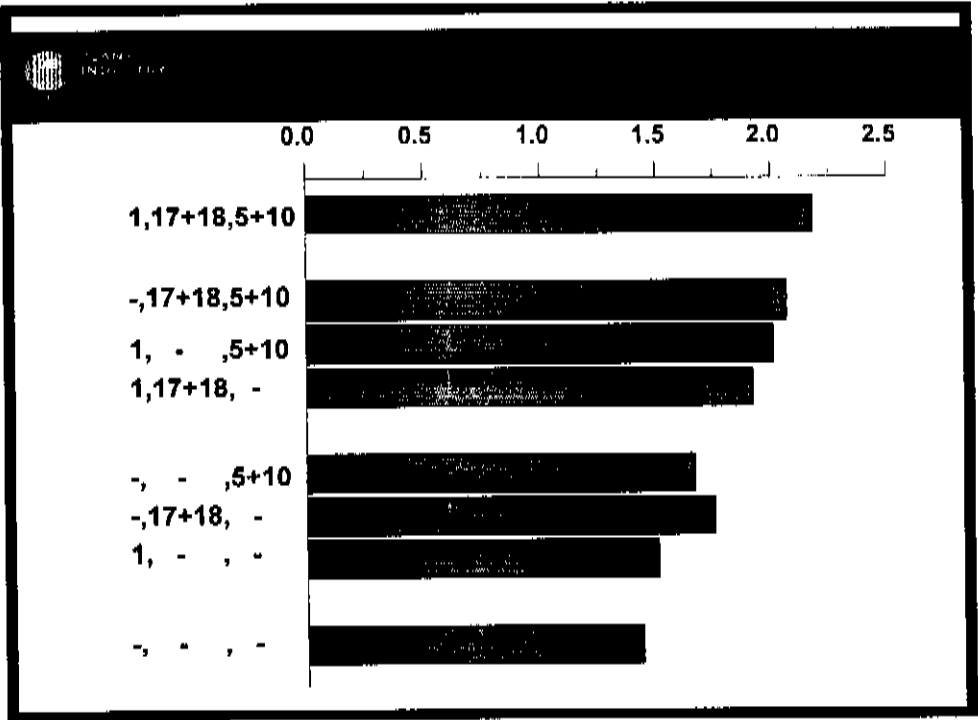


■ 1,17+18,5+10    ■ 1, -, ,5+10    □ 1,17+18, -  
 ■ -,17+18,5+10    □ -, 17 + 18, -    □ 1, -, -, -  
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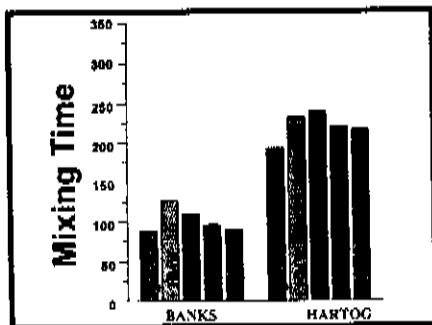
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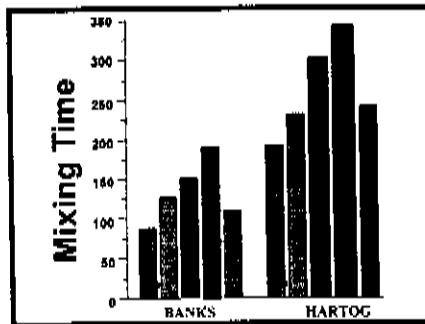




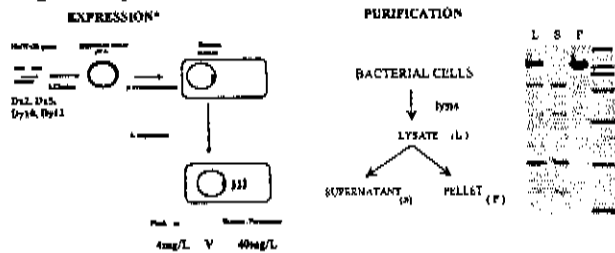
### Bx7+By8



### Dx5+Dy10

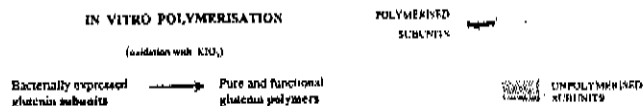


### A. Large-scale production and purification of HMW-glutenin subunits



\*The vectors used in this project were provided by Olin Anderson

### B. Creating functional glutenin polymers from glutenin subunits



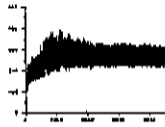


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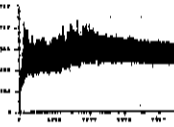
### C. Examining glutenin polymers in model dough systems

#### DOUGH ANALYSIS (MIKROGRAPH)

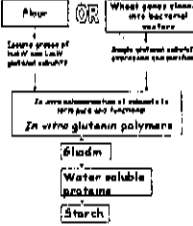
Reconstituted native  
glutenin polymer



Glutenin polymer enriched  
with bacterial Dy12



#### THE MODEL DOUGH SYSTEM



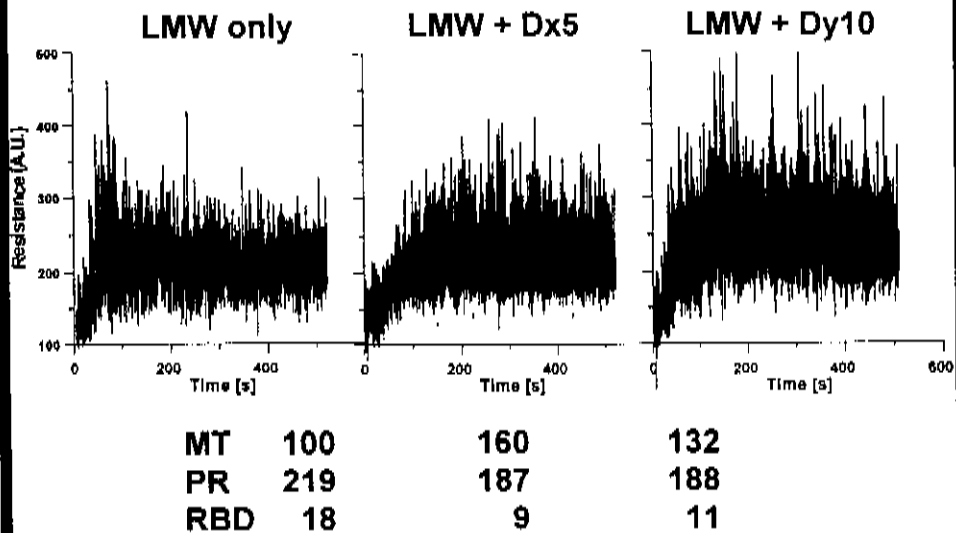
**RESULT:** Establish specific effects of glutenin proteins on dough properties and wheat quality

**VALUE:** An assay system for determining effects of proteins on dough processing

Clear information on which proteins and genes should be targeted for breeding



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**Aims and Purpose:**

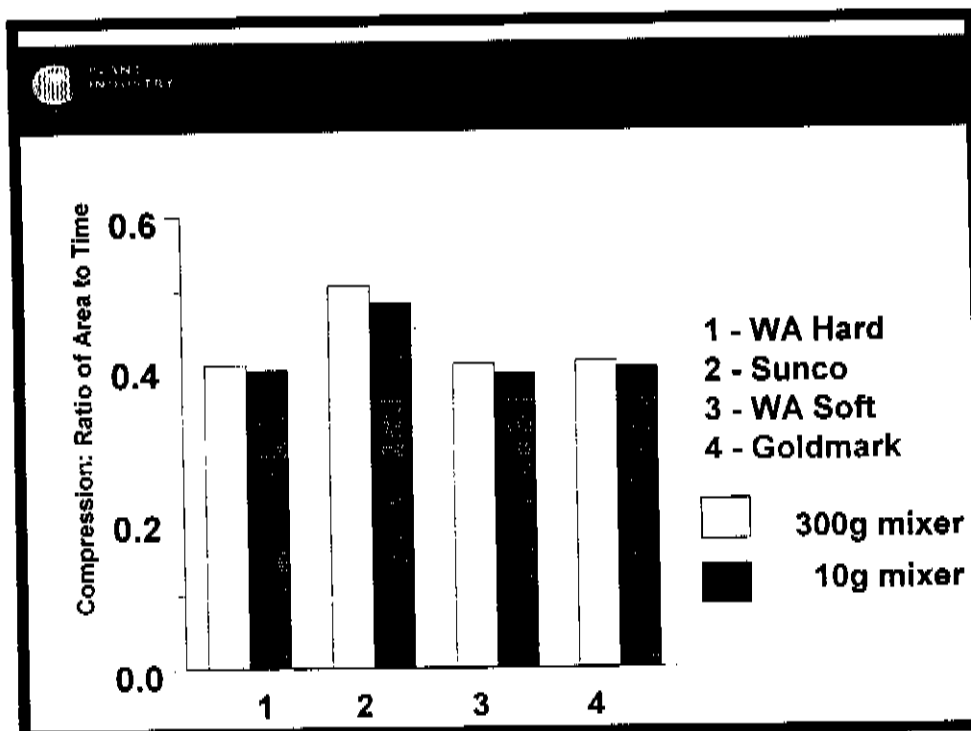
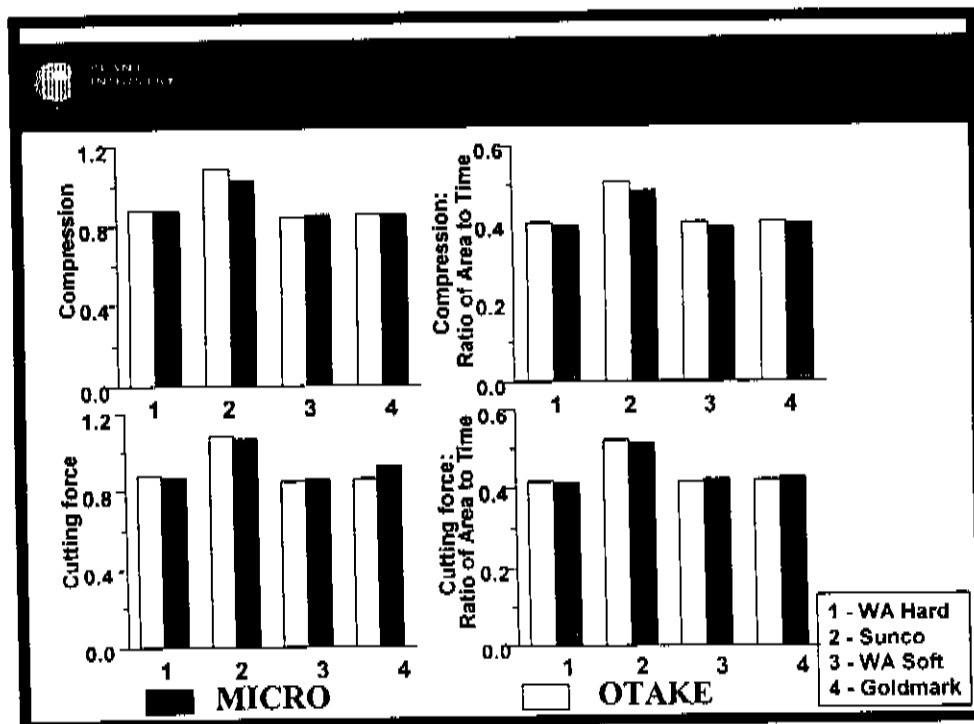
Understand role of particular flour components in determining key structures within pan bread and noodle end-products to help relate variability in wheat flour function in dough products to structure, and contribute to an understanding of the basis of out-of-specification performance of doughs.

**Anticipated Outcomes:**

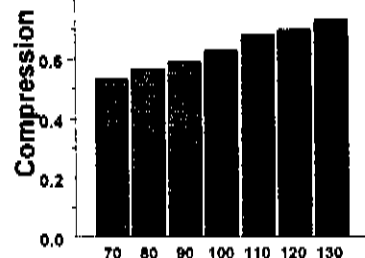
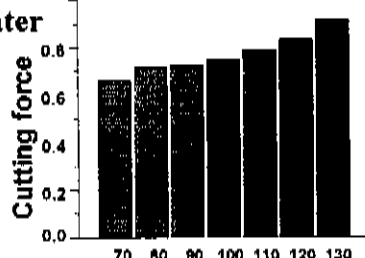
The work has the potential for being able to identify flours and doughs which perform anomalously in industry, develop diagnostic tests for troubleshooting, and understand the role of particular components in two major groups of end-products (pan breads and noodle types). Better understanding of structure of noodles will facilitate evaluation / selection of wheat types in breeding programs, focus genetic selection for texture/structure. This should help us understand raw material requirements for end-products and to understand the basis of out-of-specification performance of doughs.

**What is the Commercial Application?**

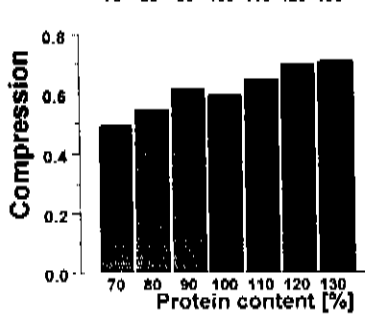
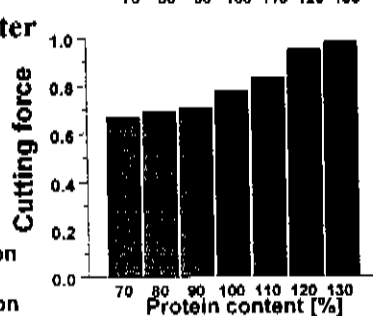
Information on why particular flours do not perform to specification in noodle and pan bread manufacture. Simple trouble-shooting tests for product performance.



32% water

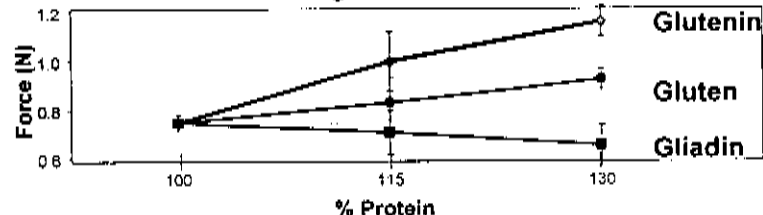


34% water

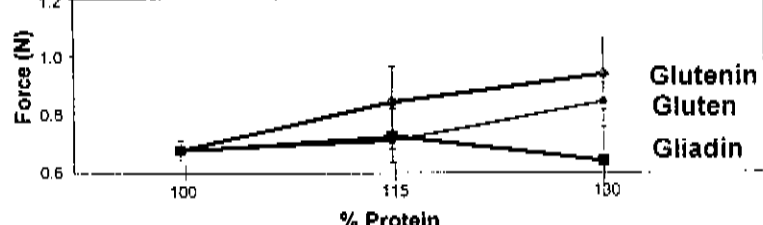


Starch addition  
Original flour  
Gluten addition

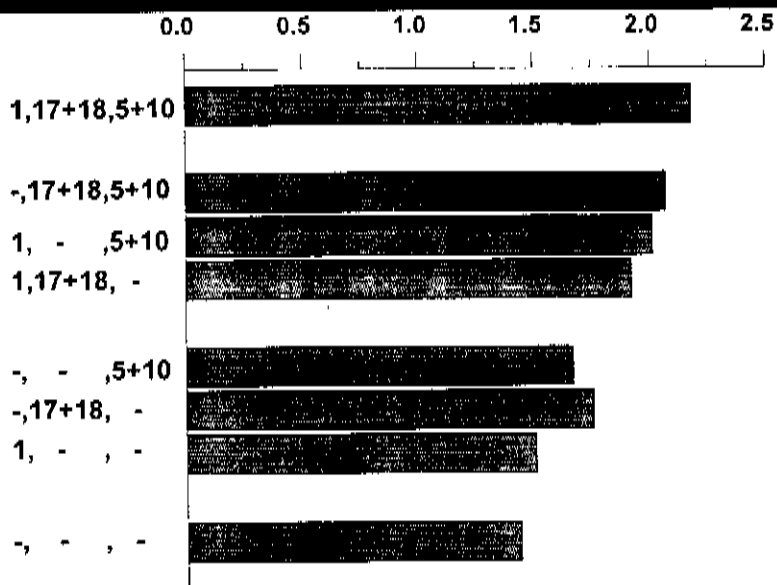
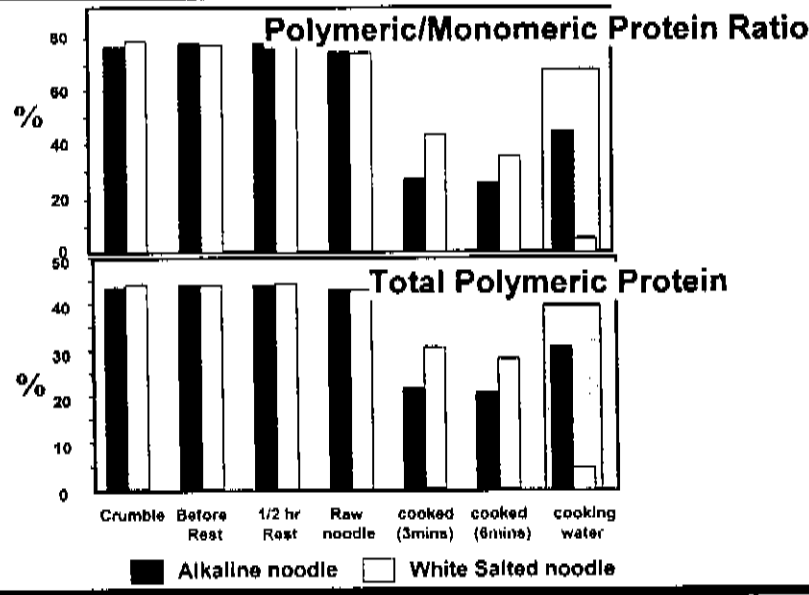
Cutting Force - Cooked Noodles

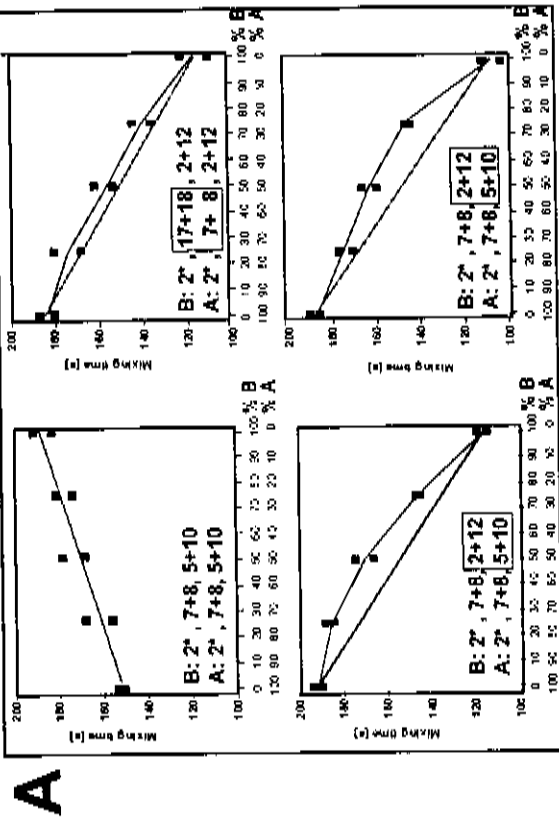


Compression Force - Cooked Noodles



AWS Noodle Flour - Cooked Noodles





- The nature of the nonlinear behavior of functional properties of flour blends has been investigated (A).
- Guidelines have been developed for the industry (B)
- A mathematical model is under development to predict functional properties from chemical data (C).

