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Research Paper

Associations of sulfur content and protein molecular weight distribution with bread-making quality for patent and mill stream flours in hard red spring wheat grown under sulfur fertilization at two locations

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Abstract. The individual flour mill streams (FMS) are combined to obtain flour blends that meet customer's specifications. The measurement of quality differences among FMS is essential to attain specific flour blends. Hence, information is needed for the effect of growing condition on biochemical components and quality characteristics in FMS. This research aimed to evaluate variations of sulfur (S) content and protein composition and their associations with dough rheology and bread-making quality for FMS in a hard red spring wheat cultivar, Glenn that was grown under different levels of S fertilization (0, 28 and 56 kg/ha) at two locations, Oklee and Perley, in USA. The results showed that the variation of patent flour S content was dependent on growing location while S fertilization had non-significant influence. For FMS, the S fertilization increased S content in primary break mill streams. Growing locations differed for reduction streams in most bread-making parameters while S fertilization did not have noticeable effect. When compared to reduction streams, the primary break mill streams exhibited longer dough development time and extensibility, and larger bread loaf volume, all of which were positively related with the higher N and S contents. The FMS were analyzed for protein molecular weight distribution (MWD) using a size exclusion high performance liquid chromatography. The FMS showed big difference for MWD parameters, especially between break and reduction streams. Multivariate approach using a principle component biplot analysis indicated that sodium dodecyl sulfate buffer unextractable polymeric proteins were primary components which improved bread-making quality in the break streams.

Keywords: Hard red spring wheat, sulfur fertilization, flour mill streams, protein molecular weight distribution, bread making.

INTRODUCTION

Wheat (*Triticum aestivum* Linn.) is used to produce diverse end-products including bread, roll, pizza, pasta, cake, crackers, cookies, pastries, noodles, and many other foodstuffs (Rasper and Walker 2000). Wheat varies for processing quality and performs differently in the end-product production. Therefore, wheat-based industry seeks wheat that has high processing and end-use quality characteristics. Wheat end-use quality characteristics are highly affected by growing conditions. Wheat requires about 15 to 20 kg sulfur (S)/ha for optimum growth (Zhao *et al.*, 1999a). Soil S deficiency is one of the limiting factors for production of bread wheat (Wrigley *et al*., 1984; Byers *et al*., 1987; Schnug *et al*., 1993; Zhao *et al.*, 1999a; Zhao *et al*., 1999b). Several factors may be responsible for the soil S deficiency: the use of concentrated nitrogen fertilizers lacking S; changes in the timing of fertilizer applications; declining soil organic matter levels; and introduction of high yielding cultivars that deplete the reserve of soil S.

Soil S deficiency leads to shifts in protein composition in wheat that resulted in degradation of flour dough quality such as increase in toughness and decrease in extensibility (Byers *et al*., 1987; Zhao *et al*., 1999a; Zhao *et al.*, 1999b). In wheat grain, most of the S is in amino acids such as cystine and methionine that are found in protein molecules. The soil S deficiency decreased cystine and methionine content in wheat grain (Schnug *et al.*, 1993). The S-containing amino acids, especially cystine forms intramolecular or intermolecular disulfide bonds in proteins that have critical influence on functionality of gluten proteins in wheat. A decrease in S supply resulted in an increase in high molecular weight glutenin subunits (HMW-GS) and a decrease in low molecular weight glutenin subunits (LMW-GS), which led to tough and inextensible doughs (Wrigley *et al*., 1984; Byers *et al*., 1987; Zhao *et al*., 1999a; Zhao *et al*., 1999b). Wheat flour samples that show tough or tenacious dough texture are hard to mix to the optimum consistency required to produce quality bread. The S fertilization has been known to ameliorate soil S deficiency, which improves flour dough quality, and leads to increased bread loaf volume (Wrigley *et al.*, 1984; Byers *et al*., 1987; Zhao *et al.*, 1999a; Zhao *et al*., 1999b). The improvement of flour bread-making quality by S fertilization is related to shifts in gluten protein composition in wheat. The late S fertilization was found to increase S-rich proteins such as LMW-GS that was primarily associated with the improvement of bread-making quality (Zörb *et al*., 2009).

Wheat kernels are milled into flour and used to produce end-products. In common wheat flour milling, the tempered wheat kernels are gradually reduced in a series of break and reduction rolls, which yields flour mill streams (FMS) containing endosperm, bran, and germ in varying proportions (Fustier *et al.*, 2009; Brutsch *et al.* 2017). The wheat FMS are combined to make various flour blends that meet specifications for different end-products. The flour end-use quality is highly influenced by biochemical components in FMS such as protein content and composition, ash content, activity levels of various enzymes, oxidized and reduced glutathione, and other factors. The FMS differ in the biochemical components and hence, vary highly in functional properties. A previous study also found that FMS were significantly different in S content, protein composition, and free amino acid content, which were highly associated with dough properties and bread-making quality in wheat (Liu *et al*., 2011). Therefore, the information on biochemical components in FMS is essential in flour blending.

