

Effects of Nitrogen and Sulfur Fertilizer on Protein Composition, Mixing Requirements, and Dough Strength of Four Wheat Cultivars

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ABSTRACT

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Two field trials using four New Zealand wheat cultivars were undertaken to observe the effects of nitrogen and sulfur fertilization on protein composition, mixing requirements, and dough strength and to compare the results with that observed with a single cultivar, Otane. The results confirmed that adequate sulfur fertilization was necessary to ensure lower dough mixing requirements. The existence of a nexus between mixing requirements and dough strength was confirmed and genotype has significant effects on it. Variation in the content of HMW-GS in the protein corresponded to changes in dough mixing requirement of Otane. Across the four cultivars, dough mixing requirements (mechanical dough development work input and mixograph development time) and dough strength

(Extensigraph resistance to extension) depended on different aspects of protein composition. As the content of polymeric proteins increased, MDD work input increased, but mixograph development time decreased, while the effect on Rmax was small. Rmax, however, was more affected by either the content of small monomers in the flour or the ratio between HMW-GS peak area to total gliadin peak area. The ratio of MDD work input to Rmax was largely explained by the gliadin content of the flour. Thus, depending on the genetic background, it should be possible to adjust dough mixing requirements by modifying overall HMW-GS, LMW-GS, or gliadin content while maintaining dough strength.

The conditions under which a crop is grown are known to alter the flour functionality and breadmaking quality (Fowler and de la Roche 1975) through changes in protein composition (Randall et al 1981, Barber and Jessop 1987, Stevenson 1987, Bruckner and Morey 1988, Bunker et al 1989, Marchylo et al 1990, Randall and Moss 1990, Anderson et al 1991, Borghi et al 1995). Long and Sherbakoff (1951) showed that late nitrogen fertilizer application influenced the quality of wheat flour. Subsequent research has shown that late applications of nitrogen (N) increased individual kernel weight as well as grain N concentration and improved bread quality (Finney et al 1957, Langer and Liew 1973, Pushman and Bingham 1976, Altman et al 1983, Darwinkel 1983). Nevertheless, Tipples et al (1977) reported that protein concentrations >17% were associated with a weakening of the dough and a deterioration in baking quality. Nitrogen fertilizer did not directly affect either the quantity of HMW glutenin subunits (HMW-GS) relative to other storage proteins (Fullington et al 1983, Levy et al 1985) or the proportion of glutenin isolated on the basis of solubility (Doekes and Wennekes 1982). Timms et al (1981) suggested that late N application in the absence of sulfur (S) may sufficiently alter the balance between these two nutrients so that S levels become inadequate for normal grain protein development. Sulfur deficiency during grain filling has been associated with alterations in the ratios between groups of storage proteins (Wrigley et al 1984, Castle and Randall 1987, Fullington et al 1987). Thus, Wrigley et al (1984), using densitometric analysis of storage proteins separated by gel electrophoresis, demonstrated that, with increasing S deficiency, there was a corresponding decrease in the LMW-GS, the sulfur-rich gliadins (α , β , and γ), and some metabolic proteins, whereas the relative content of HMW-GS and ω -gliadins increased. Increasing S fertilization was associated with reduced resistance to extension (Rmax) and increased extensibility (Ext) in three Australian cultivars, one high-protein

hard, one multipurpose, and one low-protein soft (Moss et al 1983). In an accompanying article, we reported that maintaining the N-S fertilizer ratio at 3:1 minimized mechanical dough development (MDD) work input (WI) during dough mixing of Otane, while other dough properties were maintained (Wooding et al 2000).

Wheat cultivars have an inherent range of WI for mixing dough to optimum consistency because breeding programs use dough strength as a major selection criterion. Low-strength dough makes poor bread but when strength is too high it is difficult to manage in industrial MDD bakeries. For these experiments, we chose four New Zealand bread wheat cultivars: Otane, with a relatively low MDD work input of ≈ 14 Wh/kg; Monad, with a high work input of ≈ 23 Wh/kg; and Domino and Endeavour, with intermediate values. They were grown under varying S and N fertilization conditions and their protein composition, mixing requirements, and dough strength were determined. The aim was to obtain insights into manipulating or breaking the nexus between mixing requirements and dough strength.

MATERIALS AND METHODS

Two trials were planted in successive years at Lincoln, Canterbury, New Zealand. Soil N and S levels before sowing are shown in Table I. The four cultivars Otane, Domino, Endeavour, and Monad composed the main plots of a split-plot design, with two replicates in the first year and three in the second. In the first year (SN3), a factorial $3 \times 3 \times 2 \times 2$ set of fertilizer treatments composed the subplots, with three levels of N (0, 75, and 150 kg/ha) and three of S (0, 25, and 50 kg/ha) applied at sowing and two of N (0 and 75 kg/ha) and two of S (0 and 25 kg/ha) at booting. In the second year (SN4), the intermediate levels of the fertilizer treatments were omitted so that the structure was $2 \times 2 \times 2 \times 2$ and, for some analyses, only the 0N/0S, 0N/75S, 225N/0S, and 225N/75S plots were selected. In addition, materials from SN1 and SN2 trials (Wooding et al 2000) were examined.

TABLE I
Soil Test Results Before Sowing Nitrogen (N) and Sulfur (S) Fertilizer

	Soil Depth (cm)	pH	N (ppm)	S (ppm)
SN3	0-15	5.8	1	8
	15-30	5.9	4	16
SN4	0-15	6.4	2	5
	15-30	6.2	4	14

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The SN3 trial was harvested at 18–20% moisture content (mc) and dried to 14–16% mc in a small grain drier. Grain samples were conditioned to 15.5% mc for 16 hr before milling on a Buhler experimental mill. The bran was fed through a bran finisher to bring the white flour extraction rate to ≈75%. HMW-GS composition of all samples was checked with SDS-PAGE (Gupta and MacRitchie 1991) to ensure that there was no detectable cross-contamination and that there were no biotypes. Chemical tests and dough tests were conducted according to the randomization of the field trials.

Materials

Flour nitrogen content at 14% mc was determined by the Kjeldahl method (ISO 1975) and flour sulfur content was determined by X-ray fluorescence spectroscopy (Randall and Sakan 1983).

