

# Influences of cultivar, cultivation year and fertilizer rate on amount of protein groups and amount and size distribution of mono- and polymeric proteins in wheat

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## SUMMARY

Influences of cultivar and environment, i.e. cultivation year and fertilizer rate, on amount of protein groups and amount and size distribution of mono- and polymeric proteins, were investigated in four sets of wheat (*Triticum aestivum* L.). The cultivars were chosen in order to obtain a high range of variation in protein concentration and gluten strength. Environmental influences on protein concentration and gluten strength were investigated, as well as relations between variation in protein concentration and gluten strength and variation in protein groups and amount and size distribution of mono- and polymeric proteins.

The results showed that cultivar and environmental influences giving rise to variation in protein concentration also gave rise to variation in most of the investigated protein components. Protein concentration was significantly positively correlated to the total amounts of glutenins and gliadins and amounts of most mono- and polymeric proteins. However, the correlation with the amount of gliadins and sodium dodecyl sulphate (SDS)-soluble mono- and polymeric proteins were often higher than the correlation to the glutenins and the SDS-insoluble mono- and polymeric proteins. Cultivar influences giving rise to variation in gluten strength were found to influence the relation between SDS-soluble and -insoluble polymeric proteins, leading to a significant positive correlation between the gluten strength and the percentage of total unextractable polymeric protein (TUPP) in the total polymeric protein and large unextractable polymeric protein (LUPP) in the total large polymeric protein. Environmental variation in gluten strength was found to be significantly positively correlated to SDS-insoluble proteins and negatively correlated to SDS-soluble proteins. This also led to a significant positive correlation with the percentage of LUPP and/or TUPP.

## INTRODUCTION

Both genotype and environment influence gluten strength and bread-making quality of wheat (Peltonen 1992; Peterson *et al.* 1992; Johansson & Svensson 1998, 1999; Mladenov *et al.* 2001). Many have studied the genetic background for variation in gluten strength and bread-making quality. Most well known are the established correlations between particular proteins and protein subunits and bread-making quality (Payne *et al.* 1983, 1987; Sontag *et al.* 1986; Lawrence *et al.* 1987; Uhlen 1990; Johansson *et al.*

1993, 1994; Johansson & Svensson 1995; Johansson 1996). Furthermore, genetically determined variation in protein concentration (Finney & Barmore 1948) and ratios of different protein compounds influence the bread-making quality (Field *et al.* 1983; Sutton 1991; Gupta 1994; Wieser *et al.* 1994; Johansson *et al.* 2001).

The environment does not influence the specific composition of wheat grain proteins. Instead it influences protein concentration (Sosulski *et al.* 1963; Benzian *et al.* 1983; Stapper & Fischer 1990; McDonald 1992), and the amount of different protein groups and amount and size distribution of polymeric proteins (Graybosch *et al.* 1996; Johansson *et al.* 2001, 2002). Environmental influences can be of different types and various effects on proteins have been

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reported depending on the type of environmental influence, e.g. variation in fertilizer rate influences the amounts of different protein groups (Wieser & Seilmeier 1998; Johansson *et al.* 2001), while weather variations influence the amount and size distribution of polymeric proteins (Graybosch *et al.* 1995; Zhu & Khan 2001; Johansson *et al.* 2002).

For wheat growers, and the milling and baking industries, it is of high importance to have wheat that is not only of good quality but also of even quality independent of cultivation environment. The wheat grain proteins are one of the factors of highest importance in determining bread-making quality (Wall 1979). In order to be able to breed for and grow wheat of high and even quality, it is important to know what cultivar and environmental variation creates which protein characteristics. In Sweden it has been shown that one of the environmental factors leading to the highest variation in gluten strength is cultivation year (Johansson & Svensson 1998, 1999). One smaller study of four Swedish wheat cultivars grown during 3 years showed that the background for seasonal variation in bread-making quality might be variation in amount and size distribution of polymeric proteins (Johansson *et al.* 2002). Further studies on combined influences of cultivar, year and fertilizer rate have not been carried out.

