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Maillard crosslinking of food proteins III: the effects of glutaraldehyde, formaldehyde and glyceraldehyde upon bread and croissants

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Abstract

The Maillard reaction influences not only the colour and flavour of foods, but also their texture. One of the mechanisms by which this occurs is via protein crosslinking. In the preceding paper, the capacities formaldehyde, glyceraldehyde and glutaraldehyde to crosslink wheat proteins were compared in vitro and in situ. All three molecules crosslinked wheat proteins in vitro, but only glutaraldehyde crosslinked the proteins when added to wheat flour dough. Here the effects of this crosslinking on dough, bread and croissants are reported. The effect of glutaraldehyde on the dough properties was marked. Upon baking, addition of glutaraldehyde was shown to alter crumb strength and texture of bread, but had no perceivable effect on croissants. A comparison to previous results, comparing enzymatic crosslinking, suggests that crosslinking of specific wheat proteins can be correlated with particular properties of cereal foods. This suggests that the Maillard reaction may be harnessed by food processors to manipulate the texture of foods.

Keywords: Protein crosslinking; Maillard; Glycation; Cereal foods; Food texture

1. Introduction

In the preceding two papers, we have discussed the importance of protein crosslinking in influencing the texture of foods and established that glutaraldehyde, glyceraldehyde and formaldehyde are able to crosslink wheat proteins in vitro. Of these, glutaraldehyde was also found to crosslink one fraction of wheat proteins—the albumins and globulins—in situ in a dough. In this paper, we explore how this crosslinking influences the properties of the dough, and also the properties of the final baked product.

Previous work in this laboratory has established that crosslinking with the enzyme transglutaminase has a large influence on the properties of bread (Gerrard, Fayle, Wilson, Newberry, Ross, & Kavale, 1998) and croissants (Gerrard, Newberry, Ross, Wilson, Fayle, & Kavale, 2000). Specifically, transglutaminase was found to increase the relaxation time of a bread dough, a specific property of dough believed to give a measure of dough development (Frazier, 1992), improve the crumb strength of white pan bread, without influencing firmness, and dramatically improve pastry lift and the volume of croissants. Recently, these effects have been found to correlate with the crosslinking of two types of wheat protein—the albumin and globulin and the SDS-insoluble glutenins (Gerrard, Fayle, Brown, Sutton, Simmons, & Rasiah et al., 2001).

The discovery that glutaraldehyde crosslinked only the albumins and globulins of dough, whilst transglutaminase crosslinked both the albumins and globulins and the HMW glutenins, suggested that we may be able to explore how particular quality parameters are influenced by specific crosslinking patterns of the wheat proteins. Glutaraldehyde had previously been reported to crosslink wheat gliadin proteins by reacting at lysine and tyrosine residues (Ewart, 1968), and to influence dough handling properties and loaf volume (Wrigley, Lee, & Gras, 1972; Simmonds, Wrigley, & Gras, 1972), but the area does not seem to have been explored since. In this paper, we describe the influence of glutaraldehyde, glyceraldehyde and formaldehyde on dough, bread and croissants, and compare these effects with those of the enzyme transglutaminase, at both the micro- and macroscopic levels.

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Nomenclature

SDS	sodium dodecyl-sulfate
ppm	parts per million

2. Materials and methods

2.1. General

Unless otherwise stated, all chemicals were obtained from Sigma Chemical Company (St Louis, MO, USA). Flour was obtained from a local mill. Doughs, bread and croissants were made according to standard in-house methods, as previously described (Larsen & Greenwood, 1991; Gerrard et al., 2000).

2.2. Dough properties

Dough properties were measured by a modification of the method of Frazier (1992), as previously described (Gerrard et al., 1998). Unless otherwise stated, crosslinking agents were added at a concentration of 100 ppm to the water used to make the dough. Glutaraldehyde was further examined using 30, 50 and 200 ppm. Experiments were repeated five times.

2.3. Bread properties

Loaf volume was measured by rapeseed displacement. The texture of the loaf was visually assessed, 1 day after baking, by a trained operator and rated on a scale of 1–11, using a Crop and Food Research Ltd in-house method. Crumb strength was measured with a Textron, using a modification of the methods of Dahle and Montgomery (Dahle & Montgomery, 1978) and Morgenstern, Newberry, and Holst (1996) as previously reported (Gerrard et al., 1998). Twelve measurements were recorded for each treatment (four replicates in each of three loaves).

