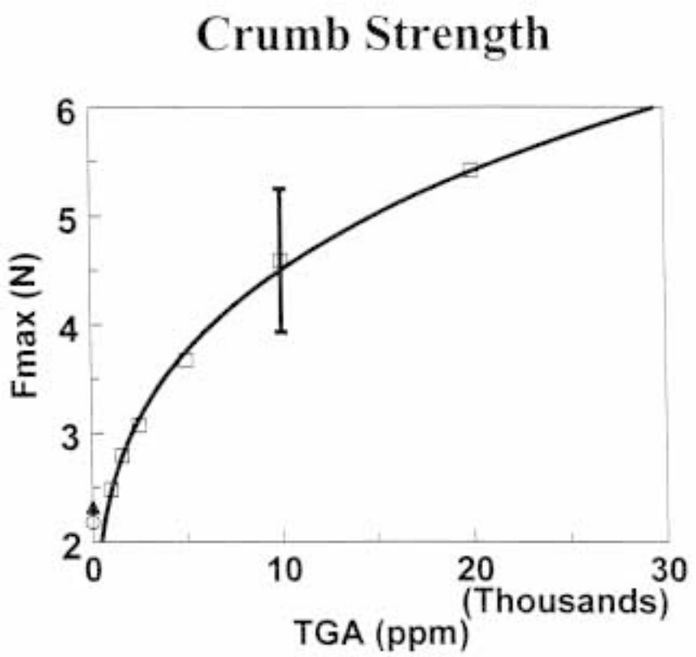

Figure 8: the effect of TGA on the crumb strength of white pan bread. (The o represents the crumb strength of control bread, the •, the crumb strength of ascorbic acid treated bread, and the 9, the crumb strength at increasing doses of transglutaminase.)

TGA has also been shown to improve the crumb strength of white pan bread, as shown in Figure 8.

Conclusions

The commercial potential of TGA as a flour improver is still being investigated, but clearly represents an exciting development for the baking industry. This discovery of a new class of flour improver emerged from fundamental studies of the chemistry of protein crosslinking in dough. Such improvers do not depend on oxidation. Therefore, they should solve an important technological problem for the industry, and result in cheaper, more efficient bread making processes. We are optimistic that future scientific studies may yield more results with commercial applications.

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This Research Bulletin is published to convey research findings to the flourmilling and baking industry. The Grain Foods Research Team of Crop & Food Research, Private Bag 4704, Christchurch, NZ, ph 03 325 6400, fax 03 325 2074.

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Understanding protein crosslinking - New approaches to flour improvers?

Understanding the chemical reactions that take place in dough is a challenge. In 'test tube chemistry' the chemist limits the 'ingredients' and controls conditions strictly. In contrast, the chemical reactions taking place in dough are complicated by the large number of ingredients and varying conditions of temperature and water level. This makes the reaction brew incredibly complicated so it is no surprise that some of the chemistry involved remains poorly understood. However, detailed examination of the chemistry that occurs in these systems can lead to new insights that, in turn, assist in the quest for new flour improvers. Some of the Grain Foods Research Team (GFRT) have been examining a specific aspect of dough chemistry - crosslinks between gluten proteins. This bulletin describes some of the fundamental science that has been carried out during Siân Fayle's PhD studies, a collaborative project between GFRT and the University of Canterbury. We also describe how this apparently theoretical or 'blue sky' research relates directly to dough chemistry, and how this understanding has enabled us to discover a new class of flour improver for the baking industry.

Introduction

Most flour improvers were discovered by trial and error and added to dough because they achieved a desired effect. How they achieved this effect was less important to bakers, who understandably focused on the end product. When scientists looked at the chemicals that were successful flour improvers, such as potassium bromate, they noticed that they were often oxidising agents. So, how do these oxidising improvers work?

Product quality is often associated with glutens, the protein component of flours, and gluten proteins contain chemical groups (thiol, -SH groups) that react with oxidising agents. Therefore, scientists have generally attributed the effect of oxidising improvers to their oxidation of the thiol groups of proteins, as shown in Figure 1. The important thing to notice is that the improver has 'stitched together' the proteins with crosslinks, resulting in larger molecules. This has explained the improvement in product quality at a molecular level.