Total unreduced proteins were extracted from 10 mg of flour for size-exclusion HPLC with 1 mL of 0.5% SDS in 0.05M sodium phosphate buffer, pH 6.9 (Batey et al 1991). The suspension was sonicated for 15 sec at 10W using a 3-mm microtip probe in a 1.5-mL microfuge tube (Singh et al 1990b). Samples were then centrifuged for 20 min at 16,000 × g. The supernatant was passed through a 45-μm polyvinylidene difluoride (PVDF) filter before injection into a Waters Protein-Pak 300 column with 50% (v/v) aqueous acetonitrile containing 0.1% trifluoroacetic acid (TFA) as the elution solvent and a run time of 35 min (Singh et al 1990a). The areas of three peaks were determined as peak I, containing polymeric proteins, primarily glutenins, triticins, and HMW albumins; peak II, containing large monomeric proteins, primarily gliadins; and peak III, containing small monomeric proteins, primarily albumins and globulins (Singh et al 1990b). Peak areas were summed to give a total peak area, and each peak also was expressed as a proportion of the total peak area. Ratios of peak areas also were calculated.

Flour proteins were sequentially extracted from 20 mg of flour for reversed-phase HPLC (Sutton et al 1992; K. Sutton, *personal communication*). The flour was dispersed in 0.5 mL of 50% (v/v) aqueous propan-1-ol in 1.5-mL microfuge tubes for 30 min at room temperature, with vortexing every 10 min. The suspension was centrifuged at 2,200 × g for 2 min and the supernatant, containing gliadins, was removed for use directly on the HPLC. The residue was extracted twice further with propanol to remove residual gliadins. The residue was treated with 0.25 mL of 0.08M Tris (hydroxymethyl)methylamine-HCl, pH 7.5, containing 50% propan-1-ol and 1% (w/v) dithiothreitol (DTT), for 1 hr at 60°C with vortexing every 10 min. A further 0.25 mL of 0.08M Tris (hydroxymethyl)methylamine-HCl, containing freshly added 3% (v/v) 4-vinylpyridine, was added to each tube, which was then vortexed and incubated for 15 min at 60°C with vortexing at the midpoint and at the end.

The samples were centrifuged at 9,000 × g for 5 min, and the supernatant, containing glutenins was removed for use on the HPLC. RP-HPLC was conducted on a Brownlee RP300-C8 column and 30-mm guard column maintained at 70°C and the solvent gradient was 75% double-deionized water with 0.1% TFA and 25% acetonitrile with 0.1% TFA for the first 40 min, 65:35 for the next 30 min, and 50:50 for the final 1 min (Sutton 1991). Gliadins formed three peaks: ω-gliadins first, α and β in the middle peak, and γ in the third (Wieser et al 1994, Lafiandra et al 1994). Glutenins formed two peaks, HMW-GS first and LMW-GS second (Bietz 1986, Wieser et al 1990). The areas of the peaks were determined and were summed to provide glutenin subtotal, gliadin subtotal, and total protein. Each peak also was expressed as a percent of its corresponding subtotal and of the total protein content. Each peak also was converted from a percent of total protein to a percent of the flour by multiplying by the concentration of protein in the flour.

Mixing requirements were measured on the mixograph (National Manufacturing Co., Lincoln, NE) using a 5-kΩ linear taper potentiometer attached to the rotating bearing shaft of the mixer arm (Wooding and Walker 1992). In this way, mixograph development time and mixograph water absorption were determined using Approved Method 54-40A (AACC 2000). Farinograph development time, water absorption, stability, and breakdown were determined using Approved Method 54-21 (AACC 2000) on a farinograph (Brabender OHG, Duisberg, Germany). Breakdown was defined

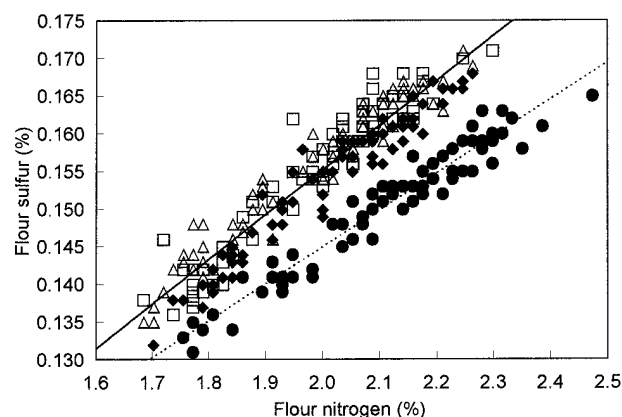


Fig. 1. Relationship between flour nitrogen content and flour sulfur content in SN3 trial. For cultivars Otane (□), Endeavour (Δ), and Monad (◆) pooled line of best fit was $S = 0.0593 \times N + 0.0365$ ($R^2 = 0.90$); for Domino (●) $S = 0.0470 \times N + 0.0490$ ($R^2 = 0.95$).

TABLE II
Effects of Nitrogen (N) and Sulfur (S) Fertilizer Treatments on Flour Nitrogen (FN) Content, Flour Sulfur (FS) Content, and N-S Ratio in Trials SN3 and SN4

Cultivar	S (kg/ha)	N (kg/ha)	FS (%)		FN (%)		N-S Ratio	
			SN3	SN4	SN3	SN4	SN3	SN4
Otane	0	0	0.139	0.145	1.74	2.21	12.5	15.2
	75	0	0.141	0.153	1.79	2.12	12.7	13.9
	0	225	0.163	0.161	2.16	2.49	13.3	15.5
	75	225	0.168	0.169	2.14	2.40	12.7	14.2
Endeavour	0	0	0.142	0.139	1.77	2.02	12.5	14.5
	75	0	0.147	0.156	1.82	2.11	12.4	13.5
	0	225	0.165	0.156	2.19	2.28	13.3	14.6
	75	225	0.167	0.158	2.16	2.26	12.9	14.3
Domino	0	0	0.137	0.142	1.84	2.12	13.4	14.9
	75	0	0.138	0.147	1.86	2.06	13.5	14.1
	0	225	0.159	0.162	2.33	2.60	14.7	16.0
	75	225	0.161	0.163	2.30	2.55	14.3	15.6
Monad	0	0	0.143	0.162	1.81	2.33	12.7	14.4
	75	0	0.144	0.166	1.84	2.30	12.9	13.9
	0	225	0.164	0.176	2.19	2.63	13.4	14.9
	75	225	0.164	0.183	2.19	2.58	13.4	14.1
SE	0.004	0.003	0.07	0.05

as the difference in Brabender units (BU) from the 500 BU line to the center of the curve 10 min after the start of mixing. MDD work input and water absorption were determined using two 125-g MDD mixers (Crop & Food Research, Christchurch, NZ) (Wooding et al 1999). Both bromated and nonbromated doughs were prepared (Wooding et al 1999). Maximum resistance to extension (Rmax) and extensibility (Ext) were measured by extensigraph (Brabender) using Approved Method 54-10 (AACC 2000). Bread quality was evaluated in terms of loaf volume, crumb grain, and bake score according to Swallow and Baruch (1986).

