Glutenin Protein Changes During Breadmaking of Four Spring Wheats: Fractionation by Multistacking SDS Gel Electrophoresis and Quantification with High-Resolution Densitometry[1]

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ABSTRACT

A multistacking SDS-PAGE procedure coupled with high-resolution densitometry was used to fractionate, characterize, and quantify unreduced glutenin proteins at various stages of the breadmaking process of four wheat cultivars differing in functional characteristics. Unreduced protein aggregates of the highest molecular weight (4% origin) showed an initial decrease after mixing and a subsequent increase during fermentation. Proteins retained at the 6% origin remained unchanged while protein aggregates retained at 8 and 10% origins decreased during breadmaking. Proteins retained at the 12 and 14% origins tend to increase during breadmaking. At all multistacking gel origins, subunits 2* and 5 (wheat cultivar Len) increased initially after mixing and then remained constant. Glutenin subunits 2*, 5, and 7 of cultivar Marshall exhibited the same pattern of change during breadmaking in all the protein aggregate groups (4–14%). Their proportion initially decreased (after mixing) and then increased in later stages. In contrast, subunits 9 and 10 of Marshall increased in the initial stages (after mixing) and then decreased in the later stages. Len, a good quality cultivar, contains gluten aggregates of higher molecular weight than the poorer quality cultivar, Marshall. The changes that occurred in the high molecular weight glutenin fraction revealed that Len had fewer changes in the proportion of the high molecular weight glutenin subunits distributed at various multistacking gel origins throughout the breadmaking process. The high molecular weight glutenin subunit stability for Len was greater than for a poorer quality cultivar such as Marshall. This may have enhanced the performance of Len during breadmaking.

Numerous investigations have been conducted to establish the relationship among different protein classes and breadmaking quality. Gliadins and glutenins were found to be more related to breadmaking quality than albumins and globulins. It is now well established that the glutenin fraction is the most important fraction related to breadmaking quality (MacRitchie et al 1990).

After 100 years of research on wheat protein composition, the tremendous complexity of these biological components has been partially elucidated. Wrigley and Bietz (1988) and MacRitchie et al (1990) have published excellent reviews on wheat protein research. The presence or absence of certain high molecular weight glutenin subunits (HMW-GS) in relation to breadmaking quality has been well documented (Payne et al 1987, Shewry et al 1992). Efforts have been made to explain variations in baking quality based on the presence or absence of certain subunits. However, as many authors have pointed out (Lawrence 1986, Lukow et al 1989, Khan et al 1989), the presence or absence of certain subunit does not fully explain the differences observed in breadmaking quality among wheat cultivars.

Limited information exists regarding quantitation of HMW-GS. Sutton (1991) studied differences between two wheat cultivars differing in breadmaking quality. The author found that the good quality wheat cultivar had greater absolute and relative concentration of HMW-GS, and that there were more HMW-GS associated with good breadmaking quality. Kolster et al (1992) used densitometry to quantify HMW-GS, showing that genetic and environmental factors contribute to the variation in the amounts of different HMW-GS.

Hou and Ng (1995) quantified HMW-GS and LMW-GS by a sequential acetone precipitation method (SAPM) and by SDS-PAGE coupled with densitometry (SDS-PAGE/DENS). They analyzed 17 soft wheat patent flours, finding that the HMW average of glutenins was 8.46% (SAPM) and 7.26% (SDS-PAGE/ DENS), while that of the LMW was 15.29% (SAPM) and 17.07% (SDS-PAGE/DENS). The authors found that the quantities of HMW-GS and LMW-GS determined by the two different methods were highly correlated. An important development in this study was the preparation of a standard from wheat gluten proteins for densitometric analysis. The presence of a standard in each gel allows for some reduction of the variability of the quantification procedure from gel to gel.

Huang (1995) used 13 wheat genotypes to evaluate the relationship between the quantity of HMW gluten protein and of individual HMW-GS to breadmaking quality. Significant differences in gluten protein solubility (in 2% SDS) were found between different wheats. Stronger flours gave less soluble protein and less soluble HMW-GS. In contrast, the weakest flours had the highest gluten protein solubility and the highest proportion of soluble HMW-GS. Huang (1995) also found that subunit 5 (which has a higher apparent molecular weight among HMW-GS) increased during dough overmixing (defined as 2 min after optimum mixing), while subunit 10 decreased. Changes in salt-soluble, SDS soluble and insoluble (residue) proteins during fermentation and baking (ovenspring) were also investigated. Salt-soluble, SDS-soluble gluten proteins, and HMW-GS increased solubility during fermentation. During ovenspring, salt-soluble proteins decreased, SDS-soluble proteins increased, and total HMW-GS also increased.

Recently, Huang and Khan (1997a–c) investigated the relationship between the molecular weight of glutenin subunits, structure, and functionality. They studied native glutenin aggregates of two different quality flours containing the same HMW-GS composition. They used a multistacking gel electrophoresis technique (SDS-PAGE) developed by Khan and Huckle (1992). They found differences in the time required to solubilize similar amounts of protein from two different flours. It took 8 hr to solubilize 91% of the protein in cultivar Len, while it took only 2 hr for weak flour sample 205. Huang and Khan (1997a) quantified the proportion of glutenin at each of the multistacking gel origins. They found that, as the duration of extraction increased, both total glutenins and glutenins at the 4% origin increased. They also found that Len had a higher total glutenin protein (3% more) and higher proportion of glutenin with the largest molecular size (also 3% more) at the 4% origin than did 205. After reduction, glutenin aggregates were analyzed by SDS-PAGE. Based on the results, Huang and Khan (1997a) postulated a randomized structure model for native glutenins.

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Huang and Khan (1997c) investigated quantitative effects of individual HMW-GS on breadmaking characteristics. Native glutenin proteins were separated by multistacking SDS-PAGE procedures. Glutenin aggregates from different origins were reduced with mercaptoethanol and the composition of HMW-GS was analyzed by densitometry. Aggregates with the largest molecular weight (4% origin) had a higher proportion of glutenin subunit 5 (18.8%) than those at the 12% origin (9.2%). The effect of extended mixing (2.5 min after optimum) was also studied. HMW-GS 5, the subunit with the highest molecular weight, increased in solubility in SDS buffer, while subunit 10 (with lower molecular weight) decreased its solubility in SDS-buffer. Huang and Khan (1997c) concluded that the functionality and aggregative properties of individual HMW-GS may depend mainly on molecular weight.

The objective of this study was to evaluate changes in the distribution of protein composition during breadmaking. The multistacking gel electrophoresis technique devised by Khan and Huckle (1992) for separation of nonreduced glutenins coupled with high-resolution densitometry was used to characterize and quantify different nonreduced protein groups at various breadmaking stages.

MATERIALS AND METHODS

Wheat Samples

Four hard red spring wheat cultivars were used in this study. Len, Marshall, and Butte 86 have the same HMW-GS composition (2*, 7+9, 5+10), and 215 has a different but similar HMW-GS composition (2*, 7+9, 2+12). Wheat cultivars were supplied by the North Dakota Agriculture Experimental Station (R. Frohberg). Wheat cultivars were grown at the same location during 1992 and 1995. During 1995, the wheat cultivars were grown in two different plots to replicate the experiment. Although three of the wheat cultivars have the same HMW-GS composition, they differ in rheological and breadbaking properties.

