Journal of Cereal Science **34** (2001) 233–242
doi:10.1006/jcrs.2000.0393, available online at http://www.idealibrary.com on IDEAL®

Distribution of Enzymes in Wheat Flour Mill Streams

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Received 13 June 2000

ABSTRACT

The distribution of *alpha*-amylase, protease, lipoxygenase, polyphenol oxidase and peroxidase in wheat roller flour mill streams was studied. Break flours had relatively less *alpha*-amylase and protease activity than reduction flours both on flour weight and a protein basis. Among the different flour streams, the 5th and 6th reduction passage had the highest *alpha*-amylase activity, while the 4th reduction passage had the highest protease activity. The lipoxygenase activity was concentrated mostly in the last break and the reduction streams, whereas polyphenol oxidase activity was highest in break flour streams. Peroxidase activity was distributed unevenly among the different mill streams.

The lipoxygenase, polyphenol oxidase and peroxidase were highly concentrated in different bran fractions. Except for protease, the other enzymes were concentrated in the 'atta', a milling by-product comprising refined flour, bran and shorts; and are least active in semolina (farina).

Keywords: wheat flour, mill streams, *alpha*-amylase, protease, lipoxygenase, polyphenol oxidase, peroxidase.

INTRODUCTION

Wheat flour contains several technologically important enzymes such as amylases, proteases, lipoxygenase, polyphenol oxidase and peroxidase. In the wheat grain *alpha*-amylase is located mainly in the pericarp with small quantities present in the aleurone layer and the seed coat[1]. Protease is concentrated in the endosperm, germ and aleurone layer[2]. The scutellum and embryo are rich in lipoxygenase[3,4]. Polyphenol oxidase and peroxidase are predominant in bran layers[5]. Although these enzymes are inactive during storage of grain and flour, when water is added they become active and play a significant role in determining the functional attributes of flour.

The aim of roller flour milling is the gradual reduction of the wheat kernel through a series of break and reduction rolls. This results in the production of different types of flour streams containing endosperm, bran and germ in varying proportions. Therefore, the levels of activity of various enzymes differ in different flour mill streams and hence their functional properties are different. Several reports are available on the protein and ash contents in different flour mill streams[6–11] and a few reports are available on the suitability of various mill streams for different bakery products[6,9,12]. Some information is available on the activity of individual enzymes in mill streams. Finney *et al.*[13] reported variations in distribution of *alpha*-amylase in various mill streams of soft winter wheats. Monnier and Godon[14] reported that proteolytic activity was found to be higher in tail end reduction streams. Hatcher and Kruger[15] reported that polyphenol oxidase activity was linearly correlated with ash content and it was also reported that PPO was most active in bran- and germ-rich milling fractions[16]. The distribution of lipoxygenase[4] and peroxidase[5] in flour streams has also been reported.

ABBREVIATIONS USED: AT = atta; B1–B5 = breaks 1–5; BD = bran delux; BF = bran flakes; BR = bran rough; C1–C6 = reductions 1–6; CMD = coarse middling diviser; LOX = lipoxygenase; POD = peroxidase; PPO = polyphenol oxidase; SO = semolina.

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Apart from these reports for enzymes in individual fractions, no information is available on the distribution of functionally important enzymes in mill streams collected from a commercial roller flour mill. This was the aim of the present work. Such information would be useful in the preparation of blends either by selecting or omitting particular stream/streams for the preparation of specific mill fractions for use in bakery products.

EXPERIMENTAL

Materials

Flour mill streams

Commercially available medium strong *Punjab* wheat was used in the study. The wheat was from crop sown in late November and harvested in May/June 1998 (*rabi* crop). The moisture, ash and protein contents were 9·3, 1·50 and 11·5%, respectively. The wheat had a Falling Number value of 440 s indicating that it was unsprouted[17]. After conditioning to 15·5% moisture and tempering for 24 h the wheat was milled in a roller flour mill (Buhler, Switzerland) of 60 tonne capacity per day.

The fractions collected were break flours (numbers B1 to B5); reduction flours (numbers C1 to C6); a coarse middling diviser (CMD), the middling fraction from the break section that goes for grading; a resultant 'atta' flour which accounts for 5–15% of the total milled products and contains a mixture of refined flour, bran and shorts; a semolina (farina) sample; and three bran samples, viz., 'bran flake' (overs of 1000 μ), 'bran rough' (throughs of 1000 μ and overs of 530 μ) and 'bran deluxe' (throughs of 530 μ and overs of 243 μ). The ash content in three bran samples increased with increase in particle size whereas the endosperm content decreased. Bran flakes were removed at the end of break rolls while 'bran rough' and 'bran deluxe' were removed at the end of reduction system.

