

Effects of Wheat Cultivar and Nitrogen Application on Storage Protein Composition and Breadmaking Quality

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ABSTRACT

Cereal Chem. 78(1):19–25

Influences of cultivar and nitrogen application on protein concentration and composition, and amount and size-distribution of different protein components, were investigated in 10 spring wheat cultivars (*Triticum aestivum* L.) with widely varying gluten strength, grown under four nitrogen fertilizer conditions. The results showed that cultivar differences in gluten strength were determined by storage protein composition, differences in total amount of HMW glutenin subunits, the glutenin-to-gliadin ratio, and the relationship between SDS-soluble and SDS-insoluble protein polymers. Negative correlations were found between protein

parameters related to gluten strength and bread volume. No cultivar stability for gluten strength in relation to differences in nitrogen application was found. Thus, the gluten strength was influenced by the nitrogen application in all the investigated cultivars. Increased nitrogen supply correlated significantly to an increase in all protein components containing gliadins and glutenins, but not to those containing albumins and globulins. The increase in protein components containing gliadins and glutenins correlated significantly with an increase in protein concentration and bread volume.

Protein concentration and composition of specific protein subunits influences the breadmaking quality of wheat (*Triticum aestivum* L.) (Finney and Barmore 1948; Sozinov and Poperelya 1980; Payne et al 1984; Branlard and Dardevet 1985a,b). Increased protein concentration leads to an increase in loaf volume (Finney and Barmore 1948; Tipples and Kilborn 1974). Correlations have been established between particular proteins and protein subunits and breadmaking quality (Payne et al 1983; 1987; Sontag et al 1986; Lawrence et al 1987; Uhlen 1990; Johansson et al 1993, 1994; Johansson and Svensson 1995; Johansson 1996).

Ratios of different protein compounds influence breadmaking quality (Field et al 1983; Sutton 1991; Gupta 1994; Wieser et al 1994). The amount of proteins that are most difficult to extract is of major importance in determining the gluten strength (Bietz and Wall 1975; Huebner and Wall 1976). In several investigations, the relative proportion of large polypeptides have correlated with breadmaking quality (Dachkevitch and Autran 1989; Singh et al 1990; Gupta et al 1993). Also, the quantity of specific glutenin protein fractions (Sutton et al 1990) and the quantity of total HMW and LMW glutenin subunits (Skerritt 1991; Andrews et al 1994) have correlated with rheological parameters.

Dough properties and baking performance of wheat are strongly dependent on both genotype and environment (Peterson et al 1992; Johansson and Svensson 1998, 1999). The protein concentration is determined by the genetic background, but also, to a large extent, by environmental factors such as nitrogen, water access, and temperature conditions (Sosulski et al 1963; Benzian et al 1983; Stapper and Fischer 1990; McDonald 1992). The composition of proteins and protein subunits is genetically determined (Payne et al 1987; Johansson et al 1993). However, the relative quantity of specific proteins, protein subunits, and protein groups, as well as amount and size-distribution of polymeric proteins varies due to environmental conditions (Wieser and Seilmeier 1998), and genetic determination (MacRitchie 1999).

The aim of the present investigation was to study the influence of the cultivar and nitrogen application on the protein concentration, protein composition, and the amount and size-distribution of dif-

ferent protein components. The influence of these different components on breadmaking quality was also investigated.

MATERIALS AND METHODS

Materials

The plant material included 10 spring wheat cultivars with widely varying gluten strength (Table I) from the combined cultivar nitrogen fertilizer trials in 1998 at Weibullsholm, Svalöf Weibull AB, Landskrona, Sweden. For each cultivar, four different nitrogen levels were applied: 55 kg N/ha (at sowing); 75 kg N/ha (55 kg N/ha at sowing and 20 kg N/ha before heading); 155 kg N/ha (55 kg N/ha at sowing and 100 kg N/ha before heading); and 215 kg N/ha (55 kg N/ha at sowing, 100 kg N/ha before heading, and 60 kg N/ha at grain filling). The cultivars were screened for quality at Svalöf Weibull AB, Svalöv, Sweden according to Johansson and Svensson (1995). Quality parameters in terms of glutograph (Brabender OHG, Duisburg, Germany) dough deformation times (Glut), mixograph (National Mfg. Co., Lincoln, NE), dough development times (Mix), and bread volumes (Johansson and Svensson 1995) are presented in Table I.