Milling and Quality Assessment

Wheat samples were milled on a Bühler laboratory experimental mill (Bühler Inc., Minneapolis, MN) according to a standard procedure currently used at the Department of Cereal Science at North Dakota State University based on Approved Method 26-20 (AACC 1995).

Flour moisture, protein (N × 5.7), ash, and falling number were determined using Approved Methods 44-15A, 08-81, and 56-81B (AACC 1995). Rheological properties were assessed using farinograph and mixograph techniques (Approved Methods 54-21, and 54-40). The baking test was a straight 3-hr fermentation (Approved Method 10-09) using 25 g of flour at 14% mb. The formulation included yeast (instant dry yeast, 1.0%), sugar (5.0%), salt (1.0%), and yeast food (ammonium phosphate, 0.1%). No other additive was used.

Dough Sampling

Doughs were collected at different stages of the breadmaking procedure. The stages considered for dough sampling were after mixing, before second punch, after second punch, after fermentation, and after proofing. Dough at each stage of the baking procedure was collected in plastic containers and immediately frozen at –40°C. The frozen doughs were freeze-dried and ground using a coffee grinder. Samples were stored in a freezer (–40°C) until needed.

Protein Extraction

Proteins were extracted in sodium phosphate buffer (0.05*M*) containing 0.5% SDS without reducing agents. Flour (2 g) or freeze-dried dough was extracted in 40 mL of phosphate buffer for 12 hr with constant stirring at room temperature (18–20°C). After extraction, soluble proteins were separated by centrifugation at 27,000 × *g* for 20 min (20°C). Extracts were immediately analyzed or kept at 4°C until needed (not more than 24 hr). These extracts were used in multistacking SDS-PAGE to fractionate the nonreduced glutenins as explained below.

Multistacking SDS-PAGE of Unreduced Proteins

A multistacking gel procedure in SDS-PAGE devised by Khan and Huckle (1992) was used to analyze unreduced gluten proteins. Five stacking gels (pH 6.9) of 4, 6, 8, 10, and 12% (w/v) acrylamide concentration (constant bis-acrylamide of 0.6%) 0.5 cm high were laid on top of a resolving gel 11 cm high (14% acrylamide, 0.28% bis-acrylamide). Gel plate dimensions were 18 cm high × 16 cm wide. Gels were 1.5 mm thick and made on a vertical electrophoresis apparatus (Hoefer Scientific, San Francisco, CA).

The 14% resolving gel solution was poured into the plate, and water was laid on the surface for a smooth surface formation. After 1 hr of polymerization, a 12% stacking gel was added and a frosted polyethylene plastic plate 1.5 mm thick was used to form a smooth surface on the stacking gel. This process was repeated with the 10, 8, and 6% stacking gels. Finally, the 4% stacking gel was added and a slot former (15 wells) was inserted.

Proteins dissolved in a sample buffer (62.5 m*M* Tris-HCl, pH 6.8, 20% glycerol, 2% SDS) were applied to the gel. Samples were electrophoresed overnight at 7.5 mA per gel at 20°C. Gels were stained for 30 min in a 0.25% (w/v) Coomassie Blue R-250 dye containing 50% methanol and 10% glacial acetic acid. Gels were destained in a solution of 20% methanol and 7% glacial acetic acid until the gel background was clear. Destained gels were used for quantitation purposes. The total extract proteins when applied to the multistacking SDS-PAGE gels are fractionated into various molecular weight species. These nonreduced species were then quantitated.

Quantitative Analysis of Proteins on Gels

Glutenins from Len were extracted according to Hou and Ng (1995) using an acetone precipitation method and used as a stan-

TABLE I
Quality Attributes of Flours from Four Hard Red Spring Wheat Cultivars[a]

Cultivar		Moisture (%)	Protein[b] (%)	Ash[b] (%)	Falling Number (sec)	Loaf Volume (cm^3)	Farinograph WA (%)	Farinograph DT (min)	Farinograph S (BU)	Mixograph WA (%)	Mixograph PT (min)	Mixograph MH (mm)
Len	Mean	12.8a[c]	13.6a	0.46a	420a	183.3a	64.5a	9.1a	14.1a	67.0a	7.5a	79.7a
	SD	0.4	1.1	0.02	11.3	3.1	0.25	1.2	4.2	2.7	0.87	4.7
Marshall	Mean	12.7a	12.4b	0.46a	442b	164.0b	59.0b	5.5b	9.2b	62.5a	4.5b	70.3b
	SD	0.7	1.0	0.02	9.0	6.6	0.74	0.5	2.0	0.87	0.50	2.1
215	Mean	13.0a	13.7a	0.46a	436b	167.7b	66.5c	6.3bc	8.8b	68.7a	4.2b	77.7a
	SD	0.4	1.3	0.06	9.8	5.5	1.0	1.6	1.4	7.5	0.29	8.5
Butte 86	Mean	13.1a	13.7a	0.43a	470c	166.3b	66.7c	7.4c	9.6b	68.7a	5.7b	79.0a
	SD	0.3	1.5	0.03	4.3	3.5	0.25	0.5	4.1	6.7	1.5	9.5

[a] WA = water absorption, DT = dough development time, S = stability, PT = peak time, MH = maximum height.
[b] 14% moisture basis.
[c] Values are means of three replicates. SD = standard deviation. Means followed by different letters in the same column are significantly different ($P < 0.05$).

dard for quantification purposes. Len flour (5-g duplicates) were mixed in 250 mL of 60% aqueous ethanol (v/v) for 30 min. The suspension was centrifuged at 15,000 × *g* for 10 min and the supernatant was discarded. The procedure was repeated and the residue recovered for glutenin extraction. The residue was extracted in a solution of 50% propanol containing 0.08*M* Tris-HCl buffer (pH 8.0) and 1% (w/v) dithiothreitol (DTT) for 1 hr at 65°C in a water bath (constant shaking). The mixture was centrifuged and the supernatant was recovered. The extraction procedure was repeated for 30 min. Supernatants were pooled and 30 mL of the propanol/Tris HCl solution containing 1.4% 4-vinylpyridine was added to alkylate the proteins for 30 min at 65°C. After centrifugation, the supernatant was precipitated with acetone to a final concentration of 80% acetone. The precipitate was washed three times with deionized water and centrifuged to remove Tris-HCl and DTT, after which, the precipitate was freeze-dried and its protein content determined by the micro-Kjeldahl procedure.

A solution of 2.5 mg of protein/mL of the above preparation was used as a standard for quantification purposes. Protein (10 µL, 25 µg) was loaded onto each gel to quantify the different gluten proteins. An imaging densitometer equipped with a 4,000-pixel CCD detector at the transmittance mode (model GS-670, BioRad Laboratories, Hercules, CA) was used for quantification. Gels were scanned and the images were digitized and stored until needed for quantitative analysis. The software (Molecular Analyst, BioRad) uses a series of menus and mouse commands to scan, display, store, and analyze electrophoretic patterns.

