Food Chemistry **30** (1988) 231–240

Changes In Flour Thiols During Storage, and Improver Action

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(Received 5 February 1988; revised version received and
accepted 22 February 1988)

ABSTRACT

Measurements of total and accessible thiols in three wheat flours, kept at room temperature for up to six months or so after milling, showed that there was a slow loss of total thiol. The rate of loss varied with the flour. The accessible thiol fell more rapidly than the total thiol but this was partly because some became inaccessible. When a flour was treated at room temperature or at 44° C with five common additives at 10^5 ppm, no significant differences could be found by amino acid analysis compared with controls. Electrophoresis and dough tests produced no evidence for peptide chain scission caused by the improvers ammonium persulphate (APS), azodicarbonamide, potassium bromate or the bleaching agent benzoyl peroxide (BP). APS and BP caused slight crosslinking, presumably by free radicals. L-Ascorbic acid produced unexplained minor changes in electrophoretic pattern, notably an extra doublet of high molecular weight. It is very unlikely that any of these changes would be significant at the levels used commercially and the results support the idea that loss of thiol groups is the reason for both the improving effect noticed during the first few months of storage and the action of improvers.

INTRODUCTION

Wheat flour improves in baking quality during storage for several months (Kozmin, 1935; Fisher *et al.*, 1937). One explanation is that thiol groups (SH)

are slowly lost by aerial oxidation. There are, however, only a few reports on the changes of SH in flour with time. Tsen & Dempster (1963) stored flours of 8% and 14·5% moisture content for six years at 24°C. The higher-moisture-content flour lost both total and accessible SH in the first year; after that it resembled the low-moisture flour by losing SH very slowly. In both flours some accessible SH became less accessible, because the total SH fell even more slowly than the accessible. Whereas the flour of 8% moisture kept its baking quality, the other flour worsened progressively. An improving effect in the first few months of storage could have been missed, because the flours were only sampled yearly.

Bellenger & Godon (1972) noted a slight *increase* in SH in flour stored in air for 16 weeks, which is hard to explain; samples stored in sealed polythene bottles had constant SH values. Rao *et al.*, (1978) reported substantial loss of SH and gain of disulphide bonds (SS) in buffer extracts of flours milled from wheat after it had been stored for four months. The surprising gain of SS, however, may be due to contamination by mercaptoethanol: two washes specified in the original method (Beveridge *et al.*, 1974) seem to have been omitted; this would raise apparent SS values but not SH.

Yoneyama *et al.* (1970 *a*, *b*) found that storage of flour in air at 30°C caused loss of acid-soluble SH in the first 20 days with no further loss in another 70 days: an N_2 atmosphere or lower temperature greatly slowed the loss.

It was felt more data would be useful on the effect of aging on SH levels. Some tests were also done to see whether common flour additives had any action on proteins, other than by oxidising SH, since no one except Joiner *et al* (1963) seems to have studied this.

EXPERIMENTAL

Aging tests

Flours of Avalon, Galahad and Canada Western Red Spring (CWRS) wheats were used. Their protein (N × 5·7) contents were, respectively, 9·4%, 8·9% and 14·7%. During storage the flours were kept with adequate free air space in Kilner jars at room temperature (20–25 C).

Accessible SH measurement

Phenyl mercuric acetate (PMA; 2 ml of 1 mM) was measured by differential pulse polarography before and after reaction at pH 4·2 with a flour sample containing ~0·1 g protein as described by Ewart (1988), the difference giving

the accessible SH. When checked for stability against freshly prepared glutathione solution, 1 mM PMA lost $<5\%$ of its strength in 7 months.

Total SH measurement

This was done as above except that the buffer was 8 M in urea, and the flour was reduced to 0·6–0·7 g to stop the solution becoming too viscous.

Reaction with improver

Unbleached and untreated bakers' white flour (Heygates Ltd, Tring, Herts.) was used: its protein content, on an air-dry basis, was 12·2%. Soluble reagents, ammonium persulphate (APS), L-ascorbic acid (Vit C), or potassium bromate ($KBrO_3$), (4·5 g) were dissolved in water (400 ml), and flour (45 g) was sieved into the stirred solution. Azodicarbonamide (ADA) or benzoyl peroxide (BP; 4·5 g) were mixed with flour then sieved into the stirred water. The suspensions were stood overnight at room temperature, or at 44 °C, without stirring. Next day the mixture was either freeze-dried, or dialysed against water at ~ 5°C with changes then freeze-dried. Controls were done with no reagent and were stood overnight at ~ 5°C, room temperature, or 44 °C.