Methods

Wheat proteins were extracted from white flour and separated on polyacrylamide gels in the presence of SDS according to Johansson (1996). The gels were stained according to Johansson et al (1993) and destained with 10% (v/v) trichloroacetic acid (TCA) for at least one day. The HMW-GS are designated according to the numbering system of Payne and Lawrence (1983) and the D zone ω -gliadins are designated according to the numbering system of Khelifi et al (1992).

The two-step extraction procedure developed by Gupta et al (1993) was applied. The first step in this method extracts the proteins soluble in dilute SDS, while the second extract contains proteins soluble only after sonication. For the first extraction 11 mg of white flour were suspended in 1.0 mL of 0.5% (w/v) SDS-phosphate buffer (pH 6.9) and vortexed for 10 sec. Samples were then stirred for 5 min at 2,000 rpm and centrifuged for 30 min at 10,000 \times g to obtain the supernatant protein. The pellet was subsequently resuspended in SDS buffer as above and sonicated in an ultrasonic desintegrator (Soniprep 150, Tamro, Mölndal, Sweden) for 30 sec, amplitude 5, and fitted with a 3-mm exponential microtip. The samples were centrifuged as above, to obtain a supernatant of proteins. The extracts were filtered through 0.45- μ m filters (Millipore, Durapore Membrane Filters) before running on HPLC. Size-exclusion HPLC analyses were performed on a Varian HPLC system using a BIOSEP SEC-4000 Phenomenex column. Separation was achieved in 30 min

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by loading 20 μ L of sample into an eluant of 50% (v/v) acetonitrile and water containing 0.1% (v/v) trifluoroacetic acid (TFA) at a flow rate of 0.2 mL/min. Proteins were detected by UV absorbance at 210 nm. Areas of different peaks were calculated. The percentage of total unextractable polymeric protein in the total polymeric protein [(SDS-insoluble large and smaller protein polymers)/(SDS-soluble and -insoluble large and smaller protein polymers)], and the percentage of large unextractable polymeric protein in the total large polymeric protein [(SDS-insoluble large protein polymers)/(SDS-soluble and SDS-insoluble large protein polymers)] was calculated according to Gupta et al (1993).

The extraction procedure of proteins for reversed-phase HPLC analyses was developed by Wieser and Seilmier (1998). In this procedure, the proteins are extracted stepwise to isolate 1) albumins (Alb) and globulins (Glo); 2) gliadins (Gli); and 3) glutenin subunits (Glu). RP-HPLC analyses were conducted according to Andrews et al (1994) on a Varian HPLC system using a Supelcosil LC-308 column with 300 Å pore size, 5 μ m particle size, 250 \times 4.6 mm i.d. The solvent flow rate was 0.8 mL/min using a column temperature of 70°C and the effluent was monitored at 210 nm. Elution was achieved using a gradient system formed from two solvents: A, water containing 0.1% (v/v) TFA, and B, acetonitrile containing 0.1% (v/v) TFA. The first fraction (Alb and Glo) was analyzed on the HPLC using a gradient of 20–60% solvent B from 1 to 20 min. The gradient used for separation of the other two fractions (Gli and Glu, respectively) was 28–56% solvent B from 1 to 30 min (Wieser

and Seilmier 1998). For the two-step SE-HPLC and RP-HPLC analyses, three and two replicates were analyzed, respectively.

Statistical Analyses

Data from quality data, SDS-PAGE results, and HPLC analyses were evaluated using the Spearman rank correlations, analyses of variance (ANOVA), and principal component analyses (SAS Institute, Cary, NC). Stability analyses were made according to Eberhart and Russell (1966) with the MSTAT program (Michigan State University, East Lansing, MI).

RESULTS

Composition of HMW-GS and D-zone ω -gliadins in the investigated cultivars are shown in Table II.

SE-HPLC

The SE-HPLC chromatograms were divided into four sections (Fig. 1) containing the following protein types determined by SDS-PAGE (results not shown); A, large polymeric proteins (LPP); B, smaller polymeric proteins (SPP); C, large monomeric proteins (LMP), mainly gliadins; and D, smaller monomeric proteins (SMP), mainly Alb and Glo. Wheat cultivar and nitrogen application influenced the amount and size-distribution of several of the mono- and polymeric proteins differentiable with SE-HPLC (Table III). The percentages of total and large unextractable polymeric protein

TABLE I
Quality Tests^a of 10 Spring Wheat Cultivars Grown with Four Nitrogen (N) Application Rates