Especially, the proteins have been recognized as important factors that influence the variability of end-use quality in FMS. A research using a gel electrophoresis indicated that quantities of HMW-GS, LMW-GS, and the ratio of HMW-GS to LMW-GS were significantly associated with bread-making quality in FMS (Wang *et al.*, 2006). Size exclusion high performance liquid chromatograph (SE-HPLC) has been applied to analyze molecular weight distribution (MWD) of unreduced proteins in wheat (Gupta *et al*., 1993; Liu *et al.*, 2011). The polymeric proteins that form protein macropolymer by disulfide linking of glutenin subunits have been identified as main components that influence gluten quality among protein MWD parameters. The SE-HPLC research showed that polymeric proteins had different associations with gluten strength parameters according to solubility in sodium dodecyl sulfate (SDS) buffer solution. The SDS extractable polymeric proteins had negative associations with gluten strength parameters while the SDS unextractable polymeric proteins had positive associations (Gupta *et al*., 1993; Ohm *et al.*, 2009; Liu *et al.*, 2011; Ohm *et al.*, 2018). SE-HPLC was applied to analyze protein composition in FMS and high molecular weight (HMW) polymeric proteins of the SDS-unextractable fraction were identified to be a primary component that influenced bread-making quality in FMS. The S deficiency was found to shift on MWD to higher molecular weight for polymeric proteins in wheat (MacRitchie and Gupta, 1993; Zhao *et al.*, 1999b).

Information is still limited regarding variation of protein composition and bread-making quality characteristics for patent flour and FMS in hard red spring (HRS) wheat that was grown under different field conditions. Therefore, the objective of this research was to gain information on the response of S content, protein MWD distribution, dough rheological and bread-making quality characteristics for individual FMS to the application of S fertilizer in an HRS wheat cultivar grown at two different locations; and second objective was to clarify associations between S content, protein MWD parameters and bread-making quality characteristics in FMS.

MATERIALS AND METHODS

Field experiment

The field study was performed using an HRS wheat cultivar, Glenn, in 2008. Glenn is renowned for strong gluten strength and excellent bread-making quality (Mergoum *et al.*, 2006). The field trial was performed as described by Kaiser *et al.* (2019). The field experiment was performed at two locations (Oklee in Red Lake County and Perley in Norman County, MN, USA). Oklee soil exhibited lower levels for organic matter, potassium, phosphorus, and S concentration when compared to Perley soil (Kaiser *et al.*, 2019). Specifically, Oklee soil had organic matter concentration of 40 g kg^{-1} while Perley had 67 g kg^{-1} (Kaiser *et al.*, 2019). Field layout for S application treatment was a completely randomized block design with four replications in individual locations. All plots were treated with the same nitrogen (N) and phosphate fertilizer rates at 62 and 93.0 kg/ha, respectively. The S applications were done at three rates (0, 28, and 56 kg/ha) of a fertilizer form called MicroEssentials S15 ($N:P_2O_5:S$, 13-33-15) (ME S15), which combines ammonium sulfate and elemental S (Anonymous, 2000). The three rates (0, 14 and 28 kg/ha) were applied before planting and the leftovers (0, 14 and 28 kg/ha) were applied at tillering stage. Since quantity of harvested grain was not enough for experiment, grain samples were combined to attain an adequate quantity of samples for individual FMS. Replicates 1 and 2 were combined to provide a new replicate 1; and replicates 3 and 4 were combined to form a new second replicate. Grain yield and other agronomic data were reported by Kaiser *et al.* (2019). They reported that grain yield did not show significant response to S application indicating that application of S may not result in an increase in grain yield when soil organic matter concentration is over 20 g kg^{-1}.

Flour milling

Milling was performed in a Buhler laboratory mill (MLU-202) (Buhler, INC, Uzwil, Switzerland) (AACC Approved Method 26-22) (AACC International, 2000). The FMS were collected separately from three break streams (B1, B2 and B3) and three reduction streams (R1, R2, and R3). Then, the six FMS were blended to make patent flour samples. Buhler laboratory mill (MLU-202) is known to produce flours which are comparable to those produced in commercial milling systems (Rasper and Walker, 2000).

Proximate analyses

Flour moisture and ash contents were determined following AACC Approved Method 44-15A and AACC Approved Method 08-01 (AACC International, 2000), respectively. Flour N content was determined using a LECO FP-528 N analyzer (LECO Corp., St Joseph, MI) (AACC Approved Method 46-30) (AACC International, 2000). Flour S content was determined using a LECO TruSpec combustion analyzer (LECO Corp., St Joseph, MI) according to the standard procedure as described in the instruction manual.

Protein MWD analysis

The SDS buffer extractable and unextractable protein fractions were obtained according to the procedure of Gupta *et al*. (1993) with minor modification (Ohm *et al*., 2009). The SE-HPLC was performed for the protein extracts using Agilent 1100 Series (Agilent Technologies, Waldbroann, Germany). The absorbance was detected at 214 nm using an Agilent 1200 photodiode array detector. The UV absorbance data were used to calculate absorbance areas using MATLAB software (The MathWorks, Natick, MA) (Ohm *et al*., 2009). The SE-HPLC profiles were divided into four fractions: F1: 3.6-4.3 (min), HMW polymeric protein; F2: 4.3-6.0 (min), low molecular weight (LMW) polymeric protein; F3: 6.0-6.85 (min), gliadins; and F4: 6.85-8.0 (min), metabolic proteins (albumins and globulins) (Ohm *et al*., 2009; Liu *et al*., 2011). Absorbance areas were calculated at 0.01-min intervals and used to obtain absorbance area of individual HPLC fraction. The absorbance area of each individual fraction was expressed in percentage values of protein based on weight (14% mb) of flour samples (flour %) and total absorbance area (area %).