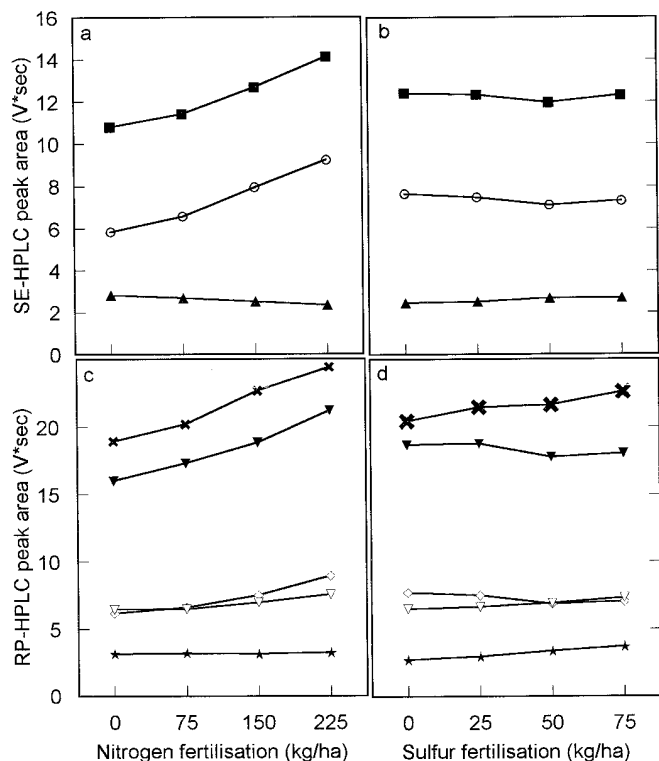


Fig. 2. Effects of nitrogen (a and c) and sulfur fertilization (b and d) in SN1 trial on protein peak areas determined by size-exclusion HPLC (a and b) and reversed-phase HPLC (c and d). Polymeric proteins (■); large monomeric proteins (○); small monomeric proteins (▲); HMW-GS (◇); LMW-GS (▼); γ -gliadins (×); ω -gliadins (∇); α + β -gliadins (★); ± 1 standard error.

Analyses

Data were subjected to analysis of variance using Genstat (Rothamsted Experimental Station, UK) and SAS (SAS Institute, Cary, NC). The two MDD mixers were treated as sub-subplots. Correlation matrices also were calculated. Regression and covariate analysis were used to investigate the relationships among quality attributes, chemical composition, and genotypes.

Stepwise regression was used to determine whether combinations of protein composition measurements could explain more of the variance in dough properties than any single measurements. Strong correlations between flour nitrogen content (FN), flour sulfur content (FS), and the ratio between them made it difficult to use more than one in the stepwise regressions, in contrast to earlier results (Wooding et al 2000). Data were pooled and the regressions tested for parallelism across trials.

Preliminary analyses indicated that total fertilizer quantity was significant and time of application was of very minor importance and usually not significant. Therefore, the split fertilizer treatments were pooled with the same total fertilizer application treated as replicates.

RESULTS

Flour N and S Content

In both trials SN3 and SN4, N fertilization led to significant increases in flour N and flour S contents, with or without additional sulfur

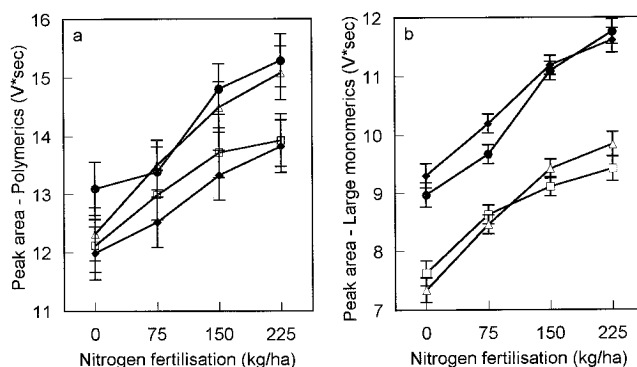


Fig. 3. Effects of nitrogen fertilization in SN3 trial on protein peak areas determined by size-exclusion HPLC: a, polymeric protein peak area; b, large monomeric protein peak area of Otane (□), Endeavour (Δ), Monad (◇), and Domino (●). Error bars ± 1 standard error.

TABLE III
Significant Correlation Coefficients Between Wheat Flour Quality Attributes in Two Nitrogen (N) and Sulfur (S) Fertilization Trials^{a,b}

	MDD		Mixograph		Extensigraph		Farinograph			Baking	
	WI	WA	MDT	MWA	Rmax	Ext	FDT	FWA	FST	FBK	LV
MDD mixer											
WI	...	0.597	...	0.503	0.499	...	0.778	0.706	0.732	-0.696	...
WA	-0.546	0.789	...	0.462	0.439	0.898	0.376
Mixograph											
MDT	0.357	-0.415	...	-0.345	0.565	-0.487	0.329	-0.386	0.505	-0.380	-0.342
MWA	0.384	0.507	-0.357	0.383	0.484	0.730	0.350	-0.379	...
Extensigraph											
Rmax	0.420	...	0.574	0.565	...	0.688	-0.627	...
Ext	-0.313	0.368	-0.779	0.235	-0.391	0.385
Farinograph											
FDT	0.528	0.468	0.223	0.344	0.356	-0.153	...	0.479	0.821	-0.847	...
FWA	0.539	0.554	-0.115	0.665	0.615	...	0.306	-0.342	...
FST	0.633	...	0.627	0.117	0.718	-0.508	0.597	0.333	...	-0.909	...
FBK	-0.548	-0.221	-0.403	-0.214	-0.571	0.267	-0.692	-0.423	-0.816
Baking											
LV	-0.145	0.357	-0.537	0.268	-0.313	0.461	-0.310	0.136	...

^a MDD, mechanical dough development; WI, work input; WA, water absorption; MDT, mixograph development time; MWA, mixograph water absorption; Rmax, extensigraph resistance to extension; Ext, extensibility; FDT, farinograph development time; FWA farinograph water absorption; FST, farinograph stability; FBK, farinograph breakdown; LV, loaf volume.