Cultivar	N (kg/ha)	Protein Concentration (%)		Glut (sec)	Mix (min)	Bread Volume (mL/100 g of flour)
		Grain	Flour			
SW 37391	55	10.8	9.4	14.9	2.7	970
	75	11.4	10.2	9.1	2.7	1,080
	155	13.3	12.3	11.1	2.9	1,260
	215	13.6	12.8	9.2	2.9	1,250
SW 37346	55	10.4	9.1	9.4	2.2	910
	75	11.0	9.6	10.8	2.8	950
	155	12.8	11.6	9.6	2.9	1,110
	215	13.3	12.4	8.2	2.8	1,210
SW 37342	55	10.8	9.1	37.7	3.5	880
	75	10.9	9.7	23.6	3.6	930
	155	13.0	12.0	17.8	3.7	1,140
	215	13.2	12.2	12.5	3.8	1,140
Velos	55	10.6	9.1	39.2	3.1	790
	75	10.8	9.6	64.3	3.3	800
	155	12.8	11.6	19.8	3.9	980
	215	12.9	11.9	24.7	3.4	1,000
Batalj	55	11.0	9.4	41.4	3.9	890
	75	11.2	10.1	15.8	3.8	960
	155	12.9	12.3	17.7	3.5	1,220
	215	13.5	12.7	15.3	3.8	1,170
Dragon	55	11.0	9.8	45.9	3.5	940
	75	11.5	10.1	23.3	3.5	950
	155	13.0	12.3	19.3	3.8	1,160
	215	13.9	13.1	17.4	3.8	1,230
Triso	55	10.7	9.4	54.0	3.9	860
	75	10.9	9.2	24.2	3.5	890
	155	12.9	11.4	31.5	4.2	1,050
	215	13.3	12.2	18.1	4.1	1,090
Vinjett	55	10.6	9.2	85.7	3.5	910
	75	10.9	9.5	24.0	3.2	890
	155	12.6	11.6	13.9	3.6	1,150
	215	13.4	12.3	13.1	3.8	1,240
SW 36188	55	11.0	9.3	123.7	4.2	870
	75	11.7	10.2	47.4	4.3	890
	155	13.2	12.2	43.9	4.9	1,140
	215	14.0	12.7	22.7	4.7	1,210
SW 37281	55	10.8	9.1	123.8	4.9	830
	75	11.3	10.0	124.0	5.1	870
	155	12.3	11.6	123.9	6.6	970
	215	13.2	11.9	124.2	6.1	990

^a Protein concentration, glutograph (Glut) and mixograph (Mix) values and bread volume.

in the total and total large polymeric protein (TUPP and LUPP) were influenced significantly by cultivar but not by nitrogen application. The cultivar differences in the TUPP and LUPP were related to differences in gluten strength between the cultivars (Table V). Increased gluten strength measured by both Glut and Mix correlated with an increase in TUPP and LUPP. The increase in gluten strength measured by Glut was due to a decrease in SDS-soluble LPP and SPP, while the increase in gluten strength measured by Mix was due to an increase in SDS-insoluble LPP and SPP (Table V). However, significant negative and positive correlations were found for SDS-soluble and SDS-insoluble LPP and SPP, respectively, in relation to both Glut and Mix, when the results from each nitrogen application level were investigated separately (results not shown). The differences in decrease and increase of SDS-soluble and SDS-insoluble LPP and SPP between the two applied methods for measuring gluten strength (Glut and Mix) was instead found to be due to differences in sensitivity for variation in protein concentration between the methods. A strong negative correlation ($P < 0.005$) was found between protein concentration and Glut, while a weaker negative correlation ($P = 0.05$) was found between protein concentration and Mix (results not shown). The amount of SDS-soluble and SDS-insoluble LPP, SPP, and LMP increased significantly with an increase in nitrogen application (Table V). The increase in amounts of these protein components was associated with an increase in total protein concentration with increased nitrogen application. The amounts of SDS-soluble and SDS-insoluble SMP (Alb and Glo) were not significantly influenced by nitrogen application. Neither TUPP nor LUPP were influenced by nitrogen application (Table V).

