

Molecular Level Protein Composition of Flour Mill Streams from a Pilot-Scale Flour Mill and Its Relationship to Product Quality

K. H. Sutton^{1,2} and L. D. Simmons¹

ABSTRACT

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Flour mill streams prepared from two Australian and two New Zealand wheat cultivars using a pilot-scale roller mill were analyzed for rheological and baking quality characteristics and for protein composition using size-exclusion HPLC. Differences in mill stream protein composition, on an industrially relevant scale, and the relationships between the distribution of proteins (and their degree of thiol exposure) and the technological quality of the flour mill streams were examined. Consistent, significant differences were observed in the physicochemical and processing characteristics of the flour streams. Between mill streams, changes in the quantities of the storage protein groups were more marked than for

nonstorage protein groups. Changes in protein composition differed between the break and reduction stream flours. In contrast, the degree of exposure of thiol groups on the various protein groups followed different patterns between mill streams. Numerous significant relationships were observed between dough mixing and product baking tests and the composition and thiol exposure state of the various protein classes. These relationships are discussed in context of manipulating the processing quality of flour-based products using mill streaming. A possible role for exposed thiol groups on storage proteins in the phenomenon of flour “aging” is suggested.

Commercial wheat flour milling produces a large number of flour fractions, or mill streams, that are recombined to provide flours with specific processing characteristics. Each mill stream varies in its composition and functional characteristics as a result of the irregular distribution of components within the wheat kernel (Pomeranz 1988). Variation in total protein and ash content (Nelson and Loving 1963; Nelson and McDonald 1977; Prabhasankar et al 2000; Loza-Garay and Flores 2003), flour color (Oliver et al 1993), lipids (Morrison and Hargin 1981; Prabhasankar and Haridas Rao 1999), enzyme activity (Rani et al 2001), and endoxylanase inhibition activity (Gebruers et al 2002) of mill streams have all been reported. Recently, it has been reported that variations in the composition of four mill fractions produced on a Buhler MLU-202 laboratory mill could be correlated with baking performance differences (Menkovska et al 2002). The diversity in protein composition of mill streams is reflected in the large variation in processing characteristics described by various workers (Dick et al 1977; Badi and Hosney 1978; Holas and Tipples 1978; Vera-verbete et al 1999; Feillet et al 2000; Villanueva et al 2001). The storage proteins (as defined by Osborne 1907) are one group of compounds that make a significant contribution to flour processing character (Preston et al 1995) and it has been recognized that different protein fractions contribute different processing effects (Dick et al 1979; Khan et al 1989; Gupta et al 1993; Lu and Grant 1999). The study of Menkovska et al (2002) illustrated that differences in molecular composition of mill fractions from a laboratory mill could be seen using techniques such as gel electrophoresis and that many of these compositional differences could be linked to variations in processing qualities of the flour streams. However, there has been little investigation of the effects of industrial-scale milling processes on the distribution of molecular level protein components within discrete mill streams from single cultivars, and the effect of this varying distribution on processing quality. The aim of this study was to measure the nature of the distribution of molecular level protein components occurring during the milling of four diverse wheats on an industrially relevant, pilot-scale roller mill, and to determine whether these differences in distribution could be related to the processing characteristics of the resulting

mill streams. As well as quantitating the amount of each protein class in a particular sample, the degree of thiol exposure (a measure of the extent of oxidation of the flour) on the protein was also measured using a recently published method (Sutton et al 2003). The wheats were milled as pure cultivars rather than mixed grists to ensure sample purity and avoid possible nonlinear effects of cultivar blending.

MATERIALS AND METHODS

Wheat Samples

Two New Zealand commercial wheat cultivars (Sapphire, chosen as a weaker cultivar; Monad, chosen as a strong cultivar) were grown in New Zealand during the 2000-2001 season, and two Australian commercial cultivars (Janz, normally a strong cultivar; Frame, normally a weaker cultivar) were grown in Australia during the 2000-2001 season. However, after we sourced the samples, we found that the Frame sample was high in protein and stronger than the Janz sample. For all four cultivars, 2,000 kg was milled using established procedures at 75% extraction on a 650 kg/hr pilot roller mill at BRI Australia into 14 separate mill streams: 1st, 2nd, 3rd and 4th break flours, break middlings (BM), sizings (SZ), and A, B, C, B2, D, E, F, and PF reduction flours (Moss et al 1991). In addition, a straight-run flour was collected, consisting of all the streams in proportion to production on the mill. After milling, the individual fractions were stored at 10°C to await further analysis.

