Effect of Nitrogen Fertilizers Differing in Release Characteristics on the Quantity of Storage Proteins in Wheat

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ABSTRACT

Sodium-dodecyl sulfate polyacrylamide-gel electrophoresis and laser-scanning densitometry were used to quantify storage proteins of spring wheat (*Triticum aestivum* L.) fertilized with various granular NH_4NO_3-N fertilizers, differing in their mode and rate of N release. Kadett, Ruso, and Reno cultivars were used in field trials. Their flour had the same high molecular weight glutenin subunit composition but differed in gliadin composition. Nitrogen fertilizer application improved breadmaking quality of wheat flour, mainly by increasing the quantity of low molecular weight gliadins. However, ω-, α-, and β-gliadins also increased in Kadett. The most positive effect on flour protein concentration and loaf volume was obtained with an application of granular, dicyandiamide-regulated, slow-release N fertilizer.

The gliadins and glutenins in wheat (*Triticum aestivum* L.) flour are the major components of gluten, which determines the quality of the flour when used for various technological processes, including breadmaking (Miflin et al 1983). Considerable quantitative variation among gliadins (Huebner and Bietz 1988, Marchylo et al 1990) and glutenins (Marchylo et al 1990) can result from differences in environment. Such variation among storage proteins may have important implications in marketing and classifying wheat for technological purposes.

The dough and baking properties of wheat gluten can be influenced by nitrogen (N) fertilization strategies (Parades-Lopes et al 1985) such as manipulating gluten quantity and quality with inorganic granular or foliar N fertilizers (Doekes and Wennekes 1982, Martin et al 1992, Peltonen 1992) or with organic N fertilizers like manure (Salomonsson and Larsson-Raznikiewicz 1985). According to Pugar and Sasek (1970), the application of N fertilizer consistently led to a large increase in the gliadins, a smaller increase in the glutenin, and a slight increase in the albumins and globulins. Conversely, Jahn-Deesbach and Jürgens (1973), Shipper and Jahn-Deesbach (1981), and Doekes and Wennekes (1982) found that an increase in protein content obtained from wheat cultivars fertilized with granular N increased only the gliadin content and loaf volume; the glutenin, albumin, and globulin contents did not change. Salomonsson and Larsson-Raznikiewicz (1985) reported that both the gliadins and the glutenins were positively correlated with the grain protein concentration, but the albumin and globulin content did not change. These results contrast those of Tanaka and Bushuk (1972), who indicated that all protein fractions varied in proportion to the total protein content of the flour, with no net change in the composition of protein. These contradictory reports probably arise from the differences between the fractionation method based on the solubility of the protein and the different quantities of protein found. Therefore, Shewry et al (1986) reported that the classification of storage proteins should be based on their biological and functional properties rather than on their solubility characteristics. This classifies storage proteins into high molecular weight glutenins (HMW subunits), sulfur-poor prolamins (ω-gliadins), and sulfur-rich prolamins (low molecular weight [LMW] subunits of glutenin, α-, β-, and γ-gliadins). The HMW-subunit composition of glutenin at the *Glu-1* locus has a strong influence on rheological and breadmaking properties of bread wheats (Payne et al 1979; 1980; 1981a,b; 1987a).

Only a few reports exist on the quantitative variation of HMW-glutenin subunits and the relation of this characteristic with breadmaking quality (Huebner and Bietz 1985, Kruger et al 1988, Sutton et al 1989, Marchylo et al 1990). In turn, the effect of different sources of N fertilization on storage proteins, classified as suggested by Shewry et al (1986), was examined by Timms et al (1981), Martin et al (1992), and Peltonen (1992). According to Timms et al (1981), a very heavy application of urea (370 kg ha^{-1}) three weeks after anthesis increased the proportions of ω-gliadins and decreased those of HMW subunits. Peltonen (1992) reported that foliar application of urea (43 kg ha^{-1}) improved the baking quality of wheat flour but decreased the proportion of HMW subunits of flour storage proteins. The effect of urea on other storage proteins was not significant. Conversely, Martin et al (1992) reported that granular ammonium sulfate (NH_4SO_4) increased both HMW and LMW subunits. There is no information available on the effect of granular ammonium nitrate (NH_4NO_3) fertilization on the amount of storage proteins, particularly the HMW-glutenin subunits. For an accurate means of improving baking quality of wheat through N fertilization, the present study was undertaken to examine the effect of granular NH_4NO_3 fertilizers, differing in their mode and rate of N release, on wheat protein quality.