Volume analysis that determines the concentration of proteins expressed as total o.d. or volume (average o.d. × area) of a protein band was conducted on different groups of gluten proteins such as nonreduced HMW-GS (all the multistacking gels 4–12%, including the 14% origin of the resolving gel), medium molecular weight (MMW) proteins (upper half of 14% resolving gel of multistacking gel, excluding the 14% origin), and low molecular weight (LMW) proteins (lower half of 14% resolving gel of multistacking gel). Quantification of the protein retained at each of the stacking gel origins was also performed. Quanitification of total and individual HMW-GS was also performed on reduced glutenin.

Preparative Multistacking SDS-PAGE

The same procedure devised by Khan and Huckle (1992) was used to isolate and separate gluten proteins on a preparative scale for further analysis. Gels 3 mm thick with 10 slot combs were used. Stacking gels of 4, 6, 8, 10, and 12% were laid onto a 14% separating gel as described above. Electrophoresis (constant current) was conducted under the same conditions, except that the current was doubled for the thicker 3 mm gel. No staining was performed. Gels were cut (1 mm below) at each of the stacking gel origins (4, 6, 8, 10, 12, and 14%) and placed in 1.5-mm plastic test tubes. Sample buffer (62.5 m*M* Tris-HCl, 20% glycerol, 2% SDS, and 1% DTT) was added to each test tube (700 mL) and incubated overnight at 50°C. Test tubes were centrifuged at 14,000 rpm for 30 min (Eppendorf model 5415C). Supernatants were used to analyze glutenin subunit composition.

SDS-PAGE of Proteins Isolated from Multistacking Gel Origins

Protein extracted from the different multistacking gel origins were loaded (80–85 µL) on 0.75 mm thick polyacrylamide gels containing a stacking gel (4% acrylamide) and a resolving gel (14%). Gels were electrophoresed overnight at 5 mA per gel at 20°C. Gels were stained with Coomassie Blue R-250 (0.25% in 50% methanol and 10% acetic acid) for 30 min and destained (7% acetic acid, 20% methanol) until a clear gel background was obtained. Gels were scanned and proteins quantitated.

Experimental Design and Statistical Analysis

A randomized complete block design (Cochran and Cox 1950, Steel and Torrie 1980) was used in this study. Three true replicates (blocks) were analyzed. Each block represented a different environment (a combination of location and year of planting) where the

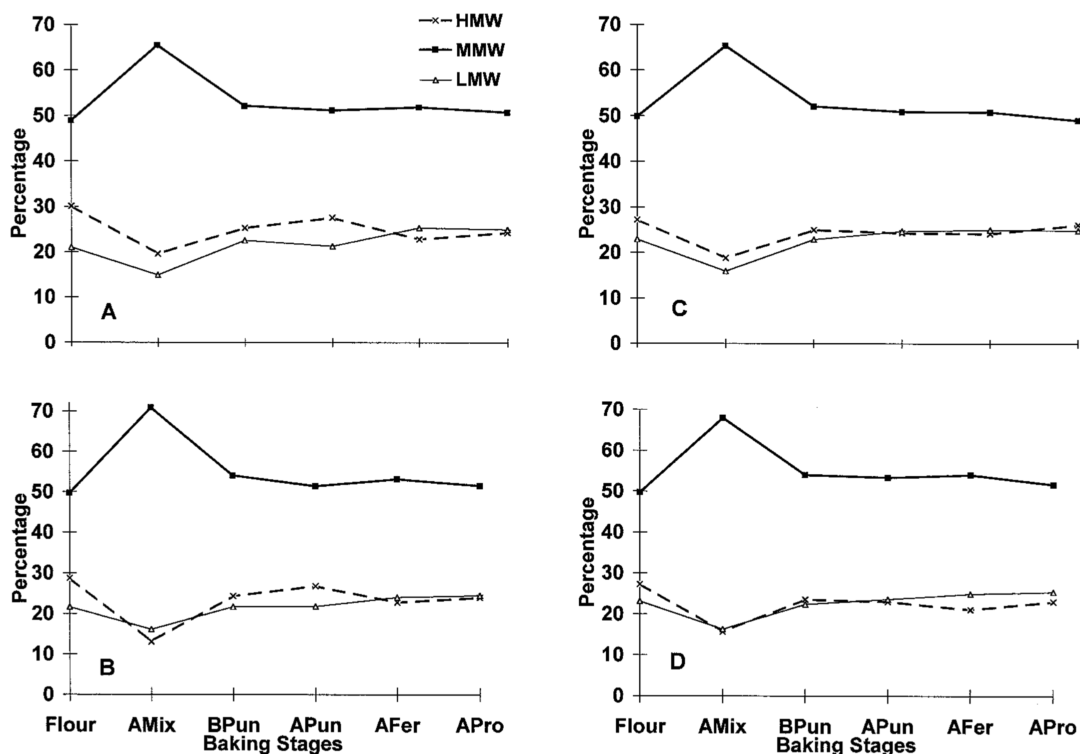

Fig. 1. Changes in the distribution of major protein groups during breadmaking (**A–D,** Len, 215, Marshall, and Butte 86 wheats, respectively). AMix = after mixing; BPun = before punching; APun = after punching; AFer = after fermentation; APro = after proofing; HMW = high molecular weight proteins; MMW = medium molecular weight proteins; LMW = low molecular weight proteins. Maximum standard deviation (as a percentage of the mean) observed among replicates was 4.9%.

four wheat cultivars were grown (all cultivars were grown with the same environmental conditions within each block). Means, standard deviations and errors, and correlation coefficients were calculated (Analytical Tool Pack, Microsoft Excel). Analysis of variances (ANOVA) and least significant differences (LSD) were made using the SAS statistics software version 6.11 (SAS Institute, Cary, NC). A level of significance of 95% was used on all statistical analyses.

RESULTS AND DISCUSSION

Flour Quality

Physicochemical (moisture, protein, and ash), rheological (farinograph and mixograph), and baking quality (loaf volume) characteristics were determined for the wheat flours used in this study (Table I). Moisture and ash content were essentially the same for all flours. Protein content ranged from 12.35 to 13.70%. Only Marshall had a statistically significant lower protein content. Farinograph and mixograph data showed that Len is a strong wheat cultivar with high values for dough development time (DT), stability (S), peak time (PT), and maximum mixograph height (MH). Only mixograph water absorption (WA) was statistically equal for all wheat cultivars. Loaf volume of cultivar Len was statistically higher (≈10%) than the rest. Although all wheat cultivars have similar protein content, they exhibit different rheological and functional characteristics that indicate other factors in addition to protein quantity are involved in the determination of wheat flour functionality.

Protein Changes During Breadmaking

Changes in major protein groups during breadmaking as determined by densitometry of proteins solubilized in SDS solvent. Figure 1 shows how each of the major protein groups (extracted under nonreducing conditions) changed during breadmaking for each wheat cultivar. It is evident that the changes are similar, regardless of which wheat cultivar is considered.

After flour is optimally mixed, there is an increase in the proportion of soluble MMW proteins, while there is a decrease in the proportions of soluble HMW and LMW proteins (Fig. 1 and Table II). However, the magnitude of those changes is cultivar-dependent. For example, Len and Marshall have similar percentages of decrease of HMW proteins (≈30%), but 215 and Butte 86 had percentages of decrease significantly higher (48.8 and 42.3%, respectively). In the MMW protein group, Len exhibited the lowest percentage of increase, while 215 showed the highest (Table II). In the LMW protein group, Butte 86 exhibited the highest percentage of decrease, but cultivar Len exhibited the lowest.