**B**

Variable	G M 1 A	G M 1 B	G M 1 D	G M 1 E	G M 1 F	G M 1 G	G M 1 H	G M 1 I	G M 1 J	G M 1 K	G M 1 L	G M 1 M	G M 1 N	G M 1 O	G M 1 P	G M 1 Q	G M 1 R	G M 1 S	G M 1 T	G M 1 U	G M 1 V	G M 1 W	G M 1 X	G M 1 Y	G M 1 Z	
Glutenin																										
Glutenin A																										
Glutenin B																										
Glutenin C																										
Glutenin D																										
Glutenin E																										
Glutenin F																										
Glutenin G																										
Glutenin H																										
Glutenin I																										
Glutenin J																										
Glutenin K																										
Glutenin L																										
Glutenin M																										
Glutenin N																										
Glutenin O																										
Glutenin P																										
Glutenin Q																										
Glutenin R																										
Glutenin S																										
Glutenin T																										
Glutenin U																										
Glutenin V																										
Glutenin W																										
Glutenin X																										
Glutenin Y																										
Glutenin Z																										

**C**

Mathematical model of predicting the functional properties of flour blends

Protein content:  $Q_{pr} = \sum_{i=1}^n x_i \cdot [\text{protein}]_i$

Glu/Gli ratio:  $Q_{glu/gli} = \sum_{i=1}^n x_i \cdot [\text{glu}]_i / \sum_{i=1}^n x_i \cdot [\text{gli}]_i$

HMW/LMW ratio:  $Q_{hmw/lmw} = \sum_{i=1}^n x_i \cdot [\text{HMW}]_i / \sum_{i=1}^n x_i \cdot [\text{LMW}]_i$

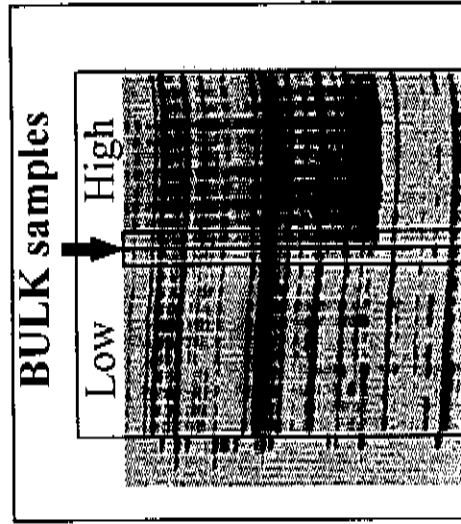
HMW alleles:  $Q_{hmw} = \prod_{i=1}^m \left( \frac{[\text{HMW}]_i}{\sum_{j=1}^m [\text{HMW}]_j} \right)^{e_{ij}} = \prod_{i=1}^m \left( \frac{[\text{HMW}]_i}{\sum_{j=1}^m x_j \cdot [\text{HMW}]_j} \right)^{e_{ij}}$

LMW alleles:  $Q_{lmw} = \prod_{i=1}^m \left( \frac{[\text{LMW}]_i}{\sum_{j=1}^m [\text{LMW}]_j} \right)^{e_{ij}} = \prod_{i=1}^m \left( \frac{[\text{LMW}]_i}{\sum_{j=1}^m x_j \cdot [\text{LMW}]_j} \right)^{e_{ij}}$

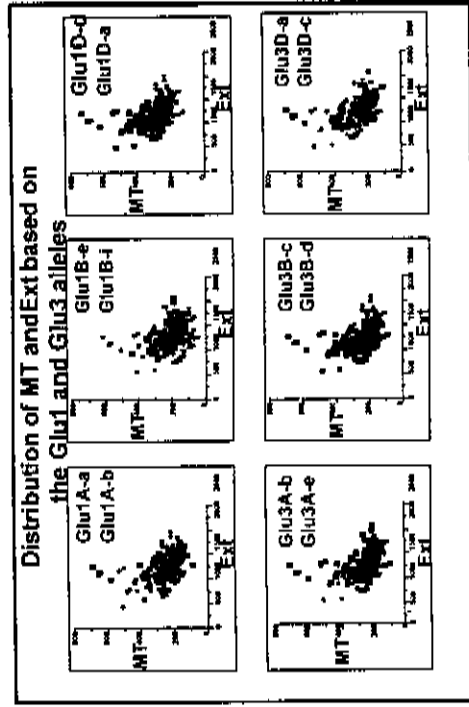
Gliadin alleles:  $Q_{gli} = \prod_{i=1}^m \left( \frac{[\text{Gli}]_i}{\sum_{j=1}^m [\text{Gli}]_j} \right)^{e_{ij}} = \prod_{i=1}^m \left( \frac{[\text{Gli}]_i}{\sum_{j=1}^m x_j \cdot [\text{Gli}]_j} \right)^{e_{ij}}$

$PV = Q_{pr} + e_{pr} \cdot \ln(Q_{pr}) + e_{glu/gli} \cdot \ln(Q_{glu/gli}) + e_{hmw/lmw} \cdot \ln(Q_{hmw/lmw}) + e_{hmw} \cdot \ln(Q_{hmw}) + e_{lmw} \cdot \ln(Q_{lmw}) + e_{gli} \cdot \ln(Q_{gli})$

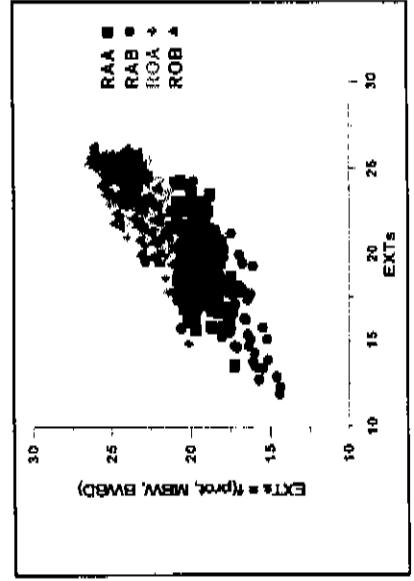
## Developing molecular markers



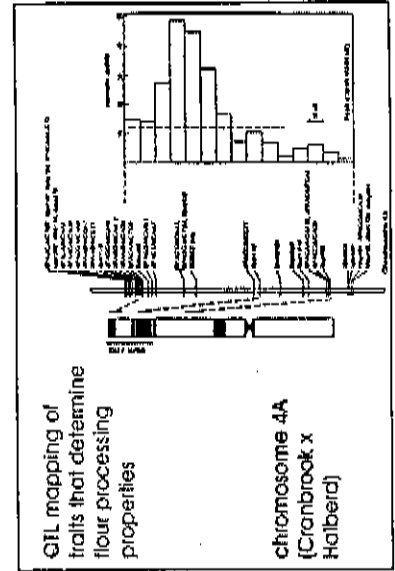
## Analysis of relationships between quality traits and storage protein composition



## Developing new predicting methodologies



## QTL analysis





# **Characterisation and introgression of novel storage protein genes**

**Dr Peter Sharp**

## PROJECT 5.1.5

### CHARACTERISATION & INTROGRESSION OF NOVEL STORAGE PROTEIN GENES

- Student appointed – Mohammad Hassani

Two main storage proteins from *Triticum tauschii*, T1 and T12.4, being introgressed into bread wheat.

**T1** – very large  $\omega$ -gliadin that may increase dough extensibility

**T12.4** – a small HMW-glutenin that may reduce excessive dough mixing times

## INTROGRESSION

### T1

AUS18913/Langdon Synthetic      X    Kite  
   ↓  
      Kite, RAC704, RAC746    x    F1  
   ↓  
  270 lines

These lines are planted for further backcrossing.

Rust resistance lines are being identified at present.

Lines segregating for T1 will be identified by PAGE over the next few months.

Selfed seed will also be collected from the lines with T1, selected for T1, and grown for production of samples for small-scale testing next year.

The synthetic was also crossed with Meering to produce BC2 derivatives. These will be PAGE analysed to detect T1 lines, and increased to produce samples for small scale testing this year.

## **INTROGRESSION**

### **T12.4**

CPI110750 was crossed as male and female to durum and to Baxter bread wheat.

The Baxter amphiploid was made, but progeny could be obtained

These crosses will be repeated this year.

## **CHARACTERISATION**

### **T1**

Placed on hold at CSIRO PI.

### **T12.4**

PCR isolation and sequencing to be restarted by M Hassani, to get identification of mutant change this year.

# **Dough Rheology Program**

**Prof. Roger Tanner**



# **DOUGH RHEOLOGY PROGRAM**

**Prof R.I.Tanner**

**Dr M. Keentok**

**Dr S.Uthayakumar**

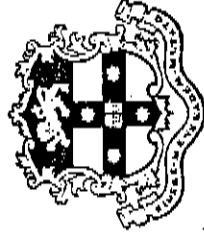
**Marcus Newberry**

**T.Hubraq**

**Prof N.Phan-Thien**

*Department of Mechanical and Mechatronic Engineering*

*The University of Sydney*



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The University of Sydney

Rheology Group



# Overview

- **Rheology of doughs made from commercial flours**
- **Effects of flour components on dough rheology**
- **Rheology of fermented dough**

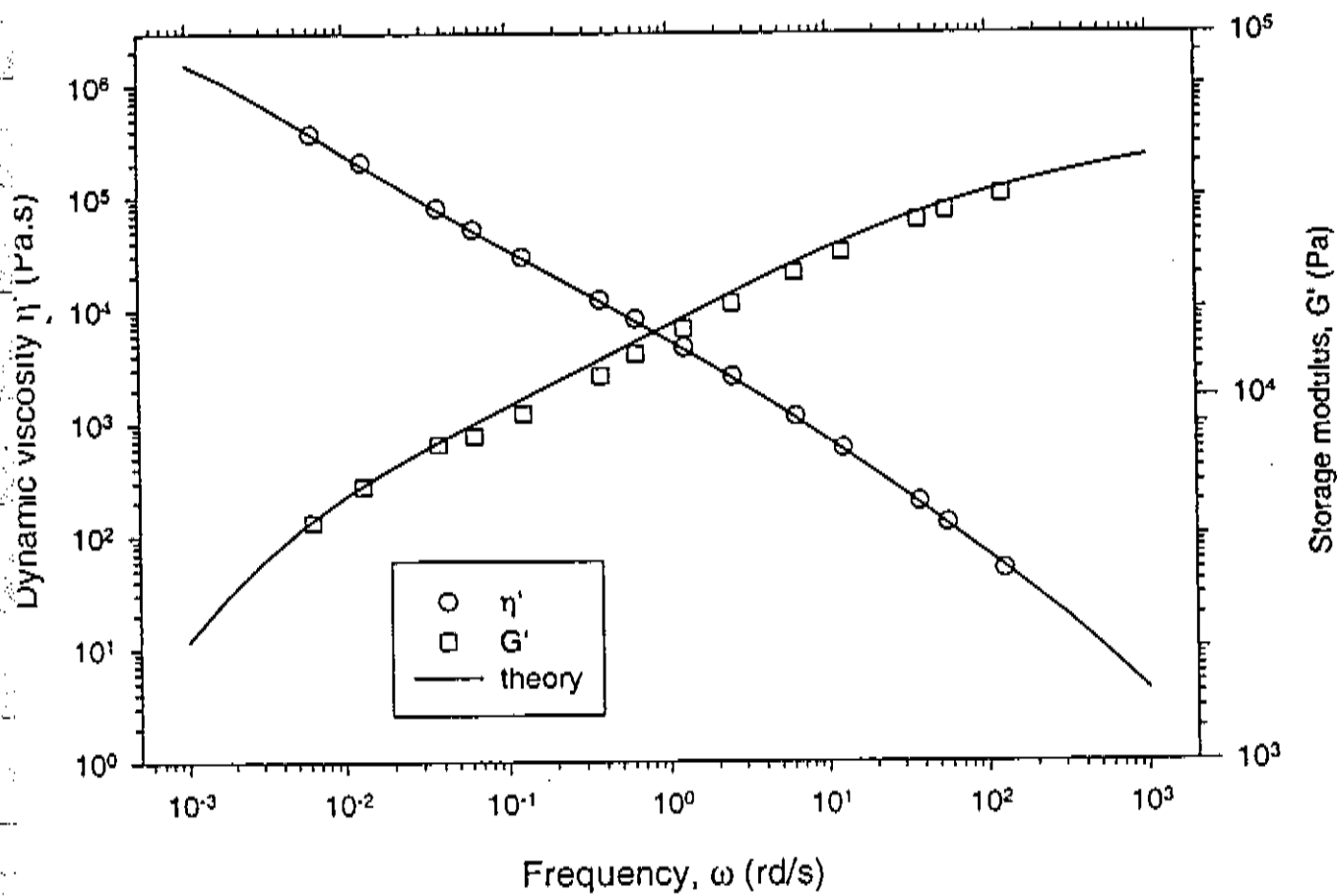
# Results: Commercial flours (M.Keentok)

## ● Rheological testing of commercial flours from

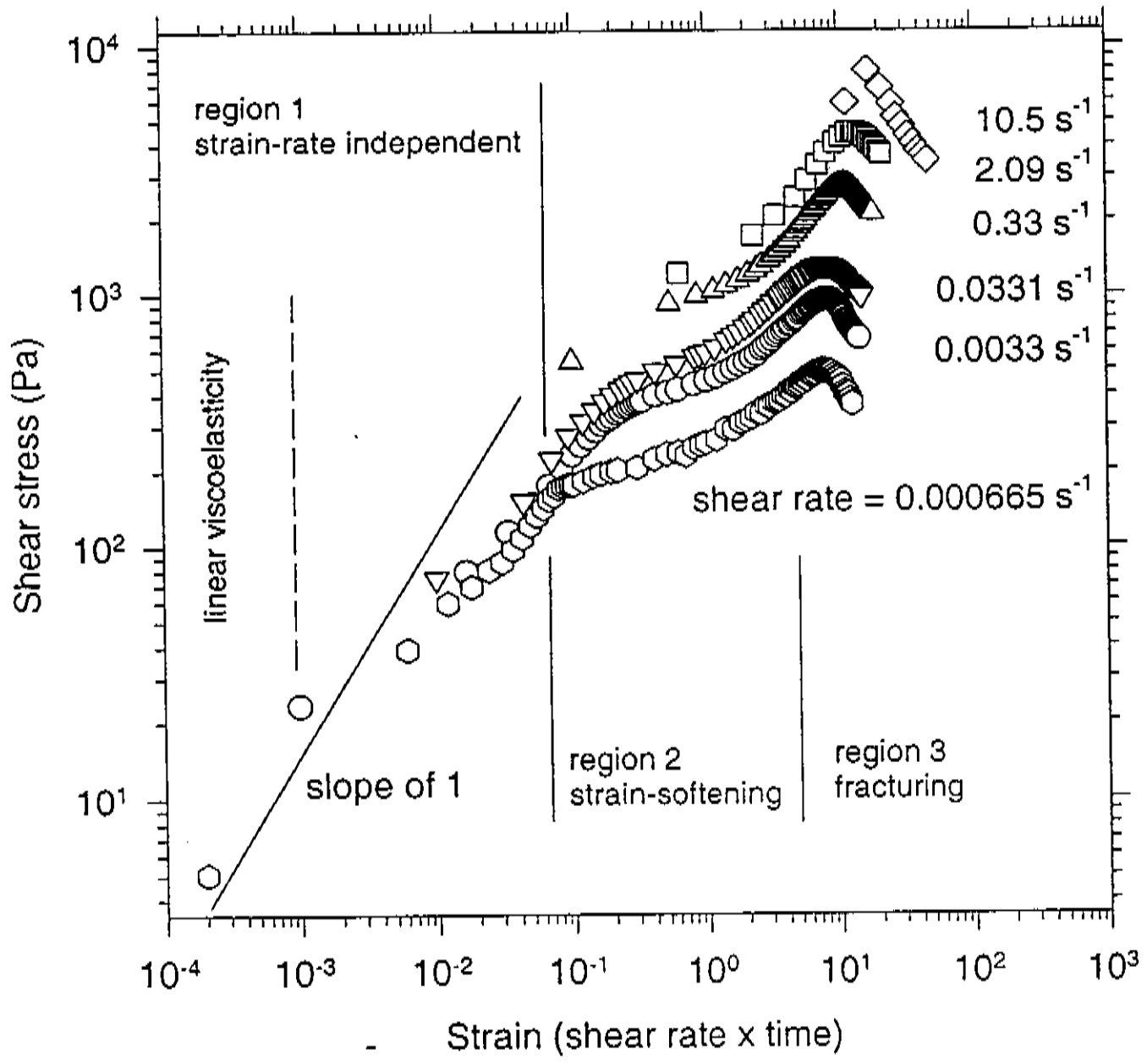
Weston:

- † A98: Promax flour
- † B98 : Bakers Extra flour
- † C98 : Eureka Medium biscuit flour
- † D98 : Everest Soft biscuit flour
- † Tests undertaken:
  - » Oscillatory shear
  - » steady shear
  - » stress relaxation
  - » creep
  - » viscometry
  - » true elongation





Dynamic viscosity and storage modulus. The solid lines represent the Maxwellian fit with 12 time constants,  $G_E = 238.9$  Pa.



