

Our starch purification process is being expanded to include starch and protein modification. We are also developing a major research collaboration on protein amyloid fibrils, which form unusual protein structures. Further research bulletins will inform you of developments.

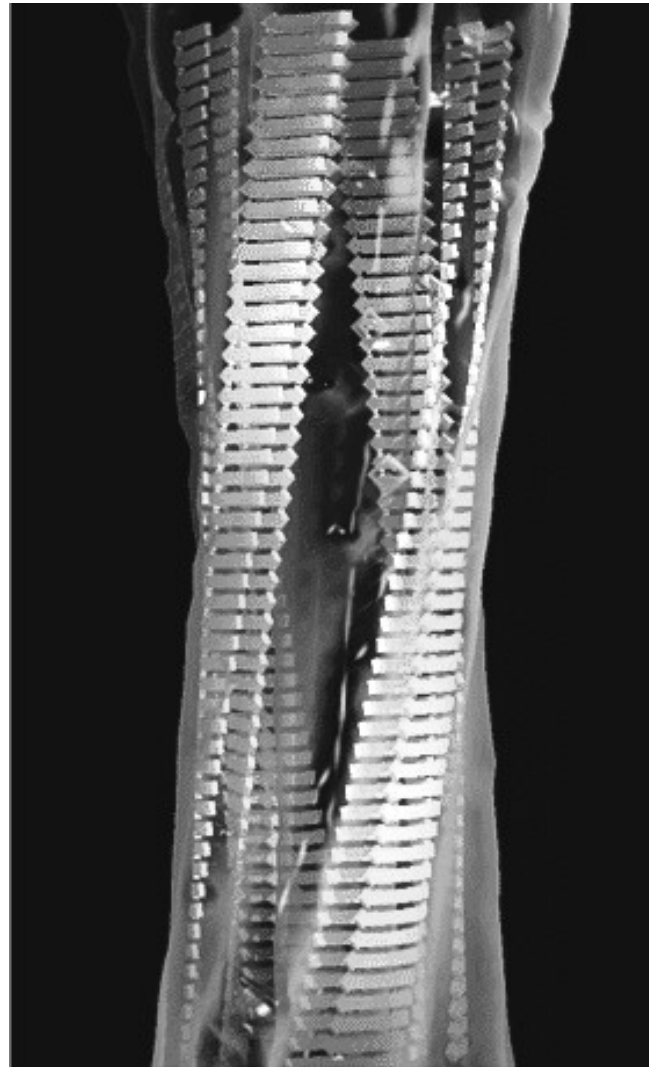


Figure 6: A model shows how proteins join to form fibrils.

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RESEARCH BULLETIN

Flour milling and baking

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Cereal science at Crop & Food Research: past, present and future

This Research Bulletin reflects on the cereal science research performed in the laboratories at Crop & Food Research and its predecessors over the last 15-20 years. It concentrates on research on the molecular constituents of grain and uses examples to emphasise the diversity of research topics. The text is based on a presentation to the RACI 2002 Cereal Science Conference. The research is covered in three periods, the years to 1997, 1997-2002, and the future.

1985-1997: Relating processing quality to composition: the beginning of the molecular level approach

From the mid-1980s the general goal of our research was to relate the processing quality of flour products to their molecular composition. In other words, if a particular flour is superior (or inferior) to the average, what are the particular protein types and other molecules causing this quality difference?

An early example of this approach was our studies of the phenomenon of 'bug damage'. Bug damage causes severe quality deterioration in baked goods by breaking down the gluten protein. In severe cases, usually following very dry seasons, bug damage can liquefy the crumb structure, causing slicing problems. The damage is caused by an enzyme in the saliva of a native New Zealand insect, *Nysius huttoni*. The enzyme's proteolytic action affects all of the gluten proteins but is highly specific for the high

molecular weight (HMW) subunits of glutenin. Similar protein subunits occur in *Nysius's* natural food source, a New Zealand native grass.