Thus, the aim of the present investigation was to study the influence of the cultivar and environment, i.e. cultivation year and fertilizer rate, on the amounts of different protein components. The objective was to gain a better understanding of the influences of different cultivars and environmental factors on amounts of different protein components in order to better understand the genetically and environmentally determined biochemical variation in bread-making quality. A better understanding of the biochemical background for variation in quality will increase the possibilities for growing and breeding wheat cultivars with a good and even quality.

## MATERIALS AND METHODS

### *Plant materials*

Four different sets of wheat cultivars were used in the present study. The cultivars within set A were chosen according to the variation in protein concentration between the cultivars and consisted of four spring wheat cultivars, Sport (around 0.18 protein), Dacke (around 0.14 protein), Dragon (around 0.12 protein) and Thasos (around 0.12 protein). Sport, Dacke and Dragon contain HMW glutenin subunits 2+12, and Thasos subunits 5+10. Set B, chosen in order to get variation in gluten strength (Johansson *et al.* 2001), comprised seven spring wheat cultivars, of which four contained HMW glutenin subunits 2+12 (Dragon, SW 37342, SW 37346 and SW 37391) and

three contained HMW glutenin subunits 5+10 (Triso, Vinjett, SW 37281). Set C contained two spring wheat cultivars Dragon (containing HMW glutenin subunits 2+12) and Vinjett (containing HMW glutenin subunits 5+10). Set D contained two winter wheat cultivars (Kosack with HMW glutenin subunits 2+12, and Tarso with HMW glutenin subunits 5+10). The grains and data from sets A, B and C were collected from combined fertilizer and cultivar trials at Weibullsholm, Landskrona, Sweden. Set D was collected from trials at different locations in Sweden (Weibullsholm, Bjertorp, K lback and Haga). The cultivars in sets B to D have normal protein concentrations during field cultivation, ranging from 0.11–0.14. Data were collected from 1991 and 1994 to 1996 for set A, 1998 to 2000 for set B, 1996 to 2000 for set C and 1998 and 1999 for set D. The sets were sown with four replications and a plot size of 16 m<sup>2</sup> each.

### *Fertilizer rates*

Four fertilizer rates were applied. During the years 1991, 1994 and 1995, the fertilizer rates were 0, 70, 70+70 (70 kg N/ha at sowing and 70 before heading) and 140 kg N/ha. In 1996, 0, 70, 140 and 190 kg N were applied. On sets B and C the fertilizer rates applied were 55, 75 (55 kg N/ha at sowing and 20 kg before heading), 155 (55 kg N/ha at sowing and 100 kg before heading) and 215 kg N/ha (55 kg N/ha at sowing, 100 kg after emergence and 60 kg before heading). For set D the fertilizer rates were 150, 75+75 (75 kg N/ha in early spring and 75 kg before heading), 75+75+40 (75 kg N/ha in early spring, 75 kg in later spring and 40 kg before heading) and 150+40 kg N/ha (150 kg N/ha in early spring and 40 kg before heading).

### *Methods*

Protein concentration was determined on a dry weight basis by near-infrared reflectance spectroscopy (calibrated with Kjeldahl analyses; Johansson & Svensson 1995).

Gluten strength was measured by using the glutograph dough deformation time and mixograph dough development times. The mixograph tests were carried out according to the AACC method 54-40A (American Association of Cereal Chemists 1983). For the glutograph test, gluten was analysed on a Brabender Glutograph (Sietz 1987). Falling numbers were determined according to standard ICC method (ICC 1968). Baking tests were carried out at the Cereal Laboratory, Sval v, Sweden applying the Farinograph treater (Thor n 1981; Johansson 1989).

For analysis of the amount and size distribution of polymeric proteins, the size exclusion–high performance liquid chromatography (SE–HPLC) method with two-step extraction procedure developed by