Amino acid analysis

The dialysed products were analysed as before (Ewart, 1987) with a 24 h hydrolysis only.

Electrophoresis

The flour samples were run on 10% polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulphate (SDS) according to Laemmli & Favre (1973), except that 2-mercaptoethanol or dithiothreitol was added to ensure at least a $10\times$ molar excess on the reagent, assuming that little or none had been lost by dialysis (as would be the case with ADA and BP).

Doughs

Flour (28 g), additive (2·8 g) and water (16 ml) were mixed in a Minorpin mixer for 1 min, stood overnight at room temperature, or at 44°C, in moisture-proof containers lined with damp filter paper and examined next morning after hand moulding.

RESULTS AND DISCUSSION

Use of acid buffer

The measurements of total SH were done at acid pH to reduce the chance of breaking labile disulphide bonds: this can occur at alkaline pH by the rapid reaction of PMA with traces of the thiol ion, S^-, produced by dissociation, so driving the dissociation forward, as for example when oxidised glutathione was shown to react with PMA (unpublished results). Bovine serum albumin, too, had higher than expected values for its SH content at pH 9.

Accessible SH

In Figs 1, 2 and 3 total and accessible SH levels are plotted against days after milling. The values are compatible with the range of 0·6 to 1·8 μmol SH g^{-1} flour quoted by Mecham (1968).

It is clear that there was a significant fall (correlation coefficient (r) −0·735 to −0·866) in accessible SH during storage for all three flours (Table 1).

Total SH

The total SH fell significantly with time for CWRS, but with Avalon and Galahad the slight negative slope was insignificant. When $\log_{10}$ days was

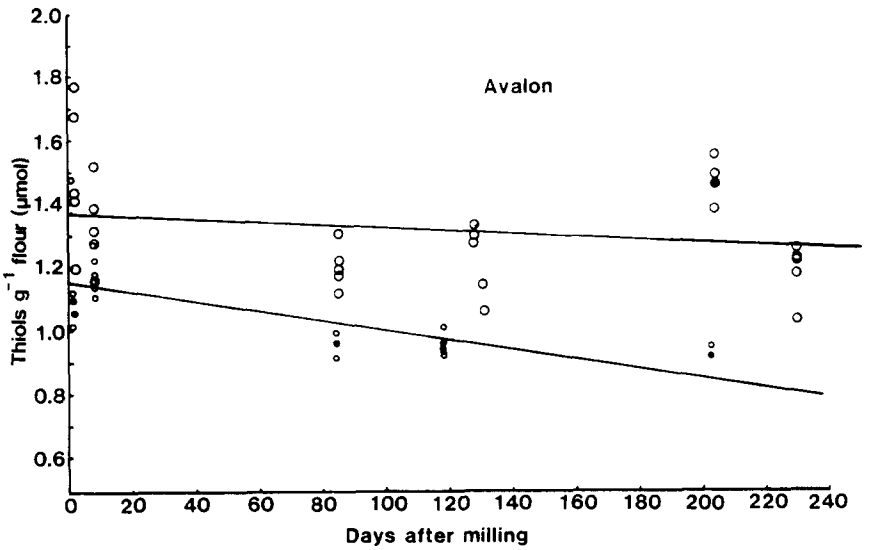

Fig. 1 Plots of total SH (○) and accessible SH (○) of Avalon flour against days after milling. The straight lines are the regression lines. Filled circles mean coincident points. Standard deviation of single titration: 0·090 (total); 0·081 (accessible).

