Distribution of Redox Enzymes in Millstreams and Relationships to Chemical and Baking Properties of Flour

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ABSTRACT

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Millstream flours, bran, pollard, and germ fractions were prepared from two Australian and two New Zealand wheat cultivars using a pilot-scale roller mill. The distribution of six redox enzymes in milling fractions and the relationship of the enzymes to baking parameters were investigated. Lipoxygenase (LOX), dehydroascorbate reductase (DAR), and protein disulfide isomerase (PDI) tended to be higher in the tail-end fractions of break and reduction flour streams, but the highest levels were in the bran, pollard, and germ fractions. These enzymes had moderate to strong correlations with ash content of flour. These results indicated that a considerable amount of these enzymes in the tail-end flour streams were likely to be derived from contamination with bran, aleurone, or germ components of grain. Peroxidase (POX) tended to be higher in the break flours, but polyphenol oxidase (PPO) and ascorbate oxidase (AOX) tended to be evenly distributed in the millstream flours. These three enzymes generally had poor correlations with ash and baking parameters. LOX and DAR had a negative correlation with the baking quality of bread made in the absence of ascorbic acid (AA) but a poor correlation with improvement of bread quality made with AA. The negative correlation probably reflects the high content of ash (hence trichomes), glutathione, and protein thiols in those fractions that have high LOX and DAR, and these high-reducing-power components and trichomes in flour may be the actual cause of poor quality bread. PDI generally had a poor correlation with bread quality in the absence of AA but a significant positive correlation with improvement in the quality of bread made with AA. It thus seems that the endogenous levels of these six enzymes were not a limiting factor in the breadmaking process, except for PDI, the levels of which may have positively influenced breadmaking in the presence of AA.

The distribution of the enzyme activities of wheat grain after manual dissection of the grain or small-scale laboratory milling or pilot-plant milling or commercial plant milling has been reported for the following oxidation-reduction (redox) enzymes: lipoxygenase (LOX; E.C. 1.13.11.12) (Miller and Kummerow 1948; Honold and Stahmann 1968; Rani et al 2001), peroxidase (POX; E.C. 1.11.1.7) (Honold and Stahmann 1968; Rani et al 2001), catalase (E.C. 1.11.1.6) (Honold and Stahmann 1968), polyphenol oxidase (PPO; E.C. 1.14.18.1) (Honold and Stahmann 1968; Marsh and Galliard 1986; McCallum and Walker 1990; Hatcher and Kruger 1993; Rani et al 2001), dehydroascorbate reductase (DAR; E.C. 1.8.5.1) (Carter and Pace 1964), protein disulfide isomerase (PDI; E.C. 5.3.4.1) (Nicolas and Drapron 1983), and ascorbate oxidase (AOX; E.C. 1.10.3.3) (Every et al 1995). As far as the authors are aware, the distribution of catalase, DAR, PDI, and AOX in all milling fractions of wheat grain after commercial milling has not been reported.

All the above enzymes have been linked to the dough and breadmaking quality of flour (Nicolas and Drapron 1983; van Oort 1996). LOX increases dough-mixing tolerance and improves loaf volume, probably by the oxidation of gluten proteins through a free-radical reaction (Nicolas and Drapron 1983; Frazier et al 1977; Faubion and Hoseney 1981). PPO appears to strengthen dough, possibly through polymerization of protein by cross-links between oxidized products of tyrosyl residues and thiol groups (Kuninori et al 1972). PPO is also responsible for the darkening of the bread crumb, particularly in whole grain and rye breads (van Oort 1996). POX and catalase destroy hydrogen peroxide, a strong inhibitor of LOX, and thus enhance the beneficial effects of LOX (Nicolas and Drapron 1983). POX has also been reported to oxidize and cross-link ferulic acid residues on arabinoxylan and produce a secondary pentosan network through the gluten network that improves dough and bread quality (Dunnewind et al 2002). The bread improving effects of LOX, POX, and PPO are most evident when exogenous enzymes are used in breadmaking. AOX, DAR, glutathione (GSH), and oxidized glutathione are important wheat components for breadmaking when ascorbic acid (AA) is used as an improver. AOX catalyzes the oxidation of AA to dehydroascorbic acid (DHA) (Sandsted and Hites 1945; Every 1999), which in turn oxidizes thiols in gluten proteins (Tsen 1965; Every 2000; Every et al 2003) and GSH (Grosch and Wieser 1999 and references therein), and thereby affects dough and bread properties. The oxidation of GSH by DHA is clearly catalyzed by DAR (Carter and Pace 1965), and it has been suggested that DHA also oxidizes PDI, which in turn catalyzes the oxidation of protein thiols to disulfide bonds (Every et al 2001, 2003).

This article reports the distribution of the redox enzymes AOX, DAR, LOX, PDI, POX, and PPO in all the milling fractions of wheat grain after samples were milled in a large-scale pilot plant according to current commercial practice. The relationship of these enzymes to the ash and breadmaking properties (with and without AA) of the millstream flours was also investigated.