Analytical Methods

Color grade, ash, moisture content, total protein ($N \times 5.7$), starch damage, farinograph, and mixograph measurements were made on duplicate samples (of the same milled sample) using a standard protocol (Holst 1993).

HPLC Analysis, Protein Thiol Labeling, and Estimation of Protein and Cysteine Content in SE-HPLC Samples

SE-HPLC analysis was performed according to the method of Sutton et al (2003), using a modification of the method of Gupta et al (1993). Flour and freeze-dried dough proteins were labeled with monobromobimane (mBrB, Sigma B-4380) according to the method of Sutton et al (2003). Estimates of protein and thiol contents were made as described in Sutton et al (2003). Reversed-phase (RP) HPLC measurements, used where necessary to elaborate SE-HPLC results, were conducted according to the method described in Bushuk et al (1997).

¹New Zealand Institute for Crop & Food Research Limited, Private Bag 4704, Christchurch 8001, New Zealand.

²Corresponding author. Phone: +64-3-3259457. Fax: +64-3-3252074. E-mail: suttonk@crop.cri.nz

Baked Product Quality Tests

Mechanical dough development (MDD) bread baking tests were performed in duplicate using a small-scale 125-g MDD system (Mitchell 1971, 1989). Loaf volume and crumb texture scores were combined to give an overall MDD bake score.

Biscuit quality was assessed using the cookie spread factor as measured using Approved Method 10-50D (AACC International 2000).

Pastry quality was assessed for a range of mill streams (selected on the basis of color acceptable to consumers) using the method of Hay (1993). Following a 60-min rest period after dough sheeting, the dough was cut into “vol-au-vent” rings 60 mm in diameter, baked for 11 min at 230°C, and allowed to cool. Ten replicates of each sample of baked pastry were measured for product height, lift ratio, shrinkage, and % skew (a measure of the evenness of lift = the difference between the maximum and minimum height of the baked pastry rings, divided by the average height).

Statistical Analyses of Data

Data from rheological testing, product (bake) testing, and SE-HPLC analyses were analyzed with analysis of variance (ANOVA) methods using the GenStat package (v. 6.2. VSN International, Oxford). All analyses were performed in duplicate, except HPLC protein analysis, which was performed in triplicate. Data analysis of the HPLC results revealed that the standard deviations of the different protein component (and protein thiol component) values were of similar magnitude. Therefore, the values for all levels were pooled to calculate a standard deviation and a confidence interval for the mean value. Least significant differences (LSD) were calculated at the 95% significance level for all experimental parameters. For each cultivar, pairwise correlations between various analytical and test results were calculated. Tables of raw data (as used for the statistical analyses) are available from the corresponding author, upon request.

RESULTS AND DISCUSSION

Flour Characteristics

The flour characteristics measured for each stream showed significant differences between all streams for color grade (which varied from -4.1 to +14.9 KJU), damaged starch (which varied from 3.2 to 15.3%), ash content (which varied from 0.31 to 2.54%), and total protein content (which varied from 8.3 to 29.9%). In general, the trend was for a gradual increase in these parameters over the successive break streams, followed by a decrease for the early reduction streams, and a subsequent increase in these parameters for later reduction streams. Not unexpectedly, the ash content of

