

PROTEIN CONCENTRATION, COMPOSITION AND DISTRIBUTION IN WHEAT FLOUR MILL STREAMS

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Abstract

Globally wheat is main cereal grain in terms of production, consumptions and trade. Milling is a mechanical gradual reduction process with different sets of break and reduction rolls to ensure maximum removal of bran and germ. Roller mills produce different flours from same grain stock. Chemical constituents are not evenly distributed in wheat kernel resulting variation in chemical composition of flour streams. Protein concentration, composition and fractions vary in distribution both in wheat kernel and flour streams. Salt-soluble proteins are concentrated in sub aleurone cells while endosperm contains mainly salt-insoluble proteins. Stream splitting is useful technique for production of quality flours where desired quality wheat is not available. Mill streams with similar quality and functional properties are blended to produce a variety of flour grades. Blending fulfills economic, qualitative and nutritional constraints and to get standardized flours. Difference in content and chemistry of proteins in different break and reduction flour streams is of immense importance for their characterization and selection for defined end uses. Milled fractions with higher arabinoxylan and protein polymers have a high oxidative potential. Thiol groups and oxidative cross links affect rheological and processing parameters of mill streams. Higher albumins and globulins quantity and lower degree crosslinkings results difference in rheological characters among break and reduction flour. Mill streams vary in dough rheology, baking performance and suitability for baked products. Different flour streams has diverse characteristics making these to suit bread or biscuit process. Selected mill stream blends result in different flour grades and diversified end products.

Keywords: mill streams, quality, protein fractions, protein composition, thiol groups, oxidative cross links

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1. INTRODUCTION

The main chemical constituents of the wheat are available and unavailable carbohydrates (65-70%), protein (10-15%) and lipids (1.5-2%) (Iuliana et al., 2010). Milling performance is a key trait involved in the processing and use of wheat (Martin et al., 2007). Interactions among endosperm constituents during the development and maturation contribute to its texture and play a significant role in milling of wheat into flour (Hamer and Hosney, 1998). Composition of gluten proteins also influences the milling properties of wheat (Menkovska and Zezelj, 1999). The main purpose of milling is isolation of the starch-protein matrix in

which the high fiber bran and high lipid germ are separated from the endosperm (Hamer and Hosney, 1998). Milling is achieved by the gradual reduction of the wheat grain through series of break and reduction rolls (Rani *et al.*, 2001). Under optimum processing conditions, milling yields a high quality uniformly colored flour with a relatively stable shelf life (GEAS, 2005). The size reduction facilitates the enzymatic and cooking processes to be carried out in an efficient way. The separation techniques are used in order to reduce the level of indigestible bran in the end product. Milling converts 70 to 75% of the wheat grain in to flour while remaining 25 to 30% as wheat by-products used for livestock consumption

(Blasi, et al., 1998). The milling process removes many important nutrients when white flour is produced (Iuliana et al., 2010). Significantly higher concentration of crude protein, fat, crude fiber, macro and trace minerals are removed from wheat as bran, aleurone cell layer and germ components of the wheat kernel. Bran, germ, shorts and red dog mill streams are produced from those portions of the wheat kernel richest in proteins, vitamins, lipids and minerals. These are eliminated during wheat milling to get white flour and results in decrease in its nutritional value for human (Blasi, et al., 1998). Highly refined (patent) flour may contain only 10 to 12% of the total thiamine and niacin, 20% of the phosphorus and 50% of the calcium of the parent grain (Shellenberger, 1970).

Roller Milling

Wheat milling is a mechanical gradual reduction process (Ruffet, 1994; Hareland and Shi 1997; Campbell, 2007; Sarakatsanis 2007) and sequence of many kinds of milling and filtration processes (Yahata et al., 2006). Flour is obtained by a gradual size reduction through different sets of break and reduction rolls (Prabhasankar and Haridas Rao, 1999) to ensure maximum removal of bran and germ (Prabhasankar et al., 2000) and the resulting endosperm particles are subsequently separated from the bran and germ particles using plan sifters and purifiers to get fine white flour.

Sequence of different sieving procedures followed in a milling diagram (Fustier et al., 2009) generates different flour fractions (Gomez et al., 2009). About 75% of the wheat is recovered as flour at the end of the milling process (Dornez *et al.*, 2006). Flour obtained after removing maximum bran and germ, represents 72% of wheat kernel and called straight grade flour (Fustier et al., 2009). In milling endosperm is first separated from the bran layers and then ground into a high number of milling streams flour (Ruffet 1994; Hareland and Shi 1997; Campbell, 2007; Sarakatsanis 2007).