For many years an important research topic has been the difficult relationship between protein content and baking/processing quality. Investigations correlating the presence or absence of particular HMW glutenin subunits with baking quality were carried out in the late 1980s, in parallel with similar work in the UK. Several high quality New Zealand wheat varieties perplexed cereal scientists because they produced better bread quality than was predicted from their combination of protein subunits, especially the (2+12) HMW glutenin subunit pair. The high quality of these varieties was eventually explained when HPLC analyses found they had higher levels of subunit 7 than the UK varieties.

The development of small scale tests for protein quality intensified during the 1980s as the cost of test baking breeders' wheat lines increased. Attempts to develop quantitative gel electrophoresis were unsuccessful. However, we used early HPLC methods to predict the test bake loaf volume for a wheat from its content of HMW-glutenin subunits, despite the poor resolution of the method.

Around this time, several new separation methods for cereal proteins arrived on the scene, including HPLC, capillary electrophoresis (CE) and 2-dimensional PAGE (polyacrylamide gel electrophoresis). These new methods greatly improved resolution so individual protein subunits could be distinguished. Suddenly, subunits that had seemed to be homogeneous by SDS-PAGE could be resolved into even smaller sub-groups by RP-HPLC or CE. However, these new methods and their increased resolution were somewhat of a double-edged sword because wheat protein chemistry suddenly seemed even more complex. Around the same time, the development of the complementary size-exclusion HPLC technique enabled us to separate aggregated glutenin proteins with high resolution. This was a breakthrough because it enabled cereal scientists to study gluten proteins in terms of their polymer chemistry. The significance of this is that much of the rheological behaviour of doughs can be explained by their elastic nature, which is essentially a property of polymers. This breakthrough thus aligned protein chemistry with rheology and provided new insights to explain dough behaviour. This approach continues to bear fruit.

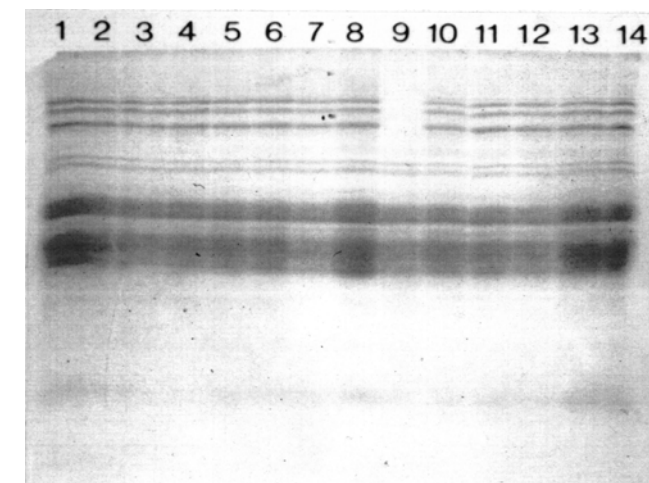


Figure 1: Electrophoresis shows bands of proteins are missing in a bug damaged sample, number 9.

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Other projects investigated various effects of protein composition. For example, a study of the effect of grain kernel weight on flour quality found that the reason that high kernel weight grains generally produce higher baking quality flours is that larger kernels contain higher concentrations of storage proteins.

The reduction of bread dough work inputs was a strong direction in industry-related research since the late 1980s. This was just about a U-turn because until that time our aim had been to increase quality, which usually means increasing work input. However, by the mid-1980s some processing problems were appearing, particularly overheating of doughs. We then returned to study in more detail how protein composition affects product quality. About this time we also developed a continuous dough mixer.

We also collaborated with CSIRO Plant Industry during the mid-1990s in an investigation of the relationships between the application of nitrogen and sulfur fertiliser, wheat protein composition and the work input of the resulting flour. This study showed that high quality flours with lower work inputs could be produced by controlling the timing of fertiliser applications and so controlling the deposition of certain protein groups in the grain.