Methods

Analysis

Moisture, ash, falling number in wheat flour were carried out using standard AACC methods[18]. Protein was estimated by the micro Kjeldahl method using a conversion factor of 5·7[18].

Enzyme extraction for alpha-amylase activity determination

Flour (1·0 g) was stirred for 2 h with 5·0 mL of acetate buffer (pH 7·5) and centrifuged for 10 min at $8000 \times \boldsymbol{g}$. The supernatant was used for the analysis of *alpha*-amylase.

Enzyme extraction for protease, lipoxygenase, polyphenol oxidase and peroxidase activity determination

Flour (1·0 g) was stirred for 2 h at 4 °C with 5·0 mL of 50 mM sodium phosphate buffer (pH 7·5) and centrifuged for 15 min at $8000 \times \boldsymbol{g}$. The supernatant was used for the analysis of protease, LOX, PPO, and POD.

Measurement of enzyme activity

Alpha-amylase was assayed as described by Bernfeld[19] with some modifications. Substrate (soluble starch; 1 g/100 mL; 1·0 mL) was incubated with 1·0 mL of appropriate concentration of the enzyme for 3 min. The enzyme reaction was stopped by the addition of 2·0 mL of dinitrosalicylic acid reagent. Colour was developed by heating the tubes for 5 min in boiling water bath followed by subsequent cooling. The solution was made to the required volume and the optical density measured at 540 nm. Maltose was used as the standard. The results were expressed as mg maltose liberated in 3 min at 37 °C by 1·0 mL of enzyme solution.

Protease assay was as described by Sarath *et al.*[20] with some modifications. Azocasein substrate (25 mg) was dissolved in 1 mL of 50mM sodium phosphate buffer (pH 7·5). Sodium phosphate buffer (50 mM, pH 7·5; 450 μL), was added to 50 μL of substrate solution and pre-incubated for 10 min at 37 °C. Enzyme extract (200 μL) was added and the mixture was incubated for 30 min at 37 °C. The reaction was terminated by adding 0·5 mL of 10% TCA and the precipitate removed by centrifugation at $8000 \times \boldsymbol{g}$ for 10 min at 4 °C. NaOH (40 μL, 10 M) was added to the supernatant and the absorbance was read at 440 nm using a Shimadzu UV-vis spectrophotometer (UV-160A). One unit of activity is defined as the change in absorbance of 1·0 unit.

To measure lipoxygenase activity, the linoleic acid substrate was prepared according to Shiiba *et al.*[21]. The reaction mixture consisted of 50 mM sodium acetate buffer (pH 5·5; 950 μL), linoleic acid substrate ($7{\cdot}5 \times 10^{-3}$ M, 30 μL) and enzyme extract (20 μL). Enzyme activity was expressed in

Table I Proximate compositions of wheat flour roller mill streams

Streams	Moisture (%)	Ash* (%)	Protein* (%)
Wheat	$9{\cdot}32 \pm 0{\cdot}05^{a}$	$1{\cdot}50 \pm 0{\cdot}06^{i}$	$11{\cdot}50 \pm 0{\cdot}080^{def}$
B1	$12{\cdot}85 \pm 0{\cdot}08^{jklmn}$	$0{\cdot}60 \pm 0{\cdot}021^{bcd}$	$11{\cdot}67 \pm 0{\cdot}07^{fgh}$
B2	$12{\cdot}70 \pm 0{\cdot}06^{jklm}$	$0{\cdot}68 \pm 0{\cdot}055^{e}$	$11{\cdot}79 \pm 0{\cdot}082^{hi}$
B3	$11{\cdot}83 \pm 0{\cdot}08^{d}$	$0{\cdot}95 \pm 0{\cdot}053^{f}$	$11{\cdot}90 \pm 0{\cdot}346^{l}$
B4	$12{\cdot}20 \pm 0{\cdot}10^{f}$	$1{\cdot}02 \pm 0{\cdot}042^{g}$	$12{\cdot}15 \pm 0{\cdot}132^{j}$
B5	$12{\cdot}33 \pm 0{\cdot}075^{h}$	$2{\cdot}07 \pm 0{\cdot}05^{j}$	$12{\cdot}26 \pm 0{\cdot}153^{j}$
C1	$12{\cdot}26 \pm 0{\cdot}06^{g}$	$0{\cdot}51 \pm 0{\cdot}026^{a}$	$10{\cdot}62 \pm 0{\cdot}057^{b}$
C2	$11{\cdot}86 \pm 0{\cdot}09^{e}$	$0{\cdot}52 \pm 0{\cdot}026^{ab}$	$10{\cdot}78 \pm 0{\cdot}071^{b}$
C3	$12{\cdot}87 \pm 0{\cdot}085^{klmn}$	$0{\cdot}49 \pm 0{\cdot}025^{a}$	$11{\cdot}76 \pm 0{\cdot}066^{ghi}$
C4	$12{\cdot}35 \pm 0{\cdot}05^{l}$	$0{\cdot}51 \pm 0{\cdot}026^{a}$	$11{\cdot}38 \pm 0{\cdot}666^{cde}$
C5	$11{\cdot}68 \pm 0{\cdot}076^{c}$	$0{\cdot}64 \pm 0{\cdot}021^{cde}$	$12{\cdot}53 \pm 0{\cdot}067^{k}$
C6	$13{\cdot}02 \pm 0{\cdot}10^{no}$	$0{\cdot}64 \pm 0{\cdot}021^{cde}$	$11{\cdot}45 \pm 0{\cdot}050^{cde}$
CMD	$12{\cdot}71 \pm 0{\cdot}09^{jklm}$	$0{\cdot}57 \pm 0{\cdot}032^{abcd}$	$13{\cdot}86 \pm 0{\cdot}056^{l}$
Atta	$11{\cdot}28 \pm 0{\cdot}10^{b}$	$1{\cdot}30 \pm 0{\cdot}061^{h}$	$11{\cdot}55 \pm 0{\cdot}050^{f}$
Semolina	$13{\cdot}44 \pm 0{\cdot}07^{q}$	$0{\cdot}57 \pm 0{\cdot}03^{abcd}$	$9{\cdot}85 \pm 0{\cdot}050^{a}$
BF	$13{\cdot}20 \pm 0{\cdot}06^{op}$	$5{\cdot}36 \pm 0{\cdot}045^{m}$	$14{\cdot}12 \pm 0{\cdot}062^{m}$
BR	$12{\cdot}95 \pm 0{\cdot}05^{mno}$	$4{\cdot}43 \pm 0{\cdot}066^{l}$	$15{\cdot}92 \pm 0{\cdot}067^{n}$
BD	$12{\cdot}89 \pm 0{\cdot}11^{lmn}$	$3{\cdot}40 \pm 0{\cdot}072^{k}$	$15{\cdot}82 \pm 0{\cdot}076^{n}$