Mill streams

The granularity, damaged starch, protein content, ash level and fat content, as well as

intensity of the enzymatic activity and fiber content vary with the type of mill stream (Ruffet 1994; Prabhasankar et al 2000). Flour from each break and reduction stream vary in portion of endosperm (Prabhasankar and Haridas Rao, 1999) and hence differs in quality. Each mill stream contain material whose composition vary by changing processing conditions like roller settings, purification conditions and the way in which the mill streams are combined (Matz, 1991). Flour mills produce different types of flours to satisfy the diversified process needs of end products (Miralbes, 2004). Flour specifications are different for the preparation of various bakery products (Prabhasankar et al., 2000). Split run milling provides millers more flexibility in selecting suitable wheat varieties for minimizing production costs (Villanueva *et al.*, 2001). The quality and quantity of the milling fractions obtained depend on the wheat quality and flow sheet followed in the mill (Prabhasankar et al 2000; Iuliana et al., 2010). Flour stream blending is based on the desired characteristics (Ramseyer et al., 2011a) as each flour fraction has different characteristics to suit bread or biscuit process (Miralbes, 2004). Selected mill stream blends can produce a variety of flour grades (Ramseyer et al., 2011a, b). All flour streams collected from each roll during milling are blended to get straight grade flour (Prabhasankar and Haridas Rao, 1999; Prabhasankar et al., 2000; Dornez *et al.*, 2006) while streams constituting this flour are separately known as patent, middle-cut and clear flour (Hoseney, 1994). Millers combine different flour fractions to get specific flour that satisfies customer needs. Patent flour is a combination of milled fractions having similar colour and low in ash contents (Hareland, 2003).

Quality parameters of milled fractions like ash content, color and texture are major attributes observed for their selection for blending (Hareland, 2004). Flour fractions with known quality parameters are combined in the pre-determined proportions to get flour specified for particular end product (Mirić and Pejin, 2008). Optimal blending of functional flour

fractions may eliminate or reduce the need for further treatments and decrease labeling requirements (Ramseyer et al., 2011b). A group of mill streams with similar quality (Prabhasankar et al., 2000) and functional characteristics (Ramseyer et al., 2011b) or complementary streams are combined for particular end use (Dube *et al.*, 1987) to produce commercial flour. Certain flour fractions separated at different technological stages are blended in different possible combinations to get whole mill flour to fulfill economic, qualitative and nutritional constraints and to get standardized types of superior and inferior flours (Iuliana et al., 2010) and to improve both end use and nutritional quality (Okrajková et al., 2007).

The quality of the resulting flour is determined by the composition of different mill streams selected for blending and the functionality of the different components (Iuliana et al., 2010). Technical information about the composition (Gomez et al., 2010) and quality characteristics of mill streams is very important for effective commercial milling to produce a high-quality flour with high yield (Yahata et al., 2006) and helpful in obtaining standardized flours with intended suitable qualities for specific applications (Iuliana et al., 2010) and particular product formulation (Gomez et al., 2010). Quality evaluation of mill streams and their relationships with flour chemical components is essential requirement to ensure production of high quality flour (Gomez et al., 2009). The knowledge regarding different quality traits is useful for millers to select the specified streams with desirable quality characteristics for blending to produce high quality flour (Liu et al., 2011) and optimal functionality of the different end products (Prabhasankar *et al.*, 2000) to meet customer specifications for baked products (Liu et al., 2011).

Shorts consist mostly of fine particles of bran and germ with small amounts of wheat red dog. Red dog is the by-product from the “tail of the mill,” consisting chiefly of the aleurone layer with small particles of bran, germ and flour (Blasi, et al., 1998). Wheat bran is commercially available byproduct, produced in

huge quantities in conventional milling industry. Bran is rich source of most of the minerals, vitamins and fibers present in the wheat grain (Barron, 2011).

Protein Content

The protein concentration and composition is different in various parts of the wheat grain (Okrajková et al., 2007; Daniel and Triboi 2001) and in the different milling streams (Okrajková et al., 2007; Menovska et al. 2002; Ruffet 1994; Prabhasankar et al 2000). The protein content is lower in the sub-aleurone layer in soft wheat than for hard wheat (Kent and Evers, 1969). This protein gradient resulted in different qualities and quantities of flour protein in different mill streams (Ranhotra et al 1990; Ciacco and D’Appolonia, 1982; Nelson and McDonald, 1977; Orth et al 1976). The concentration of total flour protein showed significant variation among mill streams (Wang et al., 2007). The protein content increased along the milling process in rye mill streams (Gomez et al., 2009; Nilsson et al. 1997 and Wannerberger and Eliasson, 1993). Second and third break flours contained very high protein content (22 and 26%) (Every et al., 2002).