RP-HPLC

Before running RP-HPLC, the proteins were extracted sequentially (Alb and Glo, Gli, and Glu). The third extraction (which was supposed to contain glutenin subunits) running SDS-PAGE also found ω -gliadins (results not shown). The chromatogram obtained from the third extraction by RP-HPLC, was divided into three parts containing ω -gliadins, HMW-GS, and LMW-GS, respectively (determined by SDS-PAGE, results not shown). This was in accordance with results obtained by Wieser and Seilmeier (1998). Wheat cultivar and nitrogen application both influenced the

amount of Gli and Glu (Table IV). The amount of Glu in relation to Gli (Glu/Gli) was influenced significantly only by the cultivar. Cultivar differences related to the gluten strength were found for the total amount of Glu, the amount of HMW-GS, and the Glu/Gli ratio. Cultivars with high gluten strength were significantly correlated to high total amount of Glu, high amount of HMW-GS, and a high Glu/Gli ratio. Increased nitrogen applications led to an increase in the amounts of Gli and Glu (Table VI), and thereby to

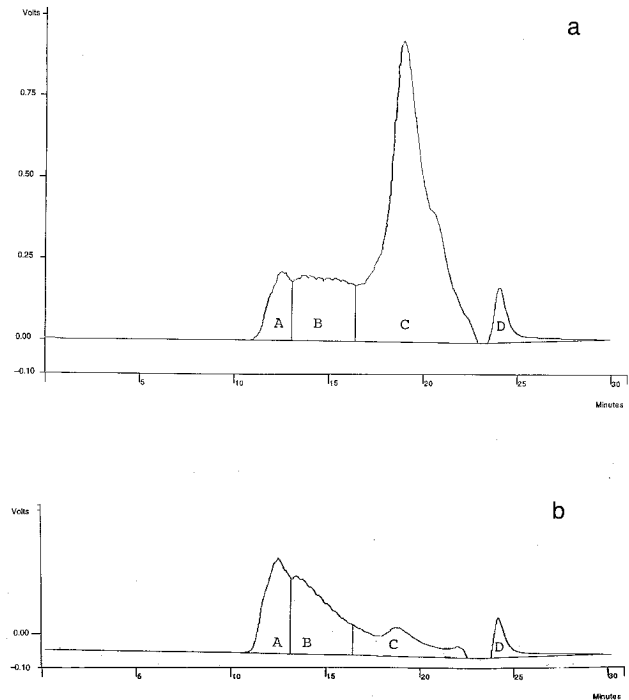


Fig. 1. Size-exclusion HPLC chromatogram of (a) SDS-soluble and (b) SDS-insoluble proteins. A = large polymeric proteins (LPP), B = smaller polymeric proteins (SPP), C = large monomeric proteins (LMP) mainly gliadins, D = smaller monomeric proteins (SMP) mainly albumins and globulins.

TABLE II
HMW-GS and D-Zone ω -Gliadins Encoded on Chromosomes 1A, 1B, and 1D

Cultivar	HMW-GS			D-Zone ω -Gliadins		
	1A	1B	1D	1A	1B	1D
SW 37391	2*	7+9	2+12	d9	d2d4	d11d12
SW 37346	2*	7+9	2+12	d9	d2d4	d11d12
SW 37342	2*	7+9	2+12	d8d9d10	d2d4	d11d12
Velos	1	7+9	2+12	d8d9d10	d2d4	d11d12
Batalj	2*	7+9	2+12	d8d9d10	d2d4	d11d12
Dragon	2*	7+9	2+12	d8d9d10	d2d4	d11d12
Triso	1	7+9	5+10	...	d3	d11d12
Vinjett	2*	7+9	5+10	d9	d2d4	d11d12
SW 36188	2*	7+9	2+12	d8d9d10	d2d4	d11d12
SW 37281	2*	14+15	5+10	d8d9d10	d2d4,d3	d11d12

TABLE III
Mean Squares from Analysis of Variance of Protein Parameters from Size-Exclusion HPLC^a

Source	DF	SDS-Soluble				SDS-Insoluble				TUPP (10 ⁻²)	LUPP (10 ⁻²)
		LPP (10 ¹¹)	SPP (10 ¹¹)	LMP (10 ¹²)	SMP (10 ⁹)	LPP (10 ¹¹)	SPP (10 ¹²)	LMP (10 ¹⁰)	SMP (10 ⁹)		
Cultivar	9	13.1	5.6***	4.8*	22.8*	11.0***	5.6	39.3***	5.5***	2.8***	4.7***
N	3	19.5	53.3***	124.6***	11.7	34.2***	2.9	32.8***	1.3	0.2	0.4
Cultivar × N	27	10.5	1.3	1.9	6.8	0.0	5.4	5.6	2.0*	0.3	0.4
Error	81	10.0	1.0	2.0	11.0	0.2	5.7	5.5	1.0	0.2	0.3

^a LPP and SPP = large and smaller polymeric proteins; LMP and SMP = large and smaller monomeric proteins; TUPP = total unextractable polymeric protein in total polymeric protein; LUPP = large unextractable polymeric protein in total large polymeric protein; N = nitrogen application.

an increase in protein concentration and bread volume. The amount of Alb and Glo were not influenced by the increase in nitrogen application, nor was the Glu/Gli ratio affected by this increase (Table VI).