Gupta *et al.* (1993) was applied. The first step in this method extracts the proteins soluble in dilute sodium dodecyl sulphate (SDS), whilst the second extract contains proteins soluble only after sonication. For the first extraction 11 mg of white flour was suspended in 1.0 ml 0.5% SDS-phosphate buffer (pH 6.9) and vortexed for 10 s. Samples were then stirred for 5 min at 2000 rpm and centrifuged for 30 min at 10 000 G to obtain the supernatant protein. The pellet was subsequently resuspended in SDS buffer as above and sonicated in an ultrasonic disintegrator (Soniprep 150, Tamro, Mölndal, Sweden) for 30 s, amplitude 5, fitted with a 3 mm exponential microtip. The samples were then centrifuged (30 min, 10 000 G) to obtain a supernatant of proteins. The extracts were filtered through 0.45 µm filters (Millipore, Durapore Membrane Filters) before being run on the HPLC. SE-HPLC analyses were performed on a Varian HPLC system using a BIOSEP SEC-4000 Phenomenex column. Separation was achieved in 30 min by loading 20 µl 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. The SE-HPLC chromatograms were divided into four parts. The different parts contained the protein types: large polymeric proteins (LPP), smaller polymeric proteins (SPP), large monomeric proteins (LMP) and smaller monomeric proteins (SMP) (Kuktaite *et al.* 2000; Johansson *et al.* 2001). Areas of the different parts were calculated. The percentage of total unextractable polymeric protein (TUPP) in the total polymeric protein and large unextractable polymeric protein (LUPP) in the total large polymeric protein were calculated (Gupta *et al.* 1993; Johansson *et al.* 2001).

To analyse the amounts of protein groups, reversed phase (RP)-HPLC analyses were used. The extraction procedure of proteins for RP-HPLC analysis was the one developed by Wieser & Seilmier (1998). In this procedure, the proteins are extracted stepwise in order to extract (1) albumins and globulins, (2) gliadins and (3) glutenin subunits.

RP-HPLC analyses were carried out according to Andrews *et al.* (1994) on a Varian HPLC system using a Supelcosil LC-308 column with 300 Å pore size, 5 µm particle size, 250 × 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 (albumins and globulins) were analysed 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 (gliadins and glutenins, respectively) was 28–56% solvent B from 1 to 30 min (Wieser & Seilmier 1998).

For the SE-HPLC and RP-HPLC analyses, at least two replicates were analysed.

#### Statistical analyses

Statistical Analysis System (SAS Institute, Cary, NC 1985) procedures and programs were used for data analysis. Evaluation of the data was carried out using Spearman rank correlations and analyses of variance (ANOVA; SAS 1985). ANOVA were calculated considering each cultivation year, and fertilizer rate as a separate environment and a random variable. Wheat sets were considered as a fixed variable.