1: A plot of the shear stress versus shear strain (in a simple shear flow) reveals three regions: 1. strain-rate independent region, which includes the linear viscoelasticity region at very small strain, 2. a softening region, and 3. a fracturing region (Phan-Thien et al [19]).



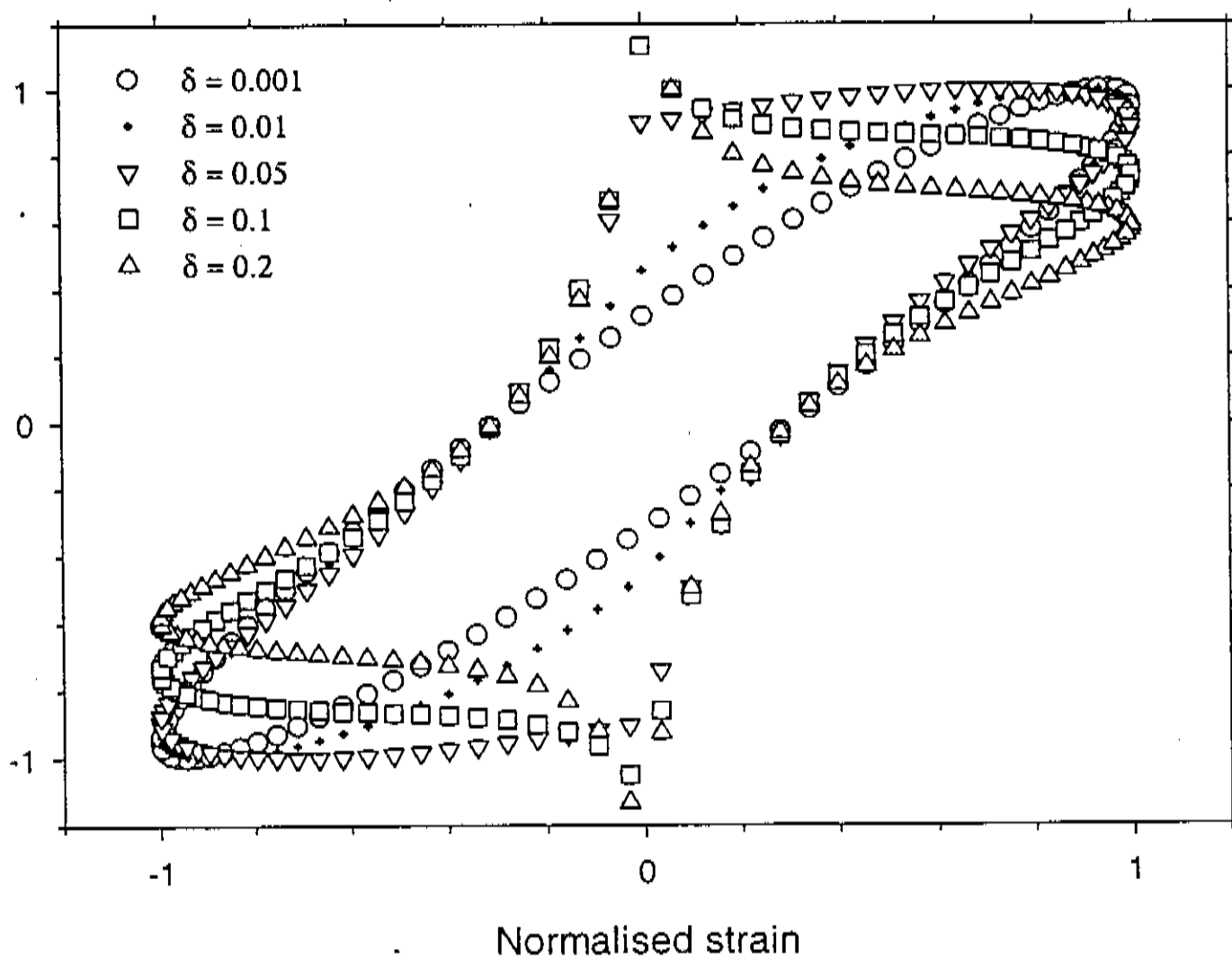
## 2.1 Oscillatory shear flow

In the case of an oscillatory flow with shear rate  $\dot{\gamma} = \delta\omega \cos \omega t = \dot{\gamma}_0 \cos \omega t$ , the particle trajectories are given by

$$X(t') = x + \delta y (\sin \omega t' - \sin \omega t), \quad Y(t') = y, \quad Z(t') = z,$$

where  $\{x, y, z\}$  are the Cartesian coordinates of the particle at time  $t$ , which at time  $t'$  is located at  $\{X, Y, Z\}$ . This leads to the deformation gradient

$$[F_t(t')] = \begin{bmatrix} 1 & \delta (\sin \omega t' - \sin \omega t) & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}.$$



Plots of normalised stress versus normalised strain at  $\omega = 1$  Hz. An ellipse indicates linearity

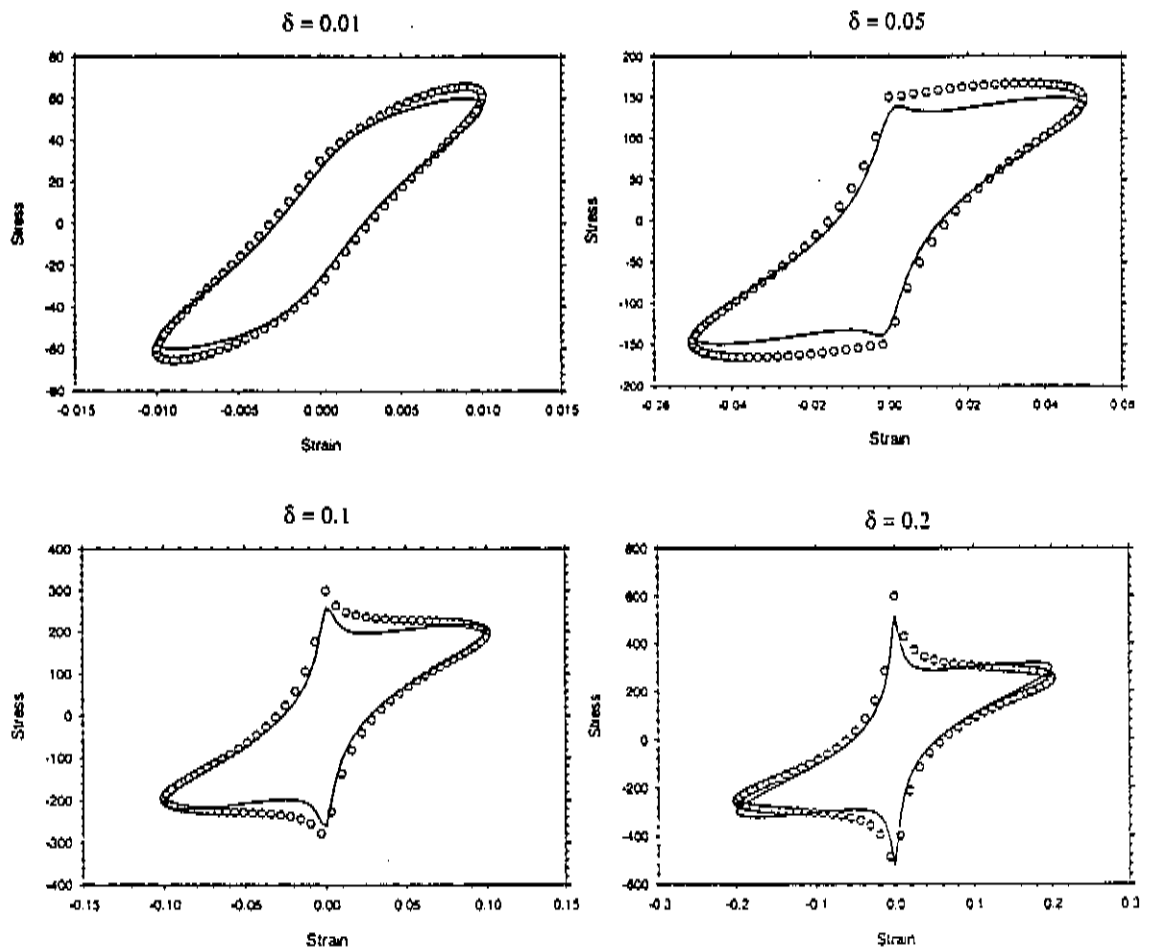
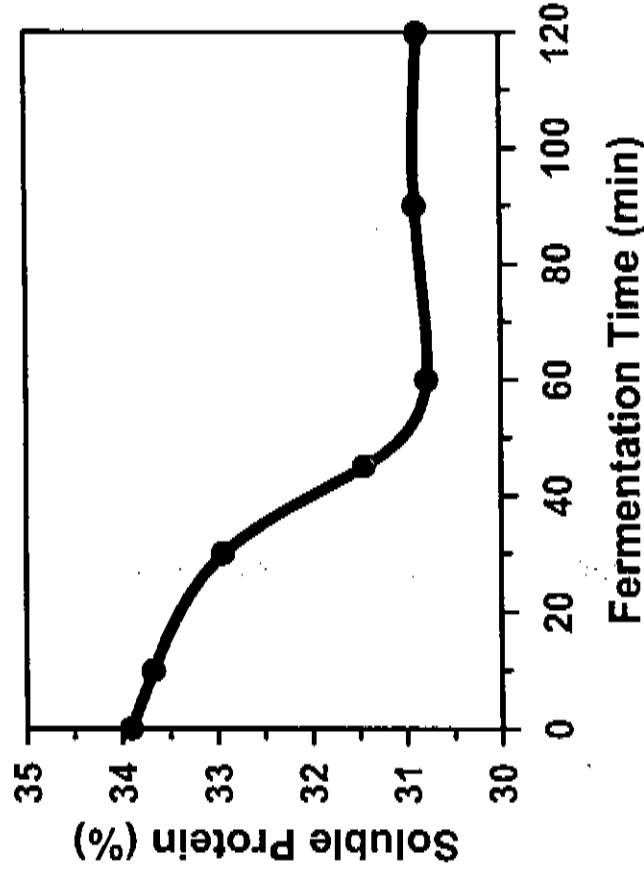


Figure 5: A comparison between the theory and the experimental data at different amplitudes and  $\omega = 1$  Hz. The symbols are experiments, and the solid lines are the predictions using shear flow parameters,  $\Gamma_1 = 300$ ,  $\Gamma_2 = 1/14$ ,  $m = 0.55$ ,  $a = 0$ , and the discrete relaxation spectrum in Table 1.

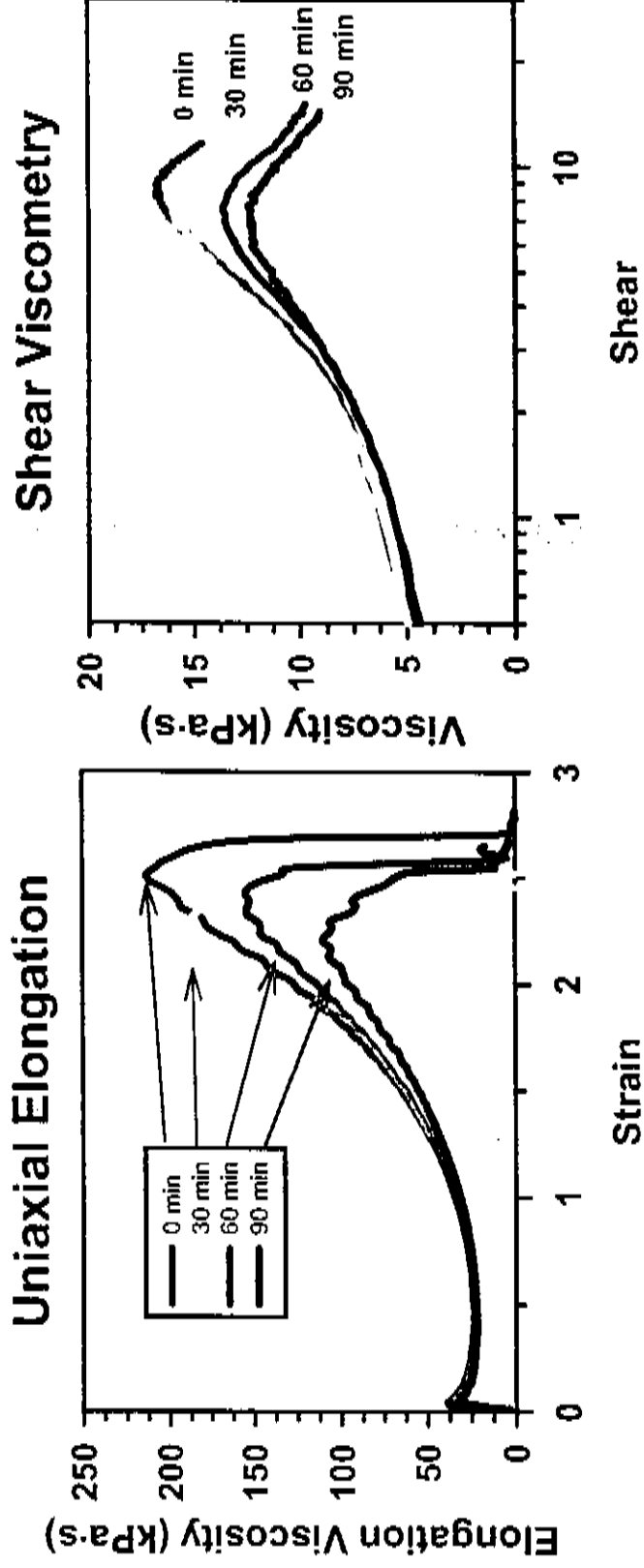
# Rheology & Structure

- Large strain changes parallel a decrease in the soluble protein aggregates with fermentation
- Fermentation forms structure on 2-D that doesn't contribute to physical strength?



# Yeasted Dough Rheology

- Fermentation has not observable effect at low strain levels
- Elongation and shear viscosities decrease with longer periods of fermentation.