Relationship Between Cultivar, Nitrogen Application, Protein Components, and Breadmaking Quality Parameters

Principal component analyses (Fig. 2) showed a close relationship between the nitrogen application, the protein concentration in the grain and in the flour, the SDS-soluble LPP, SPP, and LMP, and the bread volume. Cultivar variation was most closely related to parameters indicating gluten strength as Glut and Mix, HMW-GS encoded on chromosome 1B and 1D, D-zone ω -gliadins encoded on chromosome 1B, and TUPP and LUPP. Gluten strength expressed as Glut was most closely related to the TUPP and LUPP when applying principal component analyses (Fig. 2). The Mix was more closely related to other protein components as LPP, SPP, and LMP, and the Glu/Gli ratio.

Spearman rank correlation analyses showed strong correlation between HMW-GS encoded on chromosome 1B and 1D, and gluten strength measured by Glut and Mix (Table VII). Furthermore, a strong correlation was found between D-zone ω -gliadins encoded on chromosome 1A and 1B, and the Glut and Mix (Table VII). Spearman rank correlation analyses also showed that SDS-soluble

and SDS-insoluble LPP and SPP, as well as TUPP and LUPP were correlated to differences in HMW protein composition encoded on chromosome 1B and 1D, and D-zone ω -gliadins encoded on chromosome 1A and 1B (Table VIII). The HMW-GS and D zone ω -gliadin compositions correlated with the total amount of HMW-GS but not with the total amount of the other protein groups (Table VIII).

Variation in Stability of Gluten Strength

When investigating the stability of the gluten strength between the cultivars, SW 37281 showed a high and stable gluten strength (high mean and a low *b* value) when the gluten strength was measured with Glut. A low and stable Glut (low means and low *b* values) was found for SW 37346 and SW 37391 (Table IX). However, when the gluten strength was measured by Mix, none of these three cultivars were found to be more stable (i.e., have lower *b* values) than the other investigated cultivars. No variation in stability between the cultivars was found due to the variation in protein composition (HMW-GS) between the investigated cultivars.

DISCUSSION

In the present investigation, different wheat cultivars had different HMW-GS and D-zone ω -gliadin compositions. As in earlier investigations (Payne et al 1983, 1987; Uhlen 1990; Johansson et al

TABLE IV
Mean Squares from Analysis of Variance of Protein Parameters from Reverse-Phase HPLC^a

Source	DF	Alb+Glo (10 ¹²)	Gli (10 ¹²)	Glu (10 ¹³)	ω (10 ⁹)	HMW (10 ¹⁰)	LMW (10 ¹¹)	Glu/Gli (10 ⁻²)
Cultivar	9	1.2	14.0***	73.5***	7.7	20.9*	67.3***	9.6***
N	3	1.1	42.3***	163.4***	26.1**	63.4***	95.9***	1.8
Cultivar \times N	27	2.6	3.1	9.0	2.6	4.0	6.6	1.0
Error	39	2.1	3.2	14.7	5.7	8.8	8.7	1.3

^a Alb+Glo = albumins + globulins; Gli = gliadins; Glu = glutenins; ω = omega gliadins; HMW-GS = HMW glutenin subunits; LMW-GS = LMW glutenin subunits; Glu/Gli = glutenin/gliadin ratio; N = nitrogen application.

TABLE V
Spearman Rank Correlation Coefficients from Size-Exclusion HPLC Analyses^a

	Cultivar	N	prot1	prot2	Glut	Mix	Vol
SDS-soluble							
LPP	-0.413***	0.667***	0.669***	0.701***	-0.599***	-0.194*	0.741***
SPP	-0.282***	0.550***	0.554***	0.593***	-0.443***	-0.120	0.595***
LMP	-0.126	0.789***	0.810***	0.828***	-0.470***	0.039	0.824***
SPP	-0.102	0.058	0.064	0.099	-0.182*	-0.118	0.141
SDS-insoluble							
LPP	0.376***	0.575***	0.596***	0.568***	0.172	0.605***	0.394***
SPP	0.360***	0.459***	0.492***	0.501***	0.155	0.505***	0.338***
LMP	0.142	0.286***	0.335***	0.352***	0.012	0.187*	0.303***
SMP	0.160	-0.065	-0.032	-0.040	0.166	0.164	-0.085
TUPP	0.439***	-0.012	0.034	-0.004	0.381***	0.481***	-0.129
LUPP	0.480***	-0.001	0.018	-0.036	0.470***	0.555***	-0.189*

^a N = nitrogen applications; prot1 and prot2 = grain and flour protein concentration; Glut = glutograph dough deformation time; Mix = mixograph dough development time; Vol = bread volume; LPP and SPP = SDS-soluble large and smaller polymeric protein; LMP and SPP = SDS-soluble large and smaller monomeric proteins; TUPP = total unextractable polymeric protein in total polymeric protein; LUPP = large unextractable polymeric protein in total large polymeric protein.

