

Effect of Mechanical Dough Development on the Extractability of Wheat Storage Proteins from Bread Dough

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ABSTRACT

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Six wheat cultivars covering a range of quality parameters were mixed to various proportions of their optimum work input using mechanical dough development (MDD) mixers. Mixing and baking characteristics were determined and each dough was subsampled. The proteins were extracted for analysis by reversed-phase HPLC. Considerable protein mobilization appeared to occur during the MDD process, but the changes appeared to be cultivar-specific and did not indicate how mixing or baking behavior could be predicted. Protein content in extracted fractions was lowest for the weakest, poorest quality wheat but failed to consistently rank the stronger samples. Acetic acid insoluble protein level decreased

with mixing as did extractable high molecular weight glutenin subunits. Gliadin protein level initially decreased with mixing before rising sharply with overmixing, while low molecular weight glutenin subunits displayed the reverse pattern. The rate of change of the extractability of the protein fractions with work input was greatest for the weakest samples and least for the stronger samples. However, when the protein quantity in the extractable fractions was plotted against relative work input, the rate of change of protein extractability did not appear to vary significantly between cultivars of different strengths.

The molecular structure of gluten proteins and their role in breadbaking has been extensively studied. Most of the research has focused on the glutenin proteins, especially the high molecular weight glutenin subunits (HMW-GS). In a dough, the gluten proteins form a poorly delineated matrix defined by both chemical bonds and physical entanglement. It seems increasingly likely that, in order to understand the significance of the processes taking place in mixing and baking, it will be necessary to study the polymeric glutenin protein rather than its individual protein classes and their subunits. Various researchers have used size-exclusion (SE) HPLC to study intact aggregated proteins solubilized in solutions containing SDS (Dachkevitch and Autran 1989, Lundh and MacRitchie 1989, Singh et al 1990, Batey et al 1991, Gupta et al 1993, Gao and Bushuk 1993, Benetrix et al 1994, Gupta and MacRitchie 1994), often with the use of sonication. However, it is questionable whether the glutenin aggregates remain intact during sonication, so this approach may not be valid. Additionally, size-exclusion media generally lack the high resolution required for detailed study of the molecular size range encompassing polymeric glutenin.

A number of researchers have investigated the SDS-insoluble gel protein fraction, referred to as the glutenin macropolymer (GMP). This protein fraction forms as a translucent layer above the starch after treatment with 1.5% SDS followed by high-speed centrifugation (Graveland et al 1980; Weegels et al 1994, 1995). Pritchard et al (1994) have shown that the rheological performance of the GMP mirrors the properties of the parent flour.

The phase of the breadmaking process where gluten functionality is critical is dough development. Studies of protein interactions during mixing and oxidation have been limited due to the complexity of the protein mixtures and the limited number of techniques for extracting proteins from doughs. This is compounded by the complexity of the mixing process, involving both the disruption and reformation of the protein matrix. European researchers have found that the quantity of GMP decreases during dough

mixing and increases again during dough resting, although the nature of the polymer present before mixing is somewhat different from that present afterwards (Weegels et al 1994, 1995). They found that the decrease in GMP content during mixing was faster in weak-doughed varieties than in strong-doughed varieties and that, in both cases, the process was well under way before mixograph peak time was reached. These observations have been verified by other research groups. In particular, Australian researchers have found that although major changes in GMP content occur before mixograph peak mixing time, changes in polymer size distribution and composition occur mainly after peak mixing time (Skerritt et al 1994). Thus, these effects may relate separately to dough mixing and breakdown.

This article reports on the changes in extractability of specific fractions of gluten proteins from doughs developed by the intensive (when compared to the mixograph and the farinograph) mechanical dough development (MDD) mixing process. The study focused on the total gluten complex and the response of modified Osborne fractions and subfractions to the mixing process using well-established chromatographic techniques, thus indirectly measuring the interactions between gliadin (GLI) and glutenin (GLU) proteins. In this way, the change in distribution and composition of proteins in polymeric and nonpolymeric states with mixing was estimated.

MATERIALS AND METHODS

Wheat

Six wheat cultivars were obtained: Otane and Karamu (from Crop and Food Research), Domino and Monad (from commercial wheat millers in New Zealand), and Glenlea and Katepwa (from the Canadian Wheat Board). All samples were from the 1993–94 harvest year. Cultivar identities were confirmed by acidic- and SDS-PAGE.

Sample Characterization

Flour protein was determined by the standard micro-Kjeldahl method ($N \times 5.7$) (AOAC 1981). Damaged starch and wholemeal falling number were determined using standard AACC methods. α -Amylase activity determined by the method of McCleary and Sheehan (1987). Particle-size index was estimated using the method of Laughland and Wilson (1987), and wet gluten content was determined by the ICC standard method 137 using the Glutomatic system (ICC 1982).