Mixing, dough and bread-making characteristics

Farinograph-E (C.W. Brabender Instruments, INC, South Hackensack, NJ) was performed at optimum water absorption (AACC Approved Method 54-21) (AACC International, 2000) using 10-g of flour (14% mb). This is because some mill stream samples showed extraordinarily long mixing time (longer than 20 min), they could not be determined for time to breakdown and dough stability in farinograph analysis. Dough rheology properties were also measured using the Extensigraph-E (C.W. Brabender Instruments, INC, South Hackensack, NJ) (AACC Approved Method 54-10, AACC International, 2000). Extensigraph data was presented only for 45-minute rest time since preliminary experiments showed no differences between rest periods. Experimental bread making was conducted using 25-g flour (14% mb) following AACC Approved Method 10-10B (AACC International, 2000). The bread was evaluated using C-cell imaging system (Calibre Control International, LTD. Appleton, Warrington, UK) according to the manual.

Statistical analysis

Analysis of variance was performed using the 'GLM' procedure in the Statistical Analysis System (SAS, V. 9.0, SAS Institute, Cary, NC). Split-plot layout was experimental arrangement in analysis of variance of

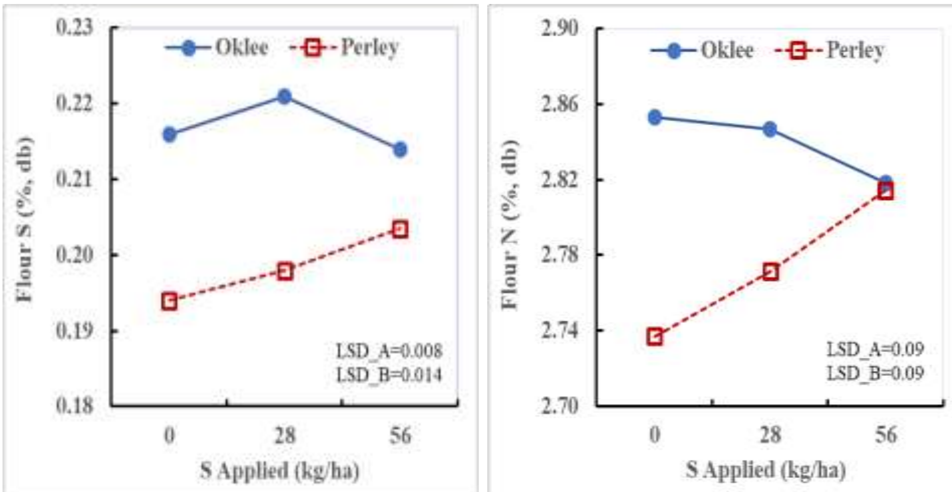

Figure 1. Effect of sulfur (S) fertilization on S and nitrogen (N) contents in patent flour samples for two growing locations (Oklee and Perley, MN). LSD_A= least significance difference between treatments within a location at P=0.05; and LSD_B= least significance difference between treatments for different locations at P=0.05.

patent flour data in which the main plot was growing locations; and the subplot was S application levels. The replications were nested within growing location. For FMS data, experimental arrangement was split-split plot layout where FMS were considered as sub-sub plot. All treatment means were tested for comparison at significance level α=0.05. Correlation coefficients were calculated using the 'CORR' procedure. Biplot analysis was performed using the 'PRINQUAL' procedure. The coordinates for the endpoints of the linear vectors (loading values) were adjusted to improve graphical display in biplots.

RESULTS AND DISCUSSION

Patent flour S and N contents

Two growing locations (Oklee and Perley) were significantly different in average values of patent flour S and N contents, with high values obtained for the Oklee samples (Figure 1). The locations also differed in response to S fertilization levels for changes in patent S and N contents (Figure 1). Oklee samples showed a decrease for S and N contents when the S application level was raised to 56 kg/ha while Perley patent samples showed a consistent increase. This could be related to translocation of S in plant. The S is stored as sulfate in vacuoles when the S level in the plant is excessive instead of translocating it into grain (Zhao *et al*., 1999a). The excessive S application appeared to restrict remobilization of S to grain, which even appeared to result in a decrease in flour N and S contents for this experiment. However, the effect of S application level was not statistically significant for N and S contents.

Patent flour bread-making quality traits

An experimental bread-making test is performed to evaluate flour bread-making quality. In the experimental bread-making test, optimum water absorption and mixing time are usually determined subjectively based on the texture of mixed dough by an experienced baker. The most important bread-making quality characteristic is bread loaf volume (LV) as it is related to the gas retention capacity of wheat gluten during bread-baking. The farinograph is also widely used to evaluate objectively flour optimum water absorption and mixing characteristics. Extensigraph evaluates dough rheological properties such as dough extensibility and resistance to extension. The bread-making quality characteristics were analyzed for patent flour samples using the farinograph, extensigraph, and experimental bread-making and the data are given in Table 1. Water absorption is an important parameter in experimental bread-making because it is related dough yield in commercial bread-making. Significant differences appeared for water absorptions obtained by farinograph and experimental bread making (FWA and BWA, respectively) between Oklee and Perley patent samples (Table 1). Flour protein content has been demonstrated to have positive associations with water absorption and LV (Bushuk, 1985). Despite the higher N and S contents, Oklee patent samples showed smaller LV than Perley samples; this was most likely due to the lower dough extensibility. Færgestad *et al.* (2000) reported that dough extensibility was positively correlated with expansion of dough, which is essential to achieve large LV during baking. Bread crumb characteristics such as cell wall thickness, diameter, and volume also had significantly bigger values for Perley samples, indicating more expansion of bubbles

Table 1. Dough rheology, and bread making quality characteristics of patent flour.