TABLE VI
Spearman Rank Correlation Coefficients from Reverse-Phase HPLC Analyses^a

	Cultivar	N	prot1	prot2	Glut	Mix	Vol
Alb+Glo	-0.077	0.160	0.123	0.132	-0.116	-0.025	0.149
Gli	-0.200	0.250*	0.252*	0.272*	-0.218	-0.072	0.313**
Glu	0.239*	0.527***	0.548***	0.577***	-0.031	0.236*	0.477***
ω	-0.054	0.480***	0.469***	0.444***	-0.169	0.093	0.445***
HMW	0.342***	0.452***	0.405***	0.349***	0.170	0.447***	0.225*
LMW	0.179	0.482***	0.508***	0.561***	-0.071	0.154	0.475***
Glu/Gli	0.347***	0.155	0.174	0.199	0.189	0.227	0.069

^a N = nitrogen applications; prot1 and prot2 = grain and flour protein concentration; Glut = glutograph dough deformation time; Mix = mixograph dough development time; Vol = bread volume; Alb+Glo = albumins + globulins; Gli = gliadins; Glu = glutenins; ω = omega gliadins; HMW-GS = HMW glutenin subunits; LMW-GS = LMW glutenin subunits; Glu/Gli = glutenin/gliadin ratio.

1993; Johansson and Svensson 1995; Johansson 1996), the protein composition of the cultivars used in this study, was correlated to variation in gluten strength. Other protein parameters such as the amount of SDS-soluble and SDS-insoluble LPP and SPP, the TUPP and LUPP, the total amount of Glu, the total amount of HMW-GS, and the Glu/Gli ratio influenced the gluten strength of the cultivars. Similar findings have been reported previously by other authors (Singh et al 1990; Sutton et al 1990; Skerritt 1991; Gupta et al 1993; Andrews et al 1994; MacRitchie 1999). In the present investigation, we were able to show that cultivar variation in gluten strength is caused not by an increase in total polymeric protein (PP) but by a shifting of PP from SDS-soluble to SDS-insoluble LPP and SPP. This shifting might be due to an increase in HMW-GS, causing an increase in Glu/Gli ratio in the cultivars with highest gluten strength compared with those with lower gluten strength. Significant correlations were found between the protein composition of cultivar and the SDS-soluble and SDS-insoluble LPP and SPP, the TUPP and LUPP, and the total amount of HMW-GS. This means that influences of protein composition on amount of different protein components could be one explanation of the cultivar variation in gluten strength. This needs further investigation because only 10 cultivars were used in the present study.

Cultivar variation in SDS-insoluble LPP and SPP, TUPP and LUPP, and total amount of HMW-GS significantly influenced the bread volume. High values of these protein parameters indicating high gluten strength of the dough were significantly correlated to low bread volumes. This was due to the Swedish breadmaking technique with a relatively gentle bread mixing and a fixed dough mixing time (Johansson and Svensson 1995). This breadmaking technique demands relatively weak wheat gluten properties.

Increased gluten strength measured by Glut and Mix correlated with an increase in TUPP and LUPP. The increase in gluten strength measured by the Glut was correlated with a decrease in SDS-soluble LPP and SPP, while the increase in gluten strength measured by the Mix was correlated to an increase in SDS-insoluble LPP and SPP. However, the variation in the determination of the gluten strength between the two methods can also be explained by differences in sensitivity for variation in protein concentration by the Glut and Mix methods. A strongly negative correlation between protein concentration and Glut has been observed in the present study as in several other investigations (Johansson and Svensson 1998, 1999), while the Mix correlated positively with protein concentration. Increased protein concentration thus results in a decrease in Glut, increase in Mix, and increase in SDS-soluble and SDS-insoluble LPP and SPP. These results counteract the cultivar-influenced results of correlations between a decrease of SDS-soluble LPP and SPP and increase of SDS-insoluble LPP and SPP, and an increased Glut. Thus, a cultivar-based increase in gluten strength leads to a decrease in SDS-soluble LPP and SPP and an increase in SDS-insoluble LPP and SPP. The changes in SDS-soluble and SDS-insoluble proteins give rise to changes in TUPP and LUPP, which in turn, lead to changes in the Glut and Mix.