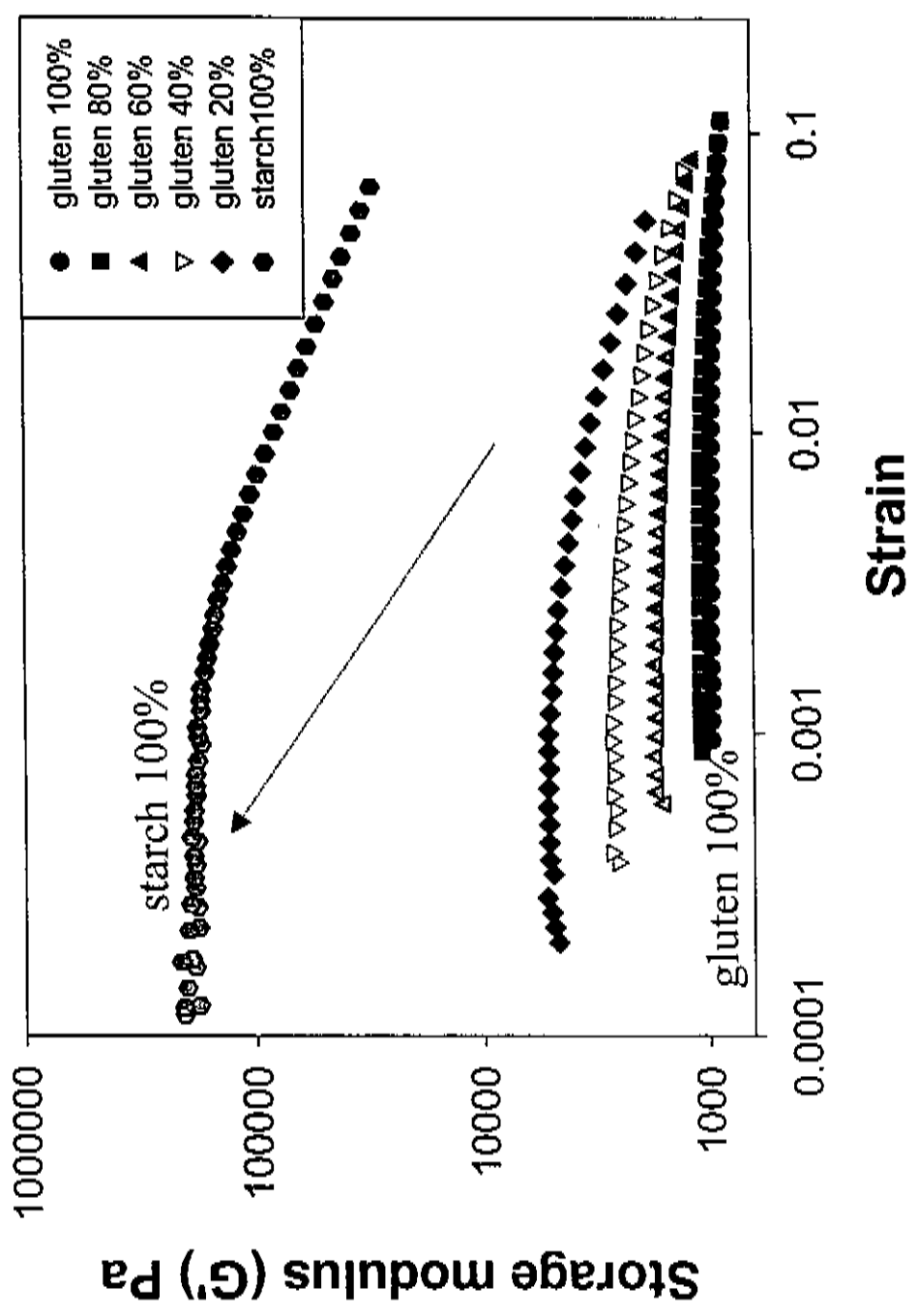
# **Effects of flour components on dough rheology**

## **AIM:**

- Determine viscoelastic properties of gluten dough and compare the properties with those of the wheat flour. Confirm that basic rheological tests can detect very significant differences in the gluten and flours studied.
- Investigate gluten-starch interaction
- Determine the effect of various HMW-GS on rheological properties
- Investigate the role of water in dough rheology

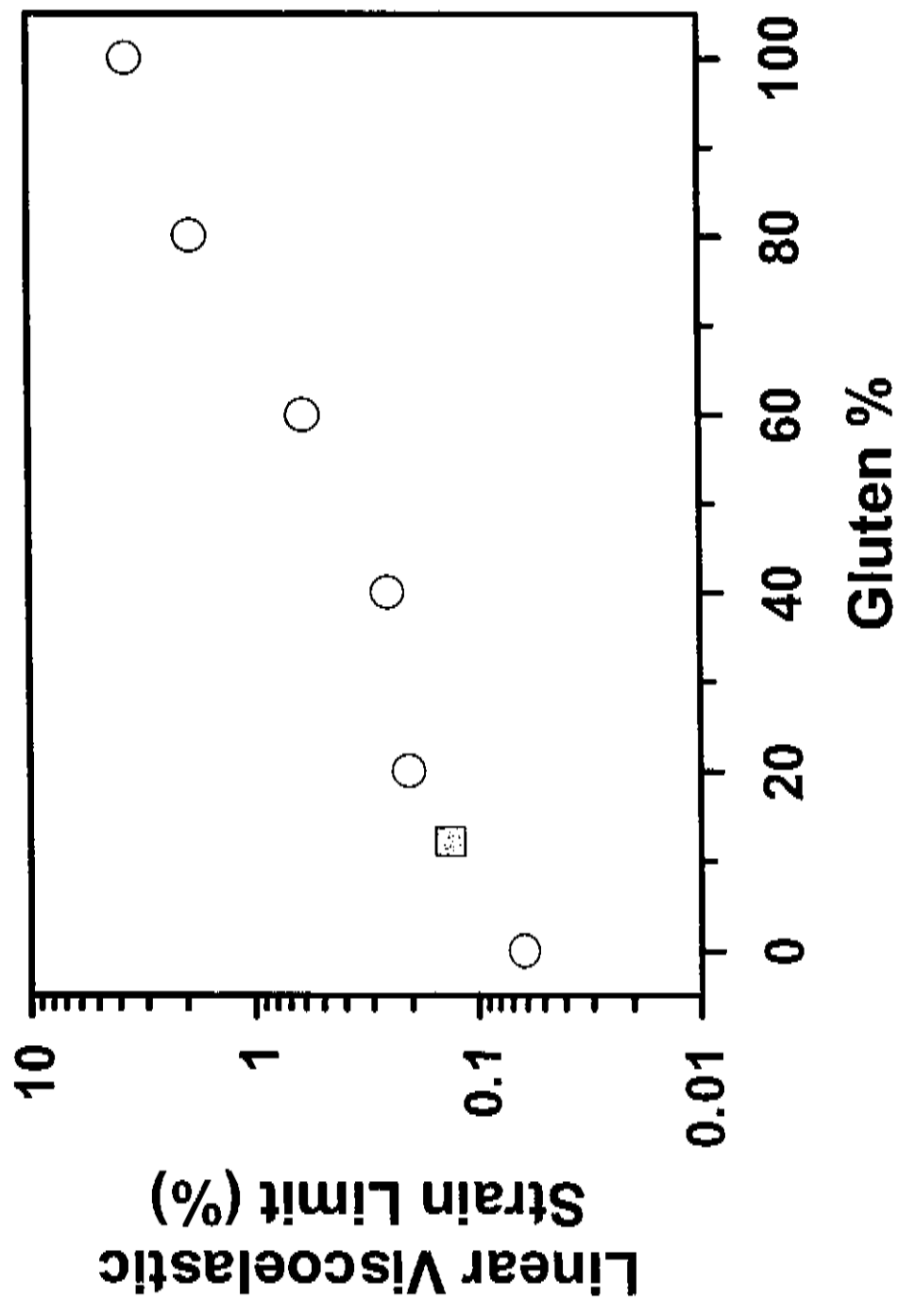


# Stress sweep experiment on starch-gluten mixtures



# Linear Strain Limit

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## SUMMARY

- **Gluten is different from flour in:**
  1. Linear visco-elastic limit for flour is below a strain of 0.1%; for gluten < 3%.
  2. Elongational viscosity of gluten > flour
- **Increase in starch in a gluten-starch complex results in a lower linear visco-elastic limit**
- **HMW-GS are major contributor to strength and stability of dough**
- **Water plays an important role in dough rheology**  
Increase in water content in dough results in decreased elongational viscosity,  $G'$ ,  $G''$  and  $\eta^*$  values

# **Diagnostics for Wheat Quality**

**Amanda Hill**

## DIAGNOSTICS FOR WHEAT QUALITY

- **Quality = suitability for a particular end product**  
**= ability of grain to meet requirements of processor**
- **Focus is on antibody-based methods - polyclonal, monoclonal and engineered antibodies**
- **Delivery systems suitable for end-use - laboratory-based test to rapid on -farm testing**
- **DIAGNOSTICS capture CRC research by development of simple methods for rapid and objective testing of quality attributes**

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R

## Diagnostics for wheat quality: Users

### Breeding programs (current users)

- **requirement: high throughput testing of large numbers of small samples**
- **examples: tests for 1BL.1RS, 1AL.1RS, 2BL.2RS translocations**
- **tests for: specific glutenin alleles**  
**rye introgressions**  
**dough properties (LMWGS discrimination Glu-A3 and Glu -B3)**  
**late maturity alpha-amylase**

### Grain receipt and processing (potential)

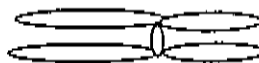
- **requirement: rapid testing for quality grade, defects**
- **must minimise sample preparation steps**
- **on-farm management for growers**
- **tests for: preharvest sprouting**  
**variety identification**  
**cereal diseases**

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## TESTS FOR 1RS TRANSLOCATIONS

Translocations of rye 1RS onto wheat chromosome 1B or 1A provide:

- rust resistances and yield increases in certain environments
- but often poor dough mixing tolerance and dough stickiness



Normal wheat

Effective early generation screening test needed:

- SDS-PAGE, HPLC and DNA-based assays have low throughput



1BL.1RS translocation

Separate antibodies detect::

- presence of Mr 40,000 gamma-secalins from rye 1RS
- absence of fast omega-gliadins from wheat 1BS

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## 1RS and 2RS assay method

ELISA kits provided to CIMMYT and Australian/NZ breeding programs  
sample preparation is extremely simple  
weighing and grinding/ crushing of grains not needed

Whole or cut wheat grains or wholemeal placed in replicate 96-well blocks

↓  
Extract overnight in 50 % isopropanol, Add to microwell plate

↓ 60 min

Wash, block non-specific binding

↓ 60 min

Add enzyme-labelled antibody

↓ 30 min

Wash, add enzyme substrate/ chromogen

↓ 20 min

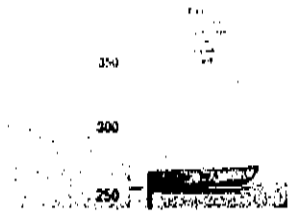
Stop. If required, read in ELISA plate reader

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## Simple field test for Pre-harvest sprouting

- New, immunochromatography test also detects alpha-amylase
  - quantitative, correlates with standard method (Falling Number)
  - results independent of variety, growth site
- Silo receival use
  - faster, less expensive than falling number machine
  - can readily be made available at all mills, silos in wet harvests
- On - farm application
  - sprouting can vary significantly within and between paddocks
  - avoid binning sound and damaged grain together



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## Late maturity alpha-amylase

- When high levels of amylase in mature grain occur without rain trigger
- Occurs in certain varieties under specific environmental conditions
- Due to the same enzyme as in sprouted wheat but under different physiological control
- A very serious quality defect - withdrawal of several advanced lines
- Current screening requires hundreds/ thousands of enzyme assays on individual seeds, tedious, low in throughput

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## Development of a new test for late maturity alpha-amylase (LMA)

CSIRO  
PLANT  
INDUSTRY

- test is a double- antibody sandwich ELISA
- higher sample throughput than enzyme assay
- suits a variety of types of sample extract
- more sensitive than enzyme assay

Method:

1. grain samples extracted in 0.5 % NaCl
2. extracts added to microwells, left 60 min. Wells washed.
3. antibody - peroxidase conjugate added, left 30 min.  
Wells washed.
4. colour developer added, left 30 min. Stopped, results scored.

*This test detects expression of LMA. LMA is recessive, so a DNA marker test for genes controlling LMA would be valuable.*

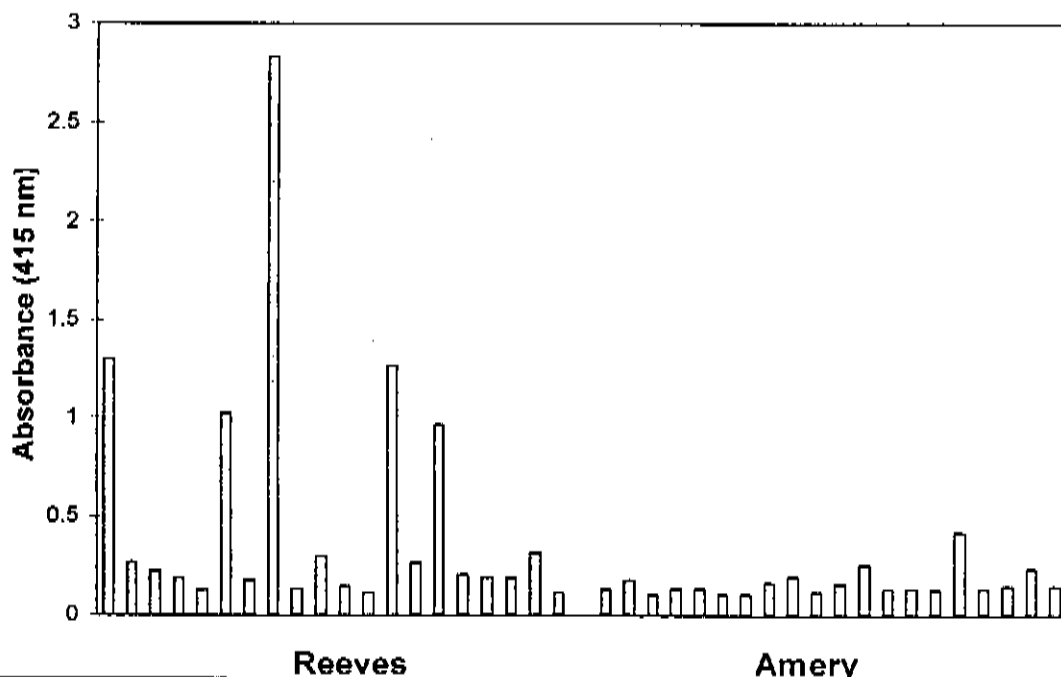
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CSIRO  
PLANT  
INDUSTRY

## Analysis of single grains of Reeves and Amery for late maturity amylase



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# **Field diagnostics for wheat varietal identification and quality traits**

**Dr Kevin Gale**



**Project Staff**

**Kevin Gale  
Malcolm Blundell  
Amanda Hill**

**QWCRC Project 5.1.8**

**Commenced April 1999**



**Aim: A rapid, field-based assay for varietal identification of wheat based on ICT card format ('WheatRite')**

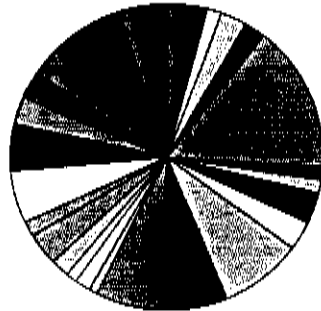
**Required for receival testing for the purposes of**

- end-use segregation**
- end-point royalty payments**
- industry domestic buyers**

**Plus quality control for breeding programs & PBR registration?**



AUSTRALIAN VARIETAL USAGE (>100K TONS) 1998/99



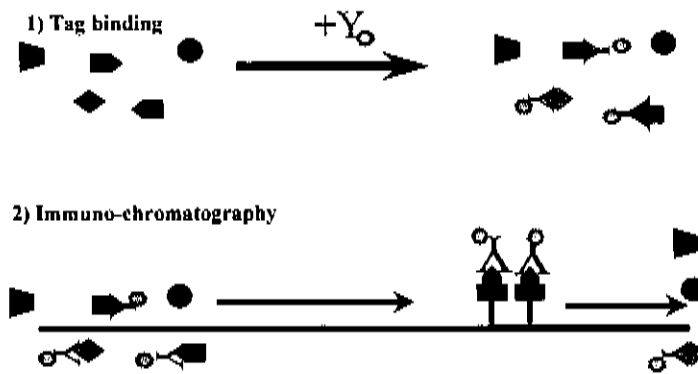
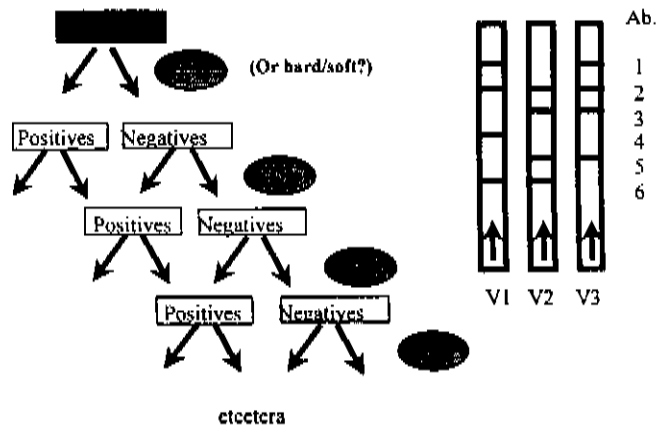
- BARKER
- BERINGA
- BATAVIA
- CUNNINGHAM
- HARTOG
- JANT
- KINGSBROOK
- KUNLU
- KRISTATE
- KUNVALL
- DOLLARBIRD
- FRANK
- HALLBERG
- LAWSON
- MDRU
- KUSSELLA
- SPICAR
- ANQUINA
- SLADE
- DAVIER
- EXCALIBUR
- KRICHALFF
- MACHETT
- STILLETU
- AMERY
- CALKNIK
- CARNAMAH
- CANADES
- CLINDRIN
- GATATINE
- BRADU
- KALANNIE
- PEREMORE
- TINEURRIN
- WILGOYNE



**-only diagnostic tools with sufficient specificity and sensitivity currently available for rapid, field-based diagnostic tests**

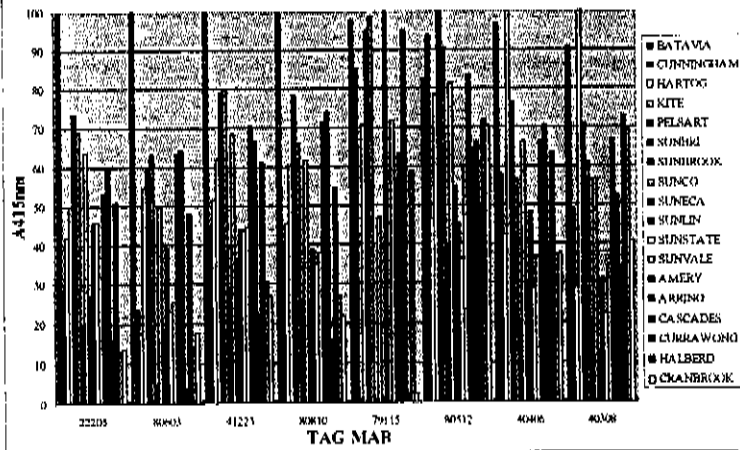
**-feasibility proven using ICT card format**