* Expressed on 14% moisture basis.
Means of same column followed by different letters differ significantly ($p<0{\cdot}05$)
B1–B5: break flours; C1–C6: reduction flours; CMD: course middling diviser; Atta: resultant atta; BF: bran flakes; BR: bran rough; BD: bran delux.

terms of hydroperoxide formed (μmol) per min using an extinction value of $2{\cdot}5 \times 10^{4}\ \text{M}^{-1}\,\text{cm}^{-1}$.

Polyphenol oxidase activity was measured by using a reaction mixture containing 50 mM sodium acetate buffer (pH 5·5; 800 μL), 0·5 M catechol (100 μL) and enzyme extract (100 μL). The increase in the absorbance was monitored at 420 nm for 180 s using a Shimadzu UV-vis Spectrophotometer (UV-160A). One unit of enzyme activity is defined as the change in absorbance of 0·001 per min[22].

The reaction mixture to measure peroxidase activity contained 50 mM sodium acetate buffer (pH 5·5, 780 μL), 1% H_2O_2 (100 μL), 0·25% *o*-dianisidine (100 μL) and enzyme extract (20 μL). The increase in absorbance was monitored at 460 nm. One unit of peroxidase activity is defined as the change in absorbance of 1·0 per min[23].

Statistical analysis

The data were statistically analysed using Duncan's New Multiple Range Test[24].

RESULTS AND DISCUSSION

Proximate composition

Table I shows the moisture, ash and protein contents of different flour mill streams. The moisture content in milled samples varied between 11·68% and 13·44%. The ash content in the initial reduction streams was lower than other streams. Protein content increased as the number of break or reduction passages increased. In general the protein content was lower in the products of the reduction passages. The protein content ranged between 10·62% and 12·53% among the different flour streams.

Alpha-amylase activity

The distribution of *alpha*-amylase in different mill streams (Table II, Fig. 1) showed that the break flours had relatively less *alpha*-amylase activity than the reduction flours. Among the break streams, B5 had higher amylase activity than the earlier break streams. A similar trend was observed in the reduction streams. The 5th and 6th reduction flours (C5, C6) showed a higher activity than the initial reductions (Table II). These two streams also had the highest activity of all mill streams. This trend is clearly indicated in Figure 2 showing cumulative *alpha* -amylase content (%) as a function of cumulative flour production (%). The curve is similar to the well known cumulative ash curve[25]. This information helps in selection of suitable streams for blending for either high or low *alpha*-amylase contents. In a fully mature wheat grain