The change in the distribution of proteins from one breadmaking stage to another is undoubtedly related to the change in solubility of the proteins from one stage to another, and changes in solubility are related to changes in the aggregation and disaggregation of the gluten polymers (polymerization and depolymerization). For example, a greater amount of soluble protein in a sample means easier solubility, perhaps due to less aggregation or more disaggregation (depolymerization). In contrast, less soluble protein means difficulty in solubilization, perhaps due to more aggregation or less disaggregation (polymerization). We have seen that the mixing operation results in a decrease in the relative proportion of soluble HMW proteins in the dough but an increase in MMW proteins. This decrease in solubility of HMW proteins (glutenin polymers) could be explained by the increase in protein aggregation that occurs during mixing (the formation of the gluten network). More aggregation means formation of large polymers that become insoluble and cannot be extracted without using more drastic solubilization conditions such as reducing agents or sonication.

After mixing, doughs were allowed to rest under controlled conditions of moisture and temperature. When proteins were quantified at the next baking stage (before the punching operation), the previously observed changes were reversed (Fig. 1). During this resting period, the proportion of HMW and LMW gluten proteins increased (solubilization, disaggregation, and depolymerization occurred), while MMW proteins decreased (decrease in solubility, polymerization). During the resting period, the dough being a dynamic and not a static system, underwent changes and rearrangements (aggregation and dissagreggation) of the proteins, thereby resulting in solubility changes. After the observed changes of mixing and resting (before the second punch), there were no major changes in the proportions of major protein groups (Fig. 1) during the remainder of the breadmaking process.

There were changes in major protein groups during breadmaking and the magnitude of those changes was not equal for all the wheat cultivars analyzed.

Changes in the proportion of glutenin protein retained at each of the multistacking gel origins of various breadmaking stages. We used the multistacking gel procedure designed by Khan and Huckle (1992) to fractionate the nonreduced HMW glutenin into subgroups according to molecular size. It is possible to estimate the molecular weight of those subgroups (aggregates) according to the position in the multistacking gel where they migrated and were retained. Aggregates retained at the 4% origin are larger and of higher molecular weights than those retained at the 6%. Those retained at 6% are larger than those retained at 8%, and so on. The lowest aggregates would be those retained at the 14% gel origin.

TABLE II
Changes in Major Protein Groups of Nonreduced Extracts from Stage to Stage of the Breadmaking Process[a]

Group	Cultivar	Percentage of Change				
		Flour to AMix	AMix to BPun	BPun to APun	APun to AFer	AFer to APro
HMW	Len	–28.2	17.2	2.2	–11.8	9.3
	Marshall	–31.1	33.3	–2.8	–0.7	8.0
	215.0	–48.8	65.4	10.2	–14.9	5.2
	Butte 86	–42.3	50.1	–2.4	–8.3	9.1
MMW	Len	25.8	–15.2	–1.9	1.3	–3.4
	Marshall	31.1	–20.3	–2.2	–0.2	–3.6
	215.0	39.2	–21.9	–4.8	3.4	–3.1
	Butte 86	36.8	–20.6	–1.2	1.2	–4.4
LMW	Len	–19.5	33.6	1.8	10.4	–1.5
	Marshall	–30.5	44.0	8.0	1.1	–0.4
	215.0	–25.5	34.6	0.6	10.4	1.8
	Butte 86	–31.1	37.5	5.4	5.5	1.8

[a] Quantification by densitometry. Values expressed as the percentage of change from one stage to the other. AMix = after mixing, BPun = before punching, APun = after punching, AFer = after fermentation, APro = after proofing. HMW, MMW, and LMW = high, medium and low molecular weight proteins, respectively.

Figures 2–4 show how the proportion of proteins retained at each multistacking gel origin (4–14%, respectively) changed during the breadmaking process. The 4% protein aggregates (Fig. 2) followed a pattern similar to the HMW protein groups (Fig. 1). There was an initial decrease of the protein retained at the 4% gel origin due to the mixing operation and then an increase in the content of these aggregates as breadmaking progressed to the before and after punching stages (Fig. 2). Another decrease occurred at the after fermentation stage and an increase after proofing.

Figure 3 shows the behavior of the 6% aggregates during breadmaking. These aggregates do not change as much as the 4% protein aggregates (Fig. 2). Mixing had no significant effect on the quantity of proteins retained at the 6% origin. The same was true for the protein retained at the 8% origin. Figure 3 shows that the tendency of the proteins retained at the 8% origin is to decrease during breadmaking. The 10% protein aggregates exhibited the same behavior as the 8% protein aggregates (Fig. 3). Changes observed in the quantities of proteins retained at the 8 and 10% origins were statistically significant.

Proteins retained at the 12 and 14% gel origins, in contrast, tend to increase during breadmaking (Fig. 4), although the tendency is more evident for the 14% protein aggregates than for the 12% protein aggregates. Marshall, 215, and Butte 86 seem to have a more stable proportion of 12% protein aggregates throughout the breadmaking process.

Changes in the relative proportions of different gluten proteins during breadmaking are related to the changes in solubility of protein aggregates from one breadmaking stage to another. Changes in solubility are also related to changes in the size and molecular weight of the gluten aggregates. During mixing, proteins retained at the 4% gel origin decreased. A decrease of HMW polymers at the 4% gel origin indicated that a decrease in solubility occurred, perhaps due to polymerization of those polymers during mixing. At the same time, during mixing, polymers of lower molecular weight (12–14% origin proteins) were increasing. This is because the lower molecular weight proteins may have been disaggregated by the shearing action of mixing, thereby becoming easily solubilized with SDS buffer or they may not have participated in interactions as much as the HMW polymers in forming the gluten network. The decrease of soluble HMW polymers (4%) combined with the increase of LMW soluble glutenin proteins (12–14% origins) during mixing denotes a process of rearrangement of the gluten proteins in which polymerization and depolymerization of proteins occur and yield less soluble large polymers (seen as a decrease in the proportion of 4% proteins) and an increase in proteins of smaller size (seen as an increase in the proportion of proteins retained at the 12–14% origins).

The magnitude of the effect of mixing on the molecular size distribution of glutenins as determined by solubility seems to be dependent on the strength of the wheat cultivar. For example, Len (a strong wheat cultivar) had the highest percentage of decrease (45%) of the 4% protein aggregates, while Butte 86 (a weaker wheat cultivar) had the lowest percentage of decrease (21%). Also, Len had the highest values of loaf volume, while Butte 86 had a lower value (Table I). Those differences in the way in which HMW gluten proteins change during breadmaking seem to be related to the performance and breadmaking quality of the flour. The theory of polymer entanglements speculates that those polymers of higher molecular weight (i.e., gluten polymers of Len) will be able to form more entangled polymers (aggregates), which in turn will be more stable and stronger (MacRitchie 1986). This seems to be an