**-some suitable antibodies already available**





### QLD. VARIETIES, 82208 CAPTURE



**GBSS is the major protein found within the starch granule of mature grain**

**Approximately 50% of Australian varieties are null for one of the three homeoalleles**

**The null phenotype is associated with enhanced suitability for Udon noodle production**

**Protein sequences for the 3 homeoalleles show approximately 97% identity**

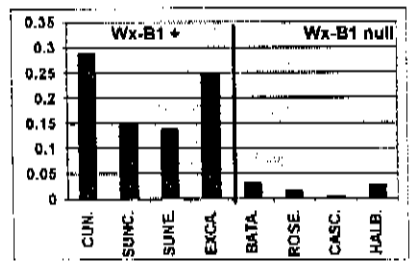


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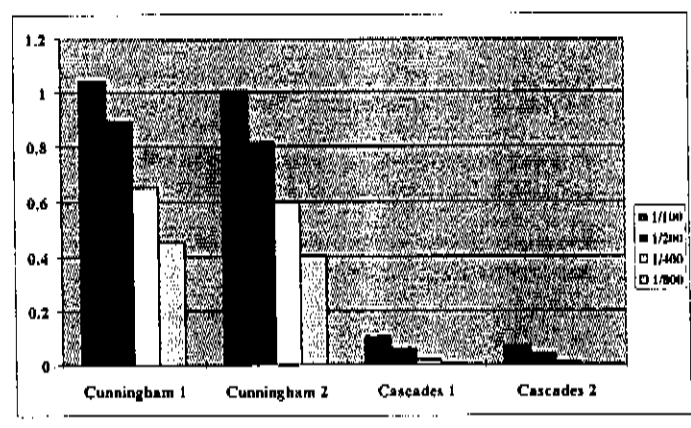
240

'D' GENOME: LEVPRILNLDNNPYFSGPYGEDVVFCNDWHTGLLACYLK  
 'B' GENOME: LEAPRILDNNNPYFSGPYGEDVVFCNDWHTGLLACYLK  
 'A' GENOME: LEVPRILDNNNPHEGSGPYGEDVVFCNDWHTGLLACYLK

EAPRILDNNNPYF[GC]--  
 SYNTHETIC PEPTIDE

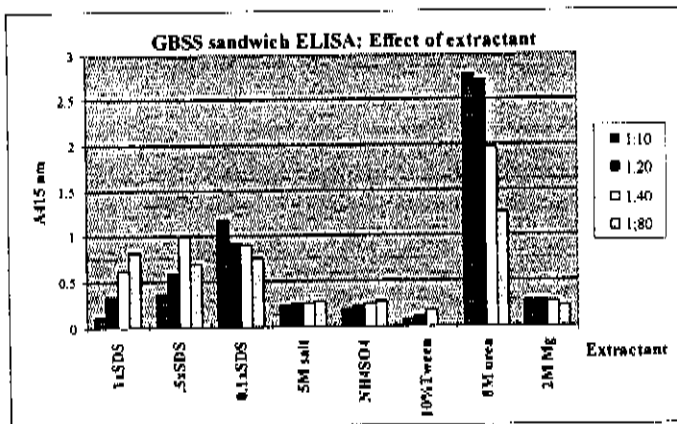


-Anti-GBSS MAb used as capture in sandwich assay





-Wholemeal flour crude extract used as antigen



	Glu-A1	Glu-B1	Glu-1D
	a (1)	a (7)	a (2+12)
	b (2*)	b (7+8)	b (3+12)
	c (null)	c (7+9)	c (4+12)
	d (5)	d (6+8)	d (5+10)
	e (1*)	e (20)	e (2+10)
		f (13+16)	f (2.2+12)
		g (13+19)	g (2 +11)
		h (14+15)	h (5+11)
		i (17+18)	
Rosella	b	b	a
Hartog	a	i	d
Frame	a	b	d
Halberd	a	c,e	d
Lawson	b	d	a
Cranbrook	b	i	a
Miskle	b	i,b	a
Falcon	b	i	a





**Variation associated with bleaching of processed durum semolina**

**Assay Lox variation to assess colour traits of new lines?**

**Progress**

- durum lox cloned and sequenced (MGF)
- expressed as fusion with INTEIN-CBP
- purification to obtain serum in progress

Durum	KEVREVGHGDIADQFWWPKMTTITELVRTCSTIITWIASALHAAVNFGQYFYAGYLPNRPT
	KEVREVGHGDI DQPWWPKMTT+ ELVR+C+TIIWIASALHAAVNFGQY YAGYLPNRPT
Bread	KEVREVGHGDIKDQFWWPKMTTVNELVRSCATTIITWIASALHAAVNFGQYSYAGYLPNRPT
Durum	VSRRPMPFPGTKEYEEAETNPDKAFIHTITSQLQSIIGVSLIEVLSHHSDEVYLGQRDE
	VSRR MPEPGTKEY+E ET+PD AFIHTITSQLQSIIGVS+IEVLS+H SDEVYLGQRDE
Bread	VSRROMPEPGTKEYEVEDEDLAFIHTITSQLQSIIGVSVIEVLSHHSDEVYLGQRDE



**1) Survey sequence variation of proteins present in mature grain between cultivars:**

- purolindoline B, lipoxygenase
- SBEI, SSIII, amylase inhibitors
- 'non-conserved' EST's -invertase

**Target polymorphisms using synthetic peptides**

**2) Microarrayed EST differential screening?**

- sequence discrimination is low
- developmental variation a problem

# **Immunodiagnosics for wheat quality and genetic screening**

**Dr Thomas Giersch**

⊕ **Why analyse HMW-GS composition?**

- ⊕ The composition of HMW-GS in wheat flours is a key property affecting flour processing and product quality.
- ✎ There is considerable interest in rapid and reliable methods to determine the allelic composition of HMW-GS in breeding programs and in industrial processing.
- ✎ Immunochemical test systems such as ELISAs are easy to perform and have the advantage of high sample throughput.

- ⊕ Development of monoclonal antibodies to analyse the high molecular weight glutenin (HMW-GS) composition of wheat flour.
- ⊕ Antibody engineering to select new specificities and to improve properties of recombinant antibody fragments.

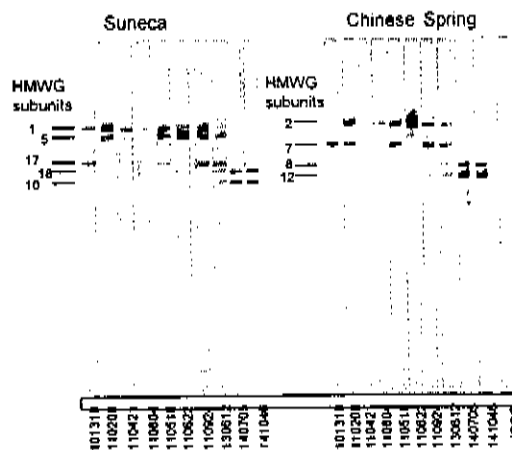


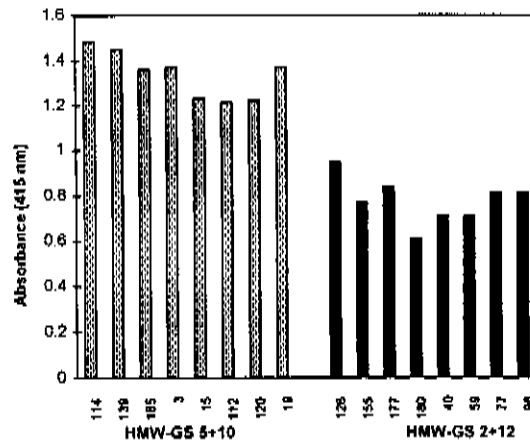
Problem

Very high sequence homology within the HMW-GS

Experimental approach

Use of purified individual HMWG subunits for the immunisation of mice to increase specificity.





- ⊕ The use of purified HMW-GS for immunisation, resulted in several monoclonal antibodies selective for particular glutenin subunits.
- ⊕ MAb 110622 could discriminate wheat varieties with the *Glu-D1* allelic pairs of subunits 5+10 and 2+12 in a simple ELISA for the analysis of flour or wholemeal.
- ⊕ This distinction is of special interest, because allelic variation at *Glu-D1* strongly influences dough properties.
- ⊕ Patent



### Why antibody engineering

- ⊕ The availability of monoclonal antibodies with required specificities is still limited.
- ⊕ cDNA expression libraries enable screening of diverse pools of antibody genes to select single chain antibody fragments (scFv) with suitable specificities to HMW-GS.
- ⊕ Further refinement can be achieved (eg. mutagenesis)

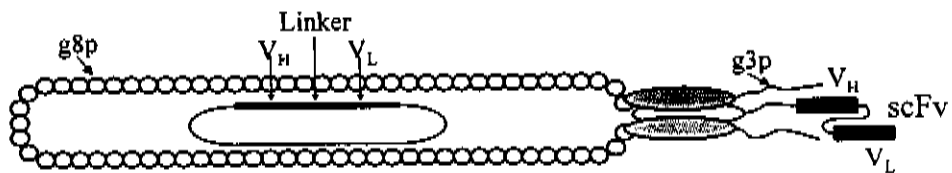


- ⊕ isolation of mRNA
- ⊕ reverse transcription into cDNA
- ⊕ separate PCR amplification of variable heavy- and light chain regions
- ⊕ assembly of H- and L-chain with linker (ScFv)
- ⊕ Sfi I / Not I - digest
- ⊕ cloning into phagemid pCANTAB 5E
- ⊕ transformation of E. coli (TG1)
- ⊕ screening: → enrichment by biopanning  
→ colony lift assay

**Cloning vectors combining features of phages (M13) and plasmids**

**phage:** M13 (single stranded) replication origin and packaging signal

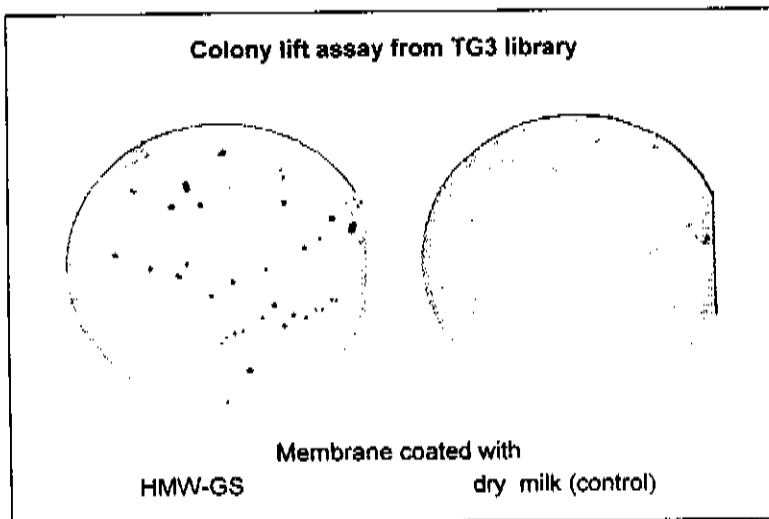
**plasmid:** origin of replication (ds)  
gene expression system  
Amp. resistance marker



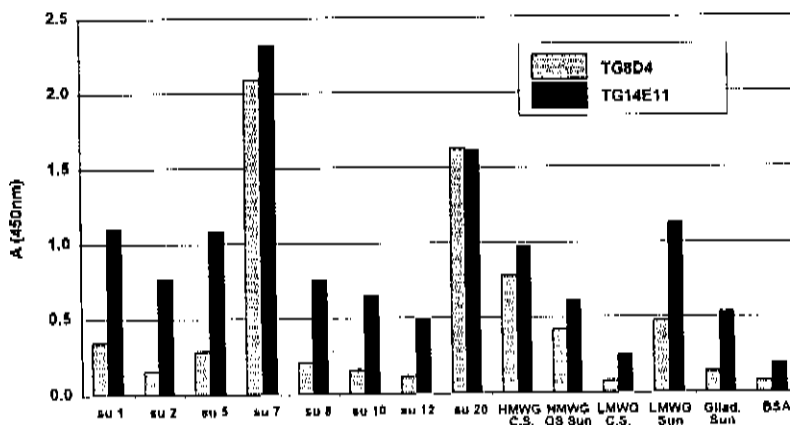
Phage display libraries have been developed from RNA of spleen cells of mice immunised with individual HMW-GS subunits

Subunit	Library
1	TG1
2	TG3
5	TG8
7	TG14
10	TG17

Colony lift assay from TG3 library

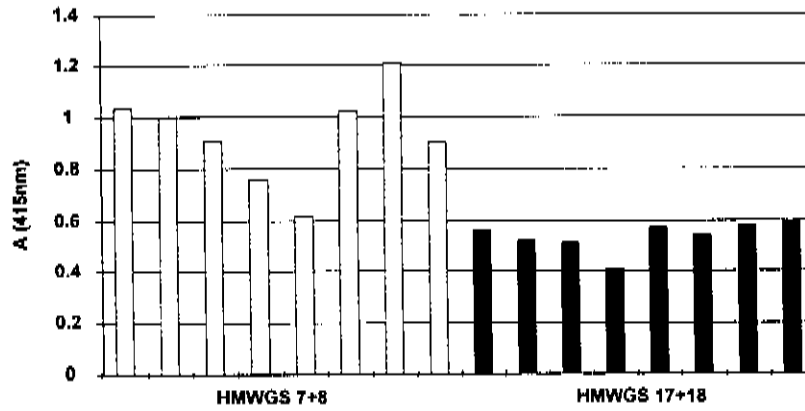


Membrane coated with  
dry milk (control)



Specificity pattern of two scFv antibodies selected from libraries derived from mice immunised with HMWG subunits 5 (TG8D4) and 7 (TG14E11)





Application of TG8D4 scFv to wheat samples of cross-bred lines from a CD87 (HMW-GS 7+8) x Hartog (HMW-GS 17+18) cross

- ④ Improve discrimination of standard wheat varieties with TG8D4
- ④ Increase expression levels and optimise purification
- ④ Stability tests an examination of suitability for different ELISA formats
- ④ Continue screening of libraries and characterise selected scFvs to find new specificities
- ④ Incorporation of scFvs in testkits for germplasm testing in breeding programs (CIMMYT)

# **Molecular Markers for Hardness**

**Ms Kym Turnbull**



**Kym Turnbull**

**PhD Student  
University of Sydney**

**Supervisors: Peter Sharp, Sadequr Rahman**



- 1) Developmental study of grain hardness
- 2) Examination of the role of puroindoline-b mutations and hardness
- 3) Characterisation of lambda genomic clones containing grain hardness markers
- 4) Use of BAC clones containing grain hardness markers for new gene and marker discovery

**Aim:** To determine when hardness can be distinguished in the developing seed

**The experiment:**

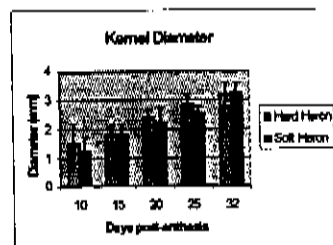
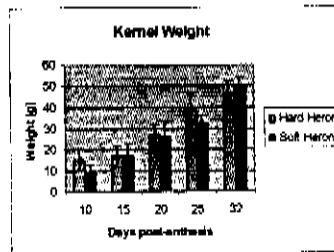
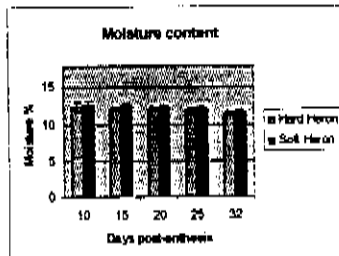
- Plant material: Near -isogenic lines Hard Heron and Soft Heron grown under controlled conditions at the CSIRO phytotron.
- Randomised block design with six replicates of each time period and each cultivar.
- Plants tagged at anthesis and harvested at 5, 10, 15, 20, 25, 30, 32 and 35 days post-anthesis (dpa).

**Mass Accumulation and Water loss during drying**

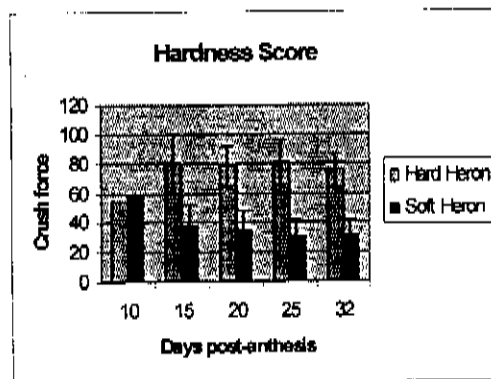
- At appropriate time periods seeds were removed from wheat heads and a wet weight (10 seed weight) taken. Samples were then air dried and weighed after 2, 3, 5, 7 and 15 days of drying. ANOVA carried out on all mass data.

