Changes in the Glutathione Content and Breadmaking Performance of White Wheat Flour During Short-Term Storage

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ABSTRACT

White flour milled from grain of the hard bread wheat cv. Mercia was stored at 20°C and used to monitor changes in the contents of free reduced glutathione (GSH), free oxidized glutathione (GSSG), and protein-glutathione mixed disulphides (PSSG). GSH levels fell from 149 to 85 nmol/g of flour during the first 10 days of storage, and they remained constant thereafter up to 40 days of storage. The breadmaking performance of the flour increased concomitantly with the fall in GSH content during the first 10 days of storage and again remained constant up to 40 days. The fall in GSH levels could not be accounted for in terms of either simple oxidation to GSSG or the formation of PSSG because glutathione levels in these two pools also fell during the initial 10-day period. The results indicate that the changes in glutathione levels in all three pools are closely related temporally to changes in breadmaking performance during the short-term storage of white flour. The nature of the reactions that lead to the drop in glutathione levels remains to be elucidated.

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Improvements in the breadmaking performance of wheat flour during short-term storage have been reported by a number of researchers (Kozmin 1935, Fisher et al 1937, Shellenberger 1939, Bothast et al 1981). The use of oxidizing improvers in breadmaking is intended to simulate these improving effects of flour aging. Despite the potential importance of short-term changes in the breadmaking quality of flour on storage, the (bio)chemistry of this effect has not been explained.

Reduction-oxidation (redox) reactions involving flour (gluten) protein sulfhydryl (SH) groups and disulfide (SS) bonds, which have an effect on the polymeric structure of the glutenin protein fraction, are generally considered important to dough rheological properties and breadmaking performance (Fitchett and Frazier 1986, Grosch 1986). In particular, there has been considerable interest in the possible involvement in the redox reactions that occur in dough of the tripeptide glutathione (γ-glutamylcysteinylglycine), which occurs endogenously in flour in the free reduced (GSH) and free oxidized (GSSG) forms, as well as in the form of protein-glutathione mixed disulphides (PSSG) (Kuninori and Matsumoto 1964; Jones and Carnegie 1969a; Tkachuk 1970; Archer 1972; Coventry et al 1972; Ewart 1985; Sarwin et al 1992, 1993; Schofield and Chen 1992, 1994, 1995; Chen and Schofield 1995).

The technological significance of glutathione and its reactions has been difficult to assess; a major factor is uncertainty about the true levels of both GSH and GSSG in flour because values reported in the literature vary by orders of magnitude (Grosch 1986; Schofield and Chen 1994, 1995). Furthermore, a convenient and reliable method for measuring GSH, GSSG, and PSSG individually was not available until recently, making it difficult to obtain a complete picture of the distribution of glutathione in the different pools in which it occurs and of the reactions that it undergoes in flour and dough.

During the course of establishing high-performance liquid chromatography (HPLC) methodology for measuring GSH, GSSG, and PSSG individually in flour, we noticed that the GSH and GSSG contents of white flour that had been stored at 4°C for 90 days before analysis were much lower than those of freshly milled flour (Chen and Schofield 1993; Schofield and Chen 1994, 1995). Sarwin and coworkers (1992) have also made similar observations with regard to the GSH contents only (not GSSG or PSSG contents, which their analytical method does not measure individually) of stored and freshly milled flours. The precise kinetics of the changes in flour GSH and GSSG contents, and the relationship of the changes, if any, to possible changes in breadmaking performance were not determined in either our preliminary work or in that of Sarwin and coworkers (1992), and neither were the effects of storage on PSSG contents. Rheological analysis (extension testing) of doughs to which GSH had been added tends to indicate that loss of GSH would be likely to increase dough strength and perhaps breadmaking performance (Grosch and Sarwin 1994).

This article reports the effects of short-term storage (up to 40 days) on the contents of GSH, GSSG, and PSSG, as well as on breadmaking performance as determined for white flour milled from a hard winter bread wheat cultivar.

MATERIALS AND METHODS

Chemicals

All the reagents used were purchased from Merck-BDH Ltd., Poole Dorset, UK, except reduced and oxidized glutathione standards, which were obtained from Sigma Chemical Co. Ltd, Poole, Dorset.

White Flour Production and Storage

Grain of the hard UK winter bread wheat cv. Mercia was purchased from Plant Breeding International Cambridge Ltd, Maris Lane, Trumpington, Cambridge, UK. The grain was milled into straight-run flour using a Brabender Quadrumat Junior Mill (Brabender, Duisberg, Germany) fitted with a 200-μm aperture size sieve. The moisture content of the flour was 14.5% (w/w). The freshly milled flour was divided immediately into 2-kg lots, packed into paper bags, and stored at a constant temperature of 20°C.