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Milling and Baking Tests

Wheat samples were tempered to 15.5% moisture before milling in a standard Buhler MLU202 laboratory mill. Break and reduction flours were collected and thoroughly blended to produce the composite flour for final analysis.

Dough production and assessment of breadbaking quality was performed using the Grain Foods Research Unit's 125-g MDD mixers and the bake test as previously described by Swallow and Baruch (1986). Optimum levels of work input (WI) and water absorption (WA) were determined before bake testing. These levels were defined as those that give maximum loaf volume. Each flour was made into duplicate doughs at one-third, two-thirds, full, and four-thirds (i.e., one-third over full) optimum WI. Dough composition was: 125 g of flour; water to optimum (~60%); 2.5 g of salt; yeast to constant activity (~3%); 0.94 g of sugar; 50 ppm of potassium bromate; 100 ppm of ascorbic acid; 0.3 g of sodium stearoyl 2-lactylate; 0.3 g of enzyme-active soy flour; and 0.225 g of diacetyl tartaric esters of distilled monoglycerides. Duplicate doughs were analyzed on the same day. Cultivar and WI level were randomized over eight days. Within 1 min of the mixer stopping, each dough piece had two subsamples removed by scalpel and accurately weighed (0.1000–0.4000 g) for immediate extraction for reversed-phase (RP) HPLC analysis. The remaining dough was then proofed and baked in the normal manner. Loaf volume was determined by rapeseed displacement, and a bake score consisting of the scaled volume measurement and a subjective crumb grain component was determined by trained personnel.

Sample Extraction Schemes for RP-HPLC Analysis

Scheme 1: Modified Osborne fractions. Gliadins were prepared by a modification of the method of Hay and Sutton (1990) and glutenins by a modification of the method of Sutton (1991). The weighed, duplicate dough subsamples were extracted for 30 min in saline solution (2 mL, 2% [w/v] NaCl), initially with the aid of gentle maceration with a glass rod and then by vortex mixing every 5 min. After centrifugation (2,000 × g, 2 min), the residual pellet was resuspended in 70% (v/v) aqueous ethanol (2 mL), and the gliadin proteins were extracted for 30 min with vortex mixing every 10 min. Following centrifugation (10,000 × g, 5 min), the clarified supernatant was removed for analysis by RP-HPLC. The residual pellet was then washed with a further aliquot of 70% (v/v)

aqueous ethanol (5 mL) for 30 min, centrifuged (2,000 × g, 2 min), and the supernatant was discarded. Glutenins were extracted from the residual pellet using a buffered reducing agent solution (1 mL of 0.08M Tris-HCl, pH 7.5, containing propan-1-ol [50%, v/v] and dithiothreitol [1%, w/v]) for 60 min at 60°C with agitation every 10 min. After cooling to room temperature, a buffered alkylating solution (1 mL of 0.08M Tris-HCl, pH 7.5, containing propan-1-ol [50%, v/v] and 4-vinylpyridine [3%, v/v]) was added and mixed well. The mixture was then heated at 60°C for 15 min to effect alkylation. After cooling to room temperature and centrifugation (20,000 × g, 10 min), the clarified supernatant was analyzed by RP-HPLC.

Scheme 2: Acetic acid fractions. Acetic acid soluble (AAS) and acetic acid insoluble (AAI) GLU fractions were prepared. Following preextraction of albumins, globulins, and gliadins as described above, the residual pellet was extracted with 0.05M acetic acid (2 mL) for 30 min with agitation every 10 min. The sample was clarified by centrifugation (20,000 × g, 10 min) and the supernatant, containing the AAS protein, was freeze-dried. Both the residual pellet (containing AAI) and the freeze-dried AAS protein were solubilized and derivatized as for the glutenin procedure in scheme 1.

RP-HPLC

A Waters 600 solvent delivery and control system with a WISP 712 automatic sample injector and a 490 ultraviolet-visible detector were used. The column was a 150 × 4.6-mm Zorbax 300SB-C8 fitted with a 12.5 × 4.6-mm guard column, which was maintained at 60°C using a Waters column heater. Solvents used were: A, water containing 0.1% (v/v) trifluoroacetic acid (TFA), and B, acetonitrile containing 0.1% (v/v) TFA. Deaeration was achieved by vacuum filtration through a 0.22-μm filter, rapid sparging with helium (100 mL/min for 10 min), and constant slow bubbling of helium into capped, vented solvent reservoirs (5 mL/min). Both gliadin and glutenin samples (10-μL injection size) were eluted with a two-step solvent gradient (25–35% B over 40 min; 35–50% B over 30 min) at a flow rate of 1 mL/min. The column was returned to the initial solvent composition over 1 min and reequilibrated for 10 min before the next analysis. Eluted components were detected at 210 nm. Chromatographic traces were recorded on a personal computer using the Waters Maxima software package.