Table II Enzyme activity in wheat flour mill streams*

Stream	*Alpha*-amylase	Protease	LOX	PPO	POD
Wheat	$4{\cdot}20 \pm 0{\cdot}032^{m}$	$165{\cdot}20 \pm 2{\cdot}10^{h}$	$13{\cdot}10 \pm 0{\cdot}14^{l}$	$35363{\cdot}0 \pm 657{\cdot}0^{l}$	$2496{\cdot}0 \pm 93{\cdot}0^{l}$
B1	$2{\cdot}74 \pm 0{\cdot}080^{c}$	$8{\cdot}68 \pm 0{\cdot}21^{ab}$	$9{\cdot}90 \pm 0{\cdot}13^{gh}$	$21846{\cdot}0 \pm 500{\cdot}0^{h}$	$751{\cdot}0 \pm 44{\cdot}0^{abc}$
B2	$2{\cdot}60 \pm 0{\cdot}050^{abc}$	$26{\cdot}54 \pm 0{\cdot}18^{c}$	$8{\cdot}17 \pm 0{\cdot}09^{d}$	$13398{\cdot}0 \pm 379{\cdot}0^{cde}$	$1893{\cdot}0 \pm 75{\cdot}0^{ij}$
B3	$2{\cdot}67 \pm 0{\cdot}060^{bc}$	$4{\cdot}78 \pm 0{\cdot}08^{ab}$	$4{\cdot}20 \pm 0{\cdot}05^{c}$	$16263{\cdot}0 \pm 480{\cdot}0^{efg}$	$1832{\cdot}0 \pm 81{\cdot}0^{hi}$
B4	$3{\cdot}12 \pm 0{\cdot}080^{d}$	$2{\cdot}22 \pm 0{\cdot}05^{a}$	$10{\cdot}86 \pm 0{\cdot}14^{jk}$	$14814{\cdot}0 \pm 411{\cdot}0^{def}$	$1468{\cdot}0 \pm 51{\cdot}0^{fg}$
B5	$3{\cdot}71 \pm 0{\cdot}080^{ghi}$	$10{\cdot}48 \pm 0{\cdot}10^{ab}$	$9{\cdot}60 \pm 0{\cdot}14^{gh}$	$19747{\cdot}0 \pm 369{\cdot}0^{fgh}$	$741{\cdot}0 \pm 38{\cdot}0^{abc}$
C1	$4{\cdot}10 \pm 0{\cdot}090^{jklm}$	$246{\cdot}00 \pm 2{\cdot}00^{j}$	$2{\cdot}70 \pm 0{\cdot}09^{b}$	$1397{\cdot}0 \pm 58{\cdot}0^{a}$	$1647{\cdot}0 \pm 52{\cdot}0^{gh}$
C2	$3{\cdot}71 \pm 0{\cdot}090^{ghi}$	$322{\cdot}00 \pm 9{\cdot}90^{k}$	$2{\cdot}71 \pm 0{\cdot}07^{b}$	$1967{\cdot}0 \pm 65{\cdot}0^{a}$	$1104{\cdot}0 \pm 28{\cdot}0^{de}$
C3	$4{\cdot}20 \pm 0{\cdot}100^{m}$	$181{\cdot}00 \pm 2{\cdot}00^{l}$	$8{\cdot}40 \pm 0{\cdot}10^{de}$	$9595{\cdot}0 \pm 206{\cdot}0^{bc}$	$2041{\cdot}0 \pm 30{\cdot}0^{jk}$
C4	$4{\cdot}30 \pm 0{\cdot}085^{m}$	$438{\cdot}40 \pm 5{\cdot}20^{l}$	$11{\cdot}30 \pm 0{\cdot}13^{jk}$	$6590{\cdot}0 \pm 170{\cdot}0^{ab}$	$923{\cdot}0 \pm 75{\cdot}0^{cd}$
C5	$5{\cdot}80 \pm 0{\cdot}120^{o}$	$85{\cdot}20 \pm 1{\cdot}50^{f}$	$9{\cdot}13 \pm 0{\cdot}12^{ef}$	$2998{\cdot}0 \pm 44{\cdot}0^{a}$	$2164{\cdot}0 \pm 28{\cdot}0^{k}$
C6	$5{\cdot}90 \pm 0{\cdot}110^{p}$	$118{\cdot}00 \pm 1{\cdot}70^{g}$	$10{\cdot}12 \pm 0{\cdot}12^{l}$	$2620{\cdot}0 \pm 48{\cdot}0^{a}$	$847{\cdot}0 \pm 40{\cdot}0^{bc}$
CMD	$2{\cdot}28 \pm 0{\cdot}040^{a}$	$187{\cdot}60 \pm 3{\cdot}10^{i}$	$1{\cdot}73 \pm 0{\cdot}04^{a}$	$20923{\cdot}0 \pm 610{\cdot}0^{gh}$	$1226{\cdot}0 \pm 69{\cdot}0^{e}$
Atta	$4{\cdot}98 \pm 0{\cdot}100^{n}$	$18{\cdot}20 \pm 0{\cdot}58^{bc}$	$20{\cdot}52 \pm 0{\cdot}20^{n}$	$38961{\cdot}0 \pm 482{\cdot}0^{i}$	$3723{\cdot}0 \pm 97{\cdot}0^{m}$
Semolina	$3{\cdot}40 \pm 0{\cdot}070^{defgh}$	$128{\cdot}00 \pm 1{\cdot}90^{g}$	$4{\cdot}86 \pm 0{\cdot}08^{c}$	$2027{\cdot}0 \pm 55{\cdot}0^{a}$	$597{\cdot}0 \pm 26{\cdot}0^{a}$
BF	$3{\cdot}88 \pm 0{\cdot}090^{ijkl}$	$47{\cdot}40 \pm 1{\cdot}80^{de}$	$19{\cdot}50 \pm 0{\cdot}60^{m}$	$82861{\cdot}0 \pm 1660{\cdot}0^{j}$	$4107{\cdot}0 \pm 74{\cdot}0^{n}$
BR	$3{\cdot}70 \pm 0{\cdot}095^{fghi}$	$54{\cdot}00 \pm 1{\cdot}70^{e}$	$27{\cdot}20 \pm 0{\cdot}42^{o}$	$103643{\cdot}0 \pm 2176{\cdot}0^{l}$	$1444{\cdot}0 \pm 60{\cdot}0^{f}$
BD	$3{\cdot}56 \pm 0{\cdot}045^{efghi}$	$35{\cdot}00 \pm 1{\cdot}60^{cd}$	$11{\cdot}00 \pm 0{\cdot}15^{k}$	$93552{\cdot}0 \pm 2000{\cdot}0^{k}$	$5183{\cdot}0 \pm 84{\cdot}0^{o}$