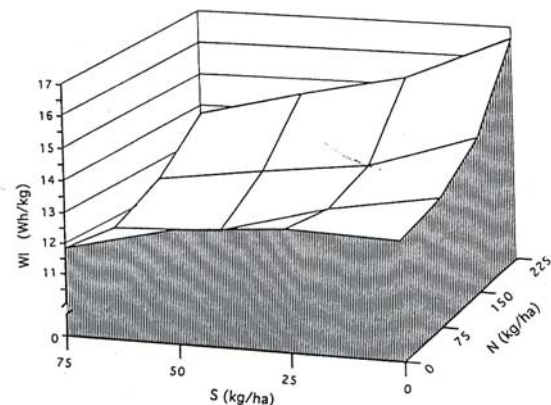


Figure 2: A graph of bread dough mixing work input versus amounts of S and N applied shows that work input is reduced by applying sulfur.

Another collaboration with CSIRO Plant Industry found that the key protein groups quantified using antibody techniques (ELISA) correlated well with the results of HPLC analysis. The ELISA method is more suitable for testing the large numbers of samples in breeding programmes and has since been developed into a commercially available test for wheat breeders.

1997–2002: High-value foods, process technologies and biomaterials

Since the late 1990s our research programmes have diversified from the traditional focus on wheat milling and baking. This was partly because government funding

priorities shifted towards research capable of creating novel industries. We continued research that aimed to generate greater value for the milling and baking industry. However, the emphasis shifted towards research on raw materials, food processing technologies for producing high-value foods, and using grains as a source of 'biomaterials'.

Protein cross-linking and its relationship to product quality was a major theme in the mid-late 1990s. Our research into the ascorbic acid improvement of bread found that some of this effect resulted from protein cross-linking. Much of our research then concentrated on the ability of small carbohydrates to link to proteins. We found that ascorbic acid and its degradation products can cross-link both model proteins and HMW-glutenin subunits to form protein polymers. Non-disulfide reactions are important, in addition to the well-known sulfhydryl-disulfide reaction. We found that cross-linking enzymes, such as transglutaminase, can greatly improve the quality and storage life of bakery products, particularly laminated pastry products.

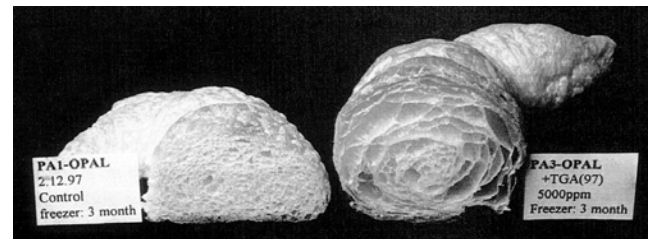


Figure 3: TGA improves the quality of frozen croissant dough.

Our research into the ascorbic acid improvement of high-value laminated pastry products expanded to investigate the role of redox chemistry in the dough making process, a collaboration with a research group at the University of Reading in the UK. This research suggested a new hypothesis for the ascorbic acid improvement of dough and showed that this was part of a larger redox chemistry cycle involving the enzyme protein disulfide isomerase. The mechanism also sheds light on the role of ascorbic acid in plants, which has puzzled researchers for years.

Another area of intensive research studied how the separation of streams of flour during milling affected their molecular composition and consequently their processing quality. Our investigation showed that various types of proteins and carbohydrates in flour were strongly segregated during the milling process. This segregation was strongly linked to differences in processing quality in a range of products. Since then we have found that this approach can be fruitfully applied to industrial processes so that individual flour streams can be used to improve specifications for product formulation.

Since 1997 we have investigated the use of sheeting technology to develop bread doughs. This is closely related

to the research to reduce work inputs because sheeting can develop doughs using much less energy than traditional Tweedy-type mixers. Various results support the idea that a great deal of the energy used in MDD developers is merely wasted as heat. The aims of our research were to determine just how little energy is actually required to develop doughs and how that energy affects protein chemistry in sheeted and Tweedy-mixed doughs. We found that sheeting can be used to develop bread flour doughs with about 1% of the energy used in a typical Tweedy-type mixer. The changes in the dough protein depolymerisation were similar for the mixing and sheeting processes. However, these doughs differed somewhat in their redox chemistry. The proteins in doughs made by high speed mixing contained much higher levels of exposed thiol groups than sheeted doughs, showing that proteins in MDD mixed doughs are changed much more than in sheeted doughs.