METHODS AND MATERIALS

Chemicals and Wheat Samples

L-Ascorbic acid, glutathione, linoleic acid, catechol, *o*-dianisidine dihydrochloride, ribonuclease A, cytidine 2':3'-cyclic monophosphate and L-dehydroascorbic acid were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals were AnalaR products purchased from Merck (Palmerston North, New Zealand).

The wheats used were the New Zealand cultivars Sapphire and Monad, and the Australian cultivars Janz and Frame. Sapphire and Janz had low protein and weak mixing properties. Monad and Frame had high protein and strong mixing properties.

Two tonne lots of each cultivar were milled on the BRI pilot mill in Sydney, Australia. This mill is set up for pilot-mill scale operations on a wide range of materials in a highly controlled way that reflects current commercial practices. Fourteen separate millstream flours were collected: 1st, 2nd, 3rd, and 4th break, break middlings (BM), sizings (SZ), and A, B, C, B2, D, E, F, and PF reduction flours. In addition to the millstream flours, a straight-run flour was collected, consisting of all the flour streams in proportion to their production on the mill. After milling, samples (20–50 kg) of each flour were stored at 10°C until analysis for total protein, ash, and bake tests. Subsamples (600 g) of each flour and samples (2 kg) of bran, pollard, and germ fractions were stored at –18°C until examination for enzyme activity. Individual

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bran and pollard fractions for cultivar Janz were not examined because these fractions were accidentally mixed at the mill.

Analytical Methods

All analytical tests were made on each of two samples for each millstream fraction and cultivar. Total protein was determined using an analyzer (CNS-2000, Leco Corporation, St. Joseph, MI) and a nitrogen-to-protein conversion factor of 5.7. Ash determinations were done by Approved Methods of AACC International (2000).

AOX and DAR were extracted from 0.3 g of flour by mixing for 40 min at 20°C with 0.9 mL of 1.7*M* $(NH_4)_2SO_4$ at pH 8.3. AOX and DAR were extracted from 0.5 g of bran, pollard, or germ by grinding in a small mortar and pestle with 1.5 mL of 1.7*M* $(NH_4)_2SO_4$ at pH 8.3 for 2 min then mixing for 40 min at 20°C. Samples were centrifuged at 15,000 × *g* for 10 min at 4°C and stored on ice for immediate use or stored at –80°C. AOX and DAR were assayed as described by Every et al (1999) by measuring the rate of change of AA in the assay reaction mixture using a Shimadzu UV-2100 recording spectrophotometer set at 265 nm. One unit (U) of AOX or DAR activity is defined as the amount of activity that oxidizes or reduces 1.0 nanomol of substrate in 1 min at 30°C.

LOX was extracted from 1 g of flour by mixing for 10 min at 4°C with 10 mL of 0.05*M* sodium acetate buffer at pH 5.5. LOX was extracted from 1 g of bran, pollard, or germ by grinding in a small mortar and pestle with 10 mL of 0.05*M* sodium acetate buffer at pH 5.5 for 2 min then mixing for 5 min at 4°C. Samples were centrifuged at 12,000 × *g* for 10 min at 4°C and then stored at –80°C. LOX was assayed as described by Hsieh and McDonald (1984) by measuring the rate of oxidation of linoleic acid to conjugated hydroperoxydienoic acid in the assay reaction mixture using the Shimadzu spectrophotometer set at 234 nm. One unit (U) of LOX activity is defined as the amount of activity that oxidizes 1.0 nanomol of linoleic acid in 1 min at 30°C.

POX was extracted as described for LOX. The POX assay method of Aparico-Cuesta et al (1992) was adapted to a kinetic assay using a microplate reader (Thermomax, Molecular Devices) set at 450 nm and 30°C. The final concentrations of substrates and buffer in the assay mixture were 1 m*M* *o*-dianisidine dihydrochloride, 0.03*M* hydrogen peroxide, and 0.04*M* sodium acetate buffer (pH 5.5). One unit of POX activity is defined as the amount of enzyme catalyzing an increase of one absorbance unit/min at 30°C and pH 5.5.

PPO was extracted as described for LOX. The PPO assay method of Coseteng and Lee (1987) was adapted to a kinetic assay using a microplate reader (Thermomax, Molecular Devices) set at 405 nm and 30°C. The final concentrations of substrate and buffer in the assay mixture were 0.063*M* catechol and 0.05*M* sodium acetate buffer (pH 5.5). One unit of POX activity is defined as the amount of enzyme catalyzing an increase of one absorbance unit/min at 30°C and pH 5.5.