MATERIALS AND METHODS

Field Experiments

Three Scandinavian bread wheat cultivars were used in this study: Kadett (Sweden), Ruso (Finland), and Reno (Norway). They were selected on the basis of their commercial importance in the local wheat market of Finland. All three cultivars have the same HMW-glutenin subunit composition (subunit 1 at *Glu-A1*, subunits 7 + 9 at *Glu-B1*, and subunits 5 + 10 at *Glu-D1*), but they have considerable variation in the relative mobilities of all gliadins. The greatest variation occurs in ω-gliadins, the least in γ-gliadins; variation is moderate in α- and β-gliadins (Peltonen and Sontag-Strohm 1992). All cultivars were grown at the experimental farm of Helsinki University (1990–91) in three replicated plots of 10 m^2. Soil pH was 5.5, and it had adequate levels of P, K, Ca, and Mg for spring wheat production (Anon. 1991). The standard N fertilization (110 kg of N ha^{-1}) was selected to correspond to current usage in Finnish wheat production. Nitrogen was applied at sowing, 7 cm deep, as granular NH_4NO_3 and granular slow-release N fertilizers in which the solubility of NH_4NO_3 was partly regulated with dicyandiamide (DCD), oxamide (OA), and isobutylidene diurea (IBDU). The concentrations of slowly soluble N in the total N were 5.4, 24.2, and 28.9%, respectively. All N fertilizer products were manufactured by Kemira Oy, Finland. The control plots were not fertilized. This program yielded series of samples with increasing flour protein concentrations.

Quality Evaluation

The grain samples were combined and mixed thoroughly for quality analyses. Subsamples (2 kg) were milled with a laboratory mill (Brabender Quadrumat Senior, model Q.U.-S., Duisburg, Germany) to obtain flour of approximately 70% extraction. The Kjeldahl method (method 46-11A, AACC 1983) was used to determine the N concentration of the flour. Protein concentration

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was determined by multiplying N values by 5.7. A sample (0.2 g) was used for determining the sulfur (S) content of flour with a Leco CHNS-1000 meter (Leco Corporation, St. Joseph, MI). Dough mixing time and stability were measured with a Brabender farinograph (method 115, ICC 1982). Test baking was performed using a Panasonic bread bakery (model SD-BT2P, Matsushita Electric Co., Japan). Zawistowska et al (1988) reported that the procedure used by this bakery is analogous to the Canadian remix baking test (Irvine and McMullan 1960). The formula used for basic baking was described by Peltonen and Salovaara (1991). Quality analyses were made on duplicate treatments.

Separation and Quantification of Wheat Storage Proteins

Storage proteins were divided into four groups: 1) HMW-glutenin subunits; 2) almost entirely ω-gliadins; 3) mainly LMW-glutenin subunits, but with some α-, β- or γ-gliadins; and 4) mainly α-, β-, and γ-gliadins, but with some LMW-glutenin subunits (Fullington et al 1987). Flour was separated using a sodium-dodecyl sulfate polyacrylamide-gel electrophoresis (SDS-PAGE) sample buffer of 3% (w/v) SDS and 7.5% (v/v) 2-mercaptoethanol, as described by Payne et al (1981a). Electrophoresis was done according to the method described by Laemmli (1970), using a vertical gel of 180 × 160 × 1.5 mm and acrylamide concentration of 14% (w/v), containing 0.3% (w/v) 1*M* Tris-HCl (pH 8.8) and 0.1% (w/v) SDS. Although the subunits can be recognized visually as separate bands using a 7.5 or 10% acrylamide gel (Lawrence 1986), the resolution and accurate quantification for subunits 9 and 10 was improved by using a 14% acrylamide gel. The gels were stained with a solution of 0.05% (w/v) Coomassie Brilliant Blue R-250, 5% (v/v) ethanol, 12.05% (w/v) trichloroacetic acid, and 75% (v/v) water for 72 hr. They were then destained in 10% (v/v) acetic acid, which was changed three or four times over a two-day period (Payne et al 1979).