* Values expressed as units/g protein.
Means of same column followed by different letters differ significantly ($p<0{\cdot}05$)
LOX: lipoxygenase; PPO: polyphenol oxidase; POD: peroxidase
B1–B5: break flours; C1–C6: reduction flours; CMD: course middling diviser; Atta: resultant atta; BF: bran flakes; BR: bran rough; BD: bran delux.

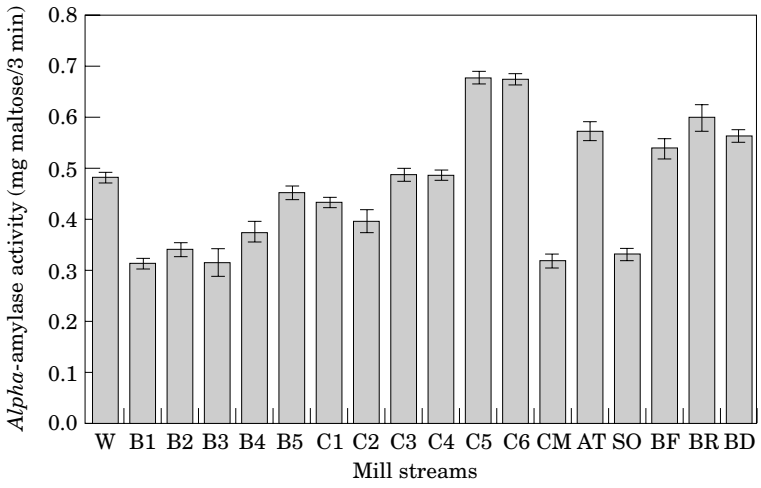

Figure 1 *Alpha*-amylase activity in flour mill streams. (W) Wheat; (B1–B5) break flours; (C1–C6) reduction flours; (CM) coarse middling diviser; (AT) resultant 'atta'; (SO) semolina; (BF) bran flakes; (BR) bran rough; (BD) bran deluxe.

alpha-amylase activity is mostly located in the seed coat, aleurone layer and scutellum[1]. The higher levels of amylase in the final break and reduction flours are not unexpected as some aleurone is present in these flour streams[26]. Kruger and Tipples[1] reported that removal of the latter reduction flour streams minimised the *alpha*-amylase content of the flour, consistent with this observation that these mill stream fractions contain relatively high levels of *alpha*-amylase activity. Germination of the grain affects the distribution of this enzyme which penetrates into the inner portion of the endosperm[27–29]. This would indeed affect the distribution of *alpha*-amylase in mill streams[27,29].