For extraction of PDI, 2 g of flour was mixed for 20 min at 4°C with 16 mL of extraction buffer (0.25*M* sucrose, 100 m*M* Tris-HCl, pH 8, 100 m*M* KCl, and 50 m*M* Mg acetate, 1 m*M* EDTA, and 1 m*M* phenylmethyl-sulfonylfluoride). PDI was extracted from 2 g of bran, pollard, or germ by grinding in a small mortar and pestle with 16 mL of extraction buffer for 5 min at 4°C. The mixtures were centrifuged at 10,000 × *g* for 20 min at 4°C and rough microsomes (endoplasmic reticulum) were prepared from the supernatants as described by Shimoni et al (1995). PDI activity was assayed in the microsome fraction using reduced-ribonuclease A and cytidine 2':3'-cyclic monophosphate substrates as described by Lyles and Gilbert (1991). Reduced-ribonuclease A was prepared as described by Grynberg et al (1977). One unit of activity is the amount of PDI producing an increase of one absorbance unit/min at 25°C and pH 7.5.

Breadbaking Tests

All baking tests were made on each of two samples for each millstream flour and cultivar. Bread was made by a mechanical dough development (MDD) system using 125 g of flour as described by Swallow and Baruch (1986) and Larsen and Greenwood (1991), except potassium bromate was not used and each flour sample was baked with or without 100 ppm of AA. Water absorption and work input values were determined for individual samples on a 125-g MDD Mitchell mixer. Loaves were evaluated for volume and crumb texture 16–20 hr after removal from the oven. Loaf volume and crumb texture scores were combined to give an overall MDD bake score (BS) as described by Swallow and Baruch (1986). In this study, the baking response to AA was measured by the difference in BS between loaves baked with and without AA.

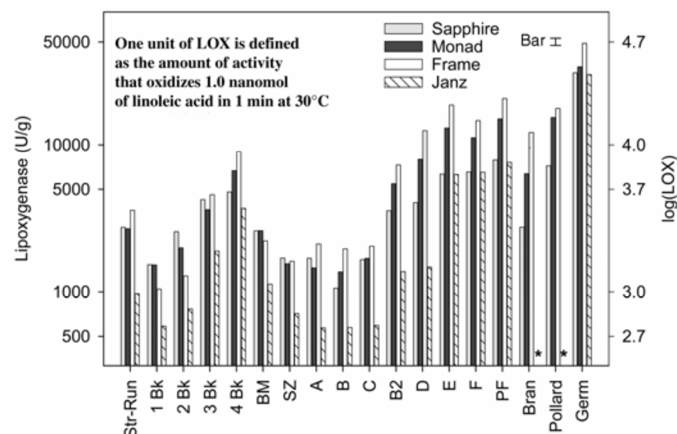


Fig. 1. Distribution of lipoxygenase (LOX) in millstream fractions and straight-run flour (Str) of four wheat cultivars. Bar indicates least significant difference at the 5% level (df 70) between two means on the log scale derived from analysis of variance. Locations marked * have no data for bran or pollard of Janz.

TABLE I
Correlation Coefficients of Redox Enzymes with Ash and Glutathione (GSH) of Millstream Flours[a]

	Sapphire		Monad		Frame		Janz	
Redox Enzyme[b]	**Ash**	**GSH**	**Ash**	**GSH**	**Ash**	**GSH**	**Ash**	**GSH**
LOX	**0.92**	**0.92**	**0.94**	**0.92**	**0.96**	**0.96**	**0.95**	**0.94**
POX	0.35	0.20	0.15	–0.01	0.07	–0.19	0.45	0.26
PPO	0.43	**0.62**	–0.04	–0.04	0.50	0.43	**–0.67**	**–0.59**
AOX	0.36	0.16	–0.38	–0.39	–0.13	–0.01	0.28	0.33
DAR	**0.60**	**0.74**	**0.63**	**0.69**	**0.85**	**0.93**	**0.70**	**0.90**
PDI	**0.79**	**0.79**	**0.56**	0.37	**0.79**	**0.76**	**0.63**	0.32

[a] Correlation coefficients (between means for 15 millstream flours) >0.52 or <–0.52 are in bold and considered to be weak, moderate, or strong. Lesser positive correlations or greater negative correlations than these values are considered to be poor.

[b] Lipoxygenase (LOX), peroxidase (POX), polyphenol oxidase (PPO), ascorbate oxidase (AOX), dehydroascorbate reductase (DAR), and protein disulfide isomerase (PDI).

Statistical Analysis

Means for all analytical and baking tests on each millstream fraction and cultivar were calculated. The various measurements were compared by calculating correlation coefficients between these means. Data presented graphically was analyzed with analysis of variance to provide estimates of variability (least significant differences). LOX measurements were logarithmically transformed before the analysis to make the variance more homogeneous across the range of the data.