**Hardness analysis**

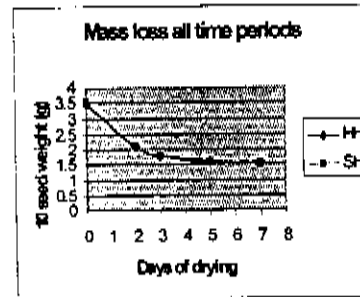
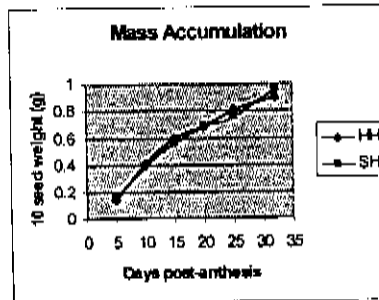
- Samples were analysed using the Perten Single Kernel Characterisation System (SKCS4100). Data generated from the SKCS
  - hardness
  - moisture content
  - kernel diameter
  - kernel weight



**Conclusion:**  
No differences in kernel weight, moisture content and kernel diameter between hard and soft lines



**Conclusion:**  
Hardness can be measured in the developing grain after 10 dpa using the SKCS and hardness occurs early in seed development



**Conclusion:** Mass accumulation and mass loss during drying does not differ between hard and soft near-isogenic lines

Fresh developing seed samples harvested at 5,10,15,20, 25 and 32 dpa and used for light microscopy



**Conclusion:** No visible difference between near-isogenic hard and soft wheat lines at light microscope level



**Grain hardness does not influence**

- Mass accumulation
- Mass loss through drying
- Kernel weight
- Kernel size
- Kernel weight

**Grain hardness occurs early in development**

- Possibly as early as 5 dpa (microscopy)
- Definitely from 15 dpa (SKCS)

**Grain hardness is mediated by the drying process**

**These results suggest a re-evaluation of current hardness theories because at early stages of seed development there are very few starch granules in the cell and very little protein**

# **Polymer size and shape in cereal processing**

**Ms Laila Daqiq**





**Supervisors**

**Dr. F. Bekes, CSIRO, PI  
Dr. F. Stoddard,  
University of Sydney**

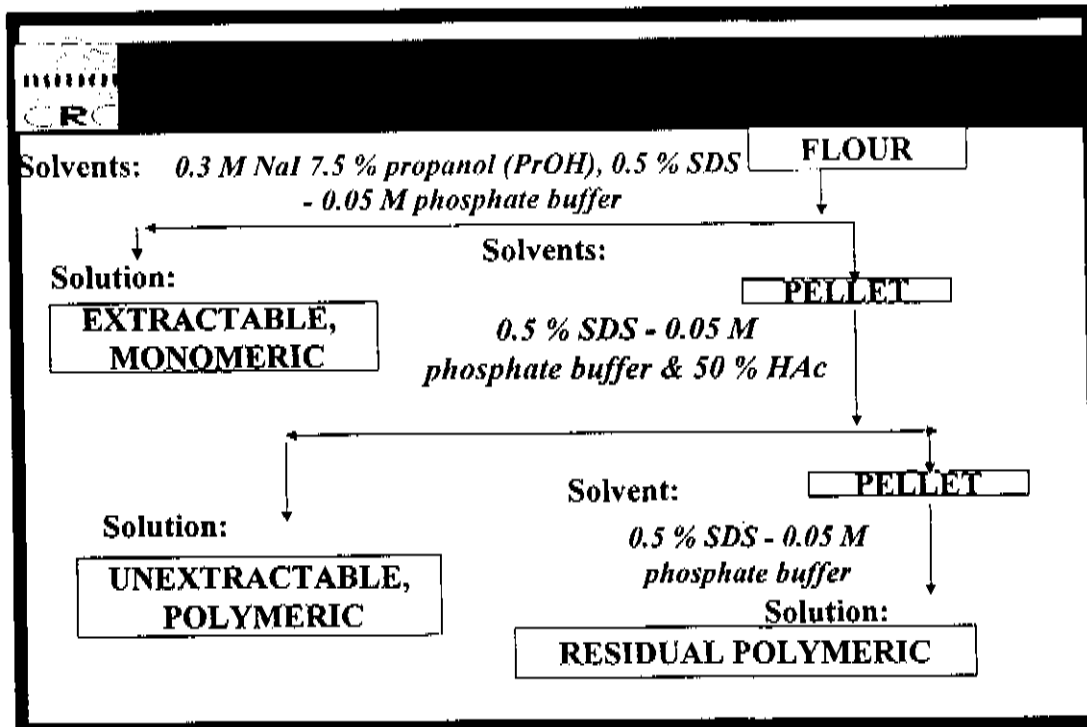
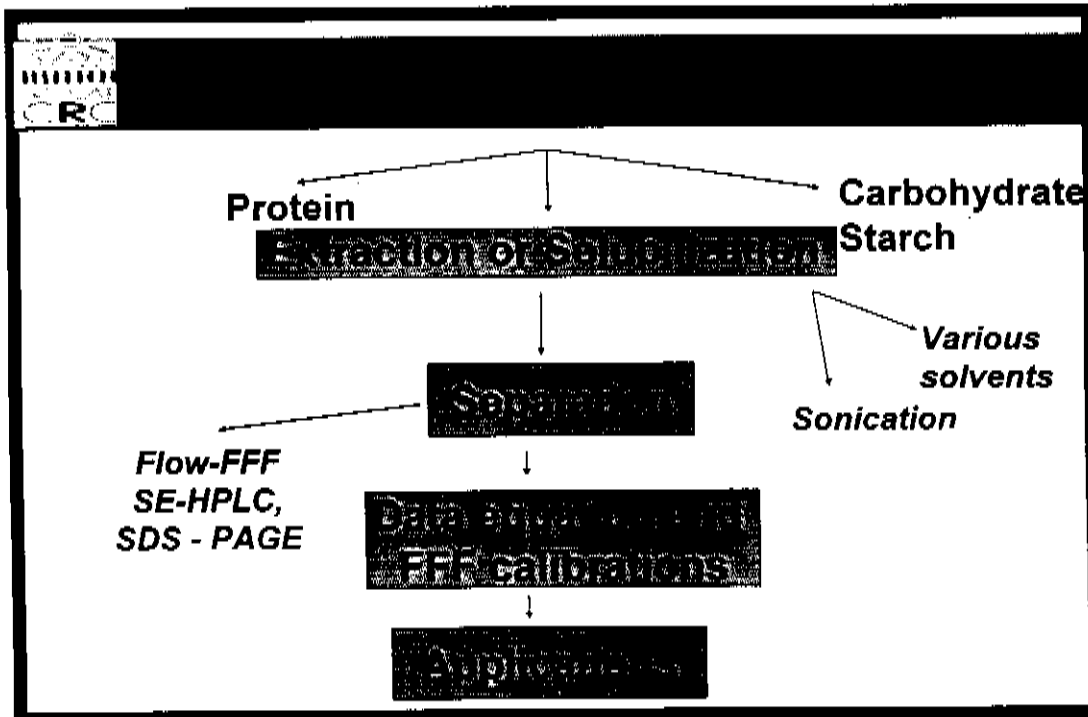
**Funded by Quality Wheat CRC**

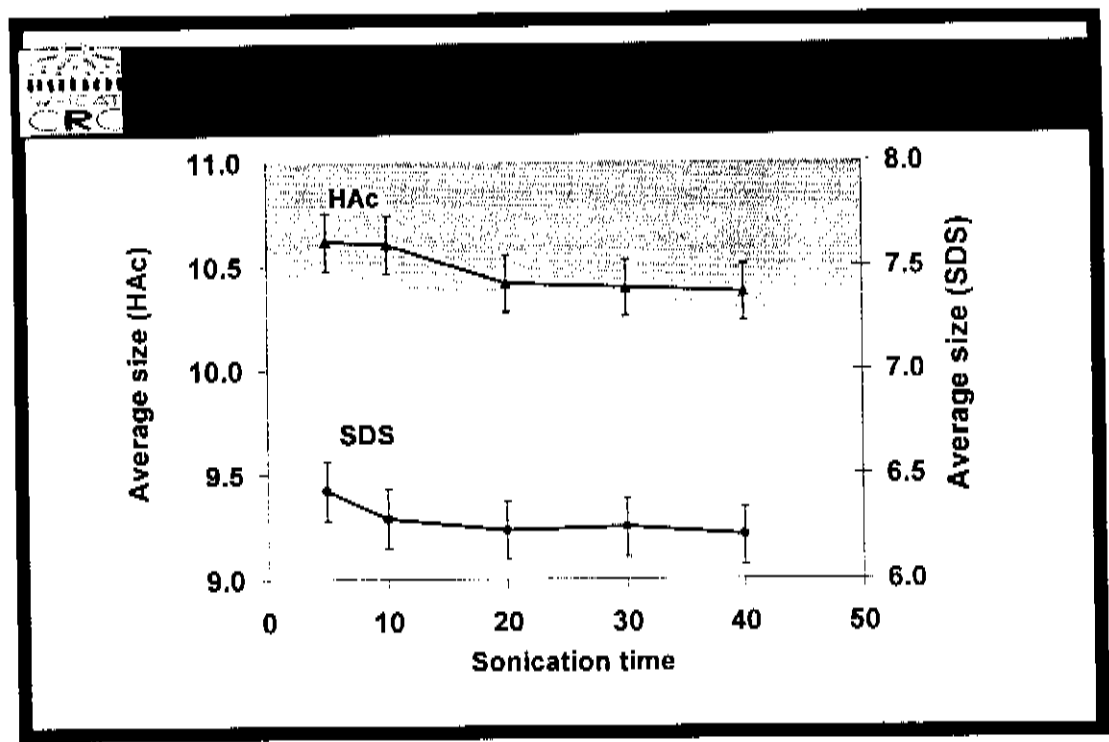
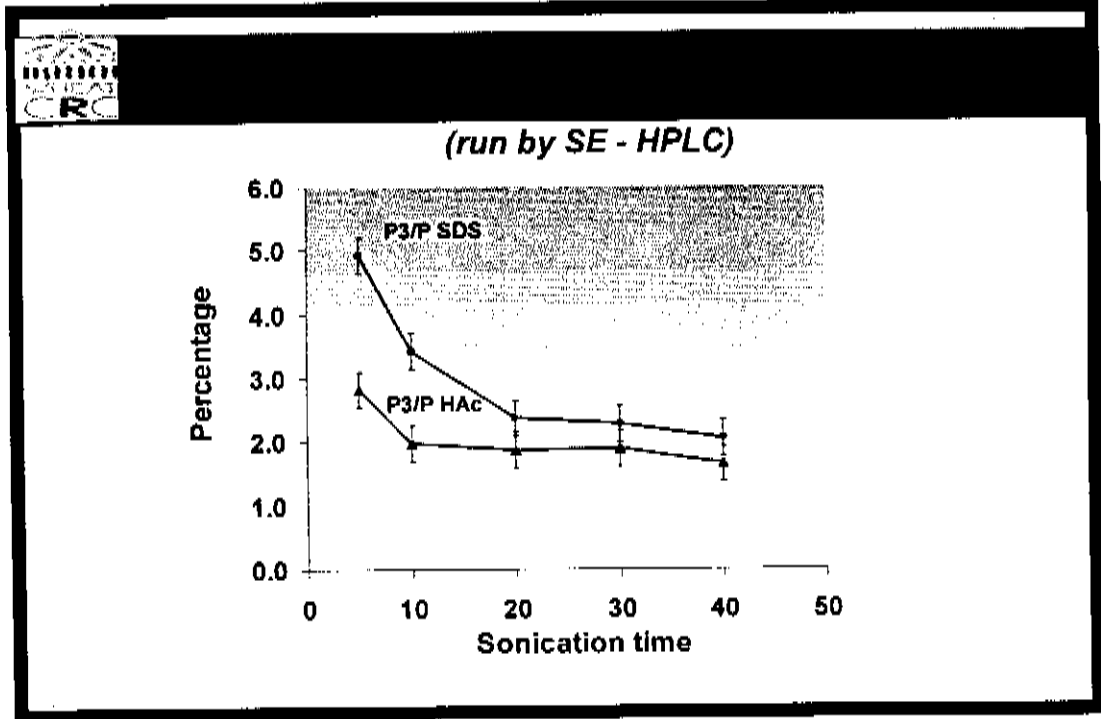
**Student**

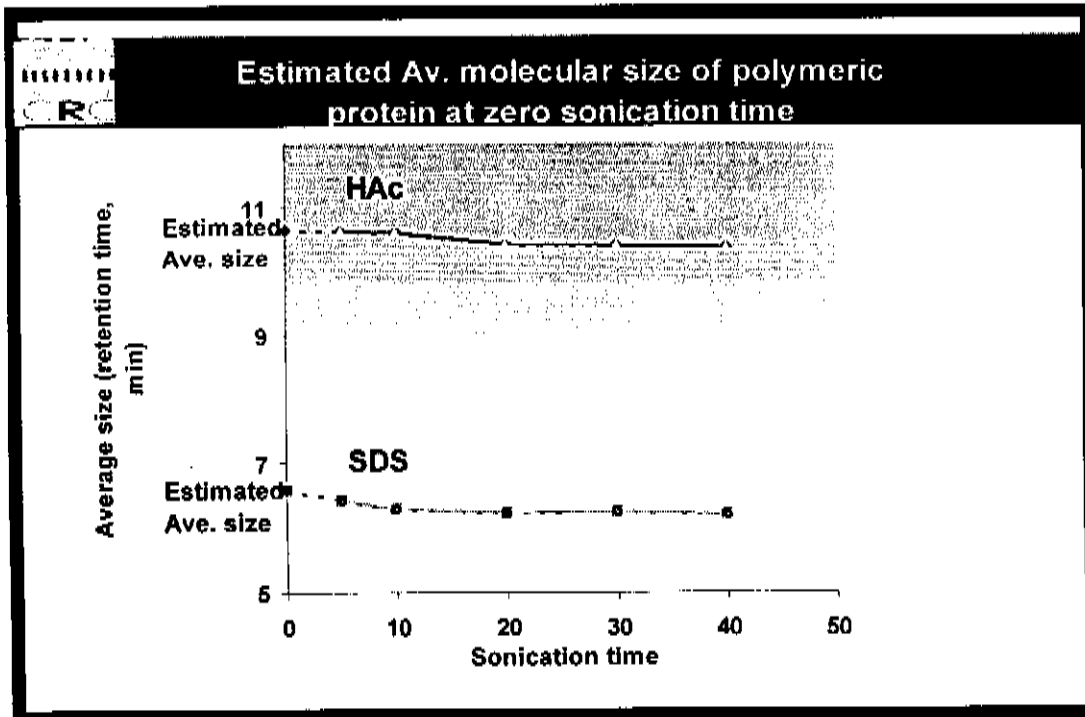
**Laila Daqiq**



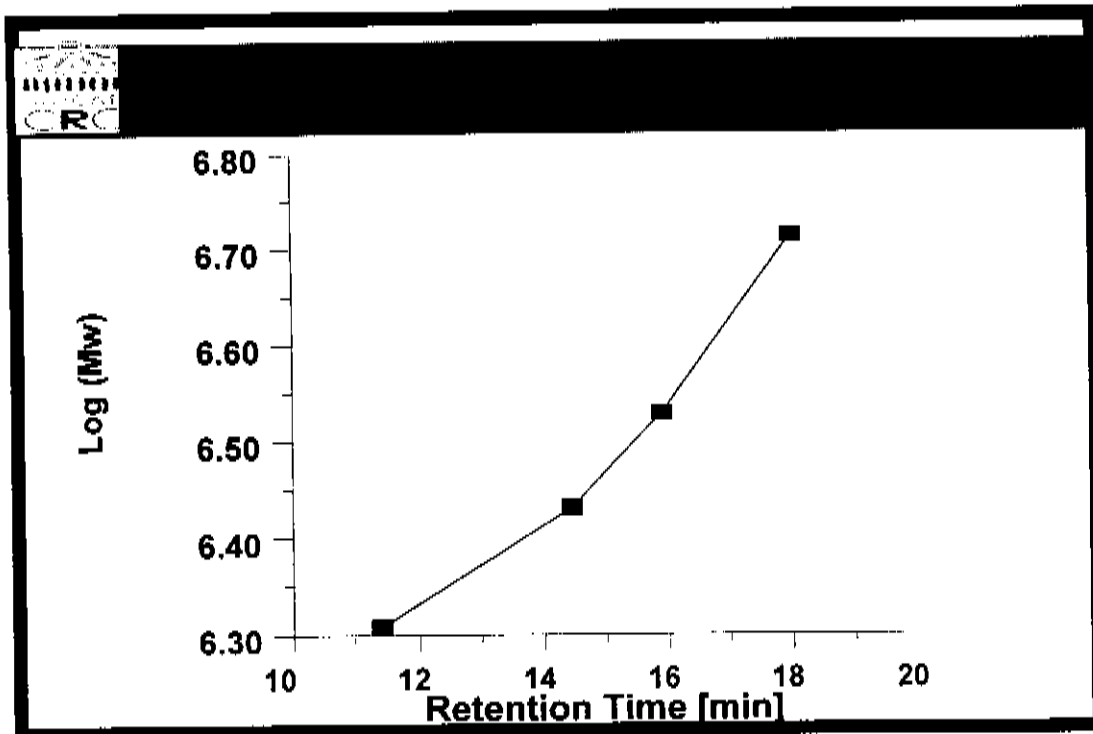
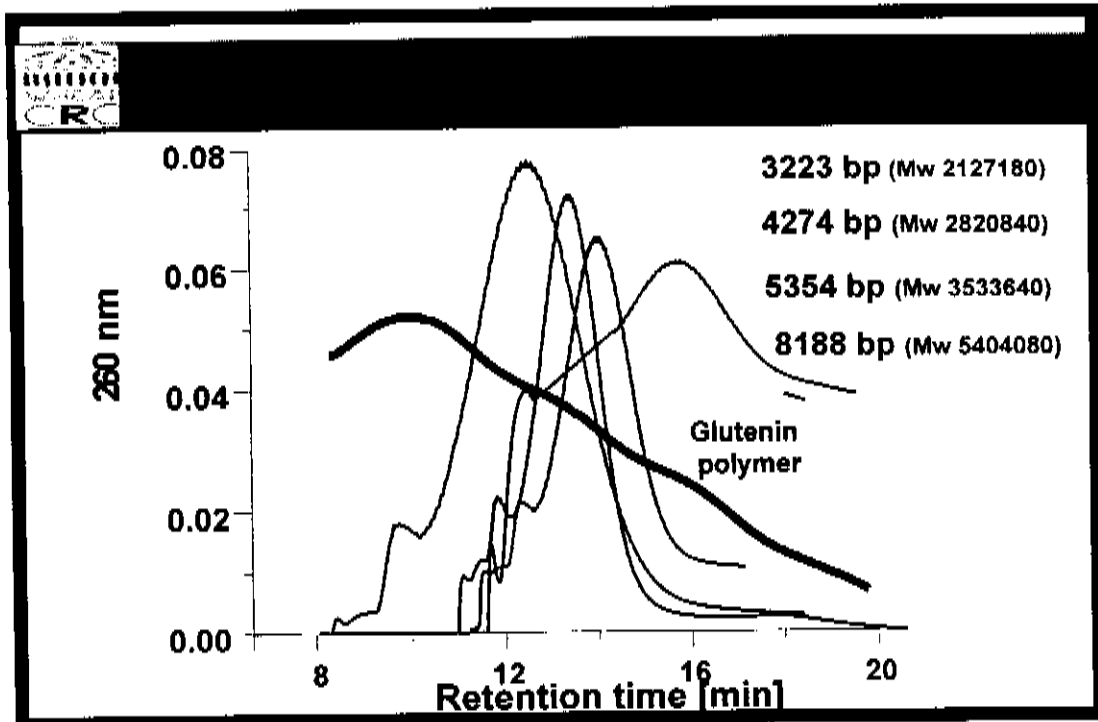
**Size distribution of wheat  
biopolymers affected their  
structure - function relationship  
and hence the quality  
of the end-use product**







1. Native size of polymeric protein can be estimated using different time of sonication and then extrapolating to zero sonication time.
2. Apparent size distribution, measured by both FFF and SE-HPLC depends on the extraction solvents.
3. Molecular sizes determined by the FFF were greater for polymeric protein extracted by acetic acid (10.4-10.6 min) in comparison to SDS (6.3 - 6.6 min).
4. Using different sonication time it was confirmed that percent of un-extracted polymeric protein(% UPP) or percent of extracted polymeric protein (% EPP) do not explain the size distribution of polymeric protein.