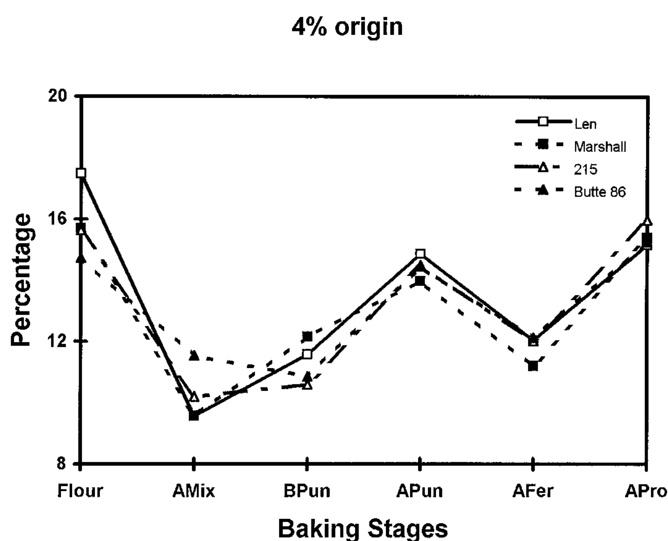

Fig. 2. Change in the relative proportions of protein retained at the 4% multistacking gel origins during breadmaking for four wheats. AMix = after mixing; BPun = before punching; APun = after punching; AFer = after fermentation; APro = after proofing. Maximum standard deviation (as a percentage of the mean) observed among replicates was 5.2%.

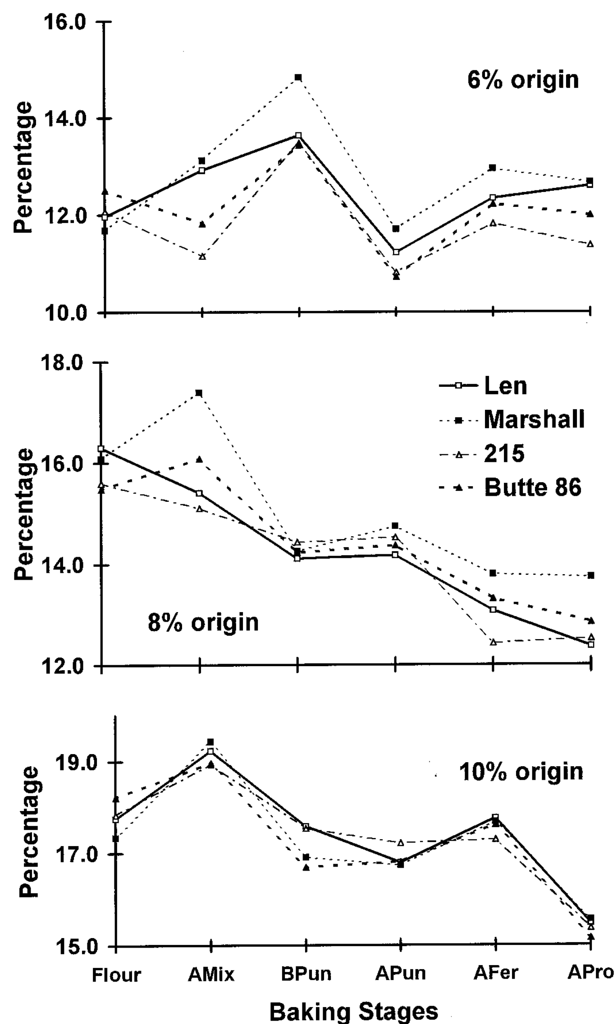

Fig. 3. Change in the relative proportions of protein retained at the 6, 8, and 10% multistacking gel origins during breadmaking for four wheats. AMix = after mixing; BPun = before punching; APun = after punching; AFer = after fermentation; APro = after proofing. Maximum standard deviation (as a percentage of the mean) observed among replicates was 5.8%.

explanation of the observed changes during breadmaking. Len has a greater proportion of HMW polymers and is able to form more entangled polymers (polymers of greater size) and a stronger gluten network, which results in good performance during breadmaking. The formation of larger polymers is confirmed by the larger decrease in the proportion of soluble HMW polymers (4% protein aggregates) after mixing in Len. The insoluble polymers remain as residue proteins (insoluble glutenin).

Changes in individual HMW-GS from glutenin polymers retained at each multistacking gel origin during breadmaking. There were significant differences in major gluten protein groups (HMW, MMW, and LMW in nonreduced SDS-soluble proteins) regarding changes during breadmaking for wheat cultivars differing in rheological and breadmaking characteristics. To further study possible quantitative differences among wheat cultivars during breadmaking, we measured the relative proportion of individual HMW-GS from the protein aggregates retained at each of the multistacking gel origins. Each origin was excised and glutenin subunits extracted with reducing agent and quantitated. This quantification was made at each of the breadmaking stages to investigate how those subunits change during the breadmaking process. We hypothesized that there should be differences in the way individual HMW-GS change during breadmaking between two wheat cultivars of differing rheological and breadmaking characteristics.

A comparison of the changes in the content of individual HMW-GS during breadmaking for Len and Marshall is shown in Fig. 5. HMW-GS (from 4% protein aggregates) of Len are more stable (less change) throughout the breadmaking process than HMW-GS of Marshall (Fig. 5). For example, subunit 2* of Len increased from 11.84% (flour) to 21.20% (after mixing) and then, remained constant at 20–21% during the rest of the baking stages. In fact, if we do not consider the flour stage as part of the breadmaking process, we would assume that there was no change in the content of this subunit during breadmaking. In contrast to Len, subunit 2* of Marshall decreased from 11.16% (flour) to 8.39% (after mixing) then increased to 10.20% in the next stage and continued to increase up to 18.79% at the end of the proofing stage. Similar to subunit 2*, the other HMW-GS (5, 7, 9, and 10) exhibited more variability during breadmaking in Marshall than in Len (Fig. 5).

In summary, HMW-GS from the 4% protein aggregates from Len are more stable (do not change as much) when compared with the HMW-GS of Marshall. For Len, subunits 5 and 9 remained unchanged, subunits 2* increased at the beginning (as a consequence of the mixing operation) and then remained constant, while subunits 7 and 10 decreased during the initial breadmaking

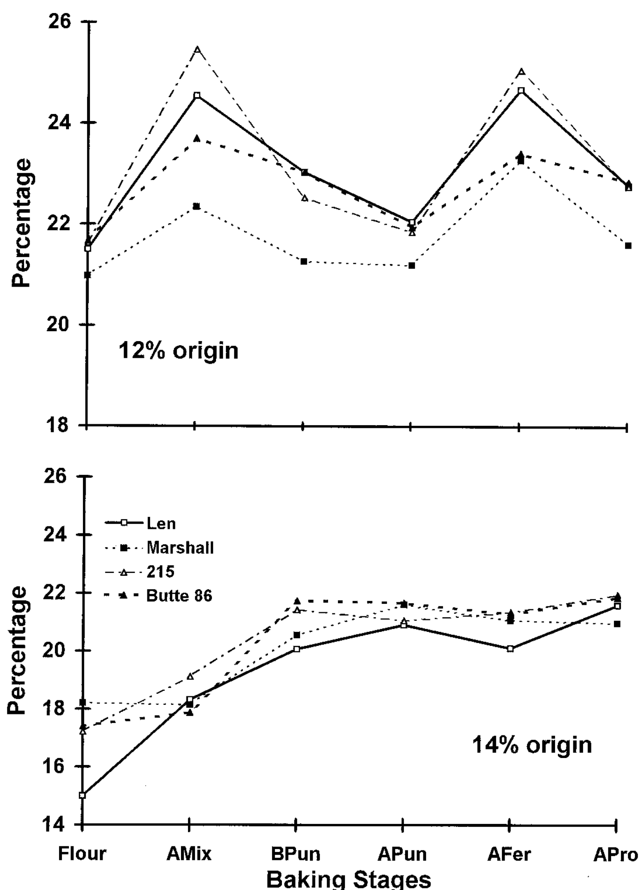

Fig. 4. Change in the relative proportions of protein retained at the 12% and 14% multistacking gel origins during breadmaking for four wheats. AMix = after mixing; BPun = before punching; APun = after punching; AFer = after fermentation; APro = after proofing. Maximum standard deviation (as a percentage of the mean) observed among replicates was 6.3%.