^b Below diagonal, SN1 laboratory-scale trial (df = 106); above diagonal, SN2 industrial-scale trial (df = 10).

fertilization, whereas S fertilization had no significant effect on the flour content of either element (Table II). The flour N-S ratio increased with nitrogen fertilization and generally decreased with sulfur fertilization, as would be expected. FN content was $\approx 0.5\%$ greater in SN4 than in SN3. The FS and FN contents were strongly correlated (Fig. 1). A common regression applied for Otane, Endeavour, and Monad and a separate one was needed for Domino, where sulfur contents were lower at any given nitrogen content. The effects of N and S fertilization on FN and FS content in SN1 and SN2 have already been described (Wooding et al 2000).

Protein Composition

In SN1, increasing N fertilization increased the peak area of both polymeric and large monomeric proteins in the SE-HPLC and of HMW-GS, LMW-GS, γ -gliadins, and ω -gliadins, but not α + β -gliadins, in RP-HPLC (Fig. 2). Increasing S fertilization did not

have consistent effects on the SE-HPLC peak areas, but led to consistent increases in all three gliadin peaks and general decreases in the two glutenin peaks in the RP-HPLC (Fig. 2).

The N \times S interaction was not significant for these peak areas. Similar trends were observed in SN2 and SN3, with the following exceptions. In SN3, increasing N fertilization had no consistent effect on the small monomeric peak area and, in both SN2 and SN3, it was associated with increasing α + β -gliadin content. Increasing sulfur fertilization increased the peak area of large monomers in SN2. In the SN2 trial, no significant difference resulted from the use of the two different (laboratory and industrial) mills. HPLC peak areas were not determined in the SN4 trial.

In SN3, the peak area of both polymeric and large monomers showed a cultivar-nitrogen fertilization interaction (Fig. 3), but it was small in comparison to the main effects.

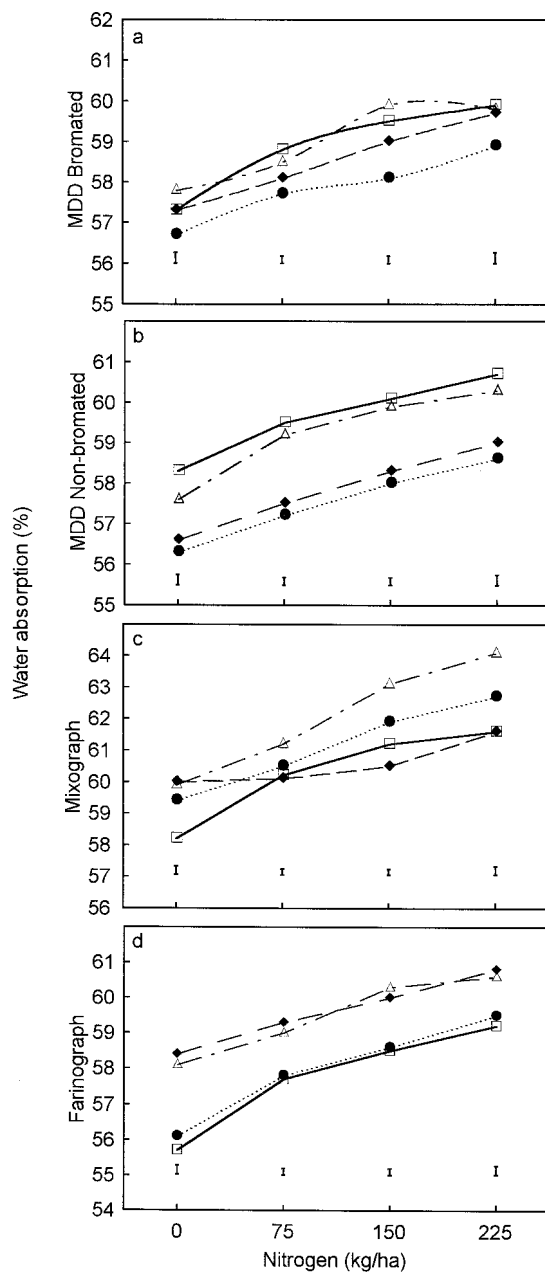


Fig. 4. Effects of nitrogen fertilization in SN3 trial on water absorption, determined by mechanical dough development (MDD) of **a**, bromated and **b**, nonbromated doughs and by **c**, mixograph and **d**, farinograph. Otane (\square), Endeavour (Δ), Monad (\blacklozenge), and Domino (\bullet). Error bars ± 1 standard error.

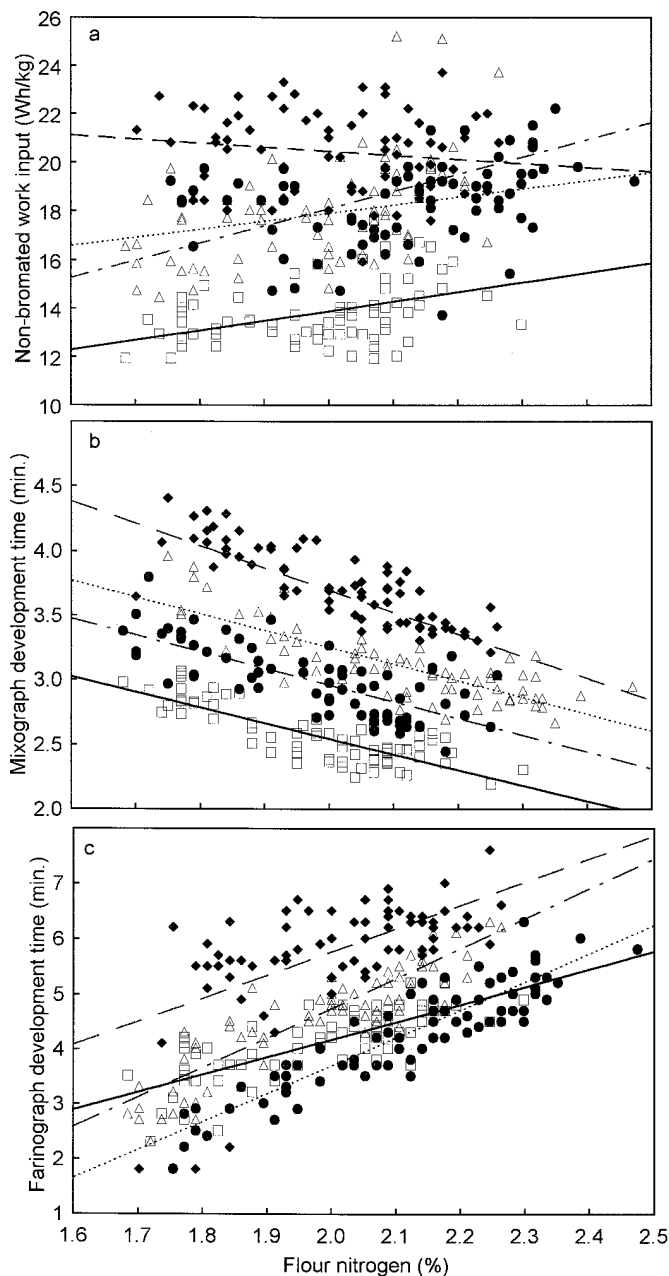


Fig. 5. Effects of flour nitrogen content on dough development in SN3 trial: **a**, mechanical dough development work input on nonbromated dough, **b**, mixograph development time, and **c**, farinograph development time of Otane (\square), Endeavour (Δ), Monad (\blacklozenge), and Domino (\bullet).