RESULTS AND DISCUSSION

Enzyme Distribution in Millstream Fractions

Figure 1 illustrates the distribution of LOX in milling fractions and straight-run flour of four wheat cultivars. LOX activity in millstream flours of all cultivars tended to be higher in the tail-end fractions of break and reduction streams. The LOX distribution in millstream flours was very similar to the distribution of ash. The distribution of ash in these millstream flours was reported by Every et al (2002). For all cultivars, there was a strong correlation (r = 0.91–0.96) between LOX activity and ash content of millstream flours (Table I). This suggested that much of the LOX activity in flour resulted from contamination by the bran, pollard, and germ fractions of wheat. Germ had at least twice as much LOX as the highest LOX levels in millstream flours, whereas bran and pollard had levels of LOX similar to those in tail-end reduction flours, which indicated that fragments of germ may have been the main contaminant contributing to LOX activity in the flour. Germ has previously been shown to have the highest LOX activity among milled wheat fractions (Miller and Kummerow 1948) and among dissected parts of wheat grain (Auerman et al 1971). The shorts fraction (pollard) of milled wheat had the next highest levels of LOX, bran had about half the enzyme level of shorts, and commercial flour had about half the enzyme level of bran (Miller and Kummerow 1948). These results are similar to the results reported here. The distribution of LOX in reduction streams from a commercial mill (Rani et al 2001) was similar to the present results, but the distribution in break streams was different in that the first two break streams had LOX levels similar to the tail-end break streams.

Figure 2 illustrates the distribution of POX in the milling fractions and straight-run flour of the four wheat cultivars. Among the millstream flours there was a tendency for higher POX activity in break streams 2–4 and the PF tail-end reduction stream. However, the pattern of higher enzyme levels in tail-end streams was not as clear as for LOX and, consequently, there was a poor correlation between POX activity and ash content in millstream flours (Table I). It therefore seems that most POX activity in millstream flours does not derive from contamination by components in the millfeeds but derives from endosperm. This suggestion is consistent with the findings of Rani et al (2002), who reported no discernable pattern of POX distribution in millstream flours. Figure 2 also illustrates that bran and pollard contained levels of POX similar to many of the millstream flours, indicating that only a little of the POX in flour would be expected to be derived from contamination by these millfeeds. Germ, however, contained over twice the POX activity of any millstream flour (Fig. 2) and some of the POX in flour might be expected to arise from contamination by germ fragments. Honold and Stahmann (1968) reported ≈5–10 times more POX activity in bran and shorts (pollard) than in straight-run commercial flour but there have been no reports of POX levels in the germ fraction.

Figure 3 illustrates the distribution of PPO in milling fractions and straight-run flour of the four wheat cultivars. There were no consistent patterns of distribution of PPO in the millstream flours. For each cultivar, the bran fractions had ≈2–3 times the activity of straight-run flour, the pollard fractions had ≈3–6 times the activity and the germ had ≈2 times the activity of straight-run flour. For Sapphire, Monad, and Frame, there were poor correlations between PPO activity and ash content in millstream flours (Table I). For Janz, there was a moderate negative correlation. It thus seems that much of the PPO in millstream flours derives from the endosperm rather than contamination by bran and germ. This conclusion is in accordance with the results of March and Galliard (1986), showing that the differences in PPO levels between bran and wholemeal were not as large as would be expected if only the bran fraction (≈15% of grain weight) were contributing PPO activity to wholemeal flour. Marsh and Galliard (1986) also showed that a commercial germ fraction had less PPO activity than commercial white flour and would contribute very little PPO activity to the flour. Similar to the present results, Rani et al (2001) showed little difference in PPO between tail-end fractions and earlier fractions, which again suggested that only a little of the PPO activity in millstream flours derives from contamination by components in the millfeeds.

Figure 4 illustrates the distribution of AOX in the milling fractions and straight-run flour of the four wheat cultivars. There were no consistent patterns of distribution of AOX in the millstream

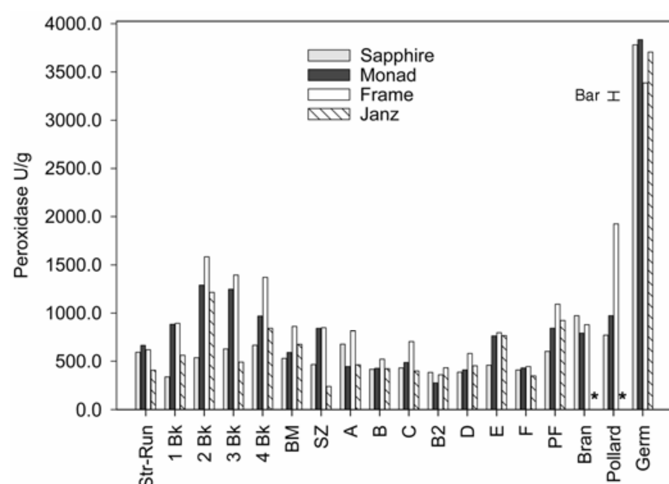


Fig. 2. Distribution of peroxidase (POX) in millstream fractions and straight-run flour (Str) of four wheat cultivars. Bar indicates least significant difference at the 5% level (df 70) between two means derived from analysis of variance. Locations marked * have no data for bran or pollard of Janz.