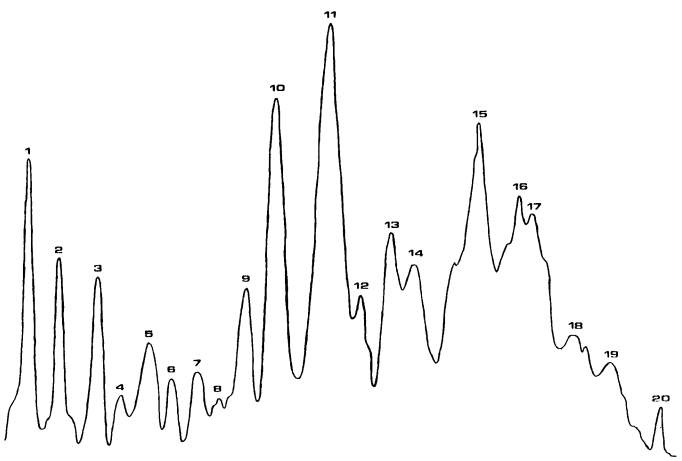

Fig. 1. Densitometric readings of total prolamins from spring wheat (cv. Ruso). High molecular weight glutenin subunits (1–5), ω-gliadins (6–8), low molecular weight glutenin subunits (9–15), and α- and β-gliadins (16–20).

The densitometric analysis of the storage proteins was done using a LKB UltroScan XL laser densitometer (model 2222-020, Pharmacia LKB Biotechnology, Sweden) in combination with GelScan XL software. Each lane was scanned twice, using different tracks of a duplicate gel. The coefficient of variation (CV) of the staining intensity was 16.9% for HMW-glutenin subunits, 23.8% for ω-gliadins, 19.4% for LMW-glutenin subunits, and 9.3% for α- and β-gliadins (n = 60) for lanes between gels. Figure 1 shows typical densitometric readings. The quantity of protein present in a particular band (x_i) was obtained by multiplying the relative concentration of the flour gluten by the subunit (% w/w, db) as described previously by Branlard and Dardevet (1985). Gluten content was determined using method 137 of the ICC (1982). Calculations were made assuming that 70% of the gluten formed consisted of glutenins and gliadins (Tatham and Shewry 1985).

Statistical Analyses

The data were analysed using ANOVA and correlation analysis (MSTAT 1989). Significantly different means were separated using Duncan's multiple range test.

RESULTS

Nitrogen Fertilization and Baking Quality

Fertilization of the wheat cultivars with N resulted in more flour protein, improved loaf volumes, and significantly increased LMW-glutenin subunits, as a consequence of increased glutenin-to-gliadin ratio, when compared with controls (Table I). Of the slow-release N fertilizers, DCD fertilizer application resulted in significantly higher flour protein concentration (12.8%) and loaf volume (1,927 ml). DCD fertilizer promoted an increase in flour protein of 2.6% and in loaf volume of 204 ml, compared with that of the control. SN fertilizer application resulted in the same flour protein content as that of DCD fertilizer, but it gave significantly lower bread volume (1,918 ml). IBDU fertilizer increased protein concentration by 2.2% and loaf volume by 175 ml, which was less than that of all other granular N fertilizers. The concentration of slowly soluble N was highest with IBDU fertilizer, which may greatly restrict the N solubility and thus reduce plant N uptake efficiency and breadmaking quality. The solubility properties of granular N fertilizers (SN, DCD, OA, and IBDU) did not, however, change the N-S ratio, HMW-glutenin subunit content, nor the amount of α- and β-gliadins.