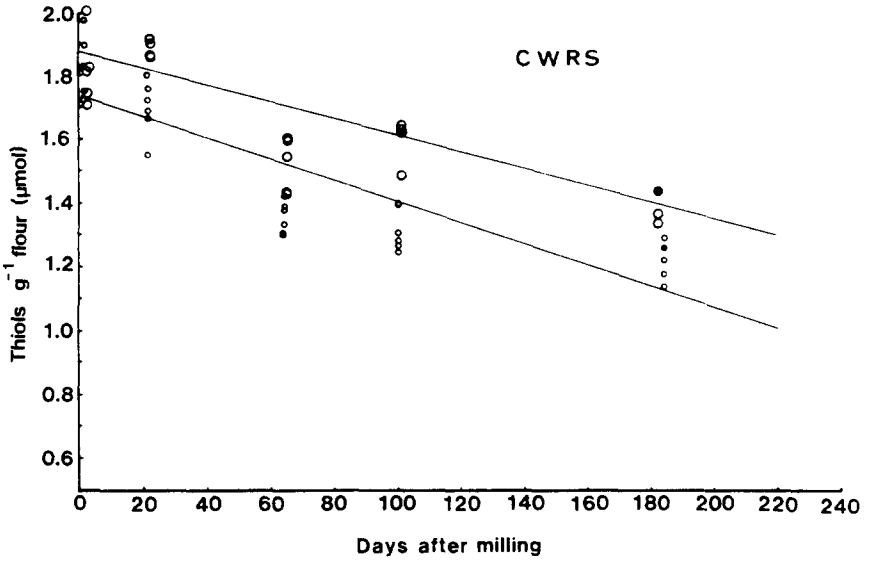

Fig. 2. Plots of total SH (○) and accessible SH (○) of CWRS flour against days after milling. The straight lines are the regression lines. Filled circles mean coincident points. Standard deviation of single titration: 0·090 (total); 0·081 (accessible).

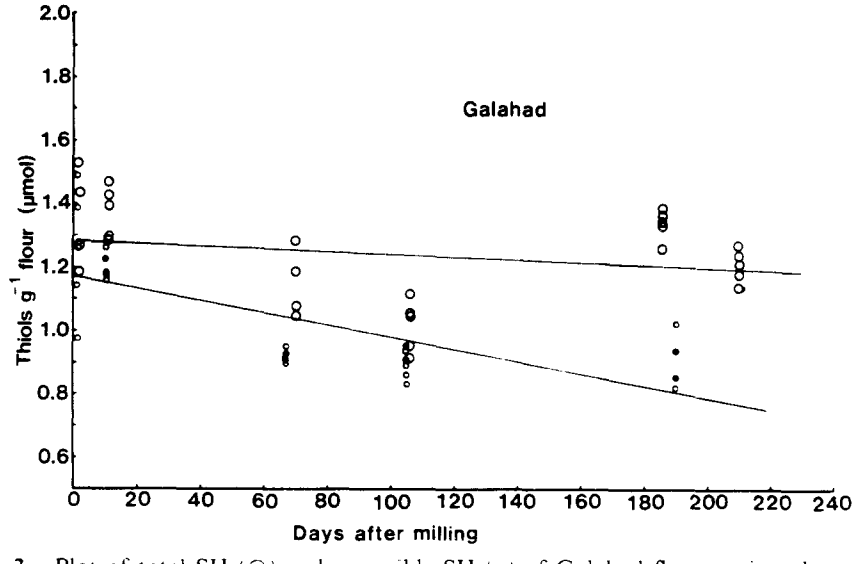

Fig. 3. Plot of total SH (○) and accessible SH (○) of Galahad flour against days after milling. The straight lines are the regression lines. Filled circles mean coincident points. Standard deviation of single titration: 0·090 (total); 0·081 (accessible).

TABLE 1
Best Straight Lines for Plots of μmol SH g^{-1} Flour *vs* Days after Milling

	Using days			*Using log_{10} days*		
	Intercept	*Slope*	*r*	*Intercept*	*Slope*	*r*
Total SH						
Avalon	1·37	$-0{\cdot}45 \times 10^{-3}$	−0·235	1·47	$-3{\cdot}93 \times 10^{-2}$	−0·407
Galahad	1·28	$-0{\cdot}45 \times 10^{-3}$	−0·235	1·40	$-4{\cdot}19 \times 10^{-2}$	−0·445
CWRS	1·88	$-2{\cdot}65 \times 10^{-3}$	−0·918	1·95	$-8{\cdot}26 \times 10^{-2}$	−0·733
Accessible SH						
Avalon	1·16	$-1{\cdot}5 \times 10^{-3}$	−0·735	1·21	$-5{\cdot}14 \times 10^{-2}$	−0·755
Galahad	1·18	$-1{\cdot}93 \times 10^{-3}$	−0·744	1·29	$-7{\cdot}95 \times 10^{-2}$	−0·838
CWRS	1·73	$-3{\cdot}29 \times 10^{-3}$	−0·866	1·89	$-12{\cdot}0 \times 10^{-2}$	−0·896

used instead of days the negative correlation became significant ($P < 0{\cdot}05$) even for these two flours (Table 1).