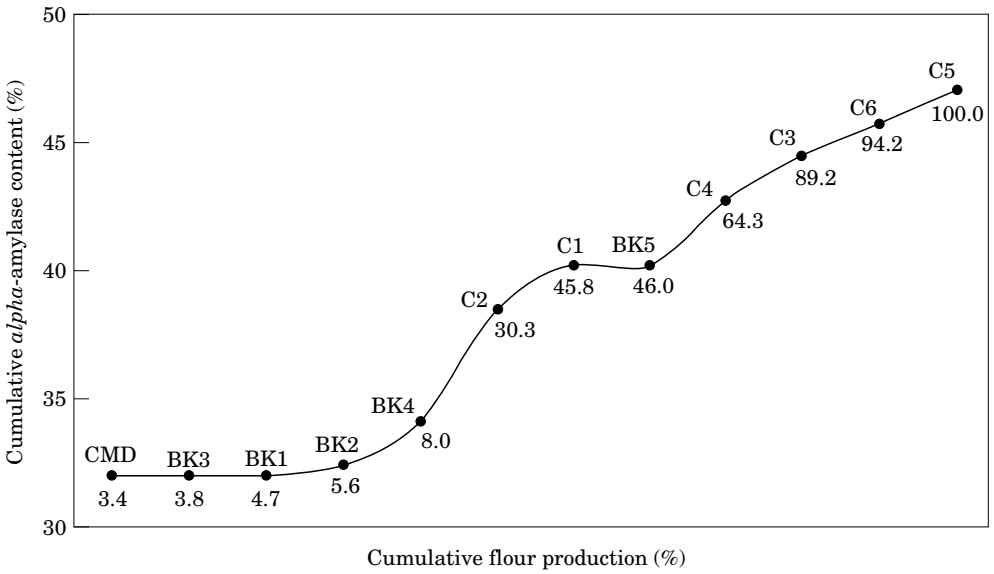

Figure 2 Cumulative *alpha*-amylase (%) content versus cumulative flour production (%). (B1–B5) break flours; (C1–C6) reduction flours; (CMD) coarse middling diviser.

The three bran samples, had relatively high *alpha*-amylase activity and the difference between these three samples was only marginal. The reason for higher activity in bran could be due to the presence of outer endosperm portion, which generally adheres to the bran[30]. The highest concentration of enzyme is found in the outer layers of the endosperm and near the germ[31]. Posner and Hibbs[30] explain that debranning or pearling the wheat kernels to remove the embryo and outer layers of bran reduces *alpha*-amylase activity in the flour.

Among the other samples resultant 'atta' (AT) had higher *alpha*-amylase activity. Semonlina (SO), which is a relatively pure endosperm fraction[32] has relatively low amylase activity because it comes from the core of the kernel where the *alpha*-amylase activity is relatively low[33].

Protease activity

The protease activity was higher in reduction streams than in the break flour streams (Table II, Fig.3). Among the reduction streams, C4 had the highest activity, which was about 2·5 times higher than that present in wheat. The C2 had the second highest activity. C1 and C3 had almost the same protease activity. End reduction streams (C5 and C6) contained relatively lower levels of protease.

Protease activity was very low in the break flour streams. Among the break flours highest activity was found in B2 and lowest activity in B4. CMD had a protease activity of 26 U/g. The presence of streams having higher protease activity (C1–C4) can reduce dough mixing time, and help in the maturation of the doughs and enhance dough extensibility[34,35]. These are important in machining of doughs.

Among the other streams SO had a value of 12·6 U/g. This was followed by BR, BF and BD. This shows that the bran portion of the wheat kernel had a lower protease activity compared to reduction streams. Streams containing more bran, such as the tail end of the break or reduction streams, also had lower protease activity. The results indicate that reduction flour streams have higher protease activity. These results are generally in accordance with those of Monnier and Godon[14]. Protease is distributed throughout the wheat kernel but is reported to be concentrated in the endosperm, germ and also in aleurone layer[2,36]. As C4 and C2 had higher protease activity and among the break flours early breaks had higher activity it is possible that reduction streams may have a higher proportion of germ and aleurone layer. The C1–C4 streams with higher protease activity could help dough softening during fermentation.

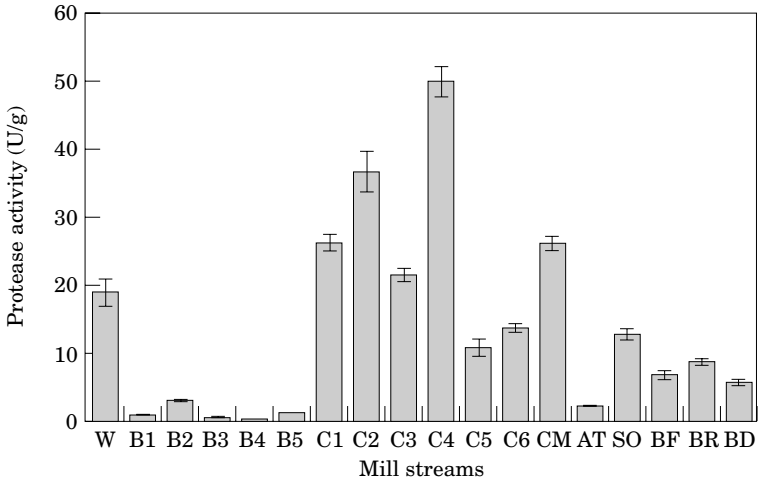

Figure 3 Protease activity in flour mill streams. (W) Wheat; (B1–B5) break flours; (C1–C6) reduction flours; (CM) coarse middling diviser; (AT) resultant 'atta'; (SO) semolina; (BF) bran flakes; (BR) bran rough; (BD) bran deluxe.