Baking Quality of Wheat Cultivars

The flour protein concentration and dough mixing time were higher for Ruso than for Reno; Kadett was intermediate (Table II). For Ruso, the loaf volume was 134–185 ml higher, the N-S ratio was 0.8–1.0% lower, and the total amount of HMW-glutenin

TABLE I
Effect of Granular N Fertilizer on Baking Properties of Spring Wheat[a]

N Fertilization Treatment[b]	Protein Content (%)	Loaf Volume (ml)	Nitrogen-Sulfur Ratio	Prolamins (g) in 100 g of Flour				Glutenin to Gliadin Ratio
				HMW-[c] Glutenin Subunits	ω-Gliadins	LMW-[d] Glutenin Subunits	α- and β-Gliadins	
Control	10.2 d	1723 d	9.5 a	1.99 a	1.21 a	9.04 b	4.11 a	2.07 b
SN	12.6 bc	1918 b	10.2 a	2.63 a	1.20 a	12.82 a	4.71 a	2.61 a
IBDU	12.4 c	1898 c	10.1 a	2.55 a	1.26 a	12.84 a	4.99 a	2.46 a
OA	12.5 c	1920 b	9.6 a	2.71 a	1.28 a	13.48 a	4.59 a	2.76 a
DCD	12.8 ab	1927 a	9.7 a	2.67 a	1.35 a	13.30 a	4.84 a	2.58 a

[a] Means of two years and three cultivars. Within columns, means followed by the same letter are not significantly different at $P = 0.05$, according to Duncan's multiple range test. Readings are the mean of 18 replicates.
[b] Control = 0 kg of N ha^{-1}; SN = 110 kg of N ha^{-1}, no inhibitor N; IBDU = slow-release N as isobutylidene diurea; OA = oxamide; DCD = dicyadiamide.
[c] High molecular weight.
[d] Low molecular weight.

subunits was 0.81–0.87 g higher in 100 g of flour, than they were for Kadett and Reno. All three cultivars investigated had similar mean quantities of ω-gliadins. Reno had a significantly higher quantity of LMW-glutenin subunits, and thus also a higher glutenin-to-gliadin ratio than that of Kadett. Conversely, the mixing stability and the amount of α- and β-gliadins were higher in Kadett and lower in Reno; Ruso was intermediate in these properties.

There was no significant correlation between baking quality properties and the quantity of HMW-glutenin subunits in the three cultivars (Table III). In Kadett, the quantity of ω-gliadins was significantly positively correlated with dough mixing time and loaf volume; the flour protein concentration and dough mixing stability were strongly correlated with the increase in LMW-glutenin subunits. In addition, the amount of α- and β- gliadins in Kadett were correlated positively with flour protein concentration. The baking properties of Ruso and Reno increased with N fertilization, probably only because of the quantitative changes in LMW-glutenin subunits.

Although the effect of N fertilization on total content of HMW-glutenin subunits was poor in this study, the improved bread-making quality after N application was related to the variation

TABLE II
Statistical Summary of Baking Properties Data from Spring Wheat Cultivars[a]

Cultivar	Protein Content (%)	Mixing Time (min)	Mixing Stability (min)	Loaf Volume (ml)	Nitrogen-Sulfur Ratio	Prolamins (g) in 100 g of Flour				Glutenin to Gliadin Ratio
						HMW-[b] Glutenin Subunits	ω-Gliadins	LMW-[c] Glutenin Subunits	α- and β-Gliadins	
Kadett	11.5 ab	3.09 ab	4.46 a	1941 a	10.2 a	2.08 b	1.16 a	15.26 b	8.47 a	1.80 c
Ruso	11.9 a	3.50 a	4.10 ab	1756 b	9.2 b	2.89 a	1.19 a	17.39 ab	6.94 b	2.49 b
Reno	11.2 b	2.88 b	3.86 b	1890 a	10.0 a	2.02 b	1.13 a	20.06 a	4.52 c	3.91 a

[a] Means of two years and five N fertilizer treatments. Within columns, means followed by the same letter are not significantly different at $P = 0.05$, according to Duncan's multiple range test. Readings are the mean of 30 replicates.
[b] High molecular weight.
[c] Low molecular weight.