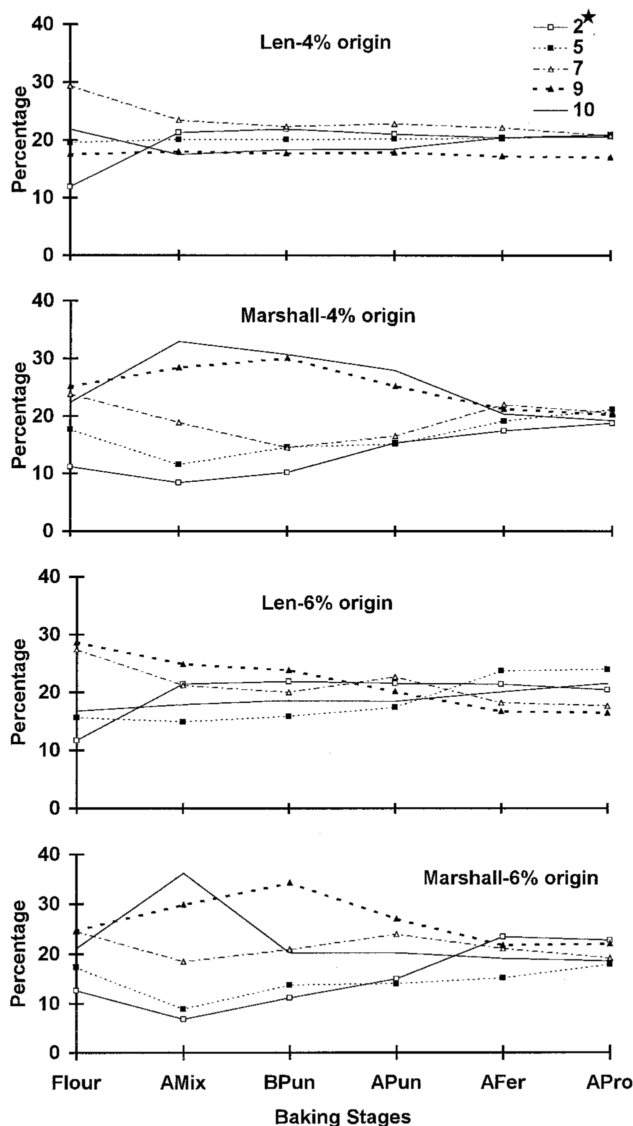

Fig. 5. Changes in individual HMW glutenin subunits during breadmaking from proteins retained at the 4 and 6% multistacking gel origins for wheats Len and Marshall. AMix = after mixing; BPun = before punching; APun = after punching; AFer = after fermentation; APro = after proofing. Maximum standard deviation (as a percentage of the mean) observed among replicates was 9.2%.

stages (after mixing) and increased afterwards. For Marshall, subunits 2*, 5, and 7 decreased after mixing and then increased in subsequent stages, and subunits 9 and 10 increased at the beginning (after mixing) and then decreased as the breadmaking process progressed.

Figures 5–7 show the changes in individual HMW-GS of proteins retained at the 6, 8, and 10% origins during breadmaking for Len and Marshall. As with the 4% protein aggregates, there seem to be more changes in the HMW-GS content during breadmaking for Marshall. There was an initial increase after mixing in the proportion of the lower molecular weight subunits 9 and 10 and a decrease in the higher molecular weight subunits for Marshall. As breadmaking progressed, there was a decrease in the solubility of subunits 9 and 10 and an increase in the solubility of the higher molecular weight subunits for Marshall. The trend was just the opposite for the better quality cultivar; Len had greater solubility of subunits 9 and 10 and lower solubility of subunits 2* and 5 as breadmaking progressed. (Figs. 5–7)

The HMW-GS of protein aggregates retained at the 12 and 14% multistacking gel origins (Fig. 7) had very similar changes during breadmaking. The proportion of subunits 2* and 5 at the 12 and 14% protein aggregates for Len increased significantly during mixing. Subunit 2* at the 12% aggregates increased from 10.99 to 14.25%; at the 14% level, it increased from 11.95 to 15.18%. Subunit 5 in the 12% aggregates increased from 9.78 to 14.6%; at the 14% level, it increased from 10.25 to 12.73%. The proportion of subunit 7 in both 12 and 14% protein aggregates decreased (0.4 and 2%, respectively) during breadmaking (Fig. 7). Subunit 9 of the 12% aggregates decreased after mixing from 29.18 to 23.52% and then remained unchanged, while subunit 9 of the 14% protein aggregates remained constant during breadmaking. With respect to Marshall, the changes observed during breadmaking for both 12 and 14% protein aggregates were the same: subunits 2, 5, and 7 decreased initially (after mixing) and then increased in the later stages of breadmaking, while the opposite effect was observed (increase, then decrease) for subunits 9 and 10.

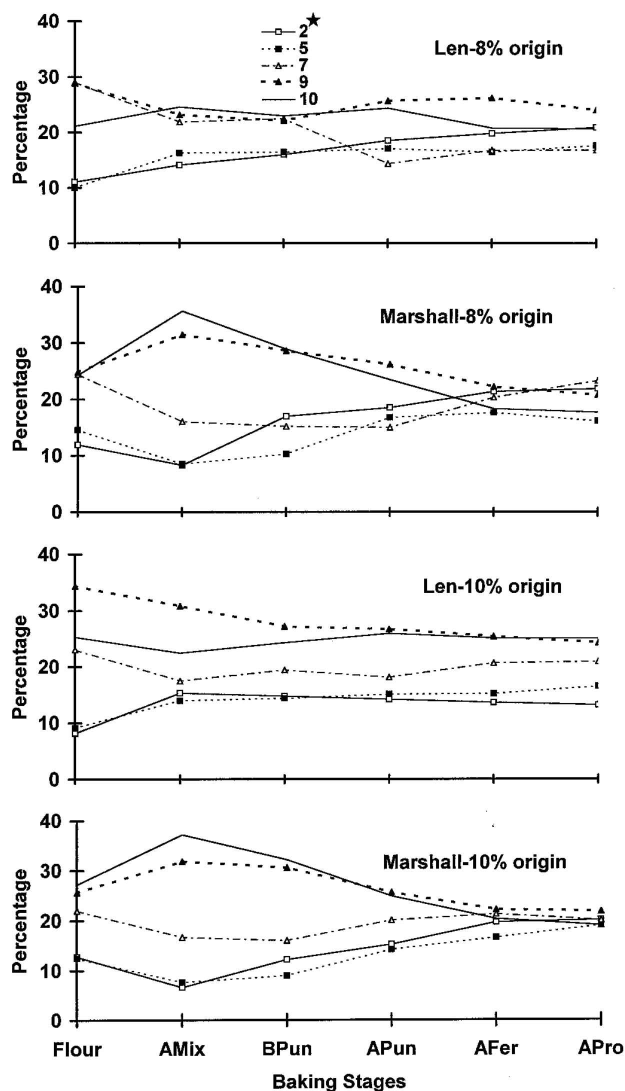

Fig. 6. Changes in individual high molecular weight glutenin subunits during breadmaking from proteins retained at the 8 and 10% multistacking gel origins for wheats Len and Marshall. AMix = After mixing; BPun = before punching; APun = after punching; AFer = after fermentation; APro = after proofing). AMix = after mixing; BPun = before punching; APun = after punching; AFer = after fermentation; APro = after proofing. Maximum standard deviation (as a percentage of the mean) observed among replicates was 8.5%.