A better knowledge and understanding about the protein characteristics in different milling streams will lead to better possibilities to produce bread of desired quality. Break streams have higher protein content than reduction streams (Gomez et al., 2010; Wang et al., 2007; Prabhasankar et al. 2000; Wang & Flores 1999 and Villanueva et al. 2001; Anjum et al., 1997, Dube et al., 1987). The break streams contained higher protein content than the middling streams of wheat milled from Bühler mill (Orth et al., 1976; Ciacco and D’Appolonia, 1982; Ranhotra et al., 1990). Protein content increase with successive break streams (Martin et al., 2007) due to incorporation of higher amount of peripheral layers of endosperm rich in protein (Prabhasankar et al. 2000; Pomeranz, 1988). Protein contents increase linearly throughout break streams (Souza et al., 2011; Iuliana et al., 2010; Fustier et al., 2009; Martin et al., 2007; Dornez et al 2006; Rani et al 2001;

Prabhasankar et al. 2000; Ziegler and Greer, 1978; Kent, 1966; Hinton, 1947) and with increasing reduction passages (Gomez et al., 2010; Iuliana et al., 2010; Dornez et al 2006; Rani et al 2001; Pomeranz et al., 1988). Total protein was low in initial break streams (Kumar et al., 2009) while highest in 4th break stream in wheat (Anjum et al., 1997) and rye (Gomez et al., 2009).

Pomeranz et al. (1988) and Simmonds and Campbell (1976) reported that reduction streams contain higher protein contents than flour from break rolls. Last break and reduction streams (B₂ and R₃) showed higher protein contents than the other streams (Gomez et al., 2010; Kumar et al., 2009; Ranhotra et al. 1990 and Prabhasankar et al. 2000b) due to contamination of the external layers of the grain (bran) containing higher protein content than endosperm (Gomez et al., 2010). Higher grade middling flours (1M, 2M and 3M) obtained from milling of hard red winter wheats were lower in protein (Wang and Flores, 1999). Protein content was lowest in sizing among different reduction streams (Anjum et al., 1997).

The protein content increases with increasing extraction rate (Ramseyer et al., 2011; Hinton 1959; Nelson and McDonald 1977; Posner and Hibbs 1997). Protein content was highest in the shorts (MF3) and bran (MF4) (Okrajková et al., 2007). Increasing trend was observed in protein content and fractions in successive break streams, followed by a decrease in early reduction streams and a subsequent increase in these parameters for later reduction streams (Sutton and Simmons, 2006). The gradual incremental trend in roller milling process is due to nature of mill where contamination of flours with non-endosperm tissue increases in later break and reduction (Nelson and Loving 1963; Nelson and McDonald 1977; Oliver et al 1993). Patent flour showed less amount of protein (Yamazaki, 1959) than flour streams but with different functional properties (Fustier et al., 2009).

Gluten Content

Gluten quality of mill streams is responsible for dough elasticity and extensibility

characteristics and flour water absorption (Hareland, 2003). There is need to evaluate the protein subunits that confer variations in gluten quality among flour mill streams and develop methods that rapidly measure gluten quality to help in preparation of flour with improved and specified end use quality (Hareland, 2003). Flour prepared from the outer layer of wheat kernels has low gluten content (Tseng and Lai, 2002). The quantity and quality of the gluten from the central endosperm is lower than those from the dorsal and ventral endosperm (Indrani et al 2003). Gluten polymer structure has prominent role in bread making quality of flour mill streams (Wang et al 2006, 2007; Liu et al., 2011). Break streams contained higher wet gluten content as compared to reduction fractions and straight grade flour (Dube et al., 1987). Wet gluten content increases with increase in the number of break (B₁-B₄) and reduction passes (C₁-C₈) (Rani et al 2001; Dornez et al 2006; Iuliana et al., 2010). Gluten content is less in initial break streams (Kumar et al., 2009). However, Anjum et al. (1997) reported lowest wet and dry gluten contents in 4th break stream. The tail end reduction streams were low in gluten forming proteins (Orth *et al.*, 1976; Indrani *et al.*, 2003).