TABLE I
Common Grain and Flour Quality Analyses^a

Sample	TKW	FN (sec)	PSI (%)	Protein ^b (%)	Wet Gluten (%)	Ash (%)	Starch Damage (%)	α-Amylase Activity (U/g)	Mill Yield ^c (%)
Glenlea	40.6	350	4.8	11.1	17	0.59	15.2	0.30	71.6
Katepwa	32.6	390	5.3	12.1	32	0.54	12.0	0.11	73.2
Domino	48.0	339	6.8	11.6	29	0.47	10.2	0.15	77.2
Karamu	47.6	416	7.3	10.2	35	0.54	12.0	0.24	75.9
Monad	45.5	276	8.2	13.2	34	0.43	11.1	0.29	74.0
Otane	55.8	399	5.7	12.4	34	0.57	11.6	0.15	74.9

^a TKW = 1,000 kernel weight (g) on as-is moisture basis. FN = falling number of wholemeal. PSI = particle size index.

^b Kjeldahl protein (N × 5.7) corrected to 14% moisture basis.

^c Adjusted to 14% moisture basis.

TABLE II
Dough Quality and Mechanical Dough Development (MDD) Breadbaking Characteristics

Sample	DDT ^a		Farinograph		MDD ^b		
	Mixograph (min)	Farinograph (min)	Stability (min)	WI (kJ/kg)	WA (%)	LV (mL)	Bake Score ^c
Glenlea	6.30	3.0	12.0	92.88	62.0	819	24
Katepwa	2.68	5.2	11.8	40.68	63.5	850	27
Domino	3.11	5.8	11.4	48.60	62.5	936	32
Karamu	1.44	2.2	2.6	15.48	67.5	729	17
Monad	3.21	7.5	13.4	55.80	63.5	918	30
Otane	2.20	5.6	17.2	41.04	68.0	891	29

^a Dough development time.

^b WI = 125 g of MDD work input. WA = 125 g of MDD water absorption. LV = loaf volume.

^c 125 g MDD bake score, including texture (grain) and scaled volume measurements (LV_{scaled} = [LV_{mL} - 540]/18).

RESULTS AND DISCUSSION

Grain and Flour Analyses

Common (non-HPLC) grain and flour analyses are summarized in Table I. Falling number and α -amylase values indicate that all samples were free from sprout damage and excessive levels of endogenous enzyme that may have affected test results. Glenlea and Katepwa were significantly harder (particle-size index) than the New Zealand cultivars, with the exception of Otane. This result is reflected in the starch damage and mill yield values; the latter values were also influenced by the New Zealand cultivars having larger kernels than the Canadian cultivars. The cultivars under study could not be classified according to either flour protein or wet gluten content (as measured using the Glutomatic system). This was particularly the case for the wet gluten measurement where the weakest, lowest protein cultivar, Karamu, returned the highest gluten content, and the strongest sample, Glenlea, returned the lowest. This result may be anomalous, as it was observed that the gluten from Glenlea did not aggregate well in the Glutomatic system and tended to wash out in small pellets.

Dough Quality and Test Baking

Dough quality and breadbaking characteristics for the cultivars under study are presented in Table II. Canadian cultivar Glenlea had a much higher dough strength than any of the New Zealand cultivars, as measured by both its MDD WI requirement and by its mixograph dough development time (DDT). Canadian cultivar

Katepwa, on the other hand, was comparable in dough strength to the mid-strength New Zealand cultivar Otane. Farinograph parameters did not parallel the MDD WI or mixograph DDT values. In particular, the farinograph DDT for Glenlea was quite low (only the biscuit wheat Karamu had a lower value), although the farinograph stability time was high. The much higher dough strength of Glenlea was not reflected in a higher loaf volume or overall bake score in the test baking procedure; it scored somewhat lower than all the New Zealand cultivars except the biscuit wheat Karamu. It seems likely that the much higher dough strength acts as an impediment to gas expansion in the loaf, resulting in a lower loaf volume.

RP-HPLC Analyses

RP-HPLC peak area data for the Modified Osborne and acetic acid schemes are presented in Tables III and IV, respectively. The proportions of x-type and y-type HMW-GS as a percentage of total HMW-GS for both extraction schemes are shown in Table V.