Quality traits	Location			S level (kg/ha)			
	Oklee	Perley	LSD[a]	0	28	56	LSD
Flour yield (%)	74.8	73.8	NS	74.8	74.3	74.0	NS
Flour ash (%)	0.5	0.5	NS	0.5	0.5	0.5	NS
Farinograph							
Water absorption (%)	68.7	65.7	2.2	66.8	67.3	67.5	NS
Development time (min)	8.7	7.7	NS	8.0	8.6	8.1	NS
Extensograph							
Resistance to Extension (BU)	325.8	283.0	NS	312.8	304.8	295.8	NS
Extensibility (mm)	203.2	216.0	NS	207.3	211.0	210.5	NS
Maximum (BU)	726.5	636.7	NS	699.8	685.5	659.5	NS
Bread-making quality							
Water absorption (%)	61.7	58.7	2.1	59.8	60.3	60.6	NS
Loaf volume (cm^3)	185.5	196.3	NS	189.8	191.5	191.5	NS
Loaf weight (g)	31.2	30.3	NS	30.8	30.7	30.8	NS
Specific Volume(cm^3/g)	6.0	6.5	NS	6.2	6.3	6.2	NS
Bread crumb characteristics							
Slice brightness	138.9	142.6	NS	142.2	138.6	141.5	NS
Cell contrast	0.8	0.7	NS	0.8	0.8	0.8	NS
Number of cells	4109.8	4122.5	NS	4052.5	4170.8	4125.3	NS
Area of cells	51.7	52.9	NS	52.4	52.3	52.3	NS
Wall thickness	2.8	2.9	0.02	2.9	2.8	2.8	NS
Cell diameter	11.9	13.2	0.8	12.6	12.5	12.5	NS
Cell volume	4.9	5.5	0.6	5.2	5.2	5.1	NS
Net cell Elongation	1.6	1.6	NS	1.6	1.6	1.6	NS
Cell alignment	0.6	0.7	NS	0.7	0.6	0.6	NS

[a]LSD, least significance difference (P = 0.05); NS, not significant (P > 0.05).

in dough during baking than observed for Oklee samples.

S application levels did not have statistically significant influence on most quality traits for patent flour samples (Table 1). The HRS cultivar, Glenn, used in this research is especially known for excellent bread-making quality and good stability over a wide variety of growing conditions (Mergoum *et al.*, 2006), that might be the cause of lack of significant effects for S application levels in this research. Significant and positive effects of S fertilization on quality characteristics such as LV were reported for a hard winter wheat grown in the U.K. (Zhao *et al*., 1997b). The results could vary due to different cultivars, locations and field condition.

Patent S content had higher correlations with FWA and BWA compared to N content (Table 2). Patent S content had also significant correlations with farinograph development time (FDT) and loaf weight while N content had nonsignificant correlations. Wooding *et al.* (2000) reported that the flour N:S ratio was a better predictor of dough physical characteristics than either N or S content. Flour N:S ratio also had a higher correlation (r = 0.687, P < 0.05) with FDT than N and S contents in this research. Bread LV had non-significant correlations with N and S contents probably due to lower dough extensibility for Oklee samples, as aforementioned.

N and S contents in flour mill streams

The N and S contents were significantly higher for break streams than reduction streams, showing the highest values for third break (B3) (Table 3). This indicates that peripheral endosperm fraction which is rich in S and protein is concentrated in tail break streams during the milling process (Every *et al*., 2002; Indrani *et al*., 2003). Locations differed significantly in N and S content for individual break steams (Table 3). Oklee samples showed significantly higher N and S contents for first and second break streams (B1 and B2) than Perley samples. The S fertilization did not have significant influence on the

Table 2. Correlation coefficients of flour nitrogen (N) and sulfur (S) contents with quality traits in patent flour.

Traits	N (%)		S (%)	
S (%)	0.816	**	-	
Flour yield (%)	-0.018	NS	0.388	NS
Farinograph				
Water absorption (%)	0.747	**	0.873	***
Development time (min)	0.337	NS	0.633	*
Extensigraph				
Resistance to Extension (BU)	0.381	NS	0.503	NS
Extensibility (mm)	0.050	NS	-0.331	NS
Maximum (BU)	0.413	NS	0.542	NS
Bread-making quality				
Water absorption (%)	0.732	**	0.860	***
Loaf volume (cm^3)	-0.366	NS	-0.435	NS
Loaf weight (g)	0.538	NS	0.678	*
Specific Volume(cm^3/g)	-0.454	NS	-0.545	NS
Bread crumb characteristics				
Slice Brightness	-0.721	**	-0.402	NS
Cell Contrast	0.806	**	0.687	*
Number of Cells	0.069	NS	0.034	NS
Area of Cells	-0.824	**	-0.800	**
Wall Thickness	-0.699	*	-0.560	NS
Cell Diameter	-0.779	**	-0.672	*
Cell Volume	-0.824	**	-0.711	**
Net Cell Elongation	-0.207	NS	-0.182	NS
Cell Alignment	-0.179	NS	-0.135	NS

*, **, and ***, correlation coefficient is significant at $P < 0.05$, 0.01 and 0.001, respectively; NS = not significant ($P > 0.05$).