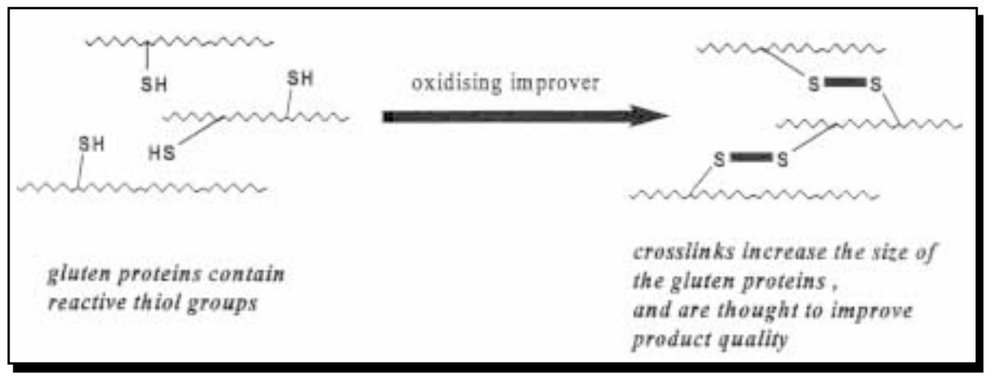

Figure 1: Proposed action of oxidising improvers on gluten proteins.

Ascorbic acid

Ascorbic acid (vitamin C) is a common food additive that consumers accept and has been used as a flour improver for many years. It remains present in most commercial flour improvers. Unlike the improvers discussed above, ascorbic acid is not an oxidising agent. How does it work?

When mixed with the oxygen in the air, ascorbic acid is converted to dehydroascorbic acid, DHA, which is an oxidising agent. Until now, scientists have assumed that ascorbic acid is converted to DHA during mixing and that DHA acts by the mechanism described above for oxidising improvers, and crosslinks gluten proteins. Scientists have shown that if ascorbic aid is converted to DHA in a test tube and then added to dough, the improving effect still functions, Figure 2. One of the problems with this process is that it is reversible. Commercial bakery doughs therefore require intensive mixing and oxidising improvers to keep the crosslinks intact.

DHA is a very reactive molecule that could react with lots of other substances in a system as complicated as dough. Siân's PhD focused on the protein component of a flour. She asked the questions, "Does DHA react with proteins in other ways and, if so, do these reactions give us any insights into the action of ascorbic acid as a flour improver?"

How to study protein crosslinking

Tracking chemical reactions in a dough is very difficult, so Siân modelled the reactions of DHA and gluten proteins in a test tube. She developed a method to study the chemical reactions of proteins that lead to crosslinking. Since the crosslinking reaction increases the size of the protein molecules, the products of the reaction were studied using a technique that separates proteins according to molecular size. One method uses an electric current to move the proteins through a material that acts like a molecular 'sieve', a method known as electrophoresis.

Figure 3 shows a typical result. The photograph on the left shows protein that has been heated in a test tube without DHA and then put through the 'sieve'. Samples removed at various times (1,2,3) are all the same size, showing that no crosslinking reactions have occurred. The photograph on the right shows that a very different pattern results when protein is reacted with DHA. The size of the molecules increases with time. This is consistent with the proposed mechanism of DHA acting as a protein crosslinking agent.

Siân also made the discovery that the same pattern resulted whether or not thiol groups (shown in Figure 2) were able to react. This is significant because it shows that thiol groups are not involved in all cross linking, as previously assumed. We concluded that in addition to the role of thiol groups in the process (Figure 2), other chemical reactions must occur that produce stable protein crosslinks.

A very detailed analysis of the reaction led us to propose a new mechanism represented in Figure 4. This mechanism involves the same sort of chemistry that occurs during the Maillard browning reaction, which produces the colour, flavour and aroma of bread. We believe that these reactions with amine groups are taking place in addition to those shown in Figure 2.

While the molecular details of the crosslinking process may seem academic, they contain an important conclusion for the baking industry: flour improvement may be achieved without forming crosslinks between thiol groups.

So what?