Modified Osborne fractions. Data presented in Table III and Fig. 1 show that, as mixing commenced, both the absolute amount and the percentage of gliadin extractable with aqueous ethanol decreased markedly. As the end of the normal mixing cycle approached (full WI), the amount and proportion of gliadin extracted began to increase. At four-thirds WI, extractable gliadin had returned to near premixing levels (Fig. 1). This is consistent with a model where gliadin becomes incorporated into the gluten matrix during mixing by either chemical or physical means. The

TABLE III
Reverse-Phase HPLC Peak Areas (mV/sec) for Extraction Scheme 1:
Modified Osborne Fractions

Sample ^a	Gliadin	Glutenin	HMW-GS ^b	LMW-GS ^b
Glenlea				
Control	22,548	22,102	6,255	15,847
1/3	20,463	22,716	5,463	17,252
2/3	7,427	21,154	4,711	16,443
Full	11,855	24,210	4,730	19,480
4/3	20,164	21,457	3,460	17,997
Katepwa				
Control	32,786	20,031	5,078	14,953
1/3	12,283	25,779	4,914	20,865
2/3	24,609	23,069	4,079	18,989
Full	10,859	46,789	6,462	40,327
4/3	11,812	30,865	3,759	27,106
Domino				
Control	26,155	22,510	4,627	17,883
1/3	8,563	33,938	4,619	29,320
2/3	15,438	24,318	4,089	20,229
Full	15,066	21,984	3,144	18,840
4/3	15,643	25,134	3,768	21,366
Karamu				
Control	25,527	12,816	2,790	10,026
1/3	10,636	16,422	3,198	13,224
2/3	8,711	15,287	2,979	12,307
Full	1,4097	16,488	2,858	13,630
4/3	20,320	16,104	2,513	13,591
Monad				
Control	34,633	22,630	4,988	17,642
1/3	14,836	31,898	5,158	26,740
2/3	9,564	37,622	4,245	33,377
Full	12,063	33,936	3,873	30,063
4/3	24,610	23,512	3,480	20,032
Otane				
Control	32,139	20,299	5,635	14,664
1/3	14,534	29,760	6,317	23,443
2/3	7,839	35,503	5,032	30,471
Full	9,181	34,674	5,283	29,390
4/3	31,414	32,191	5,573	26,617
SEM ^c	1,498	1,730	162	1,687

^a Portions of each flour made into duplicate doughs at one-third (1/3), two-thirds (2/3), full, and four-thirds (4/3) (i.e., one-third over full) optimum work input.

^b High- and low-molecular-weight glutenin subunits, respectively.

^c Standard error of the mean.

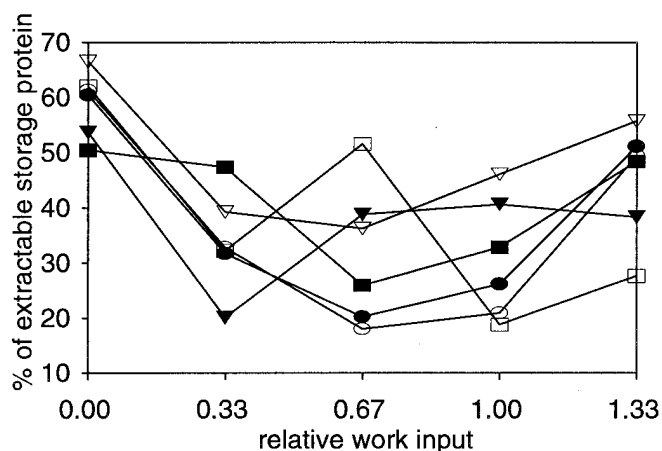


Fig. 1. Effect of mixing on gliadin as a percentage of total storage protein determined by modified Osborne fractions (scheme 1) for Glenlea (■), Katepwa (□), Domino (▼), Karamu (▽), Monad (●), and Otane (○).

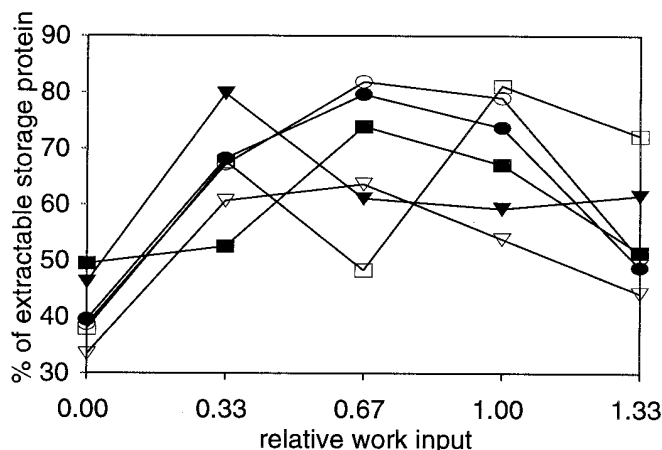


Fig. 2. Effect of mixing on glutenin as a percentage of total storage protein determined by modified Osborne fractions (scheme 1) for Glenlea (■), Katepwa (□), Domino (▼), Karamu (▽), Monad (●), and Otane (○).

release of gliadin with overmixing is presumably the result of some breakdown or rearrangement of the gluten matrix. There were two exceptions to this trend: Katepwa gliadin decreased in a somewhat erratic manner without showing the overmixing increase; and Domino gliadin changed little after initially decreasing (Fig. 1).