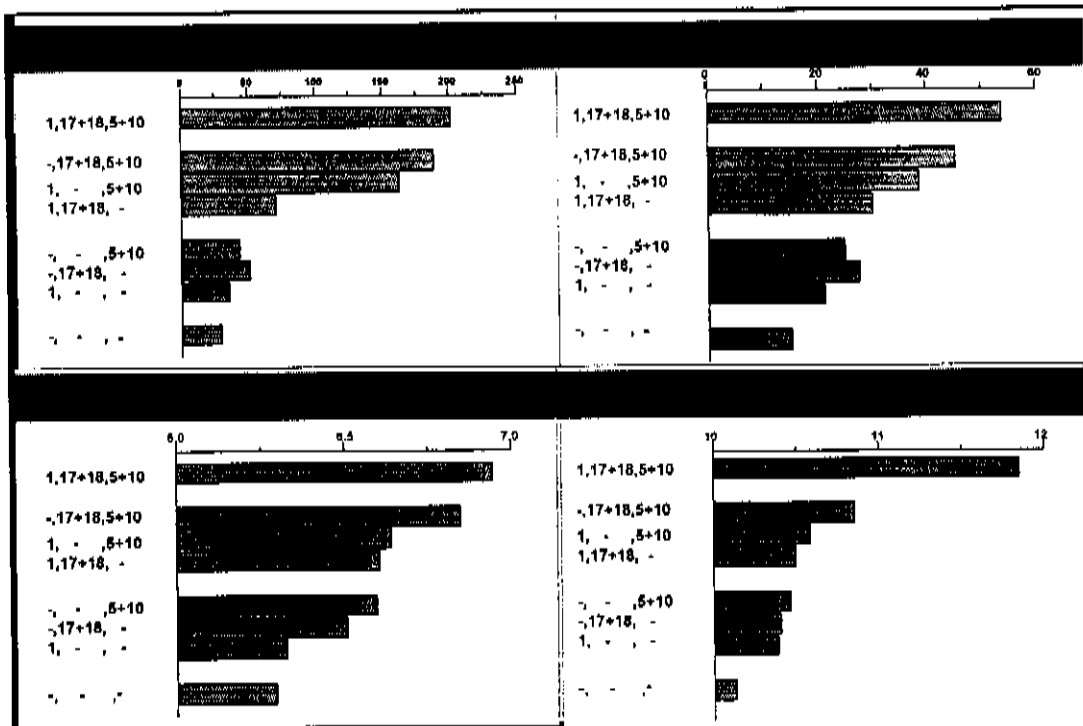


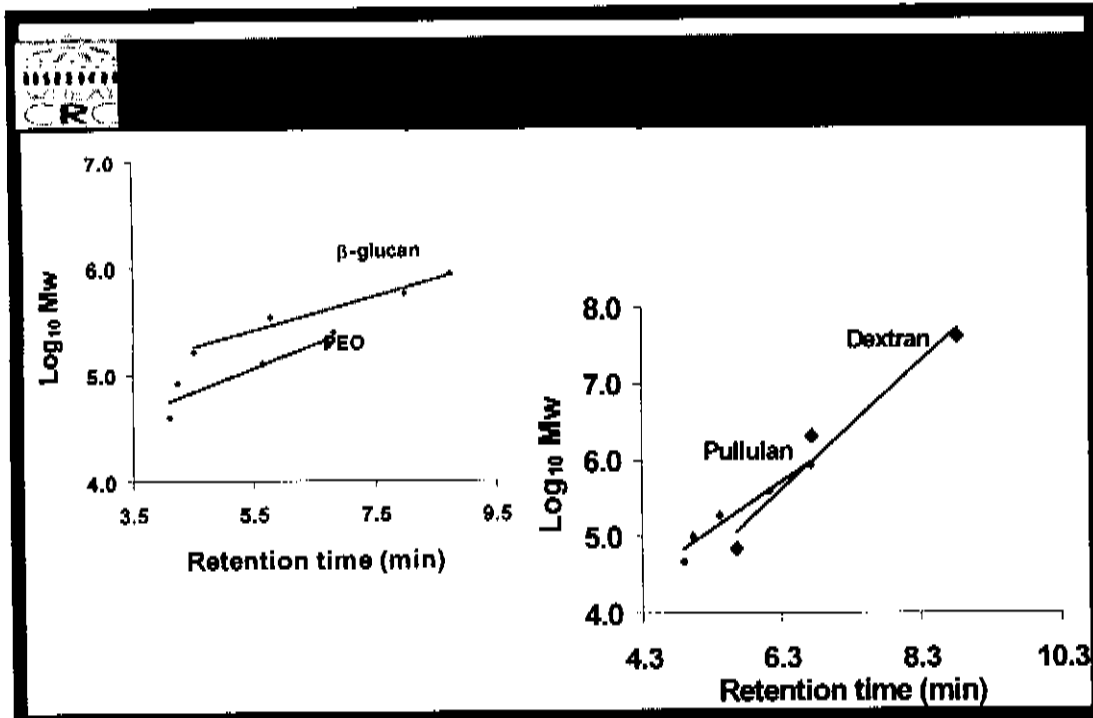


**Correlation coefficients between molecular size distribution of polymeric protein extracted from eight wheat cultivars and mixograph parameters**

	<i>MT</i>	<i>PR</i>	<i>RB</i>	<i>Ave. size</i>	<i>%EPP</i>
<b>MT</b>	<b>1</b>				
<b>PR</b>	0.098	<b>1.000</b>			
<b>RB</b>	-0.062	<b>-0.753</b>	<b>1.000</b>		
<b>Ave. size</b>	<b>0.746</b>	0.006	-0.314	<b>1.000</b>	
<b>%EPP</b>	<b>0.897</b>	-0.061	-0.005	<b>0.714</b>	<b>1</b>

The Bold values show the strong correlation between molecular size and Mixing time





Samples	Amylose content %	Elution time (min)	Molecular weight (kilo Daltons) by references to			
			β-Glucan	Dextran	Pullulan	Poly-ethylene oxides
Waxy TE-7	0.3	7.6	340	6180	3280	556
Waxy EH-4	1.6	7.8	363	6650	3940	583
Sunlin	22.1	7.6	324	4350	2890	538
Klasic	22.6	8.0	411	10600	5540	636
Spear	25.4	7.8	366	8850	4030	586
Sunstar	26.8	7.4	300	3260	2340	509
Mintou	28.9	8.0	402	9700	5190	626
Katepwa	31.4	7.6	317	3990	2710	529
Waxy Maize	<1.0	8.3	473	17800	8110	702
Amylopectin	0	8.1	430	12600	6240	656



- 1. Each standard set of carbohydrate polymers had a different linear relationship between molecular mass and retention time.**
- 2. Carbohydrates containing similar molecular weight give different retention times, demonstrating that FFF distinguished differences in shapes of biopolymers as well as sizes.**
- 3. The molecular weight estimates for cereal starches depend on standards retention times.**
- 4. Four minutes sonication gave complete solubilization of starches with <22% amylose content.**
- 5. Greater sonication time will decrease apparent molecular size of starches.**

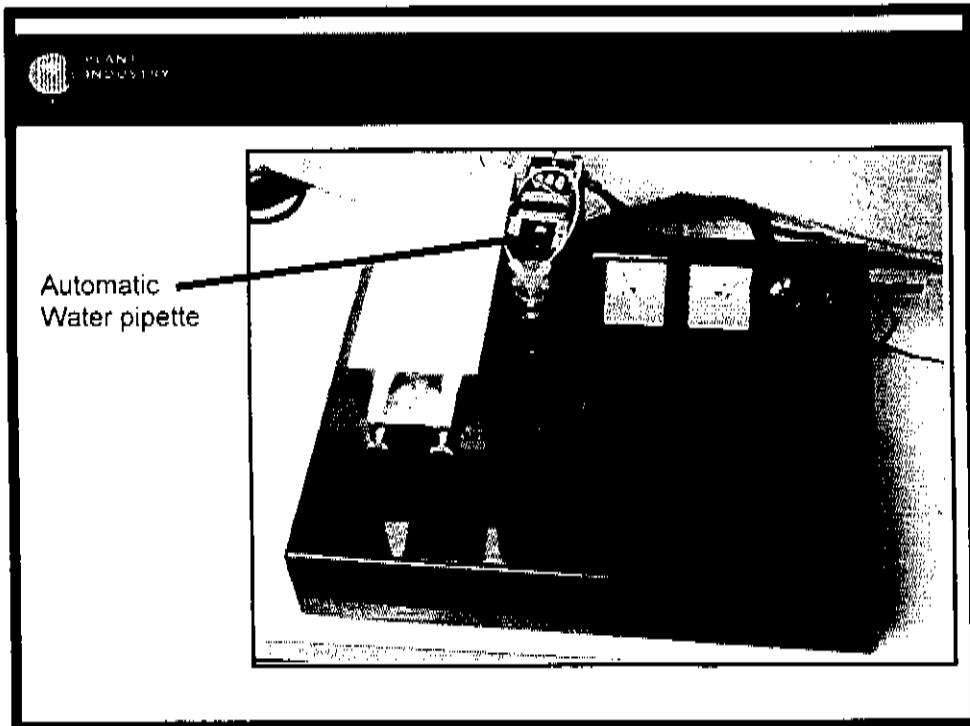
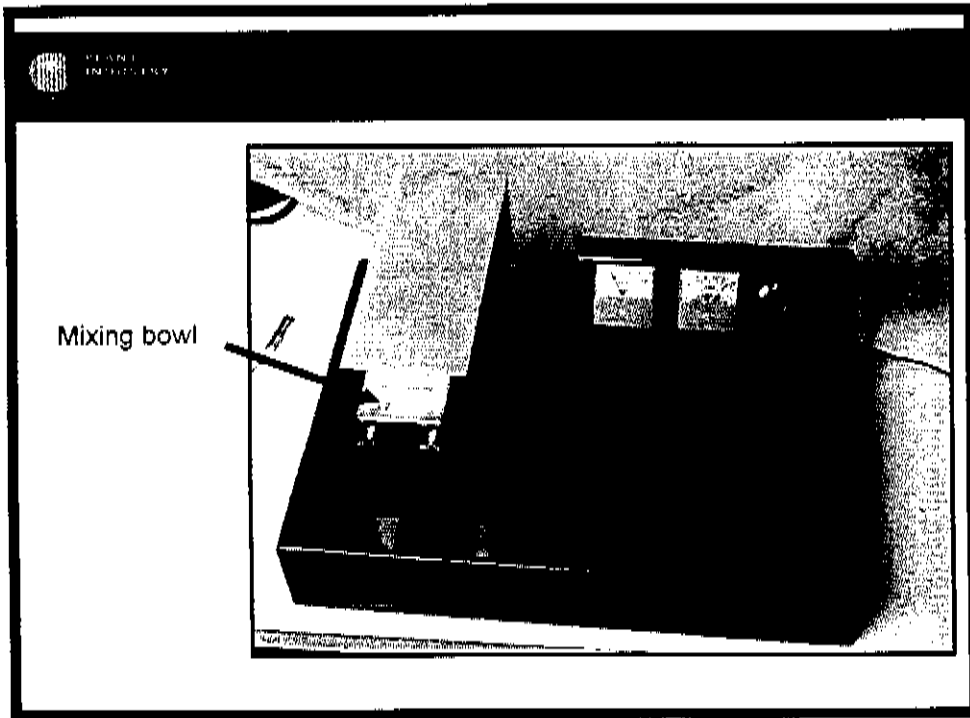


- Applications**
  - relationship between the size distribution of polymeric glutenin and its subunit composition**
  - the effect of size distribution on the non-linearity of certain quality traits in flour blends**
  - QTL analysis of size distribution**
  - relationships between quality attributes (MT, Rmax, Ext,) and size and shape of biopolymers**
- Mathematical modeling with Dr. R. Melnik (CSIRO Mathematical and Information Science).**



# **Micro- Z arm mixer**

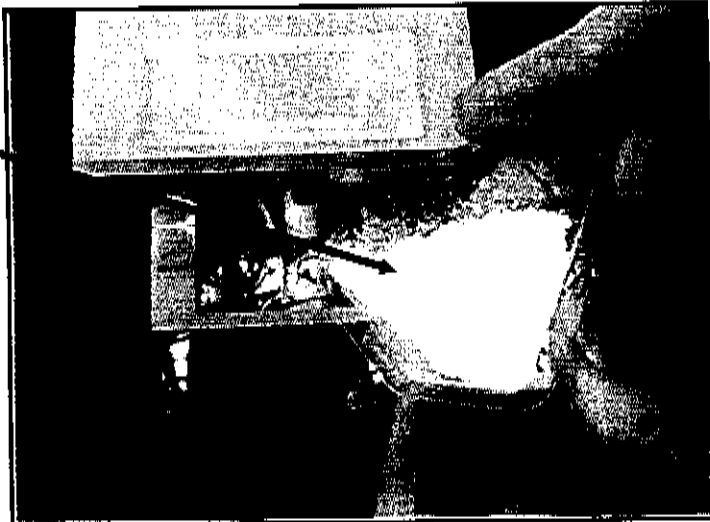
**Dr Peter Gras**

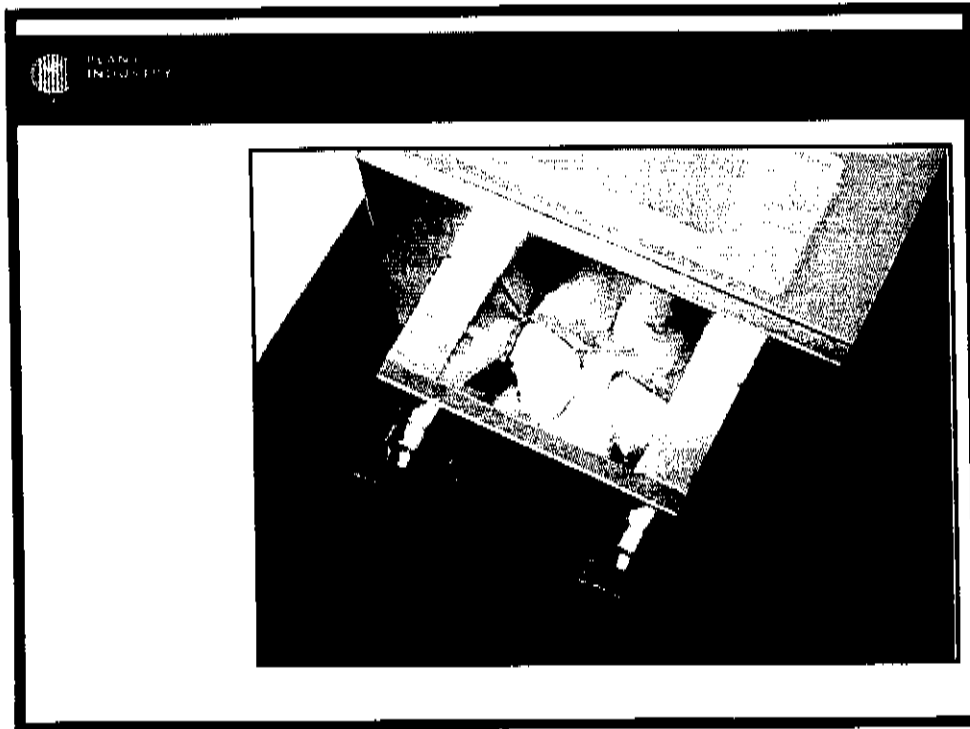
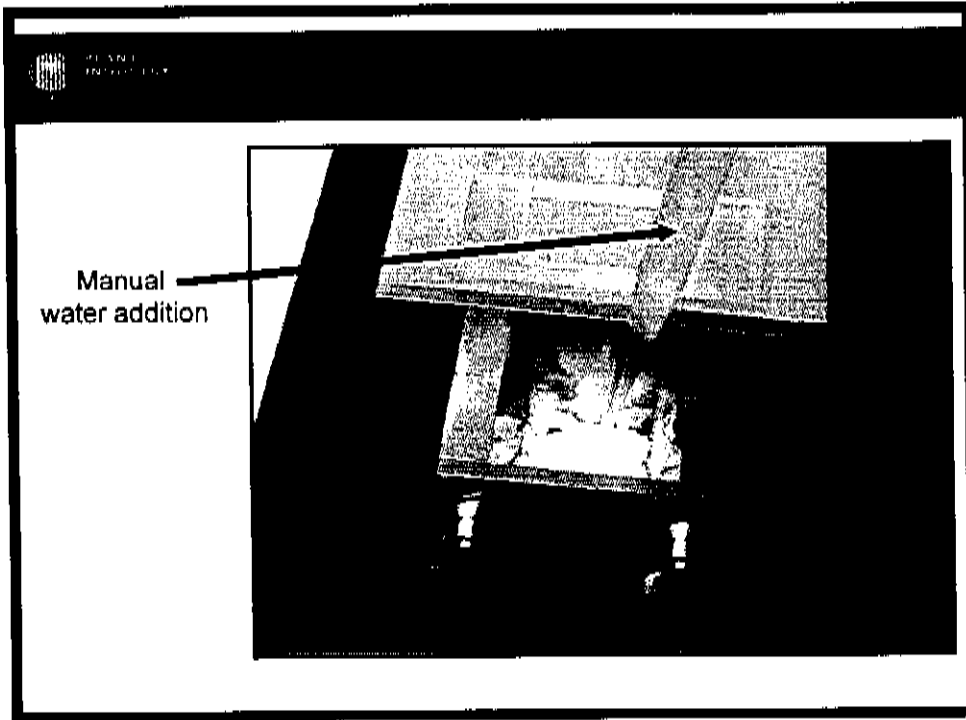