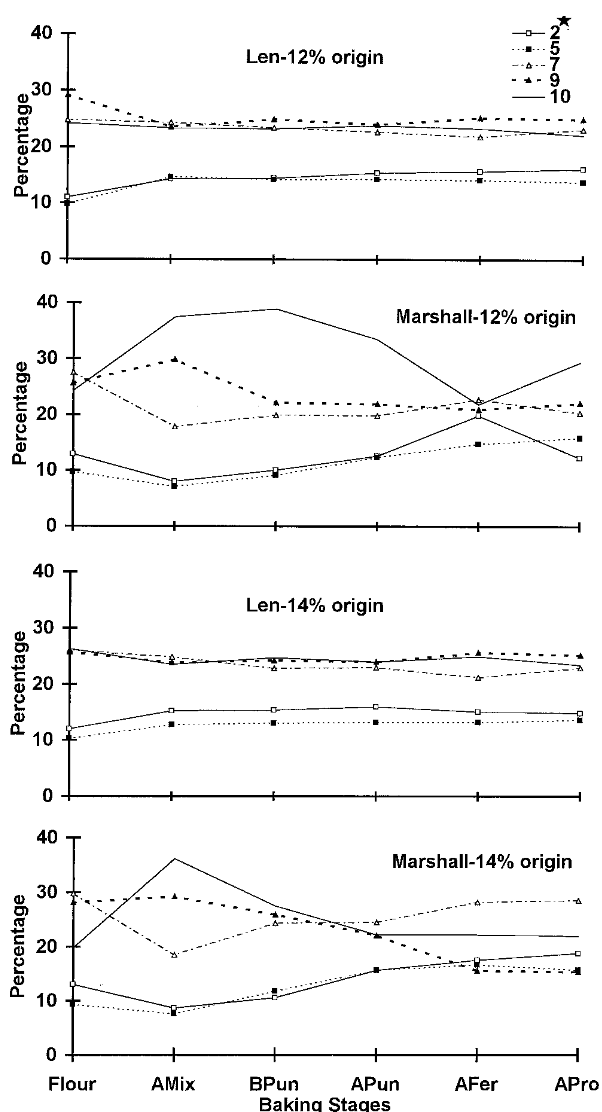

Fig. 7. Changes in individual high molecular weight glutenin subunits during breadmaking from proteins retained at the 12 and 14% multistacking gel origins for wheats Len and Marshall. AMix = after mixing; BPun = before punching; APun = after punching; AFer = after fermentation; APro = after proofing. Maximum standard deviation (as a percentage of the mean) observed among replicates was 7.9%.

Summary of Protein Changes During Breadmaking

Protein changes during breadmaking were studied by solubility changes and multistacking SDS-PAGE analysis for wheat cultivars differing in breadmaking characteristics. We studied the SDS-soluble protein fraction to obtain information on the nonreduced soluble glutenins that can be separated by multistacking SDS-PAGE into various molecular weight species and quantitated by densitometry. After characterization and quantitation of these nonreduced glutenin species, they can be further eluted from the gel origins and the subunit composition can be characterized and quantitated. In contrast, nonreduced residue proteins (insoluble glutenins) cannot be extracted but can only be characterized after reduction with a reducing agent and solubilization of the resulting subunits of glutenin. Therefore, much more information about breadmaking quality differences may be obtained from the soluble glutenin, which is actually a mirror-image of the insoluble glutenins (residue proteins). More soluble glutenin indicates less residue proteins, less soluble glutenin indicates more residue proteins.

The mixing operation in the breadmaking process is the first step in which the different gluten protein groups, including individual HMW-GS, interact to give the initial rheological characteristics of the dough. However, as the results of the study indicate, the proteins, such as the various glutenin protein species, further interact and redistribute themselves during the other stages of the breadmaking process to influence the final bread quality (loaf volume). Therefore, the mixing step may not be the best indicator of breadmaking quality (loaf volume).

After mixing, there is an increase in the proportion of MMW gluten proteins and a decrease in the proportion of HMW and LMW protein groups (Fig. 1). The magnitude of the change was cultivar-dependent. During the resting periods of the fermentation process (fermentation and proofing), no major changes in the proportion of major gluten groups occurred.

Protein aggregates of the highest molecular weight (those retained at the 4% origin) followed a pattern similar to the total HMW gluten proteins: initial decrease after mixing and subsequent increase during fermentation and proofing (Fig. 2). Proteins retained at the 6% origin remained unchanged (Fig. 3), while protein aggregates retained at 8 and 10% origins decreased during breadmaking. Proteins retained at 12 and 14% origins tend to increase during breadmaking (Fig. 4).

HMW-GS distribution from protein aggregates retained at each of the multistacking gel origins changed during breadmaking. In general, the better quality wheat cultivar Len showed more stable distributions (less change) during breadmaking than Marshall. At all multistacking gel origins of Len, subunits 2* and 5 increased initially after mixing and then remained constant. Subunits 7, 9, and 10 exhibited different patterns of change in different protein aggregates of Len (proteins retained at different multistacking gel origins). Glutenin subunits 2*, 5, and 7 of Marshall exhibited the same pattern of change during breadmaking in all the protein aggregate groups (4–14%). Their proportion initially decreased at mixing (less soluble polymers) and then increased (more soluble polymers) in later stages. In contrast, subunits 9 and 10 increased in the initial stages (after mixing) and then decreased in the later stages, indicating more interaction of the lower molecular weight subunits in the later stages of breadmaking to influence final loaf volume.

CONCLUSIONS

The importance of the molecular weight and of the molecular weight distribution of different polymer aggregates in determining rheological properties of nonprotein polymers has long been recognized (Severs 1962, Nielsen 1977, Yanovsky 1993). The effect of the molecular weight on polymer properties can be seen as a function of the length of the chain of the polymer. A LMW polymer has a shorter chain, while a HMW polymer has a longer chain (assuming none or little branching). The shorter the chain of a polymer, the less resistance there is to untangling when stress is applied. Hence, the molecules can disentangle easily under stress and produce more sites of weakness in the structure of the polymer. As the molecular weight of the polymer increases (longer chains), there are more entanglements of the chains, resulting in an increase in the strength properties of the polymer (Yavnosky 1993).