2.4. Croissant properties

Croissants were assessed as previously reported (Gerrard et al., 2000).

3. Results and discussion

3.1. Effects of glutaraldehyde, glyceraldehyde and formaldehyde on dough properties

In order to quantify any changes in dough development, brought about by the addition of carbonyl compounds, dough relaxation time was measured using a modification of a method reported by Frazier (Frazier, 1992). Relaxation time refers to the time taken by dough to recover after being compressed by a standard force, and gives a quantitative indication of the degree of dough development. This method was employed as it only requires a small sample size, and is sensitive to the state of development of the gluten proteins in dough.

Non-yeasted doughs were prepared, containing one of the three carbonyl compounds at the desired concentration. Control doughs, containing either no improver, or standard doses (100 ppm) of ascorbic acid, were also prepared. The ascorbic acid-treated dough acted as a positive control, since this compound is a known dough improver with well-characterised effects (Thewlis, 1971). Each dough was mixed to its optimum work input, as indicated by the peak of the mixing curve. The relaxation times of each dough were measured periodically throughout the resting stage of the dough.

In preliminary experiments, high doses of the most reactive compound, glutaraldehyde, were added to dough to determine whether changes in dough development might occur. Glutaraldehyde, at a concentration of 5000 ppm, produced a firm, inelastic dough. After 10 min, the relaxation time of this dough was too long to be measured, indicating over-development. The dough was observed to turn a brown colour, which suggested that Maillard-type chemistry was occurring. Similarly, an over-developed dough was produced at 800 ppm glutaraldehyde. At 100 ppm, a dough with better handling properties and dough development was produced. This concentration was therefore used for all three compounds, and provided a useful comparison to the flour improver ascorbic acid, which is commonly added to dough at 100 ppm.

Fig. 1 demonstrates that glutaraldehyde significantly increased dough relaxation times compared to both the standard and ascorbic acid-treated doughs. This suggests that crosslinking, via the Maillard reaction, increased the rate of dough development. Formaldehyde also improved the relaxation time compared to the standard dough, even though no crosslinking was visible when the proteins were extracted from the dough (see preceding paper). This was somewhat puzzling, but perhaps reflects the fact that only a few protein crosslinks are required to alter dough properties, and that those forming in the case of formaldehyde are below the detection limit in our electrophoretic protocol.

Alternatively, non-crosslinking protein modifications may be responsible for the changes observed in relaxation times on addition of formaldehyde. Glyceraldehyde did not appear to significantly alter dough development, when compared to the standard dough, despite the fact that it crosslinks proteins in vitro at a faster rate than formaldehyde; this could perhaps relate to the inability of glyceraldehyde to undergo dehydration to form malondialdehyde in a dough, thereby reducing its reactivity.

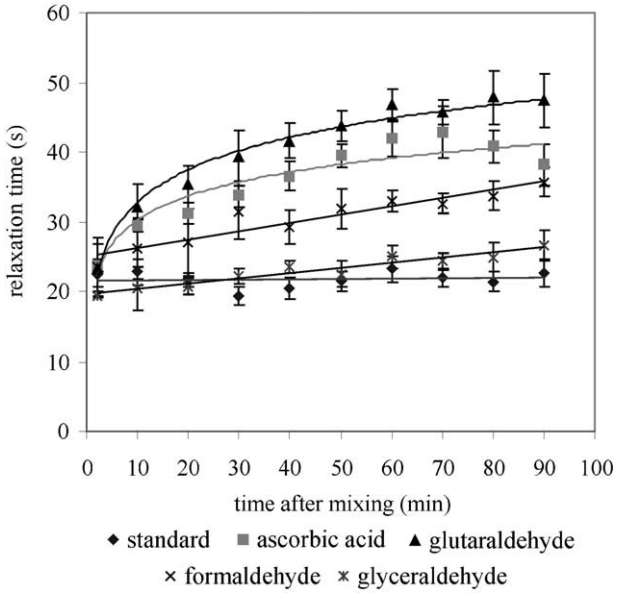

Fig. 1. The effect of glutaraldehyde, formaldehyde and glyceraldehyde on the relaxation times of dough. Each value represents the mean value of five readings. Error bars represent the standard error of the mean.