N contents of individual FMS as observed for patent samples. In contrast, S fertilization increased S content in all break streams. All break streams showed significantly lower S content for 0 kg/ha level than 28 and 56 kg/ha levels.

Quality traits of flour mill streams

The FMS differ widely in quality traits (Table 4). The FWA and BWA values were higher for B3 and R3 than for another FMS (Table 4). The tail-end mill streams usually show high water absorption due to increased damaged starch content (Brutsch *et al.*, 2017). The B1 and B2 had longer FDT, higher EXT, smaller RTE and maximum (MAX), and larger LV than reduction streams. The larger LV for B1 and B2 resulted from larger protein quantity as represented by the higher N and S contents (Table 3) as well as better gluten characteristics as indicated by the longer FDT and extensibility (EXT) (Wooding *et al*., 2000; Brutsch *et al*., 2017).

A comparison of the two locations shows similar trends for farinograph, extensigraph and bread-making parameters as already observed for patent flour samples (Table 4). Oklee FMS showed individually larger values for FWA, BWA, and FDT than Perley FMS (Table 4). Oklee R1 and R2 showed notably longer FDT than Perley samples, as observed for patent samples (Table 1). For extensigraph parameters, Oklee reduction streams R1 and R2 were lower for EXT and higher for resistance to extension (RTE) and maximum resistance (MAX) than Perley samples. The R3 showed significantly higher EXT for Perley sample than Oklee sample. This result indicated that the dough texture was tougher and less extensible for Oklee samples. Bread LV of individual FMS were larger for Perley than Oklee samples. Specifically, reduction streams R2 and R3 showed statistically significant differences for LV between Perley and Oklee. These results indicate that the lower LV for Oklee patent samples primarily resulted from their tougher and less

Table 3. Nitrogen (N) and Sulfur (S) content in flour mill streams for growing locations and S fertilization levels[a].

Location/S levels	Break mill stream			Reduction mill stream		
	B1	B2	B3	R1	R2	R3
	N content (%, db)					
Location						
Oklee	3.286a	3.613a	4.158a	2.674a	2.546a	2.614a
Perley	3.120b	3.467b	4.056a	2.647a	2.583a	2.677a
S levels						
0 kg/ha	3.190a	3.520a	4.084a	2.644a	2.556a	2.624a
28 kg/ha	3.222a	3.556a	4.130a	2.665a	2.564a	2.638a
56 kg/ha	3.197a	3.542a	4.106a	2.672a	2.573a	2.674a
	S content (%, db)					
Location						
Oklee	0.245a	0.267a	0.307a	0.206a	0.196a	0.200a
Perley	0.223b	0.244a	0.275a	0.187a	0.185b	0.191a
S levels						
0 kg/ha	0.228b	0.250b	0.285b	0.191b	0.187a	0.196a
28 kg/ha	0.238a	0.257a	0.294a	0.198ab	0.191a	0.193a
56 kg/ha	0.238a	0.261a	0.295a	0.201a	0.194a	0.198a

[a]Treatment means within columns followed by the same letters are not significantly different (α = 0.05) within location or S levels.

extensible dough characteristics of reduction streams compared to Perley samples.

Protein MWD parameters

Break streams contained larger values for all the protein MWD parameters as represented by higher flour % values than reduction streams (Table 5). The flour % values could represent quantity of individual protein fraction in FMS. The SDS extractable F3 (EF3) that is known to be primarily composed of gliadins (Ohm *et al*., 2009) showed significantly higher flour % values for break streams than reduction streams. Flour % values of SDS unextractable F1 (UF1) also showed significant difference between break and reduction streams. The UF1 is primarily composed of HMW polymeric proteins that are highly correlated with dough elasticity in HRS wheat (Ohm *et al*., 2009; Ohm *et al*., 2018). The area % values represent the proportion of individual protein fractions on total protein in FMS. The individual break streams showed lower area % values for extractable F1 (EP1) than reduction streams. The area % values of extractable F2 (EP2) showed higher values for R3 when compared to B3. For the area % value of unextractable F1 (UP1), the B2 and B3 individually showed significantly higher values than R2 and R3, indicating that the break streams had a higher proportion of unextractable HMW polymeric proteins on total proteins as well as larger quantity on flour weight. The two growing locations did not show noticeable differences in flour % values in individual FMS. The two growing locations also did not show significant difference in area % values for individual FMS except for extractable F2 and F3 (EP3) in B3. The S fertilization also did not have significant effect on protein MWD parameters for FMS (data not shown). This result did not agree with other researches (MacRitchie and Gupta, 1993; Zhao *et al*., 1997b). Kaiser *et al*. (2019) stated that the growing condition such as S application did not affect significantly grain protein concentration for the HRS wheat cultivar, Glenn, because it had high N use efficiency to produce proteins in grain. The same reason might result in non-significant effect of location and S fertilization on protein MWD parameters. In addition, the soil S level might have been more than required to produce a response to S in this experiment (Kaiser *et al*., 2019).

Biplot analyses of protein MWD parameters and quality traits

The principle component analysis biplot represents not only the relationships between variables but also the influence of individual observation elements on the relationships. To be more specific, Figure 2-A exhibits a

Table 4. Quality characteristics for flour mill streams obtained from two growing locations.