Increased nitrogen supply leads to an increase in all protein components containing Gli and Glu: SDS-soluble and SDS-insoluble LPP, SPP, and LMP, total amounts of Gli and Glu, and total

amounts of ω -gliadins, HMW-GS, and LMW-GS. The amount of protein components containing mainly Alb and Glo (SDS-insoluble and SDS-soluble SMP, and Alb+Glo) are not significantly influenced. Nor was the relationship between different protein groups containing Gli and Glu (TUPP and LUPP, and Glu/Gli ratio) influenced. Increased protein concentration enhances the bread volume because the amounts of all protein components involved in building the gluten network are increased.

Wheat cultivars with different HMW-GS composition vary in stability in relation to gluten strength due to variation in environment (Johansson et al 2000). With the cultivars used in the present investigation, no variation in stability in relation to gluten strength was due to differences in nitrogen application. Three of the cultivars showed tendencies of being more stable than the other cultivars when Glut were used for measuring stability of gluten strength. However, these three cultivars had Glut close to the limits of what is possible to measure with a glutograph. Thus, these cultivars

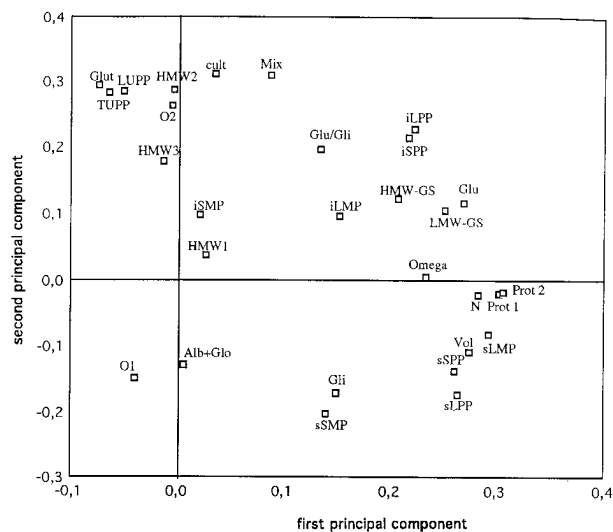


Fig. 2. Relationships among cultivar, nitrogen application, protein components, and breadmaking quality parameters based on the first two axes of principal component analysis. First and second axes explained 32.2 and 21.6% of the variances of the components, respectively. Cult = cultivar, N = nitrogen application, sLPP and sSPP = SDS-soluble large and smaller polymeric protein, sLMP and sSMP = SDS-soluble large and smaller monomeric protein, iLPP and iSPP = SDS-insoluble large and smaller polymeric protein, iLMP and iSMP = SDS-insoluble large and smaller monomeric protein, TUPP = total unextractable polymeric protein in total polymeric protein, LUPP = large unextractable polymeric protein in total large polymeric protein, Alb+Glo = albumins+globulins, Gli = gliadins, Glu = glutenins, omega = omega gliadins, HMW-GS = HMW glutenin subunits, LMW-GS = LMW glutenin subunits, Glu/Gli = glutenin/gliadin ratio, HMW1, HMW2, and HMW3 = HMW subunits of glutenin encoded on chromosome 1A, 1B, and 1D, respectively, O1 and O2 = D-zone ω -gliadins encoded on chromosome 1A and 1B, respectively, prot1 and prot 2 = grain and flour protein concentration %, Glut = glutograph dough deformation times sec, Mix = mixograph dough development times min, Vol = bread volume in mL/100 g of flour.

TABLE VII
Spearman Rank Correlation Coefficients Among HMW Glutenin Subunit (HMW-GS) and D-Zone ω -Gliadin Patterns and Breadmaking Quality Parameters^a

	HMW-GS			D-Zone ω -Gliadins	
	1A	1B	1D	1A	1B
prot1	0.161	-0.040	-0.138	-0.084	-0.069
prot2	0.175	-0.093	-0.195	-0.092	-0.153
Glut	-0.214	0.512***	0.406***	-0.387***	0.499***
Mix	-0.025	0.513***	0.400***	-0.390***	0.539***
Vol	0.279	-0.228*	-0.200*	0.118	-0.272***

^a prot1 and prot2 = grain and flour protein concentration, Glut = glutograph dough deformation time, Mix = mixograph dough development time, Vol = bread volume.