## RESULTS

### Set A

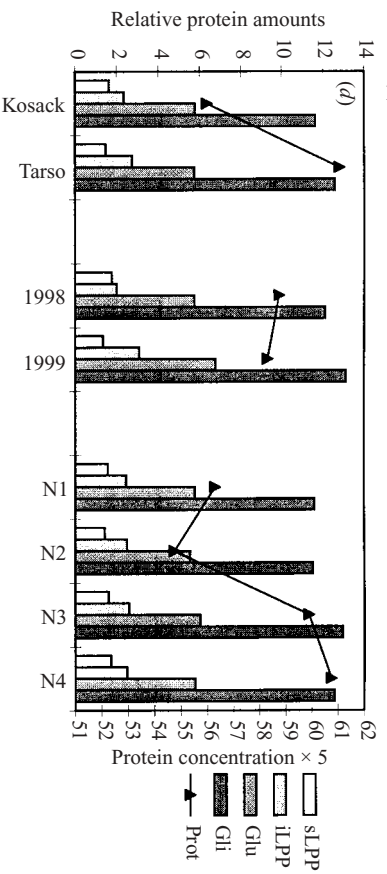
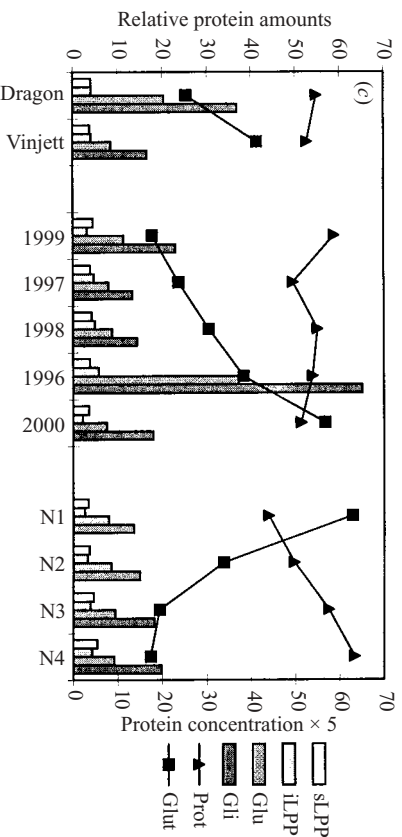
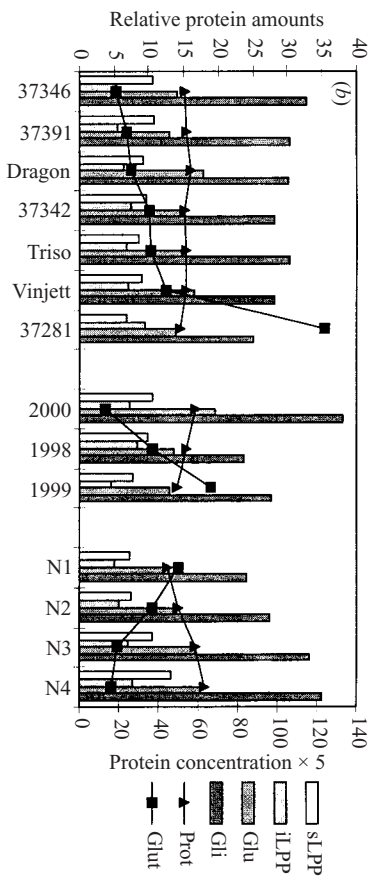
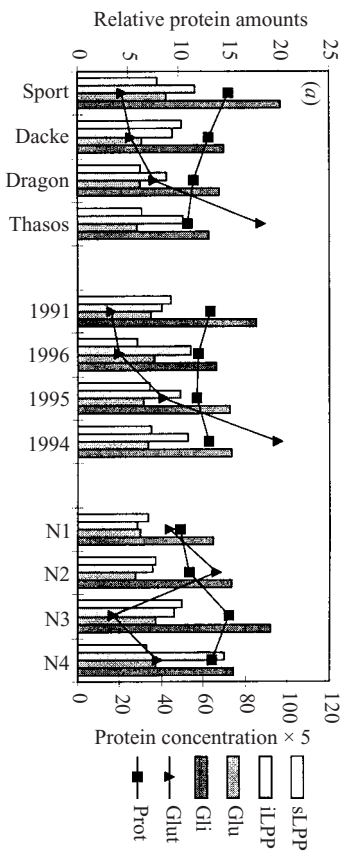
The protein concentration was found to vary significantly due to cultivar ( $P < 0.005$ ), year ( $P < 0.01$ ) and fertilizer rate ( $P < 0.005$ ). The increase in protein concentration in a cultivar as a result of increased fertilizer rate was similar for the different cultivars (results not shown). The gluten strength, measured by glutograph dough deformation time, varied significantly due to variation in cultivar ( $P < 0.01$ ) and year ( $P < 0.05$ ).

Significant influences of the cultivar and fertilizer rate on the amount of the different protein groups, albumins and globulins, gliadins, HMW- and LMW glutenins, were found ( $P < 0.005$ ). Variation in protein concentration due to cultivar or fertilizer rate was significantly positively correlated ( $P < 0.005$ ) to total amounts of gliadins and glutenins (Fig. 1*a*). Gluten strength, measured by glutograph dough deformation times, was negatively correlated ( $P < 0.005$ ) with amounts of gliadins but positively correlated ( $P < 0.005$ ) with amounts of glutenins (results not shown). The variation in year leading to variation in gluten strength was not significantly correlated to variation in amounts of the measured protein groups (Fig. 1*a*).

Variation in cultivar, year and fertilizer rate influenced the amount and size distribution of most mono (SDS-soluble and -insoluble LMP and SMP-) and polymeric (SDS-soluble and -insoluble LPP and SPP) proteins ( $P < 0.05$ – $0.005$ ). The variation in cultivar and year also influenced the TUPP and LUPP ( $P < 0.005$ ). Increased protein concentration due either to cultivar or fertilizer rate led to increased amount of SDS-soluble and -insoluble LPP ( $P < 0.05$ – $0.005$ ; Fig. 1*a*). Variation in year leading to variation in gluten strength between years was significantly positively correlated with LUPP ( $P < 0.01$ ; Fig. 2*a*).