Quality traits	Location	Break mill stream			Reduction mill stream			LSD[a]	
		B1	B2	B3	R1	R2	R3	A	B
Flour yield (%)	Oklee	9.2	4.0	2.0	35.3	18.8	5.5	1.2	1.1
	Perley	3.8	8.0	1.5	36.7	18.7	5.2		
Flour ash (%)	Oklee	0.43	0.46	0.75	0.44	0.50	0.81	0.03	0.03
	Perley	0.46	0.48	0.77	0.45	0.50	0.80		
Farinograph									
Water absorption (%)	Oklee	67.7	70.3	75.3	66.9	69.1	74.6	0.5	1.5
	Perley	63.7	66.1	72.0	64.7	66.3	70.6		
Development time (min)	Oklee	17.9	18.1	13.2	10.7	7.0	5.7	1.9	1.9
	Perley	17.1	16.7	11.3	2.4	3.6	5.6		
Extensigraph[b]									
Resistance to extension (BU)	Oklee	253.3	231.3	-	371.4	345.3	231.3	28.5	37.0
	Perley	245.3	219.3	-	313.9	269.3	229.7		
Extensibility (mm)	Oklee	243.6	241.4	-	190.0	164.2	151.2	15.6	20.2
	Perley	244.5	242.0	-	209.6	171.0	191.3		
Maximum (BU)	Oklee	755.7	720.8	-	820.3	639.1	325.6	64.6	95.2
	Perley	705.7	670.4	-	708.1	526.3	375.4		
Bread-making quality									
Water absorption (%)	Oklee	60.7	63.3	64.3	59.9	62.1	67.6	0.5	1.5
	Perley	56.7	59.1	61.1	57.7	59.3	63.6		
Loaf volume (cm^3)	Oklee	196.8	202.9	169.5	185.1	177.0	158.8	6.3	6.8
	Perley	203.1	205.9	170.7	188.8	186.3	174.8		

[a]LSD: A, least significance difference between millstreams within a location at P=0.05; and B, least significance difference between mill streams for different locations at P=0.05.
[b]Extensigraph analysis was not conducted on B3 because of low sample quantity.

biplot for relative loading values of protein MWD parameters and N, S and ash content values and PC1 and PC2 score values of FMS. Figure 2-A shows that EF3 and UF1 were the primary variables that contributed to PC1 along with N and S contents. The vector lines of N and S contents show small angles with EF3. This indicates that EF3 positively associated with the N and S contents primarily due to their quantitative increase in B1 and B2. The vector line of UF1 also shows a small angle with the N and S vector lines, suggesting that quantitative increases in N and S associated positively with unextractable HMW polymeric proteins in the break streams. In contrast, Figure 2-A indicates that proteins in R3 contained a higher proportion of extractable polymeric proteins (EP1 and EP2) and a lower proportion of unextractable polymeric proteins (UP1), which also appear to contribute more to PC2 than other MWD parameters. Figure 2-A also indicates that ash content associated positively with EP1 and EP2 and negatively with UP1.

Figure 2 also shows biplots for farinograph (Figure 2-B), extensigraph (Figure 2-C), and bread-making characteristics (Figure 2-D) together with protein MWD parameters. The biplots indicate that the protein MWD parameters, EF3 and UF1, associated positively with FDT, EXT, and LV. Other researchers have also reported significant correlations of LV and EXT with gliadin (EF3) (Klindworth *et al*., 2014) and unextractable HMW polymeric proteins (UF1) (MacRitchie and Gupta, 1993; Gupta *et al*., 1993; Ohm *et al*., 2009; Liu *et al*., 2011; Ohm *et al*., 2018). Dough rheological properties have been known to be primarily determined by monomeric gliadins and glutenin polymers as they associate with dough extensibility and elasticity, respectively (Wieser

Table 5. Percentage values of protein MWD parameters for flour mill streams and patent flour obtained from two growing locations.

Protein Fraction	Location	Break mill stream			Reduction mill stream			Patent flour	LSD[a]	
		B1	B2	B3	R1	R2	R3		A	B
Extractable (flour %)[b]										
F1	Oklee	0.4	0.4	0.5	0.3	0.3	0.4	0.3	0.1	0.1
	Perley	0.4	0.4	0.5	0.3	0.3	0.4	0.3		
F2	Oklee	2.0	2.3	2.8	1.7	1.7	1.9	1.8	0.2	0.2
	Perley	2.0	2.2	2.4	1.5	1.7	1.9	1.8		
F3	Oklee	6.9	7.7	8.5	5.4	5.1	5.0	5.8	0.6	0.6
	Perley	6.5	7.3	7.2	4.8	5.2	5.3	5.6		
F4	Oklee	1.6	1.8	2.0	1.4	1.4	1.5	1.5	0.1	0.4
	Perley	1.5	1.7	1.7	1.3	1.4	1.6	1.4		
Unextractable (flour %)										
F1	Oklee	2.6	2.9	3.4	2.1	2.0	1.9	2.3	0.1	0.5
	Perley	2.5	2.8	3.3	2.2	2.0	1.9	2.2		
Extractable (area %)[c]										
F1	Oklee	2.2	2.4	2.5	2.6	2.7	2.9	2.5	0.3	0.3
	Perley	2.5	2.4	2.4	2.5	2.7	3.1	2.5		
F2	Oklee	12.5	13.0	13.7	13.0	13.7	14.5	13.3	1.1	1.2
	Perley	12.8	13.1	11.9	11.9	13.8	14.7	12.9		
F3	Oklee	42.9	43.6	41.8	40.9	41.2	39.4	41.8	3.4	4.2
	Perley	42.2	42.8	36.1	36.9	41.3	40.5	41.1		
F4	Oklee	10.2	10.3	10.0	10.8	11.3	12.1	10.6	0.8	2.3
	Perley	10.1	9.8	8.7	9.7	10.9	12.3	10.6		
Unextractable (area %)										
F1	Oklee	16.2	16.6	16.7	16.1	15.8	14.7	16.2	0.5	NS
	Perley	16.1	16.7	16.6	16.7	15.9	14.3	16.1		