Data presented in Table III and Fig. 2 show that the extractable glutenin peak area increased with increasing mixing in samples Otane, Monad, and Domino before declining to near initial levels with overmixing. Karamu glutenin increased initially after the onset of mixing and then stabilized, while Katepwa and Glenlea glutenin increased overall but rather erratically (Fig. 2). For most cultivars, glutenin as a percentage of total extractable protein did increase before dropping with overmixing, with the exception of Katepwa, where the glutenin as a percentage of total extractable protein increased through a series of erratic steps. Table III reveals that changes in the data for extractable glutenin are largely due to changes in the solubility behavior of the low molecular weight glutenin subunits (LMW-GS). This would be unsurprising, given that LMW-GS make up between 70–85% of total glutenin. Table III also shows that protein content in each extracted fraction was lowest for the weakest, poorest quality wheat (Karamu), but that this parameter failed to consistently rank the stronger samples.

For Glenlea, Domino, and Monad, the HMW-GS peak area declined steadily with mixing (Table III); no significant shift occurred for the other cultivars. When the proportion of HMW-GS (as a percentage of GLU) were examined, Glenlea, Katepwa, and

Karamu showed a steady decrease; Otane and Monad showed a decline to a minimum at two-thirds WI before increasing again slightly with increased mixing; Domino showed an initial drop and then did not change significantly with increased mixing (Fig. 3). In most cases, the proportion of extractable x-type relative to y-type HMW-GS showed an initial increase to a maximum at approximately two-thirds to full WI. The sharp drop in the trend line for Katepwa in Fig. 4 was due to a marked increase in extractable LMW-GS for this particular sample (Table III). Incorporation of gliadin in the glutenin matrix may, in part, account for the observed increase in extractable glutenin, but it does not account for the decrease in extractable HMW-GS observed for some cultivars or the shift in proportions of x- and y-type subunits.

If it is assumed that mixing causes interchange but no loss of storage protein, the total extractable storage protein (GLU+GLI) may have been expected to remain relatively constant. In contrast, the total extractable storage protein had a pattern of extractability similar to that described for gliadin, decreasing to a minimum before rising with further mixing (Fig. 5). The position of the minimum relative to WI varied with cultivar, as did the amount of recovery. The only sample with a markedly different behavior was Katepwa, which reached a minimum extractability at one-third WI, climbed sharply to a maximum extractability at optimum WI, and then dropped with further mixing. With the exception of Otane, all samples have less extractable storage protein at four-thirds WI than in the parent flour.

TABLE IV
Reverse-Phase HPLC Peak Areas (mV/sec) for Extraction Scheme 2: Acetic Acid Fractions

Sample ^a	Acetic Acid Soluble			Acetic Acid Insoluble		
	Glutenins	HMW-GS ^b	LMW-GS ^b	Glutenins	HMW-GS ^b	LMW-GS ^b
Glenlea						
Control	877* ^c	228*	649*	17,629	5,381	12,248
1/3	2,936	331	2,605	16,037	4,225	11,812
2/3	5,135	608	4,527	19,803	4,582	15,220
Full	5,360	720	4,640	14,289	3,646	10,643
4/3	5,947	536	5,411	17,932	2,748	15,184
Katepwa						
Control	578*	31*	547*	14,000	4,030	9,970
1/3	8,037	701	7,337	16,524	4,005	12,519
2/3	4,557	481	4,076	15,247	2,719	12,527
Full	7,465	352	7,113	23,617	4,249	19,368
4/3	5,107	361	4,746	17,069	2,481	14,588
Domino						
Control	914*	112*	802*	17,001	4,108	12,893
1/3	5,864	273	5,592	24,915	3,483	21,432
2/3	6,763	488	6,275	21,243	3,405	17,838
Full	5,015	348	4,666	18,459	2,916	15,543
4/3	6,636	656	5,982	12,437	2,154	10,283
Karamu						
Control	1,389	341	1,048	11,027	2,633	8,394
1/3	3,551	524	3,027	10,960	2,462	8,497
2/3	4,409	547	3,862	11,549	2,499	9,050
Full	4,057	811	3,246	7,418	1,400	6,018
4/3	4,934	522	4,413	11,451	1,990	9,461
Monad						
Control	2,029	371	1,658	19,304	4,133	15,171
1/3	7,352	472	6,880	24,169	4,452	19,717
2/3	4,787	211	4,575	26,157	3,077	23,080
Full	11,677	710	10,967	20,881	2,355	18,526
4/3	7,087	533	6,553	19,101	3,086	16,015
Otane						
Control	2,232	569	1,663	17,253	4,771	12,482
1/3	6,635	767	5,868	18,308	4,491	13,817
2/3	5,607	487	5,120	12,256	2,335	9,921
Full	4,042	435	3,607	17,950	3,199	14,751
4/3	8,134	712	7,422	23,704	4,601	19,103
SEM ^d	844	69	793	2,133	281	1,990

^a Portions of each flour made into duplicate doughs at one-third (1/3), two-thirds (2/3), full, and four-thirds (4/3) (i.e., one-third over full) optimum work input.