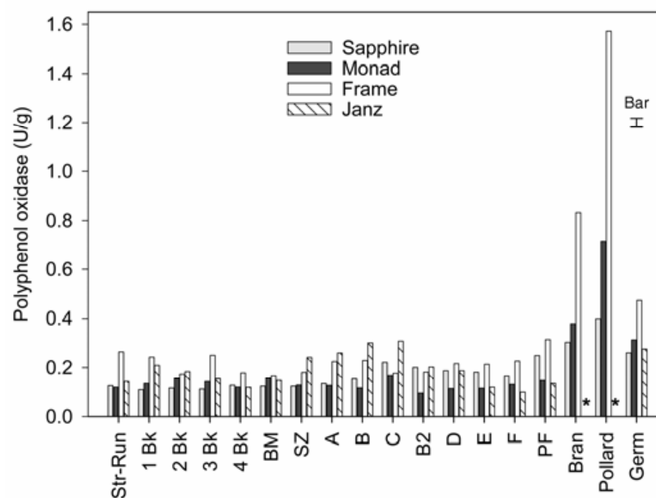


Fig. 3. Distribution of polyphenol oxidase (PPO) in millstream fractions and straight-run flour (Str) of four wheat cultivars. Bar indicates least significant difference at the 5% level (df 70) between two means derived from analysis of variance. Locations marked * have no data for bran or pollard of Janz.

flours and there was a poor correlation between AOX activity and ash content (Table I). The bran fractions had about twice the activity of the flour fractions and the pollard fractions had about four times the activity of the flour fractions. The germ fraction could not be measured by the assay method used in this study because of excessive 265-nm absorbing material in the sample that interfered with the assay. In a different assay system used by Every et al (1995) on milling fractions from a commercial mill, germ had about half the level of AOX activity of pollard, and pollard had ≈7–10 times the activity of the flour samples. The only millstream flours measured by Every et al (1995) were the 1st break flour and C-reduction flour, which had similar activity to each other.

Figure 5 illustrates the distribution of DAR in milling fractions and straight-run flour of four wheat cultivars. DAR activity in the millstream flours of all the cultivars tended to be higher in the tail-end fractions of break streams and particularly in the tail-end fractions of reduction streams. Although there was a moderate correlation between DAR activity and the ash content of millstream flours for all cultivars (Table I), the distribution patterns of DAR (Fig. 5) and ash (Every et al 2002) in the millstreams were not as similar as those for LOX and ash. This suggests that some of the DAR activity in flour may have resulted from contamination by the bran, pollard, and germ fractions of wheat, but most DAR activity probably derived from the endosperm. Bran, pollard, and germ all had high levels of DAR activity with germ having the highest. Carter and Pace (1964, 1965) reported about five times more DAR activity in the embryo and scutellum of dissected wheat grain than in the endosperm.

Figure 6 illustrates the distribution of PDI in milling fractions and straight-run flour of four wheat cultivars. PDI activity in the millstream flours of all the cultivars tended to be higher in the tail-end fractions in the break and reduction streams, but unlike LOX and DAR, the PDI activity of tail-end fractions of both break and reduction streams were similar. There were moderate correlations between PDI activity and the ash content of millstream flours for all cultivars (Table I), suggesting that some of the PDI activity in the flour may have resulted from contamination with the bran, pollard, and germ fractions. Bran, pollard, and germ all had much higher levels of PDI activity than flour. In fractions of dissected mature wheat grain, the embryo had about three times more PDI activity than the endosperm (Grynberg et al 1977).

Generally, the distribution of the redox enzymes in the millstream flours would be determined by the relative amounts of the enzymes in starchy endosperm and contaminating fragments of pericarp, seedcoat, aleurone, and embryo, as well as the extent to which the milling process denatures the enzymes in these grain parts.

Relationship of Redox Enzymes to Breadmaking Properties

Figure 7 illustrates the bake score (BS) properties of millstream flours without AA and the change in baking properties when AA was added to dough. Bread made without AA from the tail-end reduction flours F and PF had such poor quality that it was not possible to assign a value on the arbitrary bake score scale described by Swallow and Baruch (1986). Thus, correlations between enzyme levels in flour and baking properties were made without millstream F and PF flours (Table II).

In all cultivars studied, LOX levels in flour had negative correlations with the BS of bread made without AA. These correlations probably had no relation to the action of LOX but may be related to the strong correlations of LOX with the ash and GSH content

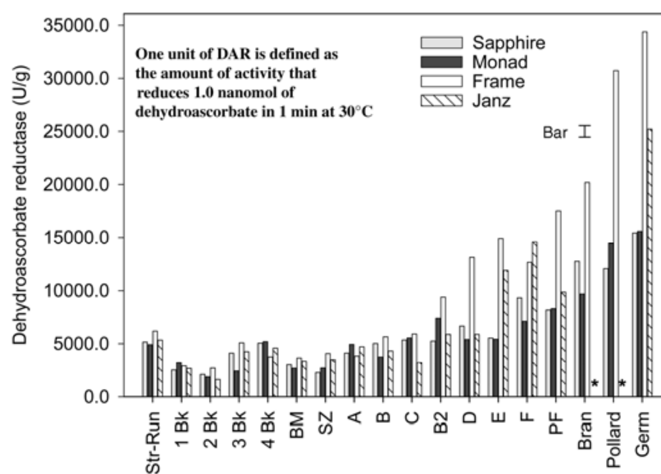


Fig. 5. Distribution of dehydroascorbate reductase (DAR) in millstream fractions and straight-run flour (Str) of four wheat cultivars. Bar indicates least significant difference at the 5% level (df 70) between two means derived from analysis of variance. Locations marked * have no data for bran or pollard of Janz.