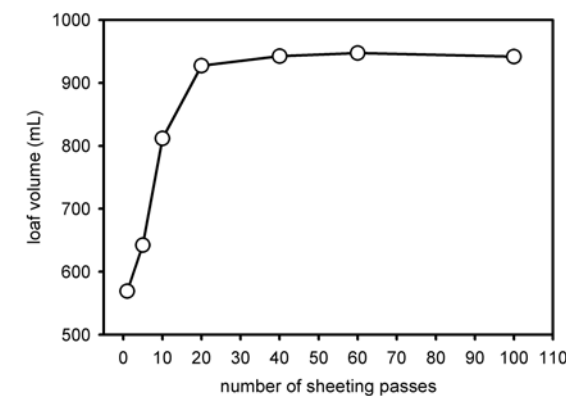


Figure 4: A graph of loaf volume versus the number of times dough has been sheeted shows that excellent bread volume is obtained after dough has been sheeted 20 times.

A related topic was research on the bubble structure of doughs and food products using confocal microscopy. This technique uses fluorescent dyes to form an image of the three-dimensional structure of "living" systems, without requiring the extensive sample preparation, freezing and fixing of conventional microscopy. This work was carried out during a Quality Wheat CRC Summer Studentship. The technique was used to form images of gas bubbles in a bread dough as it fermented. Subsequently, image analysis techniques have been used to follow and calculate the growth and decay of bubbles.

One of our new research directions since 1997 is a 'biomaterials' programme. This programme aims to use our expertise in ingredient chemistry and processing technologies to develop novel high-value products, mainly from New Zealand-sourced 'natural' (biological) starting materials, such as horticultural products. Already, we have successfully created excellent protein-polysaccharide emulsifiers by conjugating them in a solid state Maillard reaction. Conjugates produced from sodium caseinate and pectin are excellent emulsifiers and outperform traditional

emulsifiers such as gum arabic and glycerol monostearates in both emulsification power and stability.

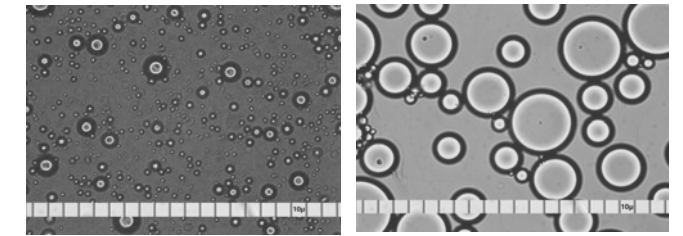


Figure 5a, 5b: Emulsions made with our new 'Emul-8' conjugate (a) had finer droplets than a sodium caseinate control (b).

We have patented a process for producing almost 100% pure starch from several plant materials without using solvents or acid-alkaline extractions. We have also carried out a detailed investigation of the structure of the key enzyme in the biosynthetic pathway for lysine (dihydrodipicolinate synthase). This could be considered basic (or pure) research work. However, work with mutant enzymes shows that it should be possible to control lysine biosynthesis in plants. This would be nutritionally significant because lysine is a limiting amino acid in many grains. The ability to control plant proteins would also expand the range of starting materials and provide more processing options for producing biomaterials.

2003 onwards: Future directions...

The immediate future presents opportunities in a number of research areas. In the grain foods area we are studying the relationships between food components and health effects of cereals in order to construct a more complete picture of cereal functionality, particularly of flour streams.

We are also looking at new industrial processes for producing flour for niche markets, for example, flours with additional health benefits and reduced environmental impacts. Working alongside the germplasm development researchers, we are developing novel, high quality wheat varieties with greatly reduced dough mixing energy requirements. A parallel project on the industrial processing front aims to develop new mixing and dough processing technologies to take full advantage of these new varieties. Unfortunately, we cannot explain these in more detail because we hope to generate patents from these projects.

In the food structure area we are developing confocal microscopy techniques for dough and food systems, including methods for imaging and measuring 3-dimensional features in confocal images. On a more fundamental level we are working to investigate the bubble structures of food systems and emulsions.

Our biomaterials research is progressing in many directions. Protein-polysaccharide conjugates present many possibilities, with the promise of novel emulsifiers.