^b High- and low-molecular-weight glutenin subunits, respectively.

^c * = Adequate quantitation not achieved due to very low protein level.

^d Standard error of the mean.

For Glenlea, Domino, and Otane, the level of extractable gliadin was the main factor in changing the level of total extractable storage protein, with the level of extractable LMW-GS varying to a lesser degree and HMW-GS varying little. For Monad, Katepwa, and Karamu, changes in the level of extractable storage proteins appeared to be linked to an possible interaction between the extractability of gliadin and LMW-GS. In these samples, extractable gliadin decreased as extractable LMW-GS increased, and vice versa. However, gliadin and LMW-GS fractions did not change by the same amount, the largest changes generally took place in the gliadin fraction. We have no direct evidence as to what happened to the storage protein that was unaccounted for. Presumably, it was either lost in the saline-soluble fraction (which was not measured) or it was rendered insoluble during the mixing process only to become soluble again upon overmixing, or a combination of these effects.

Acetic acid fractions. Analyses of AAS and AAI GLU showed that with increased mixing both the AAS-GLU peak area and proportion increase and the AAI-GLU peak area and proportion decrease (Fig. 6). This has been observed by other researchers (Mecham et al 1965, Tsen 1967, Tanaka and Bushuk 1973, Paredes-Lopez and Bushuk 1983, Sievert et al 1991). In particular, AAS-GLU increases sharply with the onset of mixing from negligible levels in the parent flour (Table IV), most notably for Glenlea and Domino. For Domino, Monad, and Otane, the proportion of AAS-GLU declined slightly on overmixing (Fig. 6).

The close linkage between total GLU-GS and LMW-GS solu-

bility noted in the modified Osborne extraction scheme was also observed for the AAS proteins. For both AAS-GLU and AAI-GLU, the balance of HMW-GS to LMW-GS shifted in favor of LMW-GS with increased mixing (Table IV), confirming the result from modified Osborne scheme (scheme 1). The trend for AAI-GLU HMW-GS peak area with mixing was erratic for Monad and Otane, but in general it showed a decline for Glenlea, Katepwa, Domino, and Karamu (Fig. 7). A more detailed analysis of the distribution of AAI-GLU between the x-type and y-type subunits is shown in Table V. The distribution of subunits differed depending on the cultivar considered. Also, the distribution of x-type and y-type subunits in the AAI-GLU did not change significantly with mixing.

More dramatic changes in the distribution of AAS HMW-GS with mixing are evident in Table V. Both Glenlea and Katepwa showed rapid initial decreases in the proportion of x-type HMW-GS but then little change after one-third optimal WI. Domino, Karamu, and Otane showed a different trend with a gradual increase in the x-type HMW-GS content over the mixing period. Monad showed no clear trend. However, it should be kept in mind that AAS-GLU was a relatively small fraction as compared to AAI-GLU. For the GLU fraction as a whole (AAS+AAI fractions), trends in the quantity of HMW-GS with mixing were dominated by changes in the quantity of AAI-GLU.

Protein Solubility and Extractability Patterns

The results presented here indicate that mixing has a subtle effect on the solubility and extractability patterns of what can be

TABLE V
Changes in Proportion (%) of x-Type and y-Type Subunits in Polymeric and Nonpolymeric Glutenin with Mixing

Sample ^a	Scheme 2 ^b					
	Scheme 1		AAS-GLU		AAI-GLU	
	x	y	x	y	x	y
Glenlea						
Control	79.4	20.6	100.0	0.0	75.7	24.3
1/3	78.2	21.8	82.4	17.6	75.9	24.1
2/3	82.6	17.4	82.3	17.7	73.1	26.9
Full	77.0	23.0	77.1	22.9	75.6	24.4
4/3	77.5	22.5	80.4	19.6	79.4	20.6
Katepwa						
Control	75.2	24.8	100.0	0.0	70.7	29.3
1/3	72.5	27.5	70.0	30.0	67.8	32.2
2/3	70.2	29.8	70.3	29.7	80.4	19.6
Full	72.3	27.7	77.6	22.4	71.0	29.0
4/3	71.2	28.8	74.2	25.8	69.1	30.9
Domino						
Control	63.6	36.4	58.9	41.1	61.2	38.8
1/3	62.1	37.9	64.0	36.0	61.2	38.8
2/3	63.3	36.7	54.1	45.9	58.8	41.2
Full	70.0	30.0	71.0	29.0	60.1	39.9
4/3	63.5	36.5	57.0	43.0	59.5	40.5
Karamu						
Control	69.7	30.3	55.4	44.6	68.7	31.3
1/3	71.1	28.9	75.5	24.5	66.7	33.3
2/3	72.1	27.9	76.6	23.4	67.6	32.4
Full	71.3	28.7	77.1	22.9	69.2	30.8
4/3	71.6	28.4	72.2	27.8	69.0	31.0
Monad						
Control	71.4	28.6	65.2	34.8	68.3	31.7
1/3	50.7	49.3	65.2	34.8	63.9	36.1
2/3	71.6	28.4	72.2	27.8	68.8	31.2
Full	70.0	30.0	64.0	36.0	69.4	30.6
4/3	68.4	31.6	69.6	30.4	64.6	35.4
Otane						
Control	78.9	21.1	72.2	27.8	75.8	24.2
1/3	77.2	22.8	77.3	22.7	75.0	25.0
2/3	78.3	21.7	78.0	22.0	78.2	21.8
Full	76.4	23.6	82.2	17.8	76.2	23.8
4/3	75.3	24.7	82.1	17.9	72.5	27.5