The measurements of total SH were less accurate (standard deviation of a single titration (sd), 0·090) than those of accessible SH (sd, 0·081) because a small unknown peak appeared before the PMA peak after the flour had been added. The position of the baseline had to be estimated, and despite attempts to be consistent in doing this, some uncertainty was inevitable.

Loss of accessibility

Though straight lines have been drawn in Figs 1, 2 and 3, there are signs that the rate of disappearance of accessible SH falls off after about 2 months.

It seems that there is also a loss of accessibility because the total SH fell less steeply than the accessible. Tsen & Dempster (1963) also found a slowing of the rate of loss of accessible SH, on a longer time scale, and assumed that some SH groups became inaccessible.

Reactions with flour additives

A temperature of 44°C was chosen to be in the 40–50° C range where $KBrO_3$ was active (Jelaca & Dodds, 1969), but starch would not gel. Microbial damage seemed negligible provided dialysis was done at ~5° C.

Doughs

At both room temperature and 44°C the control and Vit C doughs had become very sticky, weak and extensible, probably owing to microbial

proteolysis (0·02% of broken peptide bonds would have a marked effect on the dough strength but be undetectable by formol titrations, and probably not by electrophoresis). The ADA, BP and $KBrO_3$ doughs were tough if rather short. The APS room temperature dough was very short; at 44°C it was even shorter and weaker though some trace of elasticity remained. Probably ADA, APS, BP and $KBrO_3$ inhibited microbial growth, and the tests suggested that these four had not broken polypeptide chains but destroyed SH groups, as they are known to do.

Amino acid analyses

Observed differences were within the range of experimental error. This was supported by statistical analysis of some replicate data. Tryptophan was not measured.

Electrophoresis

SDS PAGE (Fig. 4) of the dialysed samples showed that APS and BP produced some insoluble matter of high molecular weight, the effect being greater at 44°C. The peroxides probably created free radicals which caused crosslinking. $KBrO_3$ and ADA had no significant changes compared with the controls.

The pattern gave no sign of the increase in polypeptides of low molecular weight that would occur if many peptide bonds had been broken. This was generally confirmed by standard formol titrations on 10 g of both dialysed and undialysed samples, but as Vit C (at 44°C) and ADA (room temperature, dialysed) showed significant increases implying a large amount of peptide bond breakage (1 to 2/chain) the results are not reported. Thick flour suspensions can easily upset the working of a combination pH electrode. (APS was unsuitable for formol titrations because of the reaction of NH_3 and persulphate with CH_2O.)

With both Vit C samples a doublet consistently appeared ahead the high molecular weight subunits. What caused this is unknown but since the initial pH was about two units lower than those of other additives, deamidation or limited fission of a labile Asx peptide bond in a high molecular weight subunit might have resulted in faster bands. If so, it would not apply in industry because the small amounts of Vit C used would not significantly lower pH. If it were due to some other reaction, e.g. dimerisation by traces of the free radicals that Vit C can form in the presence of trace metals, this would be quite negligible at the levels used in breadmaking. The formation of Schiff's bases by dehydroascorbic acid, to which Vit C is partly converted (Pfeilsticker & Marx, 1986) would not have