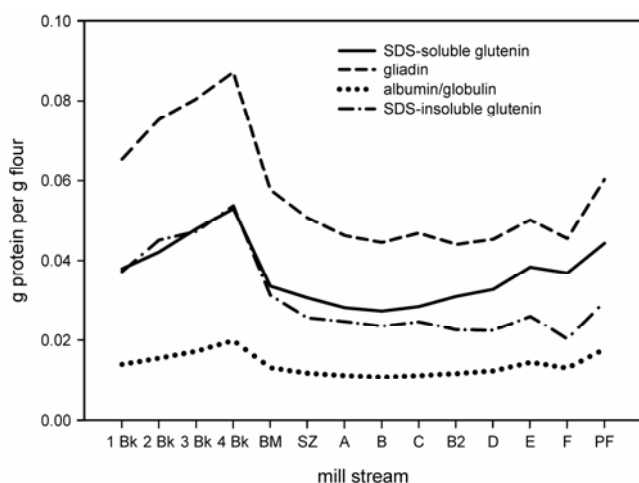


Fig. 1. Protein distribution in mill streams for cultivar Monad.

the streams was highly correlated to color grade ($r = 0.97$, $P < T = 0.001$). Similarly, but less strongly, the ash content of the streams was correlated to degree of damaged starch ($r = 0.525$, $P < T = 0.05$). The trends for these parameters were as expected given the nature of the roller milling process (that is, increasing contamination of later break and reduction flours with nonendosperm tissue) and are consistent with trends reported in the literature (Nelson and Loving 1963; Nelson and McDonald 1977; Oliver et al 1993).

Protein Distribution in Mill Streams

Detailed analysis of the protein SE-HPLC data revealed that the relative proportions of the protein subgroups did vary (although, in most cases, not greatly or significantly) during the streaming process. Changes in the quantities of storage protein groups were generally more marked than for the nonstorage (albumin and globulin) group. The quantities of gliadin, SDS-soluble glutenin, and SDS-insoluble glutenin both changed markedly during the series of break flours but were much less variable during the series of reduction flours. Figure 1 illustrates these features for the New Zealand cultivar Monad.

RP-HPLC analysis indicated that the variations in the levels of albumin and globulin proteins between mill streams of all cultivars was spread evenly across early-, mid- and late-eluting types (data not shown).

Even small changes in the ratio of SDS-insoluble glutenin to SDS-soluble glutenin can produce very large changes in the functional behavior of flours (Sutton et al 2003). Figure 2 illustrates the percentage of glutenin protein insoluble in the SDS buffer for all of the mill streams in the four cultivars under study.

Figure 2 shows that the percentage of SDS-insoluble glutenin showed similar trends for the four cultivars. The increase from 1st to 2nd break was found for all four cultivars, followed by a decline for the 3rd break and 4th break flours, except for Frame, which showed a significant increase in the 4th break flour (the increase in the Monad trend line was not significant). The percentage of SDS-insoluble glutenin decreased over the reduction flour series (streams A–F) in a similar manner for all four cultivars. The consequences of these changes in the protein profiles will be discussed later concerning the relationships of protein distribution to processing characteristics.

Detailed analysis of the protein exposed thiol SE-HPLC data revealed broadly similar trends to those seen for the protein data (above) but the relative proportions of the different protein groups varied to a greater degree than for the protein only results. Figure 3 illustrates the results obtained for the New Zealand cultivar Monad. Results for the other three cultivars showed similar trends.

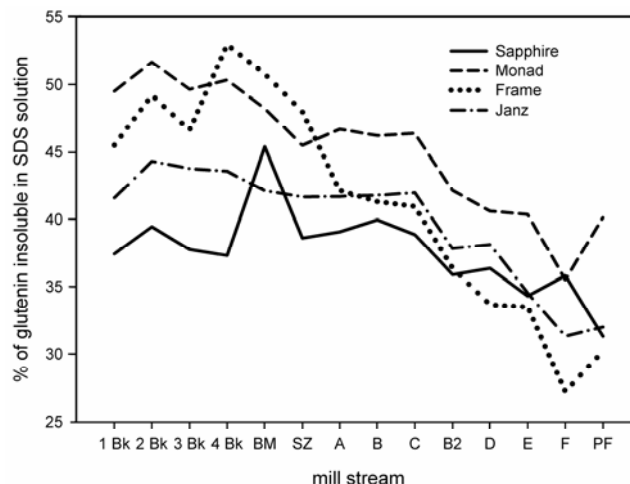


Fig. 2. Percentage of glutenin protein insoluble in SDS solution in mill streams of four cultivars.