Dough Quality

Correlations between most dough quality parameters were significant in SN3 and also in SN4 (Table III). Water absorption measured with the MDD mixer, the mixograph, and the farinograph differed, with the strongest correlation between the farinograph and the mixograph values. The cultivars performed differently when evaluated with the different instruments; Otane and Endeavour had higher water absorptions than Monad and Domino in MDD (nonbromated), but Endeavour and Monad had higher values than Otane and Domino in the farinograph (Fig. 4).

MDD work input and farinograph development time were more closely correlated with each other than either was with mixograph development time (Table III). This also was shown by correlations with FN content (Fig. 5), which were negative for all cultivars for mixograph development time, positive for all cultivars for farinograph development time, and in various directions for MDD work input.

Rmax was not significantly correlated with any measure of water absorption but showed moderately strong positive correlations with MDD work input, mixograph, and farinograph development times and farinograph stability (Table III). Ext showed weak but significant

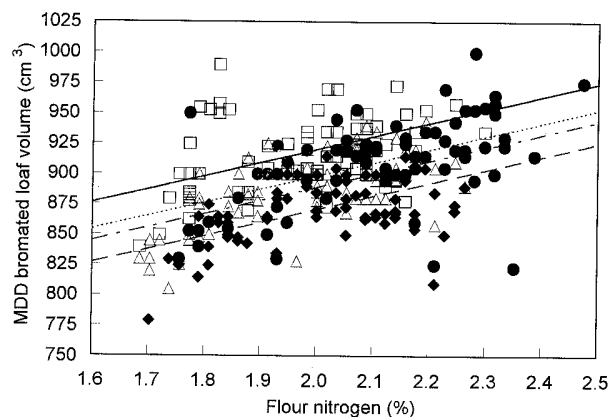


Fig. 6. Effects of flour nitrogen content on loaf volume of bromated doughs of Otane (□), Endeavour (Δ), Monad (◆), and Domino (●) prepared in a mechanical dough development mixer. Common regression slope is 109 (standard error = 10).

negative correlations with two of the three measures of dough development time in each of the two experiments.

Although crumb grain was not significantly affected by the fertilization treatments in SN3 and SN4, loaf volume (Fig. 6), bake score, and water absorption increased with FN or FS content.

Simple Relationships Between Protein Composition and Dough Quality

In all three trials, MDD work input showed a strong positive correlation with FN content (Table IV) and even stronger correlations with large monomers peak area, percent peak area due to small polymers, and ratio of polymers to large monomers. In SN1 and SN2, high correlations of MDD work input also were found with small monomers peak area, ratio of polymers to small monomers, ratio of large monomers to small monomers, HMW-GS peak area, and LMW-GS peak area. The lower correlations for these comparisons in SN3 may have been attributable to the fact that it involved four cultivars whereas SN1 and SN2 were based on just one (Otone).

Mixograph development time showed a strong negative correlation with the γ -gliadin peak area in all three trials. No other variate showed a uniform pattern across the three experiments. The strong negative correlation of MDT with FN content in SN1 was exceeded by the α + β -gliadin peak area and the γ -gliadin peak area, and that in SN2 was exceeded by several peak areas.

Only one variate, % peak area due to glutenins, showed a uniform direction of correlation with Rmax in the three trials. Several protein measurements in SN2 had strong correlations with Rmax but none had significant correlations with the ratio of MDD work input to Rmax in that trial. In SN1 and SN3, the strongest correlations with this ratio were from FN; no protein-group peak area exceeded these.

The ratio of mixograph development time to Rmax showed strong negative correlations with FN and stronger ones with the HMW-GS peak area. Polymeric peak area showed stronger correlations in two of the three trials and the ω -gliadin peak area also had consistent negative correlations. Several protein measurements showed strong correlations with this ratio in one direction in SN1 and SN2 and a weak opposite correlation in SN3. These were percent peak area due to small monomers, ratio of polymers to large monomers, ratio of polymers to small monomers, ratio of large to small monomers, α + β -gliadin peak area, and percent peak area due to ω -gliadins.

TABLE IV
Significant Correlation Coefficients of Selected Size-Exclusion HPLC and Reversed-Phase HPLC Protein Measurements with Mixing Requirements and Dough Strength in Three Sulfur (S) and Nitrogen (N) Fertilization Trials^a

Measurements ^e	MDD WI ^b			MDT ^c			Rmax ^d			WI to Rmax			MDT to Rmax		
	SN1	SN2	SN3	SN1	SN2	SN3	SN1	SN2	SN3	SN1	SN2	SN3	SN1	SN2	SN3
FN	0.631	0.597	0.384	-0.852	...	-0.318	0.639	...	0.625	-0.839	-0.714	-0.245
Poly PA	0.673	0.600	0.231	-0.804	...	-0.461	0.636	...	0.518	-0.827	-0.729	-0.437
LM PA	0.703	0.800	0.514	-0.798	...	0.207	...	0.674	...	0.608	...	0.524	0.862	-0.845	0.176
SM PA	-0.702	-0.781	-0.281	0.427	-0.792	-0.389	-0.386	-0.782	-0.299	0.750	...	-0.476
PA due to SM (%)	-0.722	-0.879	-0.436	0.701	...	-0.247	...	-0.818	...	-0.499	...	-0.485	0.863	0.790	-0.296
Ratio of poly to LM	-0.646	-0.853	-0.414	0.757	...	-0.537	...	-0.808	-0.189	-0.538	...	-0.309	0.835	0.857	-0.492
Ratio of poly to SM	0.767	0.926	0.298	-0.653	0.584	0.858	...	0.455	...	0.477	-0.872	-0.742	0.209
Ratio of LM to SM	0.757	0.931	0.388	-0.675	...	0.291	...	0.864	...	0.471	...	0.472	-0.874	-0.767	0.377
HMW-GS PA	0.805	0.961	0.179	-0.631	...	-0.487	...	0.820	...	0.503	...	0.247	-0.841	-0.808	-0.576
LMW-GS PA	0.709	0.801	0.377	-0.734	0.656	...	0.531	...	0.426	-0.855	-0.837	...
ω -gliadin PA	...	0.647	...	-0.648	...	-0.595	0.377	-0.520	-0.610	-0.740
α + β -gliadin PA	0.554	0.669	0.310	-0.857	...	0.258	0.603	...	0.299	-0.812	-0.774	0.233
γ -gliadin PA	0.345	-0.865	-0.766	-0.707	-0.425	0.565	...	0.256	-0.702	...	-0.477
PA due to glutenins (%)	0.590	0.877	0.610	0.166	0.534	0.813	0.270	-0.667	...
PA due to ω -gliadins (%)	0.602	-0.630	...	0.501	...	-0.359	...	-0.585	...	-0.444	...	-0.287	0.617	0.591	-0.559