Since glutaraldehyde gave the most dramatic results, the effect of varying concentrations of this compound, on dough development, were examined. It was observed that increasing concentrations of glutaraldehyde increased dough relaxation time (see Fig. 2). The general increase in relaxation time, mediated by the addition of glutaraldehyde, compared to the standard dough, suggests that the crosslinks introduced by these compounds, via Maillard chemistry in vitro, can also form in the more complex dough system. These results are consistent with early literature reports on the effect of glutaraldehyde on dough handling properties (Simmonds et al., 1972; Wrigley et al., 1972).

The ability of glutaraldehyde to increase relaxation time compared to the commonly used flour improver, ascorbic acid, is strongly suggestive that dough development could be accelerated by Maillard chemistry, with subsequent cost reductions associated with lowering the requirements for oxidising agents and high mixing intensity.

3.2. Effect of glutaraldehyde on the quality of baked loaves

Due to the significant impact glutaraldehyde had on dough development, its effect on the properties of baked bread, was examined as a model for the possible role of the Maillard reaction in flour improvement. The three parameters chosen to examine loaf quality were: loaf volume, crumb texture, and crumb strength. Varying concentrations of glutaraldehyde were added to 125-g yeasted doughs, which were then baked according to a Crop and Food Research Ltd standard method. Control doughs contained no additive, or ascorbic acid (100 ppm).

Loaf volume was found to decrease slightly with increasing concentrations of glutaraldehyde, as shown in Fig. 3. The loaves containing ascorbic acid were significantly larger than the control loaves, which did not contain additive, and the glutaraldehyde-treated loaves.

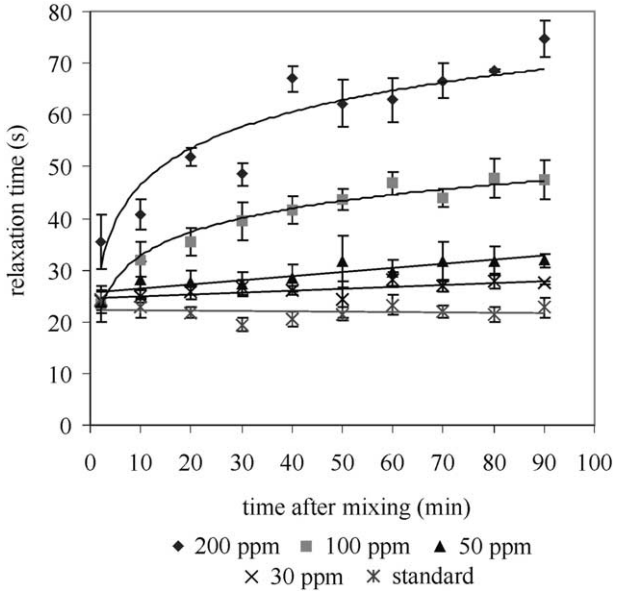

Fig. 2. The effect of different concentrations of glutaraldehyde on the relaxation times of dough. Each value represents the mean value of five readings. Error bars represent the standard error of mean.

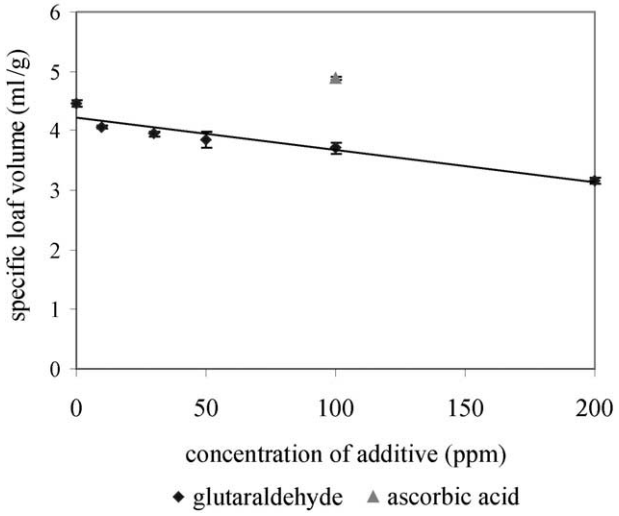

Fig. 3. The effect of glutaraldehyde on the volume of loaves of white pan bread, compared to ascorbic acid-treated loaves. Each point represents the mean specific volume of four loaves, measured in duplicate by rape-seed displacement. Error bars represent the standard error.