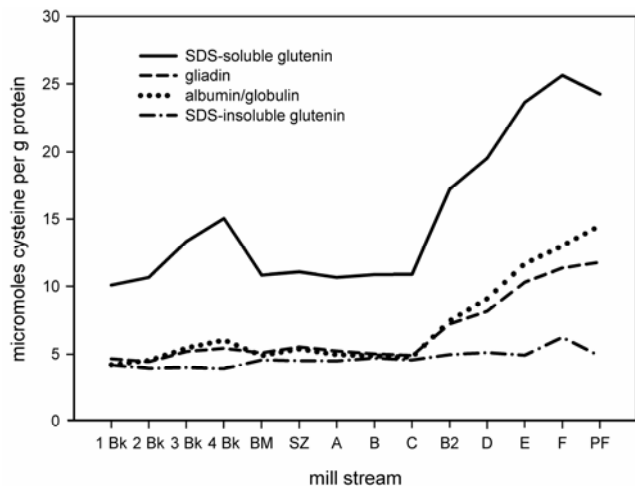


Fig. 3. Protein thiol distribution in mill streams for cultivar Monad.

Figure 3 shows that the quantities of exposed thiol on the SDS-soluble glutenin protein were significantly higher than for the other protein groups. A notable feature of Fig. 3 is that the quantity of exposed thiol on the SDS-soluble glutenin protein increased more markedly than for any of the other protein groups during the break flour series. Also notable was that the increase in the quantity of exposed thiol on the albumin/globulin, gliadin, and SDS-soluble glutenin proteins in the later reduction flours (stream B2 onward) was not found for the SDS-insoluble glutenin protein. Of interest was that the stronger NZ flour (Monad) had generally higher levels of exposed thiols on the storage protein than did the weaker NZ flour (Sapphire). A similar pattern was noticed for the stronger Australian flour (Frame) and its weaker counterpart (Janz). The consequences of these changes in the protein thiol profiles will be discussed later concerning the relationships of protein distribution to processing characteristics.

Mixing Characteristics and Relationship to Protein Distribution

Segregation of the various protein groups during the milling process should be reflected in a corresponding variation of those processing qualities that rely on protein. Mixing behavior (as measured by MDD work input, by farinograph, and by mixograph) varied with mill stream, regardless of the technique used, with MDD work input relationships being the most complex. Although MDD mixing is a combination of shear and stretch, as are farinograph and mixograph, it is conducted at higher speeds (300 rpm against 100 rpm for the farinograph) and it is difficult to relate MDD work input figures to development times obtained by farinograph or mixograph. The MDD values measured were the amounts of work input required to optimally develop the dough for each mill stream. Except for cultivar Sapphire, strong correlations across mill streams were observed between WI and the percentage of the glutenin protein that was insoluble in SDS buffer present in the flour stream ($r = 0.795$, Monad; 0.624 , Frame; 0.669 , Janz). This result was not entirely unexpected as this parameter has been associated with dough strength in the past (Gupta et al 1993). For cultivars Sapphire, Monad, and Janz, there were also highly significant correlations between WI and total protein ($r = 0.799$, Sapphire; 0.867 , Monad; 0.619 , Janz) as determined by SE-HPLC. This was also reflected in correlations between WI and the quantities of individual protein subclasses. There were no significant correlations between WI and the amount of exposed thiol on any of the storage proteins.

A relationship between total protein content (and each of the protein subfractions) and farinograph dough development time was observed for all four cultivars ($r = 0.725$, Sapphire; 0.719 , Monad; 0.921 , Frame; 0.939 , Janz). In the two strong wheats, significant

correlations were also seen between the dough development time and the percentage of the glutenin protein insoluble in SDS buffer ($r = 0.565$, Monad; 0.756 , Frame). There were no significant correlations between farinograph development time and the amount of exposed thiol on the storage proteins. For the two strong cultivars, farinograph dough stability was positively correlated with the quantity of SDS-insoluble glutenin protein ($r = 0.810$, Monad; 0.775 , Frame) but this relationship did not extend to either of the less strong cultivars. For the two Australian cultivars, farinograph dough breakdown was negatively correlated with percentage of glutenin protein insoluble in SDS buffer ($r = -0.865$, Frame; -0.873 , Janz). The same negative relationship was seen for the NZ cultivars but the relationship did not reach the 95% significance level. Similarly, for the two Australian cultivars, farinograph dough breakdown was correlated with total exposed thiol over all the dough protein groups ($r = 0.98$, Frame; 0.939 , Janz) but this relationship was not significant for the NZ cultivars.