^a Portions of each flour made into duplicate doughs at one-third (1/3), two-thirds (2/3), full, and four-thirds (4/3) (i.e., one-third over full) optimum work input.

^b AAS = acetic acid soluble; AAI = acetic acid insoluble; HMW-GS = high-molecular-weight glutenin subunits.

regarded as monomeric and polymeric proteins. Gliadins are generally regarded as being monomeric, while glutenin is regarded as being polymeric. It is possible, even likely, that mixing causes a degree of interchange between these two classifications. For the more mobile gliadin (or monomeric) fraction, the apparent reversibility of the processes taking place during mixing most likely represents entanglement and disentanglement with other large protein aggregates. This suggests that gliadins have a primarily physical role in modifying dough properties through viscosity changes in the gluten matrix.

The AAI-GLU content of a flour has been linked to potential baking quality (Mecham et al 1965, Tsen 1967, Orth and Bushuk 1972, Tanaka and Bushuk 1973, Orth and O'Brien 1976, Singh et al 1987). The role of HMW-GS in dough rheology and baking performance has also been well reported (Huebner and Bietz 1985, Sutton et al 1989, MacRitchie 1992). The changes in proportion and absolute quantity during mixing of the groups reported here strongly indicate that they are taking part in some form of interaction or interchange process. The nonreversibility of the trends observed, and the inability to account for some of the protein material, suggests that there are chemical as well as physical processes occurring.

The bulk of the protein that cannot be accounted for in the extraction schemes detailed above can be ascribed to the glutenin (or polymeric) fraction. Specifically, mixing results in a greater proportional reduction in extractable HMW-GS than in LMW-GS

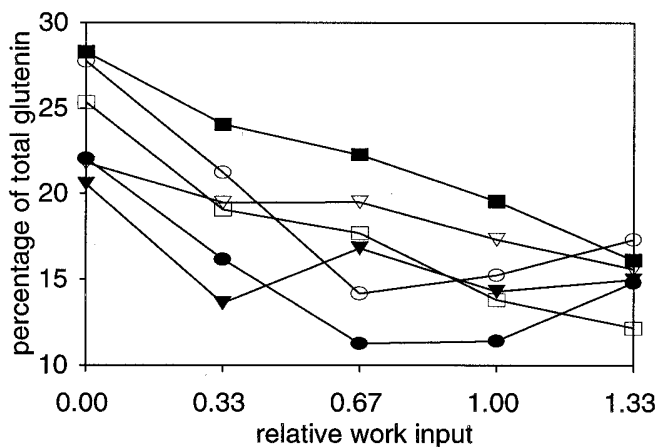


Fig. 3. Effect of mixing on high-molecular-weight glutenin subunits as a percentage of total glutenin determined by modified Osborne fractions (scheme 1) for Glenlea (■), Katepwa (□), Domino (▼), Karamu (∇), Monad (●), and Otane (○).

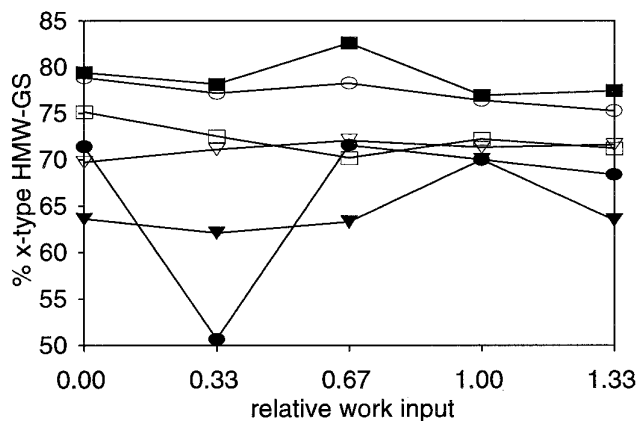


Fig. 4. Effect of mixing on x-type high-molecular-weight glutenin subunits as a percentage of HMW-GS, determined by modified Osborne fractions (scheme 1) for Glenlea (■), Katepwa (□), Domino (▼), Karamu (∇), Monad (●), and Otane (○).

which cannot be accounted for by increases in other protein fractions. At this point, we speculate that the unaccountable protein has either been converted to a water-soluble form (and so removed in the preextraction step) or converted to a form that is completely insoluble in the solvent systems used in this study.