TABLE III
Correlation Coefficients Between Baking Properties and the Content of Storage Protein Groups in Nonfertilized and N-Fertilized[a] Wheat Cultivars

Cultivar[a] and Quality Trait	HMW-[b]Glutenin Subunits	ω-Gliadins	LMW-[c]Glutenin Subunits	α- and β-Gliadins
Kadett				
Protein content	0.08	0.35	0.87*** [d]	0.95***
Mixing time	0.41	0.87***	0.64*	0.61
Mixing stability	−0.25	−0.37	0.54	0.49
Loaf volume	0.39	0.92***	0.34	0.46
Ruso				
Protein content	0.54	0.17	0.88***	0.53
Mixing time	0.60	−0.04	0.86***	0.40
Mixing stability	0.34	−0.26	0.82**	0.36
Loaf volume	0.60	0.11	0.69*	0.24
Reno				
Protein content	0.31	−0.31	0.88***	−0.25
Mixing time	0.41	−0.30	0.81**	−0.24
Mixing stability	−0.27	−0.47	0.77**	−0.56
Loaf volume	0.47	−0.41	0.67*	−0.33

[a] $n = 10$.
[b] High molecular weight.
[c] Low molecular weight.
[d] *, **, *** are significantly different at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

TABLE IV
Correlation Coefficients Between Baking Properties and the Content of the High Molecular Weight Glutenin Subunits in Nonfertilized and N-Fertilized Wheat Cultivars

Cultivar[a] and Quality Trait	*Glu-A1* (1)	*Glu-B1* (7 + 9)	*Glu-D1* (5 + 10)
Kadett			
Protein content	0.39	0.56	0.36
Mixing time	0.20	0.75** [b]	0.16
Mixing stability	0.61*	0.02	0.03
Loaf volume	0.06	0.61*	0.14
Ruso			
Protein content	0.82**	0.59	0.66*
Mixing time	0.78**	0.63*	0.59
Mixing stability	0.61*	0.45	0.26
Loaf volume	0.61*	0.91***	0.36
Reno			
Protein content	0.92***	0.53	0.66*
Mixing time	0.92***	0.49	0.59
Mixing stability	0.73*	0.40	0.26
Loaf volume	0.84***	0.42	0.36

[a] $n = 10$.
[b] *, **, *** and significantly different at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

in the quantity of HMW-glutenin subunits (Table IV). In Ruso and Reno, an increase in the quantity of *Glu-A1* (subunit 1) correlated strongly with an increase in flour protein concentration, dough mixing time, mixing stability, and loaf volume; in Kadett, however, it correlated strongly only with mixing stability. The *Glu-B1* subunits (7 and 9) correlated significantly with mixing stability and loaf volume in Kadett and Ruso, and the quantity of *Glu-D1* subunits (5 and 10) was associated with high flour protein concentration in Ruso and Reno.

DISCUSSION

There is a risk that application of high levels of a nitrogenous fertilizer (NH_4NO_3-N), in the absence of sulfur fertilization, may lead to a change in the balance between available N and available S, such that the available S level becomes insufficient for normal grain development (Timms et al 1981, Skerritt et al 1988). Under S-deficient growing conditions, ω-gliadins increased (Timms et al 1981, Skerritt et al 1988), leading to a drop in baking quality (Moss et al 1981). Byers et al (1987) showed that an N-S ratio in excess of 17:1 lowered breadmaking quality. In this study, N fertilization did not significantly influence the N-S ratio, which ranged between 9.2:1 and 10.2:1. Therefore, the effect of N fertilization on baking quality characteristics was not dependent on the limiting S status of flour, but protein composition is affected by NH_4NO_3-N fertilization.