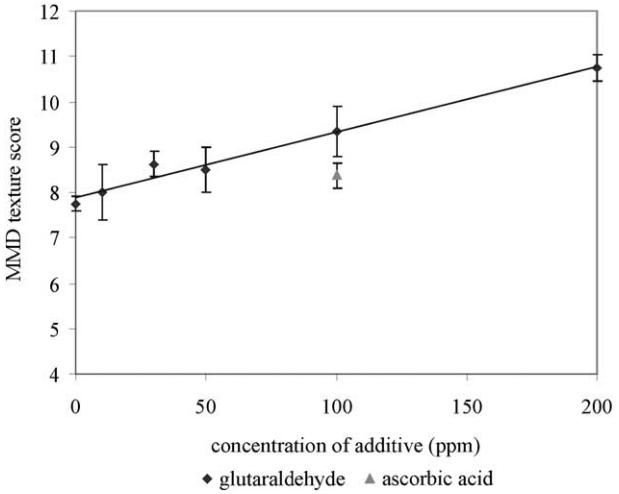

Fig. 4. The effect of glutaraldehyde on the texture of loaves of white pan bread, compared to ascorbic acid-treated loaves. Each point represents the mean loaf texture score of four loaves. Error bars represent the standard error of the mean.

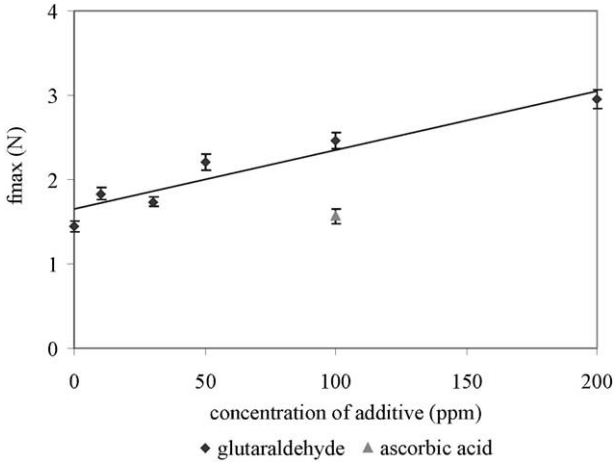

Fig. 5. The effect of glutaraldehyde on the crumb strength of loaves of white pan bread, compared to ascorbic acid-treated loaves. Each point represents the mean crumb strength measurement of four slices of bread, from three loaves. Error bars represent the standard error of the mean.

It would appear that glutaraldehyde caused the dough to become strong, but inelastic and inhibited gas cell expansion during proofing.

Consumers place value on the crumb texture of bread: hence it is an important characteristic in the assessment of bread quality. Texture is generally defined by the relative fineness of the crumb structure (Coles, 1998). The crumb texture was determined subjectively by a trained assessor using a Crop and Food Research in-house standard method. Each loaf was given a score between one and twelve, based on standardised photographs. A higher score represents a finer crumb texture. Increasing concentrations of glutaraldehyde appeared to increase crumb texture scores (Fig. 4). High doses of glutaraldehyde produced texture scores towards the top end of the scale, indicating a fine crumb structure. At 200 ppm glutaraldehyde, crumb texture was significantly improved, compared to the controls. It is recognised that the corresponding decrease in loaf volume will have contributed to the creation of fine crumb texture, due to the lack of gas cell expansion. A balance between high loaf volume and fine crumb texture needs to be achieved in order to produce loaves with high market value. Further work would be required to establish whether this could be achieved by the addition of other flour improvers, such as ascorbic acid, in concert with a Maillard crosslinking agent. Interestingly, no change in colour was observed in the glutaraldehyde treated loaves. The flavour of the treated loaves was not tested.

Crumb strength is the ability of sliced bread to withstand handling and is measured as the maximum force required to rupture a slice of bread. Lack of crumb strength is a common consumer complaint, especially with regard to fresh bread, which is difficult to butter (Gerrard et al., 1998), and is therefore an important factor in assessing bread quality. The addition of glutaraldehyde to the bread dough, at each concentration, significantly increased the crumb strength of the baked loaf, compared to the controls (Fig. 5). This suggests that the addition of glutaraldehyde results in the formation of crosslinks within the gluten matrix, adding strength to the crumb.