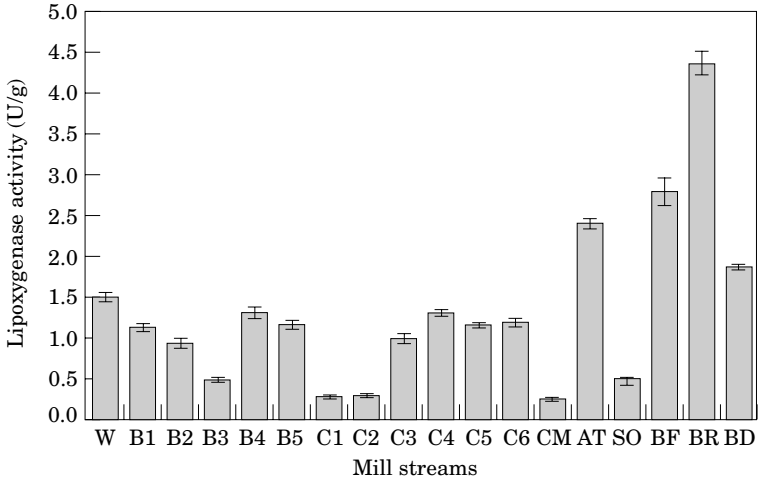

Figure 4 Lipoxygenase activity in flour mill streams. (W) Wheat; (B1–B5) break flours; (C1–C6) reduction flours; (CM) coarse middling diviser; (AT) resultant 'atta'; (SO) semolina; (BF) bran flakes; (BR) bran rough; (BD) bran deluxe.

Lipoxygenase activity (LOX)

Wheat used for milling had a LOX activity of 1·5 U/g. LOX activity varied widely in streams and the values ranged from 0·24 to 4·3 U/g. LOX activity was relatively high in the tail-end fractions of break (B4 and B5) and reduction (C4, C5 and C6) streams (Table II, Fig. 4). This is because the tail-end streams besides the endosperm contains a small amount of bran and germ, that are rich in LOX. Miller and Kummerow[4] first showed that among the milled wheat fractions, the germ showed the greatest LOX activity and later Auerman *et al.*[37] reported that wheat germ and bran contains 17-fold and 4-fold more LOX activity, respectively, than the endosperm. LOX bleaches the pigment of the flour and increases the mixing tolerance in dough[38–40] hence strengthening the dough during breadmaking.

Polyphenol oxidase activity (PPO)

In wheat-based products, particularly those based on whole wheat flour, PPO activity has been implicated in darkening reactions in the dough

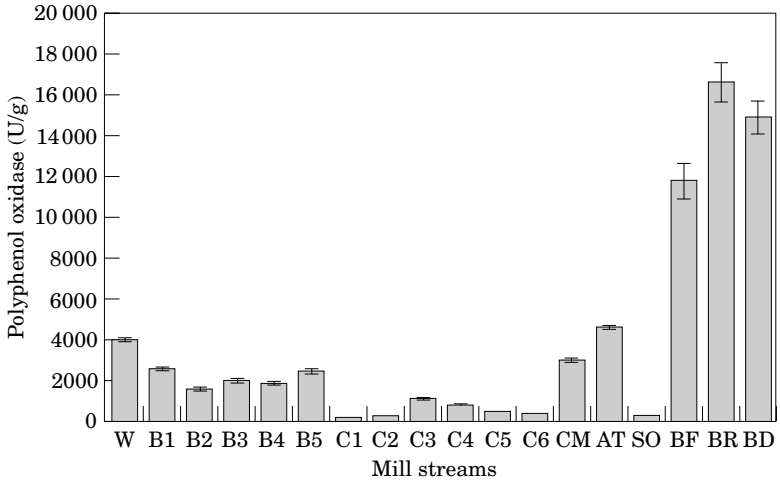

Figure 5 Polyphenol oxidase activity in flour mill streams. (W) Wheat; (B1–B5) break flours; (C1–C6) reduction flours; (CM) coarse middling diviser; (AT) resultant 'atta'; (SO) semolina; (BF) bran flakes; (BR) bran rough; (BD) bran deluxe.

that limit the acceptability of certain pasta products and chapatis[41,42]. PPO activity and distribution within the wheat grain differs with variety and the stage of its development[43,44]. Abrol and Upretty[41] reported high activity of PPO, in the grain coat.