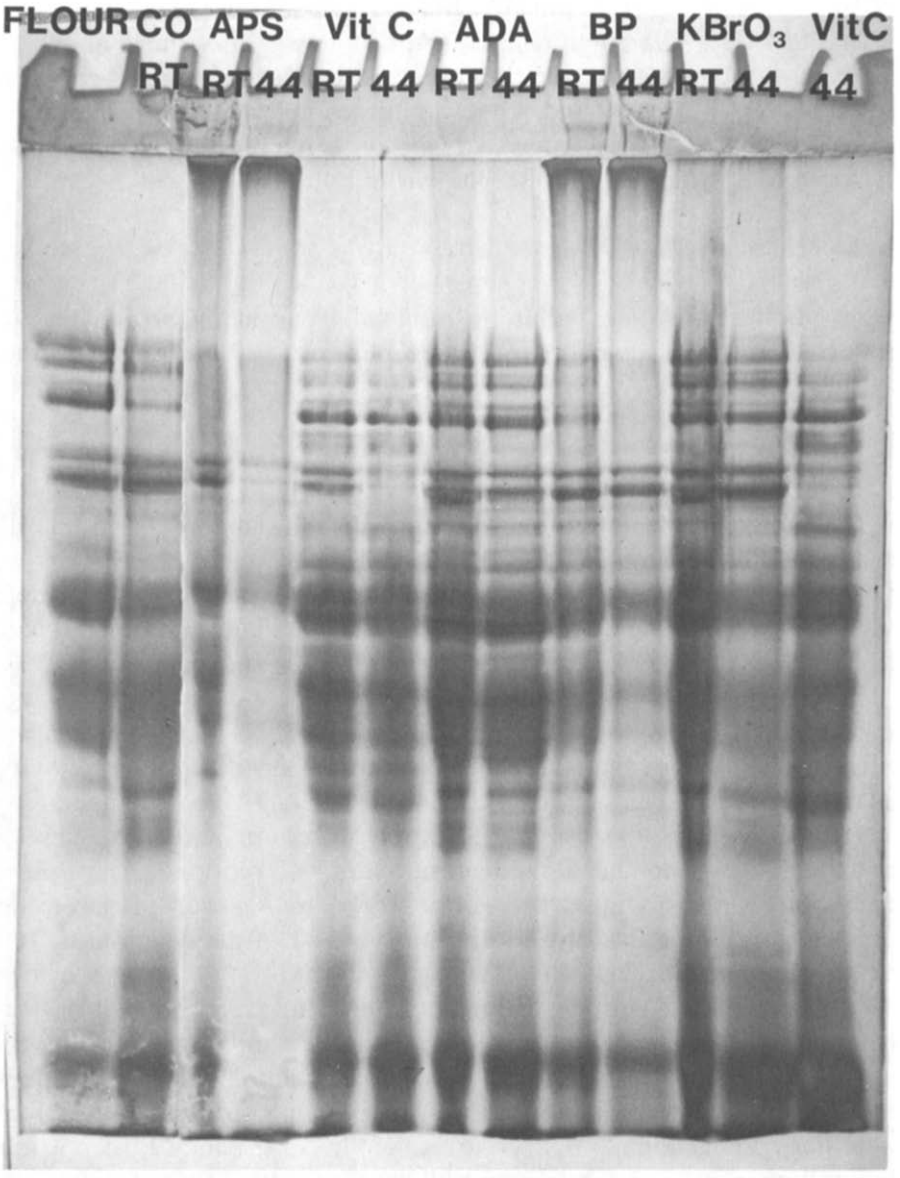

Fig. 4. SDS PAGE of improver-treated flours. FLOUR = Extract of the flour with no treatment; CO = control; RT = room temperature reaction; 44 = reacted at 44 C.

been detected. It is unlikely that this reaction, if it occurs at all in dough, affects loaf volume because it should occur equally with dehydro-D-ascorbic acid, which has little or no improving action (Maltha, 1953).

Relevance to breadmaking

BP at the levels used in bleaching flour is not known to have any improving action; hence, its action on the proteins at those levels is almost certainly nil, as was confirmed by the dough tests. APS at 44 °C had decreased protein solubility but in the dough test some trace of elasticity remained. Therefore, the level of crosslinking would not be heavy, less than one link per polypeptide chain. In that case only about one APS molecule in 200 would cause a crosslink. The proportions of improvers used here (10^5 ppm on the flour) are of the order of a 1000 × higher than those used in breadmaking; therefore, such a reaction in breadmaking would be negligible.

CONCLUSIONS

The work suggests that there is a small real loss of SH on storage for all three flours, and this varies with the flour. Accessible SH are also lost on storage, some disappearing (as seen by the fall in total SH) and some becoming less accessible.

Work of more than one author has suggested that only a small fraction of the flour SH is rheologically active in dough, of the order of 0·2 μmol g^{-1} flour (Ewart, 1988). The fall in accessible SH found here over about 6 months is of this order, as is also the level of improvers added in breadmaking. Therefore the only significant action of improvers and the improving effect of storage are likely to be to decrease the rheologically active SH, which seem to be the major determiners of wheat protein baking quality.

It may be speculated that rheologically active SH lie on small diffusible molecules and that they become inaccessible by reacting with insoluble proteins. Rao *et al.* (1978) said that soluble SH decreased after 4 months' storage, which agrees with this idea.

ACKNOWLEDGEMENT

This work forms part of a project sponsored by the UK Ministry of Agriculture, Fisheries and Food (MAFF) to whom thanks are due. The results of the research are the property of the MAFF and are © Crown Copyright 1988. The author thanks Mr P. Messenger of Heygates Ltd for a

flour sample, Dr T. Fearn for the statistical calculations and Miss W. A. Keddie for experimental assistance.

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