^a SN1, df = 34; SN2, df = 10; SN3, df = 142.

^b MDD = mechanical dough development, WI = work input.

^c MDT = Mixograph development time.

^d Rmax = Maximum resistance to extension.

^e FN = flour nitrogen; Poly = polymers; PA = peak area; LM = large monomers; SM = small monomers; HMW- and LMS-GS = high- and low-molecular-weight glutenin subunits, respectively.

Compound Relationships Between Protein Composition and Dough Quality

A high proportion of the variance in MDD work input, mixograph development time, and Rmax could be explained by cultivar-specific factors and individual protein measurements. The models explaining most of the variance with SE-HPLC measurements are shown in Table V and those with RP-HPLC in Table VI.

Nearly identical proportions of the variance in MDD work input were explained by trial, cultivar, and either the content of polymeric in the flour determined by SE-HPLC (87.7%) or the area of the HMW peak in RP-HPLC (87.4%). In both models, the slope of the regression line was significant and positive in five of the six cases, the exception being Monad in SN3, and the greatest slope was in Endeavour in SN3 followed by Otane in SN2 and Otane in SN3. The intercept was largest in Monad in SN3, followed by Domino in SN3 and, in both cases, the intercept for Otane in SN2 was not significantly different from zero. These differences in slope and intercept among cultivars help to account for the differences between experiments in simple correlations described above.

The optimum model for Rmax (75.9% of variance) fitted the ratio between HMW-GS and total gliadins, as determined by RP-HPLC (Table VI). Positive slopes were found for Otane in SN1, Otane in SN2, and Monad in SN3, but not for the other three entries. Otane in SN1 had an intercept not significantly different from zero and in SN2 it was negative, but all intercepts in SN3 were significant and positive. Nearly as good a model (75.7%) used peak area of HMW-GS as a proportion of total RP-HPLC peak area and the same pattern of significances and directions applied. The optimum model for Rmax involving SE-HPLC measurements (73.3%) used the content of small monomers in the flour (Table V). All intercepts

were significant and positive and three slopes, Otane in SN1, Otane in SN2, and Domino in SN3, were significant and negative, whereas the other three were not significantly different from zero.

Mixograph development time was extremely well characterized by the content of polymeric in the flour determined by SE-HPLC (90.3%; Table V). All intercepts were significant and positive and all slopes were significant and negative. An even better model was achieved with the content of gliadins in the flour and the ratio of HMW-GS to total gliadins (Table VI). In this case, the trial × covariate term was not significant; that is, a single slope for each of the two covariates applied across all six entries. In this case, as well, the intercepts for Otane in SN1, SN2, and SN3 were not significantly different from each other.

Manipulating the Nexus Between Mixing Requirements and Dough Strength

The ratio between MDD work input and Rmax, which was chosen as an expression of the nexus between these two traits, was not as well characterized by the covariates as were the previous measures. The optimum model (54.1% of variance) fitted the content of polymeric in the flour and, as an additional covariate with one slope applying to all entries, the ratio of the sum of polymeric and large monomers to small monomers (Table V). Intercepts were nonsignificant, except for Otane in SN2 and Monad in SN3, and slopes were significant, except for Otane in SN2. A somewhat poorer but simpler model (47.0%; Table VI) fit the total gliadin content of flour as the only covariate, with a positive slope which applied across all entries, and the intercepts varied significantly.

The ratio between mixograph development time and Rmax, the other expression of a nexus between mixing requirement and dough

TABLE V
Relationships Between Mixing Requirements, Dough Strength, and Flour Composition Measured by SE-HPLC

Dependent Variable, Covariate ^a	Cultivar, Trial	Intercept	SE	Slope due to Covariate	SE of Slope	Cumulative R ²
MDD WI						
Polymeric (% of flour)	Otane, SN1	4.94	2.15	1.50	0.40	...
	Otane, SN2	-2.85	4.21	3.16	0.70	...
	Otane, SN3	-0.73	2.98	2.84	0.49	...
	Endeavour, SN3	-4.34	2.71	4.01	0.43	...
	Domino, SN3	7.64	3.03	1.75	0.47	...
	Monad, SN3	19.91	3.33	0.23	0.58	0.877
Rmax						
Small monomers (% of flour)	Otane, SN1	303	35	-75	30	...
	Otane, SN2	521	39	-259	35	...
	Otane, SN3	281	41	-22	34	...
	Endeavour, SN3	337	61	-33	56	...
	Domino, SN3	459	94	-234	111	...
	Monad, SN3	269	86	40	88	0.733
MDT						
Polymeric (% of flour)	Otane, SN1	5.17	0.28	-0.500	0.054	...
	Otane, SN2	4.08	0.56	-0.223	0.094	...
	Otane, SN3	4.90	0.39	-0.383	0.065	...
	Endeavour, SN3	5.58	0.36	-0.415	0.058	...
	Domino, SN3	6.32	0.40	-0.498	0.062	...
	Monad, SN3	7.84	0.44	-0.714	0.077	0.903
MDD WI to Rmax ratio						
Polymeric (% of flour)	Otane, SN1	0.0030	0.011	0.0142	0.0025	...
	Otane, SN2	0.0653	0.020	0.0048	0.0034	...
	Otane, SN3	0.0079	0.014	0.0129	0.0023	...
	Endeavour, SN3	-0.0194	0.013	0.0179	0.0024	...
	Domino, SN3	-0.0023	0.014	0.0167	0.0025	...
	Monad, SN3	0.0338	0.016	0.0109	0.0030	...
Ratio of (polymeric + large monomers) to small monomers	All data	-0.00248	0.00056	0.541
MDT to Rmax ratio						
Large monomers (% of flour)	Otane, SN1	0.0185	0.0007	-0.00217	0.00022	...
	Otane, SN2	0.0287	0.0015	-0.00428	0.00039	...
	Otane, SN3	0.0178	0.0013	-0.00190	0.00032	...
	Endeavour, SN3	0.0166	0.0010	-0.00168	0.00026	...
	Domino, SN3	0.0174	0.0011	-0.00115	0.00024	...
	Monad, SN3	0.0187	0.0013	-0.00137	0.00028	0.782

^a MDD = mechanical dough development, Rmax = maximum resistance to extension. MDT = mixograph development time.

strength, was better characterized. The content of large polymeric in the flour accounted for 78.2% of the variance in this ratio (Table V), with all slopes negative and all intercepts positive. An alternative model (75.5%) was fitted using the glutenin content of flour, with all slopes negative and all intercepts positive, and the LMW-GS content as a percent of total peak area as an additional covariate with a positive slope (Table VI).