Mixing bowl



Flour addition  
(4.0 gram)

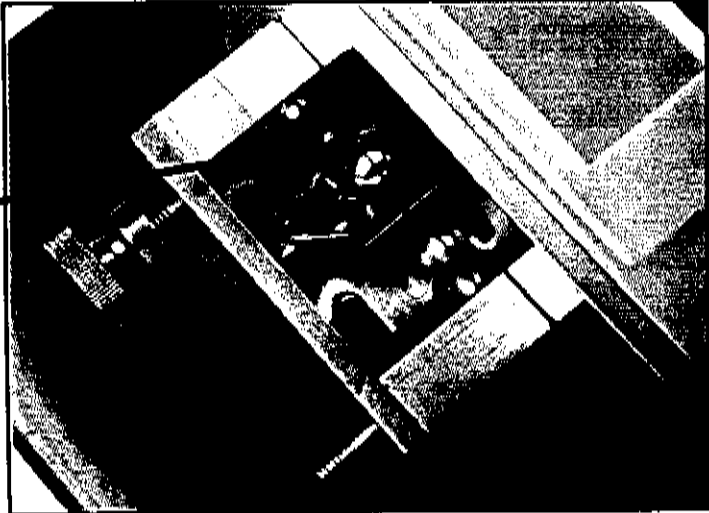




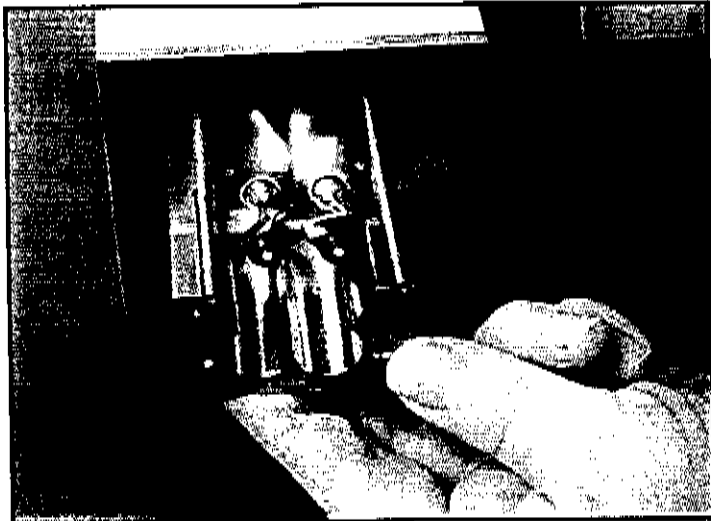


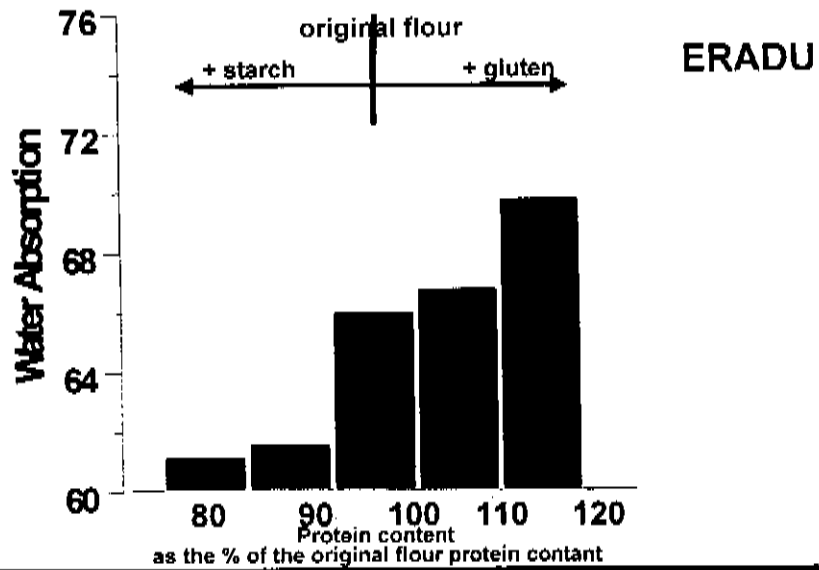
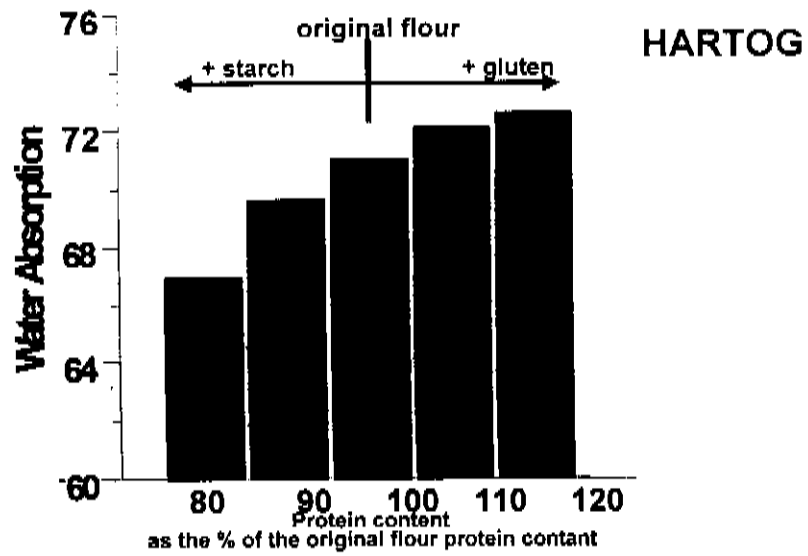
PLAN  
INDUSTRY

Mixing bowl



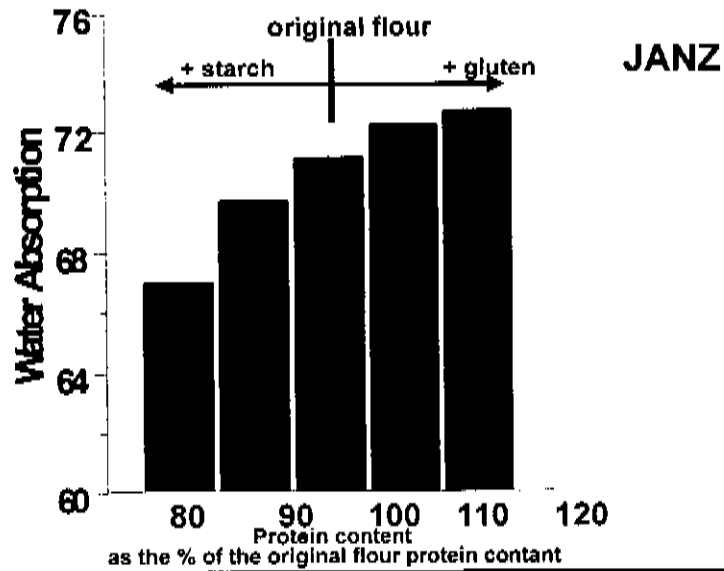
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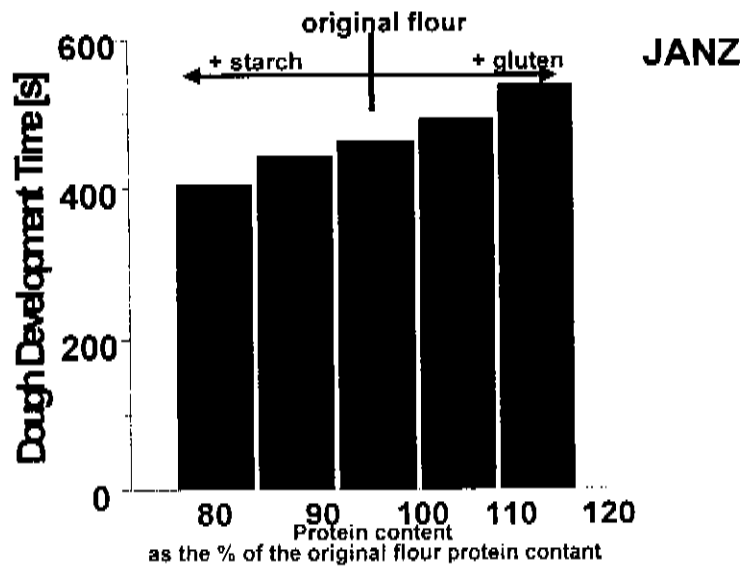


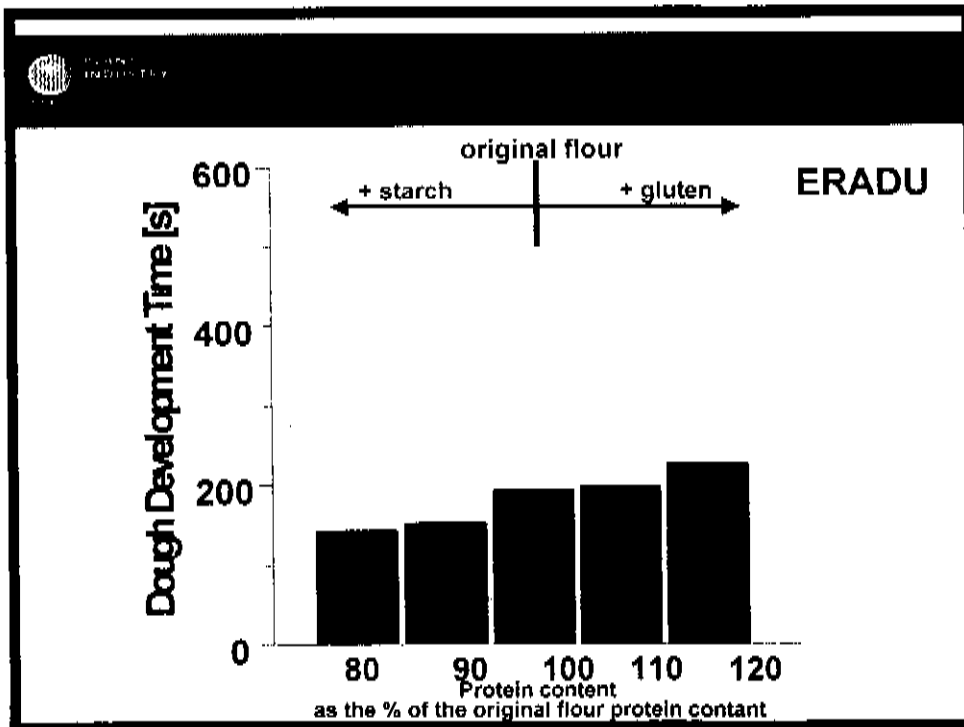
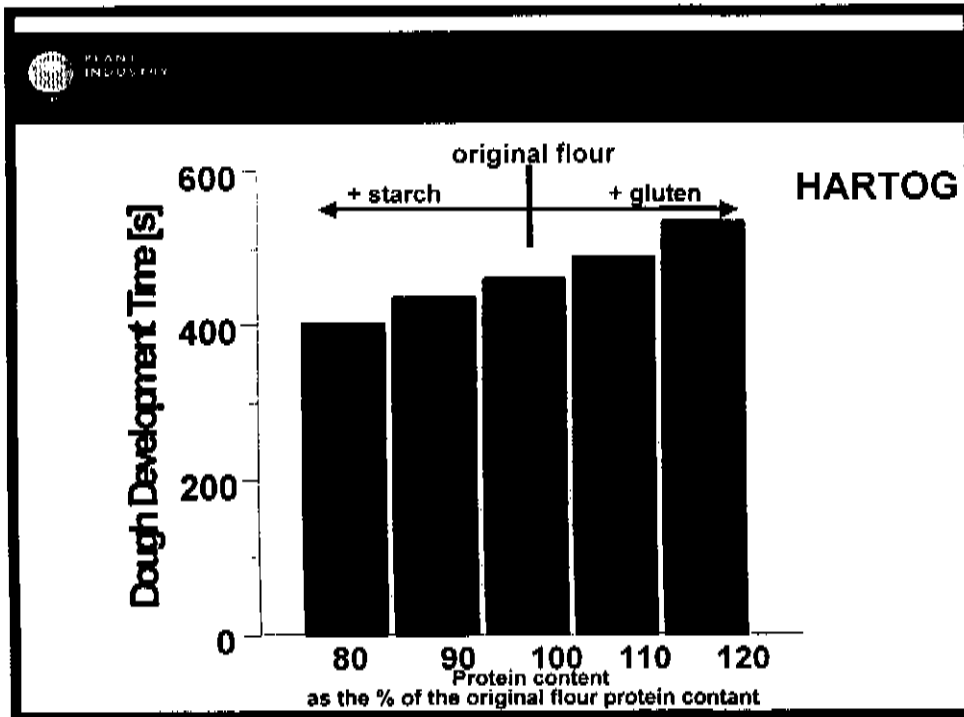


FLOUR  
INDUSTRY



FLOUR  
INDUSTRY



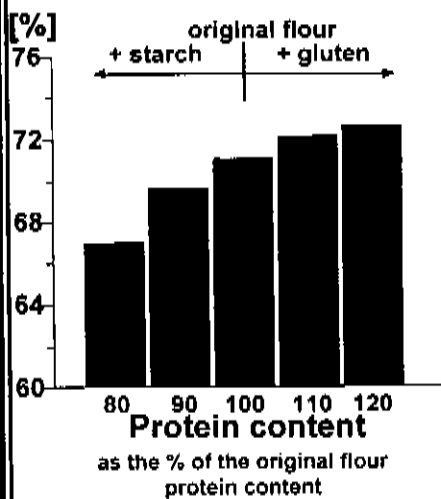




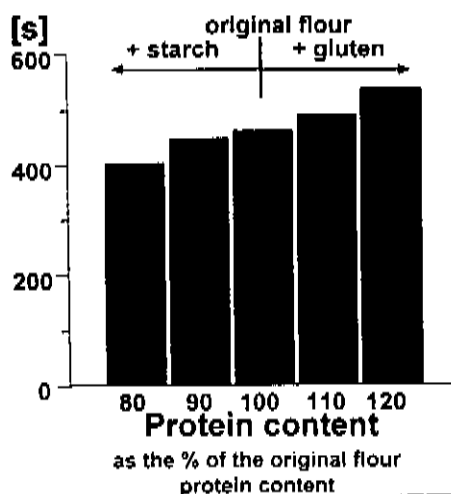


FLOUR  
INDUSTRY

### Water Absorption

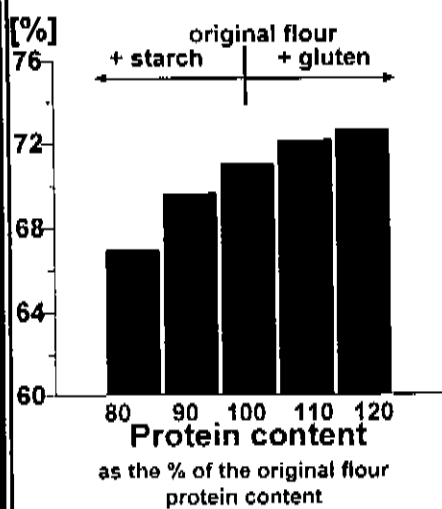


### Dough Development Time



FLOUR  
INDUSTRY

### Water Absorption



### Dough Development Time