The x-type and y-type HMW-GS interactions are harder to define because changes, when apparent, are small and also

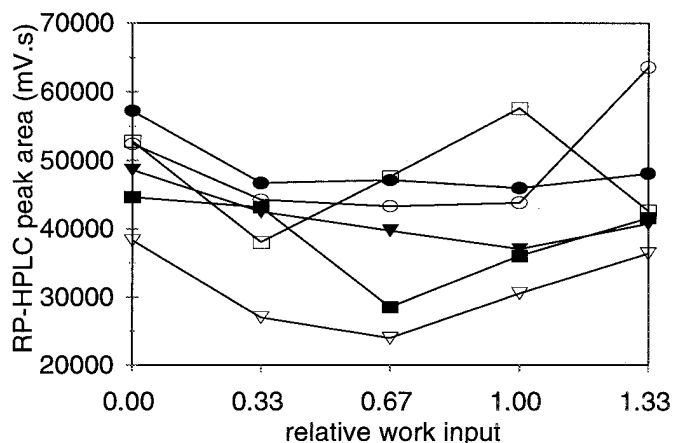


Fig. 5. Effect of mixing on total extractable storage protein RP-HPLC peak area (GLI+GLU), determined by modified Osborne fractions (scheme 1) for Glenlea (■), Katepwa (□), Domino (▼), Karamu (∇), Monad (●), and Otane (○).

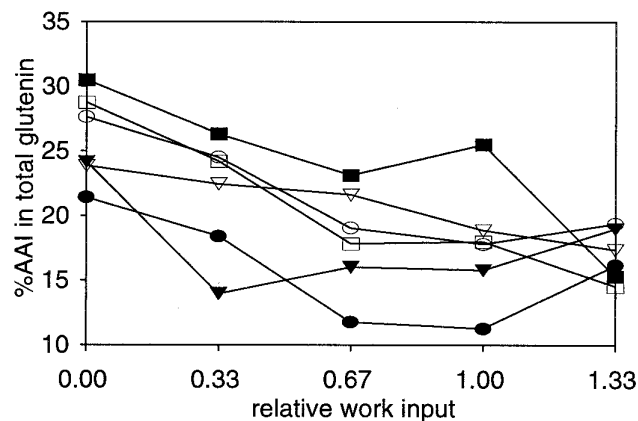


Fig. 6. Effect of mixing on percentage of total glutenin determined by the acetic acid solubility method (scheme 2) for Glenlea (■), Katepwa (□), Domino (▼), Karamu (∇), Monad (●), and Otane (○).

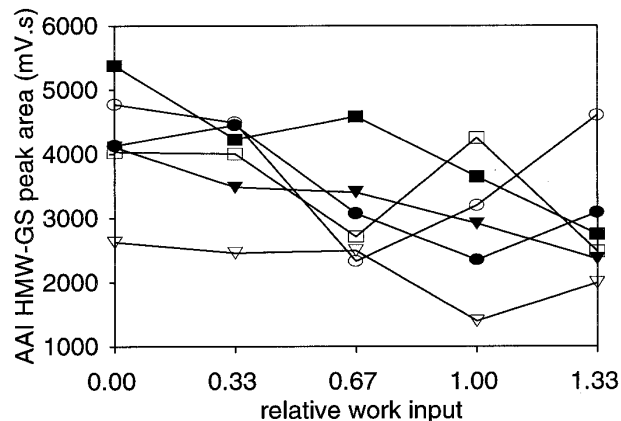


Fig. 7. Effect of mixing on reverse-phase HPLC peak area for acetic acid insoluble high-molecular-weight glutenin subunits determined by the acetic acid solubility method (scheme 2) for Glenlea (■), Katepwa (□), Domino (▼), Karamu (∇), Monad (●), and Otane (○).

because of strong cultivar effects. The predominant trend of loss of γ -type HMW-GS with increased mixing may be due to the greater ability of this subunit type to form links with other proteins, possibly water- or saline-soluble proteins, as has been suggested by Sievert et al (1991).

This study was designed to examine the protein composition of samples when they were mixed to the same relative proportion of optimal WI. The results show that, at a given stage of development, the processes taking place in the protein matrix (state of binding or entanglement) are similar, regardless of the strength of the flour sample. Differences in the rate of change of protein extractability become apparent only when absolute WI or time of mixing is considered. In general, the rate of change of the extractability of the protein fractions with absolute level of WI was greatest for the weakest samples and least for the stronger samples. This means that, at a given WI, the protein matrix of a weak dough is in a different chemical and physical state than that of a stronger dough.

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