Kumar et al. (2009) reported higher gluten levels for later breaks and middling streams. Contribution of protein to the gluten network was 83% for break flours while 69% for middling flours. Gluten content increased with an increase in number of break passages (Indrani *et al.*, 2003). The amount of dry gluten in flours showed positive correlation with protein content ($r = 0.95$). Bran flour and 3rd break stream from hard wheats contained higher gluten levels than other streams (Wang and Flores, 1999). Tail end flour streams contained lower wet gluten (Dube et al., 1987). Gluten strength of break flour fractions was higher than flour from reduction streams (Pojic et al. 2004; Sutton and Simmons, 2006). Hareland (2004) used farinograph and alveograph to quantify the gluten properties in 4 commercial varieties of hard red spring wheat (Alsen, Russ, Ember and Verde) milled to get 18 mill streams. Flour color and ash do not

show strong prediction of gluten quality (Hareland, 2003).

Sedimentation values

The sedimentation value increased from 27 to 68 ml in different break streams (Prabhasankar et al. 2000) while no definite trend observed in reduction fractions (24.0 to 54.0 ml). Lower sedimentation values were recorded in reduction streams than break streams. Sedimentation value gradually increases with increasing numbers of breaks in the flour streams (Kumar et al., 2009; Indrani et al., 2003 and Prabhasankar et al., 2000). Third and fourth breaks showed higher value (Kumar et al., 2009). The alkaline water retention capacity was found higher in break flour stream than reduction flour stream (Anjum et al., 1997).

Distribution of proteins

Protein classes of different nature vary in their distribution in various parts of wheat grain (Daniel and Triboi, 2001). Salt-soluble proteins are generally concentrated in the sub aleurone cells while endosperm contains mainly the salt-insoluble proteins, including gliadin and glutenin (Tseng and Lai, 2002). Concentration and distribution of different proteins and protein fractions vary in different in different portions of wheat grain as shown by the amino acid analyses of different sections of wheat kernel (Jensen and Martens, 1983). The quantities of storage protein groups showed marked changes than non-storage protein groups and the change in protein composition was greater in the break stream flours as compared to reduction stream flours (Sutton and Simmons, 2006).

Protein composition differed among different flour fractions (Sutton and Simmons, 2006). Albumins and globulins are generally concentrated in the embryo while gliadins and glutenin subunits are found in greater amount in the endosperm (Daniel and Triboi, 2001). The quantity of smaller monomeric proteins (albumins and globulins) was higher in shorts than other mill streams while bran contains greater amount of high molecular-weight (HMW) glutenin subunits. Reduction flour fractions showed greater quantity of albumins and globulins. Break and reduction flour

fractions contained high amount of large polymeric proteins. Concentration of albumins and globulins were highest in the shorts while maximum gliadins content was recorded in break flour, reduction flour and whole meal flour (Okrajková et al., 2007).

Protein quantity, concentration of protein groups, amount and size distribution of polymeric and monomeric proteins were similar in different milled fractions for the two cultivars despite of differences in their genetic makeup (Okrajková et al., 2007). Variation in concentration and composition of protein in different break and reduction flour fractions is of greater significance for their characterization and selection for defined end uses (Sutton and Simmons, 2006). Electrophoretic analysis of extracted protein fractions is a valuable tool for quantification and investigation of the difference in functional attributes of different flour fractions (Wang et al., 2006).

Analysis of the protein fractions in break and middling streams showed no significant differences in the levels of glutenin, gliadin and albumin (Wang and Flores, 1999). Gliadin and glutenin contents were greater in later break streams among gluten fractions. Residue proteins were lower in middling streams in comparison with break streams. SDS-PAGE of different streams indicated higher content of high molecular weight glutenin subunits in later breaks and early middling streams (Kumar et al., 2009). HMW glutenin subunits were found in higher amount in break flour, bran and whole meal flour (Okrajková et al., 2007). Gliadins as well as glutenin subunits were found to higher in variety Hana than Samantha. So the variation in milled fractions is determined by milling process and a blend of flour from various fractions could be utilized for preparation of specialty flour for specific end use (Okrajková et al., 2007).

Protein subunits

Differences in composition of proteins in different mill streams resulted in significant variation processing characteristics studied by several researchers (Dick et al., 1977; Badi and Hosney, 1978; Holas and Tipples, 1978; Veraverbeke et al., 1999; Feillet et al., 2000;

Villanueva et al., 2001). Various protein fractions contribute differently to processing effects of mill streams flour (Dick et al., 1979; Khan et al., 1989; Gupta et al., 1993; Lu and Grant, 1999). Differences in molecular composition of mill fractions showed strong relation to variation in bread making qualities of the flour streams (Menkovska et al., 2002). The quantities of protein fractions were significantly different among mill streams. The ratio of polymeric to monomeric proteins was significantly greater in break streams than reduction streams (Wang et al., 2007).