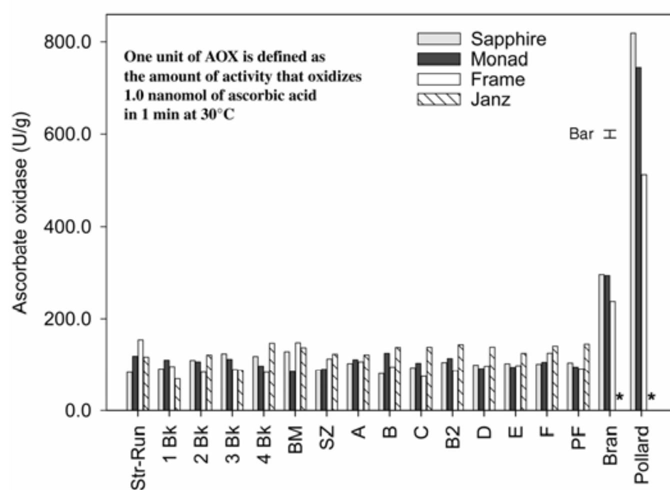


Fig. 4. Distribution of ascorbate oxidase (AOX) in millstream fractions and straight-run flour (Str) of four wheat cultivars. Bar indicates least significant difference at the 5% level (df 66) between two means derived from analysis of variance. Locations marked * have no data for bran or pollard of Janz. No data was collected for germ of any cultivar.

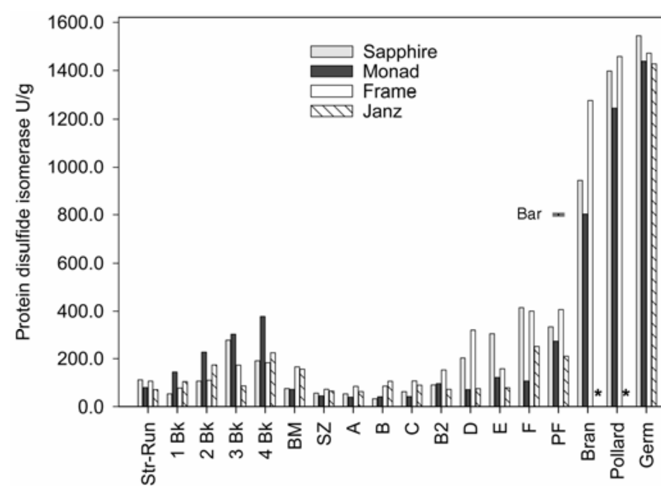


Fig. 6. Distribution of protein disulfide isomerase (PDI) in millstream fractions and straight-run flour (Str) of four wheat cultivars. Bar indicates least significant difference at the 5% level (df 70) between two means derived from analysis of variance. Locations marked * have no data for bran or pollard of Janz.