The improvements in breadmaking quality (flour protein concentration, dough properties, and loaf volume) obtained by NH_4NO_3-N fertilization were due to increases in the glutenin-to-gliadin ratio of wheat gluten, which is in agreement with the results of Martin et al (1992), who found that the increase in the glutenin-to-gliadin ratio was due to an increased quantity of HMW and LMW subunits. In contrast, our study indicated that the positive effect of N fertilization on baking quality was strongly associated with a high quantity of LMW-glutenin subunits. The effect of N application on HMW-glutenin subunits was not significant. These results contrast with those of Doekes and Wennekes (1982), who showed that the glutenin-to-gliadin ratio of gluten decreased with an increase in loaf volume.

Studies of Cressey et al (1987), Grama et al (1987), and Sutton et al (1989) did not establish any variation in glutenin-to-gliadin ratio between cultivars. In contrast, MacRitchie (1989) and Martin et al (1992) found that a cultivar with strong dough properties and good baking performance was associated with a high glutenin-to-gliadin ratio. In this study, the glutenin-to-gliadin ratio seems to have the most genotypic variability. Kadett and Reno had very different glutenin-to-gliadin ratios because of differences in the quantities of LMW-glutenin subunits and α- and β-gliadins. This, however, resulted only in differences in dough mixing properties; loaf volumes were similar. Conversely, the strong gluten characteristics (as described by Moss et al 1981, Fullington et al 1987, Lawrence et al 1988), long dough mixing time requirement, high quantity of HMW-glutenin subunits, and low N-S ratio in Ruso led to poor baking performance. We conclude that the optimum glutenin-to-gliadin ratio of a cultivar for breadmaking may depend absolutely on the particular type of wheat used, the type of bread made, and the process used. Therefore, as in previous publications (Doekes and Wennekes 1982, Cressey et al 1987, Grama et al 1987, MacRitchie 1989, Sutton et al 1989, Martin et al 1992), no apparent optimum glutenin-to-gliadin ratio for breadmaking was established. However, ratios can easily be manipulated through N fertilization strategies to become more optimal for baking, as shown by this and other studies (Martin et al 1992, Peltonen 1992). In this study, the best response to NH_4NO_3-N was obtained with DCD fertilizer, which led to high flour protein concentration and large loaf volume. Similar results were reported by Rodgers and Ashworth (1982), indicating that N uptake by wheat plants was increased using a DCD inhibitor of N fertilizer.

There may be changes in the types and groups of gliadins and glutenins between cultivars and N treatments (Timms et al 1981). We also recorded genotypic differences in response to NH_4NO_3-N fertilization. The improvements in baking characteristics of the spring wheat cultivars after N application were related to the quantitative changes in LMW-glutenin subunits and also to an increase in ω-, and α-, and β-gliadins in Kadett. This difference between cultivar response to N fertilization is not surprising, because Peltonen and Sontag-Strohm (1992) demonstrated varietal differences between these cultivars regarding ω-, α-, and β-gliadins. Sozinov and Poperelya (1984) showed how different allelic variants of gliadin were related to breadmaking quality. Payne et al (1987b) demonstrated that the differences in baking quality of genotypes (differing in the *Glu-A1* locus) primarily stemmed from the LMW-glutenin subunits. Huebner and Bietz (1988) suggested that either weather or other environmental factors during kernel development can generate quantitative variation in gliadin synthesis. According to Marchylo et al (1990), the effect of environment was strongest on ω-gliadins and weakest on HMW-glutenin subunits. Although granular N fertilization did not change total quantity of HMW-glutenin subunits, N applications caused clear quantitative variation within HMW-glutenin subunits, mainly in *Glu-A1* subunit 1. The quantity of subunit 1 had a strong positive influence on flour protein concentration, dough mixing properties, and loaf volume in Ruso and Reno.

In conclusion, these results reveal the complexity of wheat proteins after N fertilization and explain the variation in protein composition and wheat baking quality within cultivars. The results of this study indicate that knowledge of the effect of N fertilization on glutenin and gliadin characteristics may have practical applications for improving technological quality of wheat flour. However, only three cultivars were examined in detail, and all had similar HMW-glutenin subunit compositions. Therefore, studies on the effects of N fertilization on the amount of storage proteins with emphasis on the other HMW-glutenin subunit combinations are needed.

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