Gliadins appeared primarily responsible for quantitative variation of proteins among mill streams. B₃ had the highest percentage of gliadins (Liu et al., 2011). The quantity of albumin, globulin, gliadin, HMW-GS and LMW-GS showed a significant increasing trend in break streams (Wang et al., 2007). Significant differences were recorded in quantity of HMW polymeric proteins in different milled fractions (Liu et al., 2011). The quantity of gliadin showed a significant increasing trend (R₁ to R₃) while decreasing trend was found in quantity of albumin, globulin, HMW-GS and LMW-GS in reduction streams (Wang et al., 2007).

The SDS-unextractable protein fractions showed significant relation with sulfur content, dough rheology and bread making traits than other protein fractions (Liu et al., 2011). SDS unextractable high molecular weight polymeric proteins showed positive correlation bread quality parameters measured by C-cell imaging (Ohm, 2010). Compositional differences in concentration of reduced and unreduced glutenin proteins in flour mill streams showed significant relation with bread loaf volume (Wang et al., 2006, 2007). The quantity of SDS-unextractable glutenins showed significant variation among the break mill streams and strong associations with mixing parameters and bread loaf volume as analyzed by size-exclusion HPLC (SE-HPLC) (Sutton and Simmons, 2006).

Milled fractions vary with respect to the amount of unreduced SDS-soluble protein fraction at 4%, 10% and 12% origin. Polymeric

proteins retained at multistacking-SDS-PAGE were separated with reducing agent and evaluated to quantify the composition of HMW and LMW glutenin subunits. Milled fractions vary significantly for the concentration of HMW-GS 2*, 7 + 9 and 5 + 10 from the 4% and 12% while amount of LMW-GS differed significantly at 4%, 6%, 12% and 14% origins. Functional quality characteristics of milled fractions are affected by variation in concentration, distribution and composition of glutenin protein (Wang et al., 2005). Unreduced SDS-soluble glutenins and the total reduced proteins were extracted with multistacking (MS)-SDS-PAGE and SDS-PAGE and their quantity was determined by densitometric analysis. Unreduced SDS-soluble polymeric glutenin proteins (HMW and LMW) showed significant variation among the milled fractions at different origins of the MS-SDS-PAGE gels. Concentration of total polymeric proteins and ratio of high to low molecular weight glutenin subunits extracted from total reduced protein varied significantly in different flour fractions (Wang et al., 2006).

Wang et al. (2007) separated protein classes (albumin, globulin, gliadin, HMW-GS and LMW-GS) by precipitation with 0.1M NH₄Ac-MeOH or acetone and evaluated for their distribution in different milled fractions of wheat flour. Break streams contained polymeric proteins in significantly higher ratio and monomeric proteins in lesser quantity as compared to the reduction streams. Total flour protein, albumin, globulin, HMW-GS and LMW-GS in flour showed significant positive correlation with loaf volume while significant negative correlation was found for gliadin content with loaf volume (Wang et al., 2007).

Amino Acids

Concentration and distribution of different protein fractions vary as shown by the amino acid analyses of different sections of wheat kernel (Jensen and Martens, 1983). The centre of the endosperm contained more basic amino acids and less nitrogen content as compared to the periphery (Tseng and Lai, 2002). The variation in free amino acid concentration is related to distribution in wheat kernels. Amino

acids showed non uniform distribution in wheat kernel. Concentration is higher in wheat non-endosperm parts than starchy endosperm, specifically wheat germ, which is a major source of free asparagine (Fredriksson et al., 2004). Shorts and bran fractions had much greater free amino acid concentrations than other mill streams. Mill streams differed significantly for presence free amino acids. Asparagine, asparatic acid and glutamic acids were main free amino acids recorded in mill stream. Asparagine, one of major precursors for acrylamide formation during baking was found at much greater levels than other amino acids in short and bran. B3 and R3 flours generally had the highest free amino acid concentration among break and reduction flours (Liu et al., 2011). Reduction streams from reground fine middlings contained a higher lysine content which is the limiting amino acid in wheat protein (Wang and Flores, 1999).