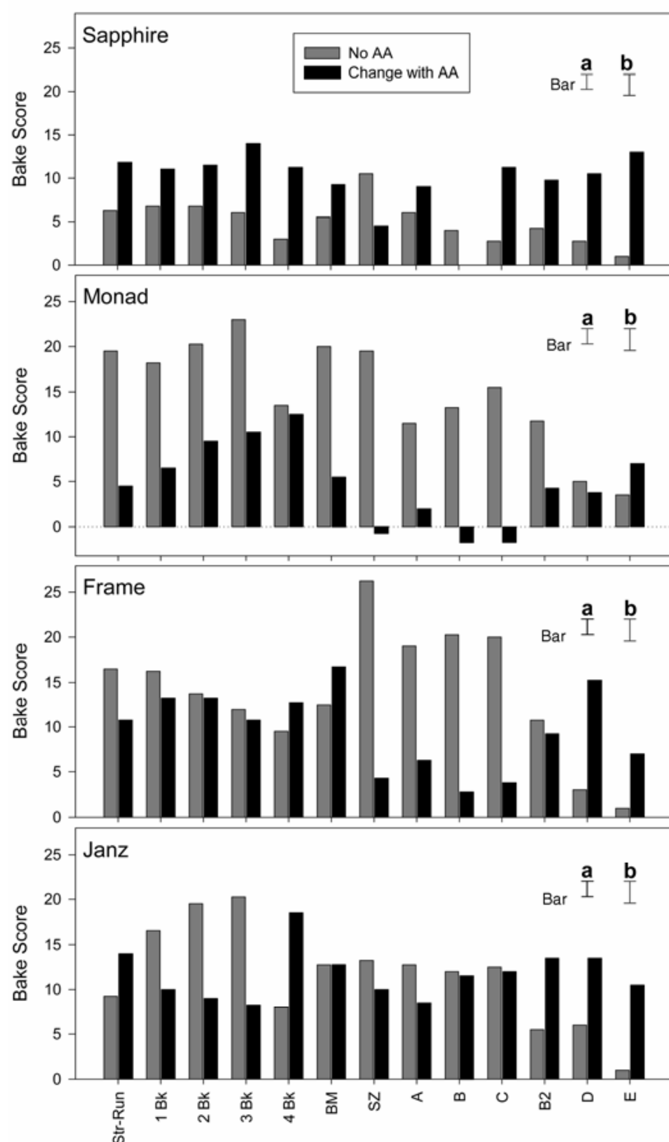


Fig. 7. Bake score (BS) of bread made from millstream flours of four wheat cultivars. Loaves made without any oxidative improver (no AA) (grey bars). Loaves made with AA and the consequent increase or decrease in BS (change with AA) (solid bars). Bars indicate least significant differences (a, no AA; b, change with AA) at the 5% level (df 104) derived from analysis of variance.

of flour (Table I). Millstreams containing high ash content are probably contaminated with high levels of brush hairs (trichomes) from the outer pericarp in bran and it is the trichomes (Gan et al 1988) rather than LOX that could cause loss of bread quality of these millstream flours. Millstream flours with high levels of GSH also had high levels of exposed thiols on all classes of flour proteins (Every et al 2006). The higher reducing potential of these flours may partly contribute to poorer breadbaking properties of the flour in the absence of AA (Every et al 2006). The BS changes in bread baked in the presence of AA had a moderate correlation with LOX activity in the Sapphire millstream flours but poor correlations with LOX activity in flours of the other cultivars (Table II). Thus the levels of LOX activity in this set of flours, and the apparent inhibitory effect of AA on LOX activity (Nicolas and Drapon 1983), probably have no bearing on the AA improver effect in baking.

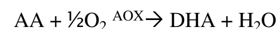
The POX level in flour was poorly correlated with the BS of bread made without AA for all cultivars and was poorly correlated with changes in the BS of bread baked in the presence of AA for all cultivars, except Monad, which had a moderate correlation (Table II). It thus seems that the redox status of dough as influenced by endogenous levels of POX has little influence on breadmaking performance with and without AA.

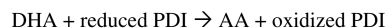
There was no consistent pattern of correlations between PPO and the BS of bread baked without AA. This suggests that endogenous levels of PPO have little influence on baking without AA. Because some plant PPO enzymes, including wheat PPO, are inhibited by AA (Mohammadi and Kazemi 2002; Yagar and Sagiroglu 2002), and some plant PPO enzymes can oxidize AA (Espin et al 2000), it is conceivable that the AA improver effect on baking is influenced by endogenous PPO levels in flour. However, it can be seen from Table II that PPO levels in flour correlated poorly with BS changes in bread baked in the presence of AA for all cultivars.

For Sapphire, Monad, and Frame there were poor correlations between AOX levels in millstream flours and baking properties. Only Janz showed a weak negative correlation of AOX with the BS of bread made without AA and a positive correlation with the change in BS of bread made with AA. It thus seems that the level of AOX is not a limiting factor in the AA improver effect for this set of samples. The level of AOX and metal ions in flour appear to be sufficient to catalyze the oxidation of AA to DHA in a well-aerated dough-mixing environment. This conclusion was the same as that of Every et al (1996). They showed that, in a set of wheat cultivars with widely varying baking quality, the level of AOX had a good correlation with baking but suggested it was the good correlation between protein content and baking quality that was more likely to be relevant to baking quality.

The level of DAR in flour was negatively correlated with the BS of bread made without AA for all cultivars (Table II). As discussed for LOX, these correlations may have no relation to the action of DAR but may reflect the moderate to strong correlation of DAR with the ash and GSH content of flour (Table II), as explained for LOX. The BS change with AA was poorly correlated with DAR for all cultivars. Thus the level of DAR in this set of flours does not seem to be associated with the AA improver effect. However, DAR activity is very high in all the flours tested and, if DAR plays a crucial role in the AA improver effect, as suggested by Grosch and Wieser (1999 and references therein), the enzyme level is unlikely to be a limiting factor in the improver effect.

There were poor correlations between PDI levels in millstream flours and the BS of bread made without AA, except for the moderate negative correlation for Frame (Table II). However, all the cultivars showed positive correlations between PDI levels and change in the BS of bread made with AA, although the correlation was weak for Janz. The results for Sapphire and Monad were similar to an earlier report on the same millstream flours but using a smaller sample set (Every et al 2001). Every et al (2001) suggested that PDI was involved in the AA improver effect in baking through the following reactions, which produce larger glutenin polymers that are associated with good bread quality:


$$AA + ½O_2 \xrightarrow{AOX} DHA + H_2O$$


$$DHA + \text{reduced PDI} \rightarrow AA + \text{oxidized PDI}$$

$$\text{oxidized PDI} + 2\ \text{glutenin-SH} \rightarrow \text{reduced-PDI} + \text{glutenin-SS-glutenin}$$