We can apply some of these findings of nonprotein polymer rheology and use them to try to explain our observed results with wheat gluten proteins. Len is composed of glutenin polymers/aggregates of higher molecular weight, while Marshall has glutenin polymers/aggregates of a lower molecular weight (Borneo 1998). Thus, the gluten network that Len will form during mixing (a form of stress) will be stronger (withstand more stresses) because the protein is composed of glutenin polymers of higher molecular weight capable of more entanglements (this stronger gluten network is seen as higher value farinograph stability). More gluten chain entanglements produce a stronger network that will hold gas and expand more (a form of stress) during breadmaking, producing larger loaf volume values. Marshall, in contrast, has glutenin polymers/aggregates of lower molecular weight (shorter chains) so fewer entanglements occur and more sites of weakness (less able to withstand stress) will be present in the network. A weaker network will not retain gas as well as the network formed by Len proteins and, hence, will produce a lower loaf volume value during baking (Table I).

We also studied the changes that occurred in gluten protein distribution by solubility procedures during various stages of breadmaking. We interpreted solubility differences in amount of protein with respect to functional properties. When polymers such as nonreduced glutenin or its various species (aggregates of different sizes) or the subunits of these reduced polymers become less soluble at a certain stage of the breadmaking process, we interpret this behavior to mean that these polymers and subunits have participated through aggregation and polymerization in forming the dough-gluten network and are less soluble. The opposite functionality is inferred (aggregation and polymerization) when the solubility of any of the protein species increased at a certain stage of breadmaking. For example, the content of the 4% protein aggregates decreased after mixing (Fig. 2) but Len had the highest percentage of decrease (45.3%) when compared with Marshall (38.1%), 215 (34.7%), and Butte 86 (21.7%). This means that polymer aggregates of the highest molecular weight (4%) in Len participated more (through polymerization and aggregation) in the formation of the dough-gluten network. The contrary is inferred for other wheat cultivars that had lower percentages of decrease (Butte 86).

A quantitative analysis of the changes that occurred in the HMW-GS fraction (regarded as the most important in determining breadmaking quality differences) revealed that Len had fewer changes in the distribution of the HMW-GS throughout the breadmaking process, while Marshall had more changes. Also, there was a greater quantity and more interaction (indicated by decreased solubility) of the highest molecular weight subunits 2* and 5 in Len, whereas there was a greater quantity and more interaction of the lower molecular weight subunits 9 and 10 in Marshall. Therefore, Len would possess polymers of higher molecular weight than Marshall. These findings confirm our previous thoughts about molecular entanglement, stability, and breadmaking. Previously, we hypothesized that, because Len is composed of gluten aggregates of higher molecular weight, it would form a stronger, more stable network (more chain entanglements). More gluten chain entanglements would produce a stronger network that would hold gas and expand more during breadmaking, producing larger loaf volume values. We found that the HMW-GS distribution changed less in Len. This indicated the formation of a more stable gluten network during breadmaking. In contrast, we predicted that Marshall would form a weaker gluten network due to the presence of lower molecular weight polymers and fewer molecular entanglements. Our results confirm these predictions. The fact that the HMW-GS distribution of Marshall changed significantly during breadmaking was evidence of the formation of less stable gluten networks and, hence, lower breadmaking performance.

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LITERATURE CITED

American Association of Cereal Chemists. 1995. Approved Methods of the AACC, 9th ed. The Association: St. Paul, MN.

Borneo, R. 1998. Identification of gluten protein quality factors during breadmaking. PhD thesis. North Dakota State University: Fargo, ND.

Cochran, W. G., and Cox, G. M. 1950. Experimental Designs. John Wiley and Sons: New York.

Hou, G., and Ng, P. K. W. 1995. Quantification of glutenin subunits by sequential acetone precipitation and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) coupled with densitometry using a known quantity of glutenins as a standard. Cereal Chem. 72:545-571.

Huang, D. Y. 1995. Quantitative determination of glutenin subunits and their relationship to breadmaking quality of hard red spring wheat. PhD thesis. North Dakota State University: Fargo, ND.

Huang, D. Y., and Khan, K. 1997a. Characterization and quantification of native glutenin by multistacking sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) procedures. Cereal Chem. 74:229-234.

Huang, D. Y., and Khan, K. 1997b. Quantitative determination of high molecular weight glutenin subunits of hard red spring wheat by SDS-PAGE. I. Quantitative effects of total amounts on breadmaking quality characteristics. Cereal Chem. 74:781-785.

Huang, D. Y., and Khan, K. 1997c. Quantitative determination of high molecular weight glutenin subunits of hard red spring wheat by SDS-PAGE. II. Quantitative effects of individual subunits on breadmaking quality characteristics. Cereal Chem. 74:786-790.

Khan, K., and Huckle, L. 1992. Use of multistacking gels in sodium dodecyl sulfate-polyacrylamide gel electrophoresis to reveal polydispersity, aggregation, and disaggregation of the glutenin protein fraction. Cereal Chem. 69:686-688.

Khan, K., Tamminga, G., and Lukow, O. 1989. The effect of wheat flour proteins on mixing and baking. Correlations with protein fractions and high molecular weight glutenin subunit composition by gel electrophoresis. Cereal Chem. 66:391-396.

Kolster, P., Krechting, C. F., and Van Gelder, W. M. J. 1992. Quantification of individual high molecular weight subunits of wheat glutenin using using SDS-PAGE and scanning densitometry. J. Cereal Sci. 15:49-61.

Lawrence, G. J. 1986. The high-molecular-weight glutenin subunit composition of Australian wheats cultivars. Aust. J. Agric. Res. 37:125-133.

Lukow, O. M., Payne, P. I., and Tkachuk, R. 1989. The HMW glutenin subunit of Canadian wheat cultivars and their association with breadmaking quality. J. Sci. Food Agric. 46:451-460.

MacRitchie, F. 1986. Physicochemical processes in mixing. Pages 132-145 in: Chemistry and Physics of Baking. M. J. V. Blanshard, P. J. Frazier, and T. Galliard, eds. R. Soc. Chem.: London.

MacRitchie, F., DuCross, D. L., and Wrigley, C. W. 1990. Flour polypeptides related to wheat quality. Pages 79-145 in: Advances in Cereal Science and Technology, Vol X. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul. MN.

Nielsen, L. E., 1977. Polymer Rheology. Marcel Dekker: New York.

Payne, P., Nightingale, M., Krattiger, A., and Holt, L. 1987. The relationship between HMW glutenin subunit composition and the breadmaking quality of British-grown varieties. J. Sci. Food Agric. 40:51-65.

Severs, E. T. 1962. Rheology of Polymers. Reinhold: New York.

Shewry, P. R., Halford, N. G., and Tatham, A. S. 1992. High molecular weight subunits of wheat glutenin. J. Cereal Sci. 15:105-120.

Steel, R. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics: A Biometrical Approach. MacGraw Hill: New York.

Sutton, K. H. 1991. Qualitative and quantitative variation among HMW subunits of glutenin detected by RP-HPLC. J. Cereal Sci. 14:25-34.

Wrigley, C. W., and Bietz, J. A. 1988. Proteins and amino acids. In: Wheat: Chemistry and Technology, Vol. 1. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul. MN.

Yavnosky, Y. G. 1993. Polymer Rheology: Theory and Practice. Chapman and Hall: London.

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