[a]LSD: A, least significance difference between treatments within a location at P=0.05; and B, least significance difference between treatments for different locations at P=0.05. NS=not significant (P>0.05).
[b]Flour %: Percent value of protein fractions based on flour weight.
[c]Area %: Percent value of protein fractions based on HPLC UV absorbance area.

et al., 2006). Specifically, the ratio of gliadin to glutenin has been known to have a negative correlation with dough development time but a positive correlation with dough extensibility (Byers *et al.*, 1987; Zhao *et al.*, 1997b). However, the ratio of gliadins to glutenin polymers which was represented by EF3/UF1 showed a significant but low correlation with EXT ($r = 0.23$, $P < 0.05$) and non-significant correlations with FDT and LV in this research. This indicates that the ratio of gliadin to glutenin quantity was not a determining factor for dough rheological properties or LV among FMS in this research. The results demonstrate that the quantitative increase of gliadin (EF3) and unextractable HMW polymeric proteins (UF1) enhanced dough elasticity and extensibility in break streams, respectively. Therefore, an increase in the two protein components resulted in larger LV for B1 and B2 than other FMS. Wieser and Kieffer (2001) also reported that quantity of both gliadins and glutenins had positive effects on LV in patent flour samples.

The Oklee reduction stream samples showed tougher and less extensible dough characteristics and lower LV than Perley samples. However, biplots indicate that