DISCUSSION

Nitrogen fertilization in the SN3 trial resulted in increases in both FN and FS, with similar ranges to those in the industrial scale SN2 trial and slightly higher than in the SN1 (Wooding et al 2000). In the SN4 trial, FN and FS contents were higher than in the earlier experiments and the ranges were lower. Finney et al (1957) reported that FN and loaf volume increased linearly with nitrogen fertilization $\leq 2.2\%$ FN and, above this value, quality decreased. In the present experiments, FN levels exceeded this critical value but loaf volume and bake score did not deteriorate, indicating that FS levels remained in balance and, hence, soil sulfur levels were not limiting, even in the presence of quite high levels of N fertilization. FS content was $\geq 0.137\%$ in SN3 and SN4; whereas, in SN1 and SN2 (Wooding et al 2000), the minimum was as low as 0.124%.

Moss et al (1981) have shown that high S concentrations in the grain occur only when both N and S are in good supply. Randall et al (1981) suggested that a flour N-S ratio $>17:1$ and an FS content $<0.12\%$ represented a critical threshold in terms of yield loss and may be useful for quality assessment. Randall and Wrigley (1986) stated that the normal N-S ratio in the grain was 10:1 to 12:1 but

could exceed 20:1 when sulfur was deficient. Byers and Bolton (1979) suggested that loss of grain yield and quality could be expected when the N-S ratio was $\geq 16:1$. Flour N-S ratios in the present experiments remained between 12:1 and 16:1. The low response to S may be attributable to a good balance of available N and S in the soil, because soil sulfur levels were eight times higher than nitrate-nitrogen in the top 15 cm of the soil, instead of being less than half of the nitrate-nitrogen levels as in the earlier SN1 and SN2 trials (Wooding et al 2000).

In the SN1 trial, bake score of bromated doughs increased with FN, not with FS; whereas, in SN2, neither element was associated with significant changes in bake score. In all four trials, bake score of nonbromated doughs showed no significant relationship with either FN or FS. These results are in agreement with the general observation that the relationship between bake score and grain nitrogen content of New Zealand wheats is either poor and nonlinear (Mitchell and Casutt 1983, Wilson 1983, Stevenson 1987) or nonsignificant (Hanson and Wilson 1985). This may be attributable to the smaller range in protein quantity in New Zealand wheats by international standards and to differences in protein quality in comparison with grain grown in other countries.

In the previous experiments, the flour N-S ratio was a better predictor of dough physical characteristics than either FN content or FS content (Wooding et al 2000). In the present experiments, however, FS content was significantly increased by both N and S fertilization. A quantitative change in the protein content of the samples may have accounted for some of the variation in physical dough characteristics. A significant change in protein composition of samples from all of the trials also was apparent, but comparable

TABLE VI
Relationships Between Mixing Requirements, Dough Strength, and Flour Composition Measured by Reversed-Phase HPLC

Dependent Variable, Covariate ^a	Cultivar, Trial	Intercept	SE	Slope due to Covariate	SE of Slope	Cumulative R ²
MDD work input						
HMW-GS peak area	Otane, SN1	6.6	1.4	0.00090	0.00019	...
	Otane, SN2	2.0	1.9	0.00154	0.00020	...
	Otane, SN3	7.5	2.2	0.00096	0.00024	...
	Endeavour, SN3	5.3	2.1	0.00167	0.00023	...
	Domino, SN3	13.2	1.9	0.00068	0.00022	...
	Monad, SN3	19.4	2.7	0.00021	0.00031	0.874
Rmax						
Ratio of HMW-GS peak area to total gliadin peak area	Otane, SN1	76	40	1180	340	...
	Otane, SN2	-104	39	2080	240	...
	Otane, SN3	270	40	-130	320	...
	Endeavour, SN3	258	59	340	460	...
	Domino, SN3	307	47	-420	430	...
	Monad, SN3	213	41	800	350	0.759
MDT						
	Otane, SN1	4.42	0.17
	Otane, SN2	4.73	0.21
	Otane, SN3	4.90	0.19
	Endeavour, SN3	5.27	0.19
	Domino, SN3	5.67	0.19
	Monad, SN3	6.11	0.18
Total gliadins (% of flour)	All data	-0.334	0.017	...
Ratio of HMW-GS peak area to total gliadin peak area	All data	2.9	1.0	0.924
MDD WI to Rmax ratio						
	Otane, SN1	0.0206	0.0048
	Otane, SN2	0.0289	0.0053
	Otane, SN3	0.0186	0.0056
	Endeavour, SN3	0.0229	0.0055
	Domino, SN3	0.0231	0.0060
	Monad, SN3	0.0228	0.0053
Total gliadins (% of flour)	All data	0.00579	0.00068	0.470
MDT to Rmax ratio						
Total glutenins (% of flour)	Otane, SN1	0.0147	0.0016	-0.00373	0.00040	...
	Otane, SN2	0.0232	0.0020	-0.00522	0.00050	...
	Otane, SN3	0.0106	0.0016	-0.00234	0.00048	...
	Endeavour, SN3	0.0110	0.0020	-0.00253	0.00048	...
	Domino, SN3	0.0099	0.0018	-0.00156	0.00042	...
	Monad, SN3	0.0149	0.0020	-0.00303	0.00054	...
LMW-GS peak area (% of total peak area)	All data	0.00358	0.00056	0.755

^a MDD = mechanical dough development, Rmax = maximum resistance to extension, MDT = mixograph development time.

variations due to N treatments in the SN3 trial were not detectable here. Salmon (1984) suggested that late applications of urea could alter the ratio of gliadins to glutenins, resulting in an increase in the proportion of the larger gliadins and producing wheat of poor baking quality, but this may have been due to the creation of an imbalance between available N and S as well as available water.