Determination of GSH, GSSG, and PSSG Contents

GSH, GSSG, and PSSG were determined in duplicate as described previously (Schofield and Chen 1995, Chen and Schofield 1995). The analytical results are expressed on an as is basis. The estimated standard deviations of single determinations are 1.7 nmol/g for GSH, 1.0 nmol/g for GSSG, and 3.1 nmol/g for PSSG.

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Breadmaking Procedure

The Chorleywood Bread Process (CBP) (Collins 1982) was used to determine the breadmaking performances of the stored flours. The recipe used contained flour (1,000 g), dried yeast (10 g), salt (18 g), Ambrex high-melting point fat (10 g), ascorbic acid (0.1 g), and water as determined using the Simon research water absorption meter (Kent-Jones and Amos 1967), with a 10-min resting period and extrusion times of 90 sec. Duplicate doughs were mixed in a laboratory-scale Morton Z-blade mixer (Morton Machine Co. Ltd.). The dry ingredients were first mixed at low speed for 30 sec. After water was added, the dough was mixed for a further 100 sec at low speed and then, after the bowl was scraped down, at high speed to a total work input of 40 kJ/kg of dough. The final dough temperature was controlled to 30 ± 1°C (Collins 1982). The dough was divided immediately after mixing, scaled at 460 g, hand-rounded, and rested for 10 min at 30°C. The dough pieces were molded in a Mono universal molding machine, placed in greased metal tins, and proved at high humidity to prevent skinning for ≈45 min at 43°C to constant height (11 cm). The proved doughs were baked for 25 min at 230°C in a Simon rotary oven. The loaves were depanned and allowed to cool for 3 hr at room temperature. After cooling, the loaf volumes were measured by rapeseed displacement.

RESULTS

During the course of our work to establish an HPLC procedure for measuring GSH and GSSG, it was noticed that a white flour milled from cv. Galahad that had been stored in the refrigerator at 4°C for several weeks before analysis had very much lower GSH and GSSG contents than flour from the same lot of grain that had been analyzed immediately after milling (Chen and Schofield 1993; Schofield and Chen 1994, 1995). The GSH content of the stored flour was 10.8 nmol/g, whereas that for the freshly milled flour was 80.7 nmol/g; the GSSG value for the stored flour was 8.5 nmol/g, whereas the value for the freshly milled flour was 16.9 nmol/g. Similar observations were also made for flour milled from cv. Haven, which has a relatively low value for GSH (17.7 nmol/g) compared with that of cv. Galahad, even for freshly milled flour; it has a somewhat higher GSSG value (20.1 nmol/g). The GSH and GSSG values for flour stored for several weeks at 4°C were 5.1 and 8.9 nmol/g, respectively. Changes in PSSG contents were not investigated in these preliminary experiments.

In the light of these preliminary observations, a flour storage experiment was undertaken in which changes in GSH, GSSG, and PSSG contents of white flour milled from grain of the cv. Mercia were monitored as a function of storage time. The content of GSH fell to 57% of the initial value for the freshly milled flour during the first 10 days of storage at 20°C (from 149 to 89 nmol/g of flour). It then remained essentially constant up to 40 days of storage (Fig. 1). But, as noted in the preliminary experiments, the decrease in the GSH content was not accompanied by an increase in the GSSG content. The GSSG content of the flour also decreased, in this case to 28% of the initial value for the freshly milled flour, over the same 10-day storage period from 61 to 17 nmol/g of flour (Fig. 1). Neither was the decrease in the GSH content accounted for by an increase in the PSSG content. The PSSG content also fell during the initial 10-day storage period, in this case, to 86% of the initial value for the freshly milled flour, from 87 to 70 nmol/g flour, and again stayed essentially constant thereafter (Fig. 1).

The fall in GSH levels could not be accounted for, therefore, in terms of either oxidation to GSSG or linkage to flour protein via disulfide bonds because glutathione levels in these two pools also