Studies on the redox metabolites and enzymes in developing wheat indicate that similar reactions may occur in immature wheat (de Gara et al 2003; Every et al 2003). For Monad, there was a strong correlation between protein and the change in BS of bread made with AA (Table II). It is possible that this relationship is more relevant to the AA improver effect than the relationship with PDI. However, for Sapphire and Frame, there was a poor correlation between protein and the change in BS of bread made with AA, and the weak to moderate correlation between PDI and

TABLE II
Correlation Coefficients for Redox Enzymes, Protein, and Breadmaking Properties of Millstream Flours of Two Australian and Two New Zealand Cultivars[a]

Enzyme or Protein[b]	Sapphire		Monad		Frame		Janz	
	BS no AA	BS Change with AA[c]	BS no AA	BS Change with AA	BS no AA	BS Change with AA	BS no AA	BS Change with AA
LOX	**–0.55**	**0.60**	**–0.74**	0.38	**–0.86**	0.12	**–0.61**	0.25
POX	0.14	0.27	0.54	**0.69**	–0.06	0.35	0.14	0.04
PPO	**–0.67**	0.00	**0.57**	0.01	–0.02	–0.06	0.25	–0.33
AOX	–0.13	0.50	0.18	–0.14	0.13	0.27	**–0.57**	**0.59**
DAR	**–0.80**	0.12	**–0.69**	–0.18	**–0.71**	–0.06	**–0.81**	0.12
PDI	–0.48	0.62	0.24	**0.89**	**–0.78**	**0.56**	0.19	0.47
Protein[c]	–0.30	0.46	0.34	**0.90**	–0.33	0.47	–0.03	0.23

[a] Correlation coefficients (between means for 13 millstream flours) >0.54 or < –0.54 are in bold and considered to be weak, moderate, or strong. Lesser positive correlations or greater negative correlations than these values are considered to be poor.
[b] Lipoxygenase (LOX), peroxidase (POX), polyphenol oxidase (PPO), ascorbate oxidase (AOX), dehydroascorbate reductase (DAR), and protein disulfide isomerase (PDI).
[c] BS change with AA = bake score (BS) with ascorbic acid (AA) minus BS without AA.
[d] Protein = % protein in flour on a 14% moisture basis.

BS change with AA may have some relevance. For Janz, the weak to poor correlations of BS change with both PDI and protein may indicate that PDI levels >50 U/g of flour, which was present in all millstreams of Janz, are sufficient to catalyze the AA improver effect, and PDI levels <50 U/g of flour may have a limiting effect on the AA improver effect.

Clearly, many factors are involved in the AA improver effect, particularly the general redox status of the flour involving free oxidized and reduced glutathione, free cysteine, protein-bound glutathione and cysteine, sulfhydryl and disulfide composition of proteins, redox enzymes and metal ions. The protein composition of flour, especially the size distribution of glutenin polymers, would also be a factor. Different factors may dominate the AA improver effect in different flours, and different mixing and proofing methods may also play a part.

CONCLUSIONS

Six redox enzymes were measured in milling fractions of two Australian and two New Zealand wheat cultivars. The enzymes LOX, DAR, and PDI all tended to be higher in the tail-end fractions of break and reduction flours, but were highest in the bran, pollard, and germ fractions. This distribution of the enzymes and the strong correlations of these enzymes with ash suggest that the main portion of the enzymes in the tail-end flour streams are derived from contamination with bran, aleurone (which is enriched in the pollard), and germ components of grain. LOX and DAR had a negative correlation with baking quality of bread made in the absence of AA but a poor correlation with the AA improver effect in baking. The tail-end flours also have higher content of glutathione, protein thiols, and trichomes (as indicated by high ash content), and it is probably these components, rather than the enzymes, that are associated with poor quality bread. PDI of Frame had a negative correlation with the baking quality of bread made in the absence of AA, but for the other cultivars the correlation was poor. PDI was the only redox enzyme to have positive correlations with the AA improver effect on bread quality.

PPO and AOX tended to be evenly distributed in the millstream flours, and, although POX tended to be higher in the break flours, all three enzymes generally had poor correlations with ash and the baking parameters. This suggests that the content and distribution of these enzymes in starchy endosperm was the main factor determining the distribution of these enzymes in millstream flours.

The relationship of the redox enzymes to the baking parameters indicates that the endogenous levels of these enzymes were not a limiting factor influencing the breadmaking process, except for PDI, the levels of which may have positively influenced bread making in the presence of AA.

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Erratum

Corrections were made to this article on April 10, 2006.

Statements were corrected at the end of paragraphs 2 and 3 on page 63:

"One unit (U) of AOX or DAR activity is defined as the amount of activity that oxidizes or reduces 1.0 nanomol of substrate in 1 min at 30°C."

"One unit (U) of LOX activity is defined as the amount of activity that oxidizes 1.0 nanomol of linoleic acid in 1 min at 30°C."

Definitions of one unit of enzyme activity were inserted into Figures 1, 4, and 5.