In a similar way to that seen for the MDD and farinograph, significant positive correlations were seen in all four cultivars between total protein content and mixograph time-to-peak, peak value, and peak width, although the relationships were more robust for the two stronger cultivars. Within the protein subclasses, the strongest relationships were between the SDS-insoluble glutenin protein group and these mixograph parameters, consistent with previous observations (Gupta et al 1993). None of the mixograph parameters were correlated with the quantity of exposed thiol on the storage protein groups.

Processing Characteristics and Relationship to Protein Distribution

MDD breadbaking. Considering the SE-HPLC protein data and data for the bread tests performed on the flour mill streams, MDD loaf volume (for all cultivars except Sapphire) was highly correlated with the percentage of the glutenin protein insoluble in SDS buffer ($r = 0.891$, Monad; 0.759 , Frame; 0.974 , Janz). Similarly, MDD loaf volume was negatively correlated with the amount of exposed thiols on the SDS-soluble protein groups, and particularly for the two Australian cultivars where the relationships were very strong (for SDS-soluble glutenin protein exposed thiol vs. loaf volume, $r = -0.726$, Monad; -0.711 , Sapphire, -0.933 , Frame; -0.879 , Janz) (Fig. 4).

Interestingly, the total protein exposed thiol was, for all cultivars except Janz, significantly positively correlated with optimum dough water absorption, whereas no relationship was found between total protein content and water absorption.

Cookie spread test. There was a significant variation in cookie spread index between mill streams. Lower spread factors (and hence negative correlations against increasing amounts of constituent groups) are considered desirable in biscuits to reduce distortion and loss of surface detail in the biscuit product. Considering the SE-HPLC protein data and data for the cookie baking tests performed on the flour mill streams, cookie spread index was highly negatively correlated with the quantity of both SDS-soluble glutenin and SDS-soluble albumin and globulin proteins ($r = -0.916$, cookie spread vs. SDS-soluble albumin/globulin for cultivar Janz). Similarly, cookie spread index was highly negatively correlated with the amount of exposed thiols on all of the SDS-soluble protein groups ($r = -0.839$, cookie spread index vs. SDS-soluble glutenin exposed thiol for cultivar Sapphire). Therefore, in high-quality cookie flour designed to give reduced distortion and loss of surface detail in the biscuit product, it is more desirable to have greater amounts of the SDS-soluble protein groups present and for these protein groups to have higher amounts of exposed thiol groups.

Pastry quality test. No significant interactions were observed between protein distribution and pastry quality for the NZ cultivars, even though pastry made from cultivar Monad mill stream fractions showed significantly greater product height than pastry