Since any protein crosslink might improve the performance of flour, we explored other possible flour improvers that could potentially crosslink gluten proteins, but which, unlike potassium bromate and ascorbic acid, did not depend on oxidation.

Top of the list was an enzyme, transglutaminase (TGA), that was known to enhance protein crosslinking and was potentially suitable for use in the baking industry. The reaction that TGA catalyses is shown in Figure 5. Notice the similarity to the chemistry that we proposed in Figure 4.

Does transglutaminase act as a flour improver?

The answer to this question was an emphatic, yes! This research led to an extensive study of the effect of TGA as a flour improver, with very exciting results. The enzyme improver has a dramatic effect on puff pastry and croissants, as shown in Figures 6 and 7, where the only difference between the two samples is the addition of TGA to the flour.

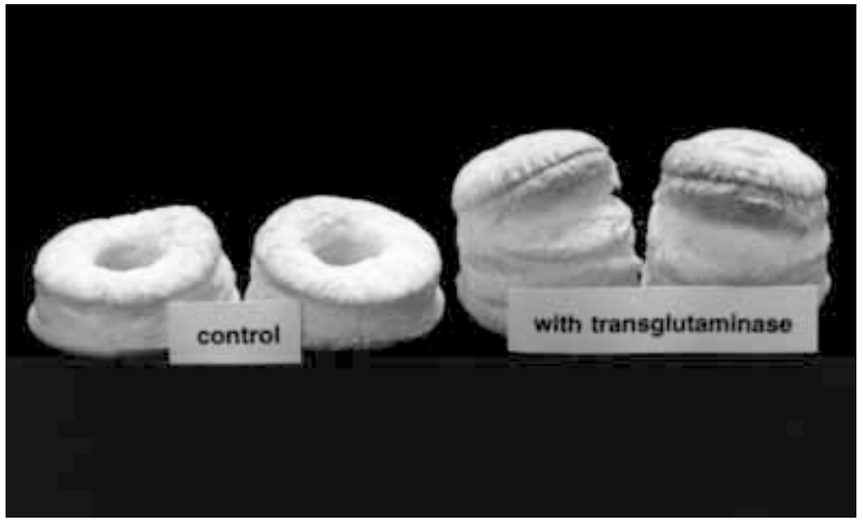

Figure 6:
Vol au vents

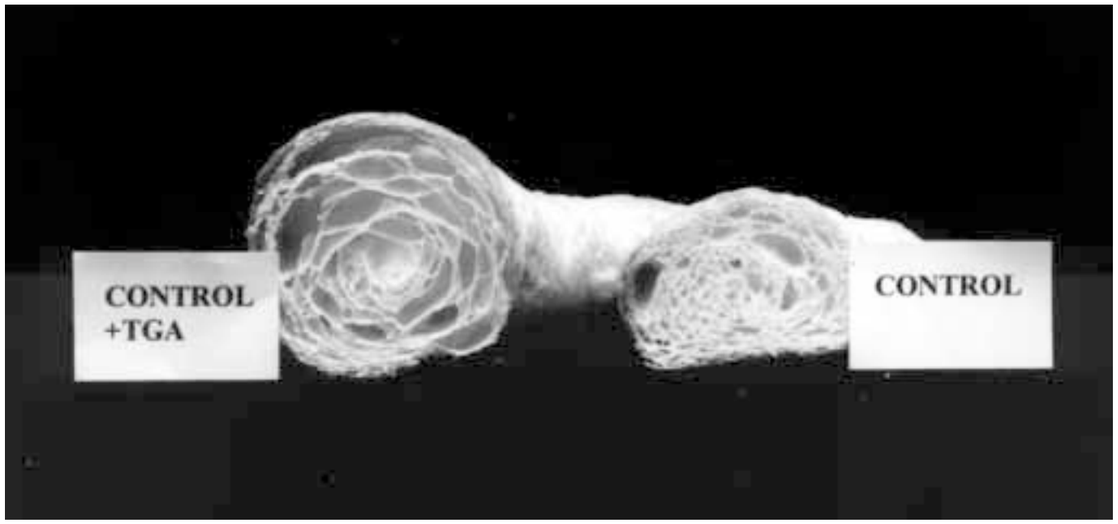

Figure 7: Croissants