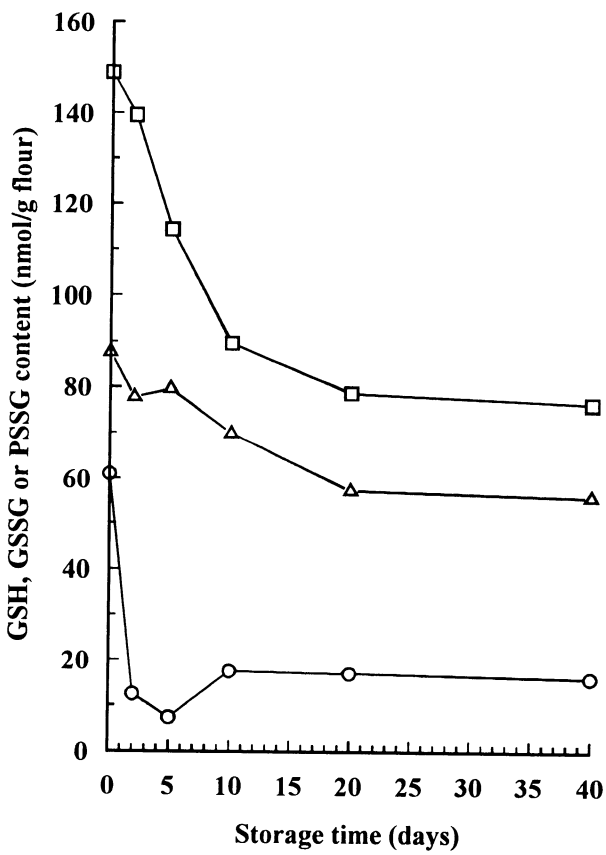

Fig. 1. Changes in the free reduced glutathione (GSH, □); free oxidised glutathione (GSSG, ○); and protein-glutathione mixed disulfides (PSSG, Δ) contents of white flour milled from the hard winter bread wheat cultivar Mercia during storage at room temperature.

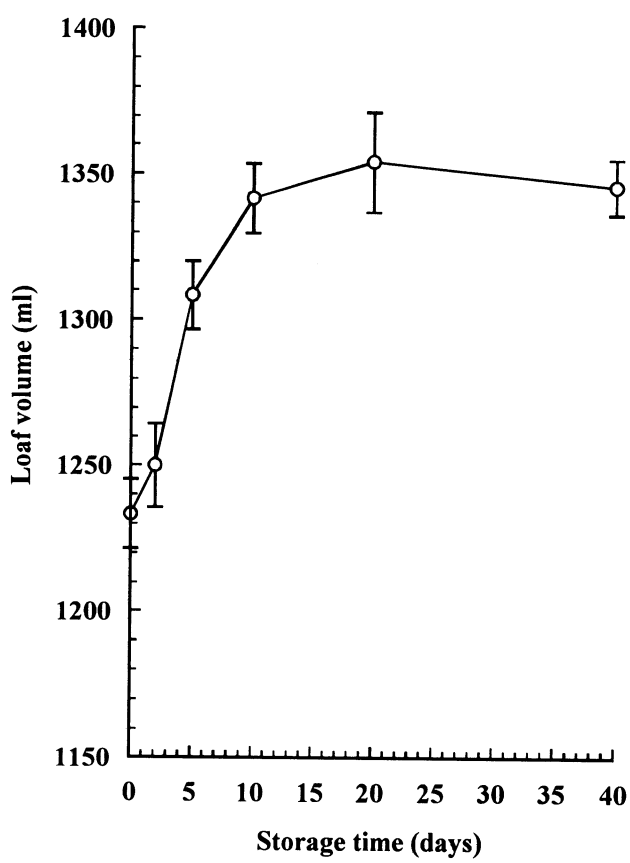

Fig. 2. Changes in the breadmaking performance in the Chorleywood Bread Process of white flour milled from the cultivar Mercia during storage at room temperature. Bars represent standard deviations of values for six loaves.

showed changes similar to that of GSH (Fig 1). The contents of glutathione in all three pools fell during the initial 10-day storage period, although the magnitudes of the changes differed, and they then remained constant up to 40 days of storage.

Changes in Breadmaking Performance During Storage

As well as measuring changes in the three glutathione pools, the breadmaking performance of the cv. Mercia flour was also monitored using the Chorleywood Bread Process. The results indicated that the breadmaking performance of the flour improved substantially during the initial 10 days of storage and then remained essentially constant thereafter (Fig. 2). Thus, the loaf volume increased from 1,250 ml for the freshly milled flour to 1,338 ml for the flour that had been stored for 10 days and remained constant up to 40 days.

DISCUSSION

Although it is widely accepted that wheat flours improve in breadmaking performance during short-term storage, relatively few studies have been reported in which this phenomenon has been investigated. We have shown here that for flour milled from the grain of UK winter bread wheat cv. Mercia, a substantial improvement in breadmaking performance occurred during the first 10 days after milling when the flour was stored at room temperature. After this, the breadmaking performance stabilized. Concomitantly with the improvement in breadmaking performance, there were substantial decreases in the flour contents of GSH, GSSG, and PSSG during the same 10-day period after milling, after which the values also stabilized. The changes in the contents of the three glutathione pools were related very closely in a temporal sense, therefore, to the improvement in breadmaking performance, although the results do not prove a causal relationship between the two.