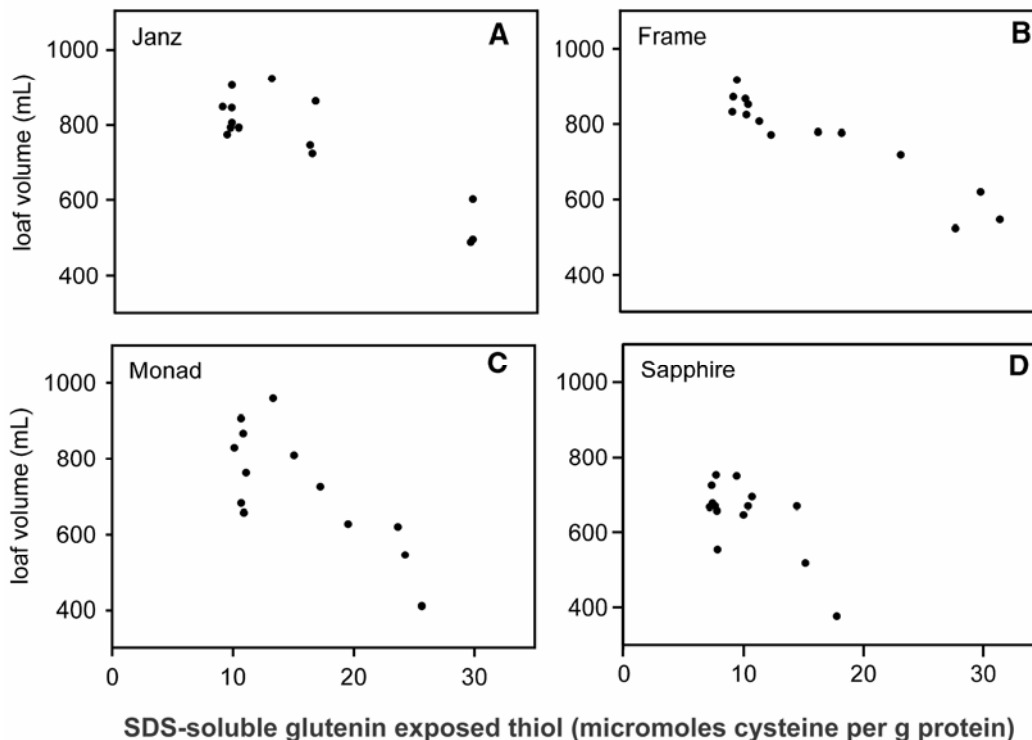


Fig. 4. Relationship between MDD loaf volume and amount of exposed thiols on SDS-soluble glutenin proteins. Cultivars: **A**, Janz; **B**, Frame; **C**, Monad; and **D**, Sapphire.

made from the other cultivars. Although most Monad mill stream fractions had more gliadin than other cultivars except Frame, there was no correlation between gliadin content and pastry height. Monad mill stream fractions were also characterized by higher MDD work input but while the protein components correlated well with MDD work input (see above), they were only weakly negatively correlated with pastry product height. For the Australian cultivars, some significant negative correlations were observed between SDS-soluble protein components and pastry height but the patterns of association were not consistent. Similar results were found when considering lift ratio, shrinkage, percentage skew, and other pastry rheological properties.

Relationship of Flour Protein Thiol Content to Processing Quality and a Possible Role in Flour Aging

As noted above, there was significant exposure of thiol groups on proteins in milled flour streams. Most notably, the later break and reduction flour streams (where a greater extent of grinding occurs and hence expectation of molecular disruption) are higher in exposed thiol content. In the discussion above, it was noted that there were highly significant correlations between the exposed thiol content of various protein subgroups and certain rheological characteristics and certain processing quality measurements. Anecdotal, it is also known that flour changes in its rheological characteristics and processing quality following milling and that it is sometimes desirable to let the flour “age” (for a period varying from several days to several weeks) before use to avoid major processing problems. This aging phenomenon is most commonly attributed to lipid oxidation processes (Gracza 1965), although some evidence has been produced that the starch granule surface proteins are involved in the process (Seguchi 1993). Our results may indicate that there is another dimension to the flour aging phenomenon involving the oxidation state of the protein thiol groups and the interactions of flour protein thiols with other proteins and dough improvers. These possibilities are being actively investigated.

CONCLUSIONS

Size-exclusion HPLC was used to determine the protein composition and degree of thiol exposure of flour mill streams prepared from two Australian and two New Zealand wheat cultivars using a pilot-scale roller mill. Significant differences were observed in both the chemical and processing characteristics of the flour streams from the four cultivars. For any of the cultivars under study, there were more marked changes in the quantities of the storage protein groups than for nonstorage protein groups and the change in protein composition was greater in the break stream flours as compared with the reduction stream flours. Contrastingly, and somewhat unexpectedly, the pattern of thiol group exposure on the storage protein groups within a set of mill streams differed to that observed for the proteins. Many significant relationships were observed between dough mixing and product baking tests and the composition and thiol exposure state of the various protein classes in the mill stream flour. This information may help to improve the control of flour milling and the development of flours with unique molecular level protein compositions. It may also provide a potential explanation of flour aging processes.

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