A significant linear correlation is recorded between free amino acid concentration and flour characteristics among flour streams. Asparagine concentration had a highly significant ($\alpha = 0.01$) and positive correlation ($r = 0.93$) with glycine concentration among FMS. Glycine contributes to decreasing acrylamide formation during bread baking (Fink et al., 2006). Some free amino acids had significant correlations with flour quality traits such as farinograph water absorption and peak time, dough extensibility, and bread loaf volume. Tyrosine concentration had significant and negative correlations with farinograph peak time and bread loaf volume. Free tyrosine also was involved in forming tyrosine cross-links between proteins (Tilley et al., 2001). Major amino acid in flour was asparagine present in highest amount in third break and third reduction FMS. Nitrogen to sulfur ratio showed significant correlation with asparagine content ($r = 0.73$) (Liu et al., 2011).

Oxidative Cross linking

A better knowledge and understanding about the protein characteristics (Okrajková et al., 2007) and variation in oxidative cross-linking potential (Ramseyer et al., 2011b) among

different milling streams results in improved flour and end-product quality by combining streams with better quality attributes (Okrajková et al., 2007; Ramseyer et al., 2011b). Higher variation in water and peroxide-peroxidase viscosity in mill streams resulted in high oxidative cross-linking potential. 1st Break, 1st and 2nd Middlings and 1st Midds Redust flour showed highest potential to form cross links while least oxidative cross-links in 3rd Break and 4th and 5th Middlings. The mill streams with arabinoxylan and protein polymers have a high oxidative potential structure more conducive to form oxidative cross-links. Oxidative cross-linking of polymers affects the end-use quality of wheat flour (Ramseyer et al., 2011b).

Break and reduction flour fractions showed high amount of disulfide cross linkings while bran contains greater amount of high molecular-weight (HMW) glutenin subunits and amount of disulfide cross linkings. Bran fraction is derived from such portions of the kernel possessing ample amount of cross-linkings in HMW glutenin subunits. Bran flour fraction is therefore valuable for utilization in whole wheat products while shorts fraction is of less value. Amount of disulfide linkings were similar in different milled fractions for the two cultivars despite of differences in their genetic makeup (Okrajková et al., 2007).

Patent flours have more potential to form oxidative cross-links than straight grade flours (Ramseyer et al., 2011c). In fact mill streams (1st and 2nd Break, 1st and 2nd Middlings and 1st Middlings redust) used for formation of patent flours are more likely to form oxidative cross-links (Ramseyer et al., 2011b). Straight grade flours formulated from same mill streams with high oxidative cross-linking potential (1st and 2nd Break, 1st and 2nd Middlings and 1st Middlings redust) showed oxidative cross links. These (3rd Break, Grader, and 3rd, 4th and 5th Middlings) mill streams in straight grade flours have a deleterious effect on oxidative cross-linking potential (Ramseyer et al., 2011c).

Thiol Groups

Flour mill streams showed a significant thiol

groups on proteins and amount was higher in both latter break and reduction flour fractions. This might be due to severity of grinding and molecular disruption. Rheological and processing parameters showed a significant correlation with thiol groups exposed on different protein subunits. Significant correlations were found between the composition and state of exposure of thiol groups on different protein fractions. Dough mixing and product baking tests unveiled the role of flour aging processes in the mill stream flours (Sutton and Simmons, 2006). Aged flour exhibited better rheological attributes and improved processing characteristics in comparison to fresh flour. Earlier scientists concluded that it is due to lipid oxidation (Gracza, 1965). However, later studies by Seguchi (1993) confirmed that the phenomenon of aging is related to the starch granule surface proteins. Recent research revealed that flour aging is due to oxidation state of the protein thiol groups and the interactions of flour protein thiols with other

proteins and dough improvers.

Australian and New Zealand wheat cultivars (2 in each) were milled from pilot-scale roller mill and evaluated through Size-exclusion HPLC for protein composition and exposure of thiol groups. The pattern of thiol group exposure on the storage protein groups differed within a set of mill streams than observed for the proteins. The information on thiol groups can help to improve the control of flour milling, the development of flours with unique molecular level protein compositions and provide a potential explanation of flour aging processes (Sutton and Simmons, 2006). Reduction flour fractions showed greater amount of free thiol groups than break streams. Amount of free thiol groups were similar in different milled fractions for the two cultivars despite of differences in their genetic makeup (Okrajková et al., 2007). Free thiol groups were found to higher in variety Hana than Samantha. Free thiol groups were higher in shorts than other mill stream (Okrajková et al., 2007).

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