TABLE VIII
Spearman Rank Correlation Coefficients Among HMW Glutenin Subunit (HMW-GS)
and D-Zone ω -Gliadin Patterns and Protein Parameters^a

	HMW-GS			D-Zone ω -Gliadins	
	1A	1B	1D	1A	1B
SDS-soluble					
LPP	-0.018	-0.356***	-0.331***	0.076	-0.363***
SPP	0.084	-0.224*	-0.278***	-0.031	-0.299***
LMP	0.089	-0.115	-0.080	0.094	-0.102
SPP	0.120	-0.108	-0.005	0.102	-0.122
SDS-insoluble					
LPP	0.103	0.347***	0.045	-0.471***	0.197*
SPP	0.080	0.277***	0.057	-0.429***	0.157
LMP	0.105	0.095	-0.028	-0.223	0.027
SPP	0.080	0.053	-0.005	-0.177	0.015
TUPP	0.063	0.392***	0.186	-0.314	0.324***
LUPP	0.101	0.472***	0.189*	-0.401***	0.360***
Alb+Glo	0.088	0.007	-0.076	-0.044	-0.048
Gli	0.055	0.006	-0.059	0.152	0.046
Glu	-0.023	0.034	0.128	-0.100	0.041
ω	-0.024	0.076	0.078	0.123	0.187
HMW	-0.195	0.211	0.318***	0.007	0.372***
LMW	0.000	-0.013	0.071	-0.114	-0.044
Glut/Gli	-0.071	0.026	0.118	-0.244	-0.018

^a LPP and SPP = large and smaller polymeric proteins; LMP and SMP = large and smaller monomeric proteins; TUPP = total unextractable polymeric protein in total polymeric protein; LUPP = large unextractable polymeric protein in total large polymeric protein; Alb+Glo = albumins + globulins; Gli = gliadins; Glu = glutenins; ω = omega gliadins; HMW-GS = HMW glutenin subunits; LMW-GS = LMW glutenin subunits; Glu/Gli = glutenin/gliadin ratio.

TABLE IX
Means, Standard Deviation (SD), Regression Coefficient (*b*), and Standard Error of Regression (SE) for Glutograph Deformation Times (Glut)
and Mixograph Dough Development Times (Mix)

Cultivar	Glut				Mix			
	Mean	SD	<i>b</i>	SE	Mean	SD	<i>b</i>	SE
SW 37391	11.1	2.7	0.17	0.06	2.8	0.1	0.51	0.09
SW 37346	9.5	1.1	0.02	0.05	2.7	0.3	0.99	0.75
SW 37342	22.9	10.9	0.78	0.07	3.6	0.1	0.39	0.18
Velos	37.0	20.8	0.48	0.97	3.4	0.4	1.28	0.62
Batalj	22.5	12.6	0.87	0.20	3.8	0.2	-0.62	0.23
Dragon	26.5	13.2	0.95	0.08	3.7	0.2	0.71	0.18
Triso	31.9	15.7	1.06	0.29	3.9	0.3	1.03	0.55
Vinjett	34.2	34.5	2.48	0.32	3.6	0.2	0.75	0.52
SW 36188	59.4	44.2	3.18	0.32	4.5	0.3	1.39	0.09
SW 37281	124.0	0.2	-0.01	0.01	5.7	0.8	3.55	1.18

might not be more stable than the others. However, for these three cultivars, it was not possible to measure stability in gluten strength with the Glut. As none of the cultivars showed a stable gluten strength (measured by Glut and Mix) for different nitrogen applications, this means that all the cultivars reacted with changes in gluten strength due to changes in nitrogen application.

CONCLUSIONS

Storage protein composition, differences in total amount of HMW-GS, Glu/Gli ratio, and differences in relative amounts of SDS-soluble and SDS-insoluble PP are important factors in determining the gluten strength of a cultivar. The gluten strength of a cultivar influences the bread volume of that cultivar. Variation in nitrogen application influences protein components containing Glu and Gli, but not protein components containing Alb and Glo. Variation in protein components containing Glu and Gli leads to variation in protein concentration and bread volume. None of the cultivars investigated showed stability in gluten strength when nitrogen application was varied. This means that nitrogen application influences gluten strength as well as bread volume.

ACKNOWLEDGMENTS

We thank Waheeb Heneen for comments on the manuscript. This work was supported by the The Cerealia Foundation R&D, The Swedish Farmer's

Foundation, The VL-stiftelsen, The Royal Physiographic Society, and Svalöf Weibull AB.

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[Received February 29, 2000. Accepted August 17, 2000.]