All measures of water absorption increased with both N fertilization and FN content. Randall et al (1990) also found that farinograph water absorption increased as N fertilization increased, but that S fertilization had no significant effect, whereas Moss et al (1981) found significant effects of both fertilizations on farinograph water absorption. Differences between the three measures of water absorption may be attributable to the different mixing actions employed and the effects on the different proteins in the doughs. Dough mixing requirements also showed differences among the three mixers employed. MDD mixers have been characterized as having a fast, intensive kneading action (French and Fish 1981), whereas the farinograph uses a gentle kneading motion (Spies 1990) and the mixograph a harsh, pin-mixing action (Spies 1990). The mixograph produces tensile stresses that are proportional to the resistance of the dough to stretching and correlate with the composition of polymeric proteins and their balance with monomeric proteins, whereas the farinograph's continuous shearing is affected more by extensibility and, hence, by the amount of glutenin in the flour (MacRitchie 1992). In the present work, MDD work input was best characterized by the content of polymeric in the flour (SE-HPLC) or the HMW-GS peak area (RP-HPLC), variates that are clearly closely related. Mixograph development time was best characterized by the content of polymeric in the flour (SE-HPLC) or by the combination of gliadin content of the flour and HMW-GS to gliadin ratio (RP-HPLC). Nevertheless, the direction of the effect of polymeric on mixograph development time was opposite to their effect on MDD work input.

Different mixers give different results. Therefore, it is clearly important to use the same sort of mixer for experimental tests as will be used on an industrial scale. Development time may not be the most appropriate measure of mixing requirements determined on a mixograph. When protein content has been increased by N fertilization, the peak height increases proportionally more than the time to maximum height; thus the area under the curve may display a significant increase. We have preliminary results showing that mixograph peak area (area under the curve until either peak height or 1 min after it) is much more strongly correlated with MDD work input (A. R. Wooding et al, *unpublished*).

Rmax showed a negative relationship with the content of small monomers in the flour and, in some cases, a positive relationship with the HMW-GS and gliadin ratio. These are different covariates than were significant for MDD work input or mixograph development time and this feature could be used to control the nexus between mixing requirements and dough strength. More information on this possibility was obtained by investigating the ratio of mixing requirements to dough strength. The ratio of MDD work input to Rmax was more than half explained by the content of polymeric in the flour and the ratio of polymeric and large monomers (i.e., largely glutenins and gliadins) to small monomers (largely metabolic proteins). An almost equivalent model, which had the advantage of being consistent across cultivars, used the gliadin content of flour. Similarly, total glutenin content and LMW-GS peak area accounted for a very substantial proportion of the variance of the ratio between mixograph development time and Rmax. Experimental manipulation of these aspects of flour composition will be an important adjunct to their evaluation from correlations.

The relevance of Rmax to the MDD process needs to be confirmed. The Approved Method 54-10 (AACC 2000) does not necessarily produce a dough of optimum consistency for the determination of Rmax and it may be more appropriate to mix the dough to optimum. Measurements such as mixing tolerance or resistance

breakdown may be more relevant to the dough strength requirements of the industrial process.

Variation in the number of sulfhydryl and disulfide groups in the protein may help to explain the changes in protein composition and bread quality brought about by nitrogen and sulfur fertilization, because they play important roles in physical dough properties and the mechanisms of oxidation. Tsen and Anderson (1963) and Tsen and Bushuk (1968) both found that the sulfhydryl content was highest in weak flours and lowest in strong flours, and that disulfide content increased with decreasing flour strength. Tsen and Bushuk (1968) found that the weaker flours had more water-soluble proteins.

Gliadins, when added to a base flour (Fido et al 1994), added to the parent flour at constant protein content (Uthayakumaran et al 1999), or added to other flours (Gupta et al 1993) reduce dough strength as measured by mixograph development time, resistance breakdown, and Rmax. The protein composition within the major SE-HPLC peaks, such as the ratio of HMW-GS to LMW-GS, may be used to manipulate the relationship between mixograph development time and Rmax. Gupta et al (1992) showed that Rmax and mixograph development time both were more strongly correlated with the glutenin content of protein than with the glutenin content of flour, whereas farinograph development time, Ext, and loaf volume were better correlated with the flour glutenin content. The authors suggested that the ratio of HMW-GS to LMW-GS was positively correlated with both Rmax and mixograph development time. In another study, mixograph development time increased with HMW-GS to LMW-GS ratio in four cultivars, but Rmax showed little effect of this ratio in two cultivars, a moderate effect in a third, and a very steep increase in the fourth (Uthayakumaran et al 2000). These results suggest that it is indeed possible to shift protein composition to reduce dough mixing requirements without adverse effects on dough strength as determined by Rmax.

Rmax is not always indicative of good dough quality. In the present experiments, Otane and Domino produced better loaf volumes than Monad and Endeavour, but it was the latter two that had the highest Rmax values across all levels of FN content. Monad differed from Otane and Endeavour at the *Glu-B1*, *Glu-A3*, and *Glu-B3* loci and from Domino at the *Glu-A1*, *Glu-B1*, and *Glu-A3* loci. Otane was in the pedigree of Endeavour and the two cultivars shared *Glu-1* and *Glu-3* alleles, but Endeavour had higher mixing requirements, water absorption, and dough strength at similar levels of FN. In SDS-PAGE, Otane showed two additional low-mobility bands which may represent a modified ω -gliadin, which would alter the physical dough properties. The lower effect of N on the MDD work input of Domino than Endeavour would make the former more industrially useful.

CONCLUSIONS

Nitrogen and sulfur fertilization may be used to manipulate the nexus between mixing requirements and dough strength to different degrees, depending on the cultivar. The variation in response of cultivars to fertilizer treatments was most likely due to genotypic differences in protein composition and the interaction of these factors with the properties of the physical tests performed. Many aspects of the nexus varied between cultivars, giving indications of how the nexus may be manipulated.

Although no single protein group was indicated that would break the nexus, keeping the gliadin content of the flour high without losing HMW-GS content could reduce mixograph development time without reducing Rmax. The application of N in the SN1 trial, however, appeared to decrease the resistance to breakdown, as observed by the gradient and thinning of the mixograph curve after maximum peak height, which is often associated with a reduction in dough strength.

Although Otane and Endeavour had the same glutenin genotype, they differed in their MDD work input requirements. The biochemical and genetic basis of this difference should be further inves-

tigated to provide insights into minimizing mixing requirements while maintaining dough strength.

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