Sarwin et al (1992) also showed that flour GSH contents decreased on storage for 60 days at room temperature, but the decreases were not of such great magnitude as observed here or in our preliminary observations (Schofield and Chen 1995). The reasons why the changes in GSH, GSSG, and PSSG contents occurred to different extents in our experiments and in GSH contents in those of Sarwin et al (1992) are not known, but a contributory factor may be that those authors used a different wheat cultivar from that used here. Effects on GSSG and PSSG contents were not reported by Sarwin et al (1992). Their method for analyzing glutathione in flour does not allow the determination of GSSG and PSSG individually. Neither did they examine the relationship of the changes in GSH content during flour storage to breadmaking performance nor the time dependency of the changes in GSH content. Grosch and Sarwin (1994) did extension tests on doughs to which GSH had been added at levels similar to those that occur naturally in flour. They found that dough properties were weakened by GSH at such levels. The removal of GSH during storage would, therefore, be expected to increase dough strength and breadmaking performance. A decrease in the GSH content of flour was also noted previously by Kuninori and Matsumoto (1964) during storage at 4°C.

The fall in GSH levels could not be accounted for in terms of either oxidation to GSSG, as suggested previously (Kuninori and Matsumoto 1964), or linkage to flour protein via disulfide bonds, because the GSSG and PSSG contents of the cv. Mercia flour also fell during the initial 10-day storage period and then remained essentially constant up to 40 days. The fate of glutathione in each of these pools is unclear. The finding by Sarwin et al (1992) that milling grain under nitrogen gave rise to flour GSH contents that were ≈50% higher than when grain was milled in air suggests that reactions involving molecular oxygen are of importance in relation to flour glutathione contents. Finley, Wheeler, and Witt (1981) showed that the cysteine and cystine residues in reduced and oxidized glutathione, respectively, were oxidized in aqueous solution by oxidizing agents such as hydrogen peroxide and potassium bromate into a number of higher oxidation states, but it is questionable whether model reactions in aqueous solutions can be compared with reactions in flour. The mechanism of the loss of glutathione in each of the three pools and the role of these reactions in the improvement in breadmaking performance during short-term storage, therefore, remain to be elucidated.

Changes in the content of SH groups during white flour storage have been reported (Joiner et al 1963; Bellenger and Godon 1972; Yoneyama et al 1970a,b). Both total and accessible SH groups were lost during the first year of storage after milling, and accessible SH groups decreased faster than total SH groups (Tsen and Dempster 1963, Ewart 1988). The rate of loss of accessible SH groups declined after about two months of storage. However, there appear to have been few detailed studies in which the short-term improvement in breadmaking performance observed here have been investigated.

Oxidants such as potassium bromate, azodicarbonamide, and ascorbic acid (after its conversion in both enzymic and nonenzymic reactions involving molecular oxygen to the oxidizing form dehydroascorbic acid) have been proposed as bringing about their bread-improving effects through oxidation of GSH to GSSG (Grosch 1986, Sarwin et al 1993). There is a rapid fall in the levels of GSH and a rapid increase in GSSG levels in dough during the early stages of mixing, and this is accentuated by these oxidants (Sarwin et al 1993; Grosch and Sarwin 1994; Chen and Schofield, unpublished). We have also observed that the formation of PSSG, presumably through SH-SS interchange reactions involving flour protein SH or SS groups, is a significant reaction during dough mixing, although it lags behind oxidation of GSH to GSSG. This reaction is also accentuated by oxidants (Chen and Schofield, unpublished). Nevertheless, we have observed that total glutathione levels (i.e., the sum of glutathione in all three forms) remains essentially constant during dough mixing in contrast to the loss of glutathione in all three forms that occurs during flour storage. The technological effects brought about by storage and those brought about by oxidizing improvers used in baking that are meant to simulate those brought about by flour storage are, therefore, caused by different types of reaction.

CONCLUSIONS

The contents of GSH, GSSG, and PSSG fell during the first 10 days of storage of white flour at room temperature, and thereafter remained constant up to 40 days of storage. The breadmaking performance of the flour increased concomitantly during the first 10 days storage period, and again remained constant up to 40 days. It is clear that changes in the contents of glutathione in these three pools are related very closely, in a temporal sense, to the changes in breadmaking performance that occur during short-term storage. The fall in GSH levels could not be accounted for in terms of either oxidation to GSSG or linkage to flour protein via disulfide bonds to form PSSG. The nature of the reactions that lead to the falls in glutathione levels remains to be elucidated.

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