3.3. Effect of glutaraldehyde on the volume of croissants

Croissants containing 100 ppm glutaraldehyde were prepared according to a Crop and Food Research Ltd

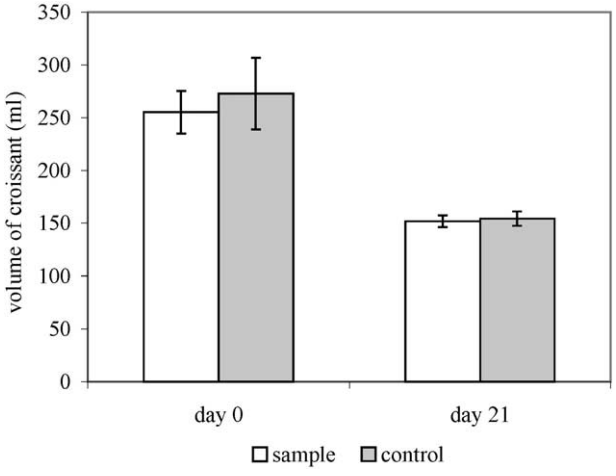

Fig. 6. The effect of glutaraldehyde (100 ppm) on the volume of croissants. Day 0 samples were prepared and baked on the same day, whilst day 21 samples were prepared on day 0 and stored at −20 °C for 21 days, before being baked. Data represents the mean of three measurements. Error bars represent the standard error about the mean.

in-house method, and compared to controls containing no glutaraldehyde. It was observed that the glutaraldehyde-treated croissant dough was thicker and stiffer than the control, and not as dry. However, upon baking, the volumes of the glutaraldehyde-treated croissants were not significantly different from the controls.

Since croissant doughs containing crosslinks introduced by the enzyme transglutaminase had previously been found to withstand frozen storage (Gerrard et al., 2000), additional unbaked control and treated croissants were stored in the freezer for 3 weeks before being thawed and baked. Again, there was no difference between the controls and treated samples. These croissants showed a large decrease in croissant volume as a result of frozen storage, irrespective of the addition of glutaraldehyde (See Fig. 6).

Thus, despite a marked effect on dough properties, and clear evidence of crosslinking in the albumin and globulin fraction, glutaraldehyde was found to have no perceivable effects on the properties of croissants.

4. Conclusion

The addition of glutaraldehyde and formaldehyde to dough increased relaxation times compared to the controls, containing no additive. This is indicative of an increase in dough development, presumably due to the introduction of additional crosslinks to the gluten network, via the Maillard reaction. This corroborates earlier work (Gerrard et al., 1998, 2000), and contributes to the growing evidence that crosslinks of any chemical nature are extremely important in dough development. The fact that the improving effect of glutaraldehyde on dough development was more substantial than ascorbic acid, suggests that the Maillard reaction, suitably harnessed, could have great potential as a mechanism for dough improvement. Whether sufficiently reactive, food-allowed, molecules, that can undergo protein crosslinking chemistry via Maillard reactions, can be generated in situ remains to be elucidated. However, the results described herein provide proof in principle, that such molecules could be used to manipulate the properties of food.

Addition of glutaraldehyde to dough, produced loaves of bread that decreased in size, with increasing glutaraldehyde concentration. These loaves were otherwise of an acceptable quality, with good crumb texture and improved crumb strength. It would therefore appear that the crosslinks produced by glutaraldehyde, strengthen the gluten network, providing good crumb strength, but reducing the ability of the gas cells to expand during proving and baking.

In contrast to the crosslinking enzyme transglutaminase, glutaraldehyde did not have a significant effect on croissant volume compared to controls. We sought an explanation for this difference in macroscopic behaviour in terms of the protein crosslinking patterns of the two crosslinking reagents. Transglutaminase has previously been shown to crosslink both the albumin and globulin fraction and the SDS-insoluble glutenin fraction of wheat proteins (Gerrard et al., 2001) and affect dough properties, crumb properties and pastry volume. Glutaraldehyde, on the other hand, was shown, in the preceding paper, to crosslink only the albumin and globulin fraction, in situ and affect dough and crumb properties, but not pastry volume. It is therefore tempting to speculate that the albumins and globulins play a role in determining dough and crumb properties, whilst the SDS-insoluble fraction, consisting largely of HMW glutenin subunits, is important in determining the characteristics of croissant pastry. This correlation between specific crosslinking patterns and particular macroscopic properties of food represents an exciting step forward in the understanding of how particular proteins influence the quality of wheat-based foods.

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