Break streams contained relatively higher levels of PPO than reduction streams (Table II, Fig. 5). In the break streams, PPO activity was between 1578 U/g and 2550 U/g. Compared to the high levels of PPO present in the bran portion, the difference amongst break streams was only marginal.

Compared with the break flours, reduction flours had very little PPO activity. PPO produces polymeric compounds responsible for browning of the wheat flour dough. Omitting the use of those streams rich in PPO could help in overcoming the dough darkening problem.

Highest values for PPO were recorded for the three bran samples. Among them BR had the highest value at 16 500 U/g followed by BD which had 14 800 U/g and then the BF having a value of 11 700 U/g. Atta had a slightly high PPO activity since it contains a reasonable amount of bran. SO had lower levels of PPO activity. The enzyme activity of wheat kernel was 4000 U/g, which was about 3–4 times lower than that found in bran samples. These results are in accordance with those of Marsh and Galliard[16] who reported that bran contained higher levels of PPO than wholemeal flour; that white flour contained relatively low levels, and that no PPO activity could be detected in germ-rich fractions.

Peroxidase activity (POD)

The activities of POD in different roller mill streams is shown in Table II and Figure 6. Among the break streams POD was relatively more abundant in B2, B3 and B4 flours. B1 and B5 had lower activity although the difference does not seem to be very significant. Among the reduction flours, no particular trend in the distribution of POD was observed. The POD values in reduction flours ranged between 97 and 271 U/g.

Highest POD values were recorded for BD, BF and AT samples. BR had a lower value, that was comparable to that of break and reduction flours. Peroxidase activity was found to be rich in bran[5]. Its presence was also reported in the aleurone layer, scutellum and endosperm. Elimination of those streams would to some extent prevent the browning of the dough as POD is known to be involved in the destruction of carotenoid pigments during dough mixing[39,41,45,46].

SO recorded low POD activity (60 U/ g), which was the least among all stream samples. POD in the wheat kernel was higher (310 U/g) than any of the flour samples- either break or reduction. Similarly Honold and Stahmann[5] showed that the whole wheat flour had higher POD than any of the other fractions.

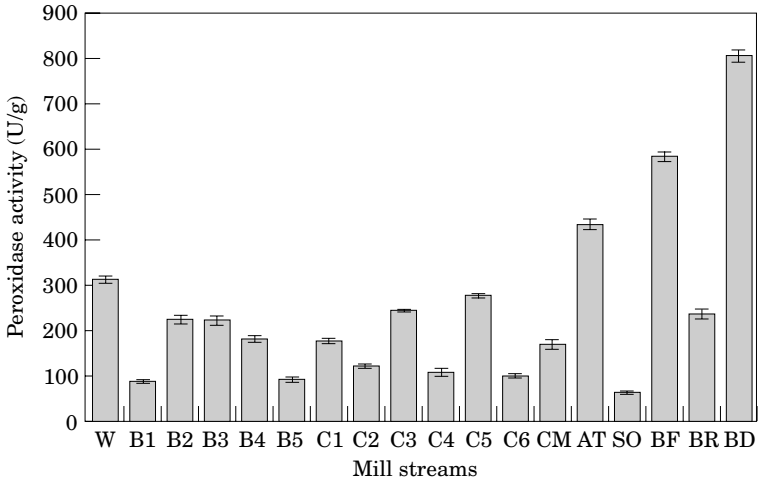

Figure 6 Peroxidase activity in flour mill streams. (W) Wheat; (B1–B5) break flours; (C1–C6) reduction flours; (CM) coarse middling diviser; (AT) resultant 'atta'; (SO) semolina; (BF) bran flakes; (BR) bran rough; (BD) bran deluxe.

Table III Comparative activities of enzymes present in wheat kernel[a] and the calculated cumulative value from streams[b]

Enzyme	Activity in wheat (units/100 g)		
	Determined value	Cumulative value	% Recovery
Alpha-amylase	48·5	45·5	94
Protease	1900	1805	95
Lipoxygenase	150	133	89
Polyphenol oxidase	390 000	370 000	95
Peroxidase	31 000	28 000	90

[a] Determined value.
[b] Cumulative value.

Enzyme activity of mill streams expressed on protein basis

It was of interest to compare enzyme activity profiles in various mill streams expressed on a protein basis and a flour basis. The results (Table II) show that, in general, the trend was the same in each case. The specific activity of *alpha*-amylase and protease was higher in the reduction streams. LOX, PPO, POD specific activities were higher in bran rich fractions and AT.

Cumulative data of enzymes in different streams

The level of individual enzymes present in the wheat grain used for milling and the cumulative enzyme content data in different mill streams are shown in Table III. The recovery of the enzymes was quite high, ranging from 89 and 95%.

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