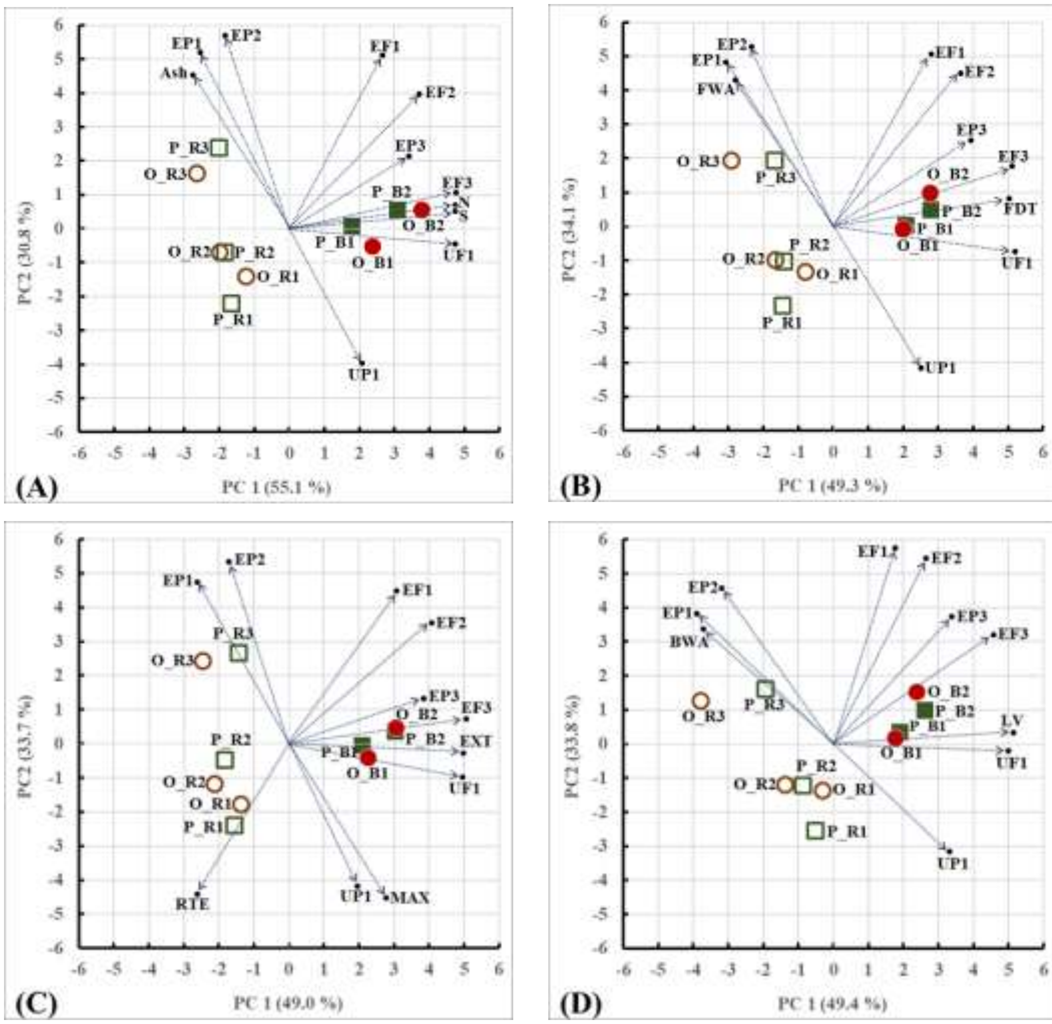

Figure 2. Principal component biplots. Rectangular and circular markers represent score values for flour mill streams and locations, respectively. Linear vector lines show relative loading values for variables including protein molecular weight distribution (MWD) parameters plus (A) ash, nitrogen, and sulfur content values, (B) farinograph (FDT, development time; and FWA, water absorption), (C) extensigraph (RTE, resistance to extension; EXT, extensibility; and MAX, maximum resistance), and (D) bread-making parameters (BWA, water absorption; and LV, bread loaf volume). Flour mill streams: O_R1, 1st reduction Oklee; O_R2, 2nd reduction Oklee; O_R3, 3rd reduction Oklee; P_R1, 1st reduction Perley; P_R2, 2nd reduction Perley; P_R3, 3rd reduction Perley; O_B1, 1st break Oklee; O_B2, 2nd Break Oklee; P_B1, 1st break Perley; and P_B2, 2nd break Perley. Protein MWD parameters: EF1, EF2 and EF3, % values of extractable fraction 1, 2, and 3 on flour weight; EP1, EP2, and EP3, % values of extractable fraction 1, 2, and 3 on HPLC UV absorbance area; UF1, % value of unextractable fraction 1 on flour weight; and UP1, % value of unextractable fraction 1 on absorbance.

growing locations did not have notable effect on protein MWD parameters, showing no noticeable influence on FDT, EXT, and LV. This indicates that there were unknown components which varied largely for growing locations, having strong influence on bread making properties other than proteins in this research. Michniewicz *et al.* (1992) reported that pentosans influenced wheat bread baking properties.

The EP1 and EP2 appear to have positive relationships with FWA (Figure 2-B) and BWA (Figure 2-D) while UP1 seems to have negative relationships. However, other research (Ohm *et al.*, 2009; Wieser and Kieffer, 2001) reported non-significant correlations. The figures show that tail reduction stream R3 majorly influenced the association. This result indicated that the proteins in the peripheral endosperm fraction contained a higher proportion of extractable polymeric proteins. One of the major factors to cause an increase of water absorption in tail-end mill streams is high damaged starch content (Brutsch *et al.*, 2017). Extensigraph RTE showed negative relationships with EF1 and EF2 (Figure 2-C). This indicated that lower quantity of extractable protein content might result in higher RTE for R1 and R2 than other streams. In contrast, MAX appeared to associate with polymeric protein composition. The Figure 2-C indicates that MAX had a positive relationship with UP1 and a negative relationship with EP1 and EP2. The figure represents that tail reduction stream R3 majorly influenced the associations. This indicates that a high proportion of unextractable glutenin polymer and low proportion of unextractable HMW polymeric proteins on the total protein resulted in low MAX values in R3 in this experiment. Similarly, Wiser and Kieffer (2001) reported a significant correlation between flour glutenin quantity and maximum resistance. The associations could be also related to high ash, fat, and damaged starch content in tail-end mill streams (R3) which interfere with the establishment of disulfide bonds and optimized gluten network development (Brutsch *et al.*, 2017).

CONCLUSION

This research investigated the responses of S content, protein MWD distribution, dough rheology properties, and bread-making quality characteristics of a hard spring wheat cultivar, Glenn, to the application of S fertilizer at two different growing locations. While growing locations had significant influence on flour S content, S fertilization did not significantly increase S content in patent flour. The flour S content and N:S ratio values were shown to be good supplementary parameters for prediction of dough quality as they have higher correlations with FWA and FDT than N content. The break and reduction mill streams showed different response to growing locations. The S content varied significantly for first and second break streams between two locations while reduction streams did not show significant difference. The S fertilization increased S content in all break streams while having non-significant influence on N content. For the quality characteristics measured by farinograph, extensigraph and experimental bread-making, reduction streams exhibited noticeable difference between two growing locations while showing non-significant influence of S fertilization. The protein MWD parameters were analyzed using the SE-HPLC for SDS-extractable and unextractable protein fractions. The protein MWD parameters did not vary significantly for growing location and S application level. This might be due to the high stability across growing environments for the HRS wheat cultivar, Glenn. It was speculated that Glenn was not responsive to growing environment because of high N use efficiency to produce proteins in grain. The soil S level might have also been superfluous and caused no response to S application in this experiment. The flour mill streams showed wide variation in N and S contents, protein MWD parameters, and dough and bread-making quality traits. The primary break streams (B1 and B2) had longer dough mixing time and extensibility, which led to larger bread LV when compared to the reduction streams. The biplot analysis clarified the associations between quality traits and S, N, and protein MWD parameters in FMS. The biplot was especially useful to see association of protein MWD parameters with bread-making quality traits as it showed the influence of individual FMS on the relationships. The quantitative variation of unextractable HMW polymeric proteins were identified as primary factors that were closely associated with the higher dough elasticity and extensibility and larger LV for break streams than other FMS. The RTE is a dough rheological property measured by extensigraph was associated negatively with quantity of extractable polymeric proteins indicating that lower quantity of extractable protein content might resulted in higher RTE for R1 and R2 than other streams. Overall, this research reports influence of growing conditions on bread-making quality traits for FMS and their associations with protein MWD parameter. Overall, individual FMS, as influenced by growing environments, exhibited variable protein compositions and bread-making quality traits. The information attained from this research will be a valuable reference in making flour blends which meet different commercial specifications for various baked products in milling and baking industry.

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