### Set B

The protein concentration was found to vary due to variation in year and fertilizer rate ( $P < 0.005$ ). Glutograph dough deformation time, falling number and



bread volume was found to vary due to variation in cultivar, year and fertilizer rate ( $P < 0.005$ ).

Variation in cultivar, year and fertilizer rate were found to influence the amounts of most protein groups ( $P < 0.05-0.005$ ). Variation in cultivar was found to influence the variation in glutenin/gliadin ratio ( $P < 0.005$ ). Variation in year was the only source influencing the variation in albumins and globulins ( $P < 0.005$ ). A significant negative correlation ( $P < 0.005$ ) was found between gluten strength of cultivar and the total amount of gliadins (Fig. 1*b*), while a significant positive correlation ( $P < 0.005$ ) was found between cultivar strength and the glutenin/gliadin ratio (Fig. 2*b*). The years, ranked according to gluten strength measured by glutograph dough development time, were negatively correlated ( $P < 0.005$ ) to total amounts of gliadins and glutenins (Fig. 1*b*). However, the ranking of years according to gluten strength also gave a ranking with a decreasing protein concentration and falling number (results not shown) over years. Fertilizer rate gave a positive correlation ( $P < 0.01-0.005$ ) with the total amount of gliadins and glutenins (Fig. 1*b*). Increased fertilizer rates led to a similar increase of all types of gliadin and glutenin proteins (Fig. 3).

Variation in cultivar year and fertilizer rate influenced the amount and size distribution of many mono- and polymeric proteins and percentage of LUPP and TUPP ( $P < 0.01-0.005$ ). Cultivar strength was negatively correlated with SDS-soluble LPP ( $P < 0.05$ ; Fig. 1*b*), but positively correlated with SDS-insoluble LPP, LUPP and TUPP ( $P < 0.01-0.005$ ; Fig. 2*b*). The years, ranked according to increased gluten strength and decreased proteins concentration, were negatively correlated with SDS-soluble and -insoluble LPP ( $P < 0.005$ ; Fig. 1*b*) and percentage of LUPP ( $P < 0.01$ ; Fig. 2*b*). The years were positively correlated with the percentage of TUPP ( $P < 0.05$ ; Fig. 2*b*). Fertilizer rate was positively correlated to protein concentration and amount of LPP ( $P < 0.005$ ; Fig. 1*b*). The percentage of LUPP and TUPP were not influenced by the fertilizer rate (Fig. 2*b*).

#### Set C

Variation in cultivar, year and fertilizer rate were found to influence the protein concentration and glutograph dough deformation times ( $P < 0.005$ ).

Variation in year and fertilizer rate influenced the variation of the amounts of most protein groups and also the ratio of glutenins/gliadins ( $P < 0.005$ ), while variation in cultivar did not. Increased fertilizer rate

led to increased protein concentration and a positive correlation with amounts of gliadins and glutenins ( $P < 0.005$ ; Fig. 1*c*). Increased protein concentration was negatively correlated with the glutenin/gliadin ratio ( $P < 0.01$ ; Fig. 2*c*).

Variation in year and fertilizer rate influenced the amount and size distribution of most mono- and polymeric proteins ( $P < 0.01-0.005$ ), while the variation in cultivar affected only a few of them. The variation in year also influenced the percentage of TUPP ( $P < 0.005$ ). Years ranked according to gluten strength were found to correlate positively and significantly with percentage of TUPP ( $P < 0.005$ ; Fig. 2*c*). Increased fertilizer rates led to an increase in protein concentration and positive correlations with amounts of LPP ( $P < 0.005$ ; Fig. 1*c*).

#### Set D

Variation in cultivar, year and fertilizer rate influenced the protein concentration ( $P < 0.005$ ).

Variation in cultivar influenced the amounts of albumins, globulins and gliadins, variation in cultivation year influenced the amounts of all protein groups, and fertilizer rate influenced the amount of gliadins ( $P < 0.01-0.005$ ). Increased protein concentration due either to fertilizer rate or cultivar led to an increase in gliadins and glutenins ( $P < 0.005$ ; Fig. 1*d*).

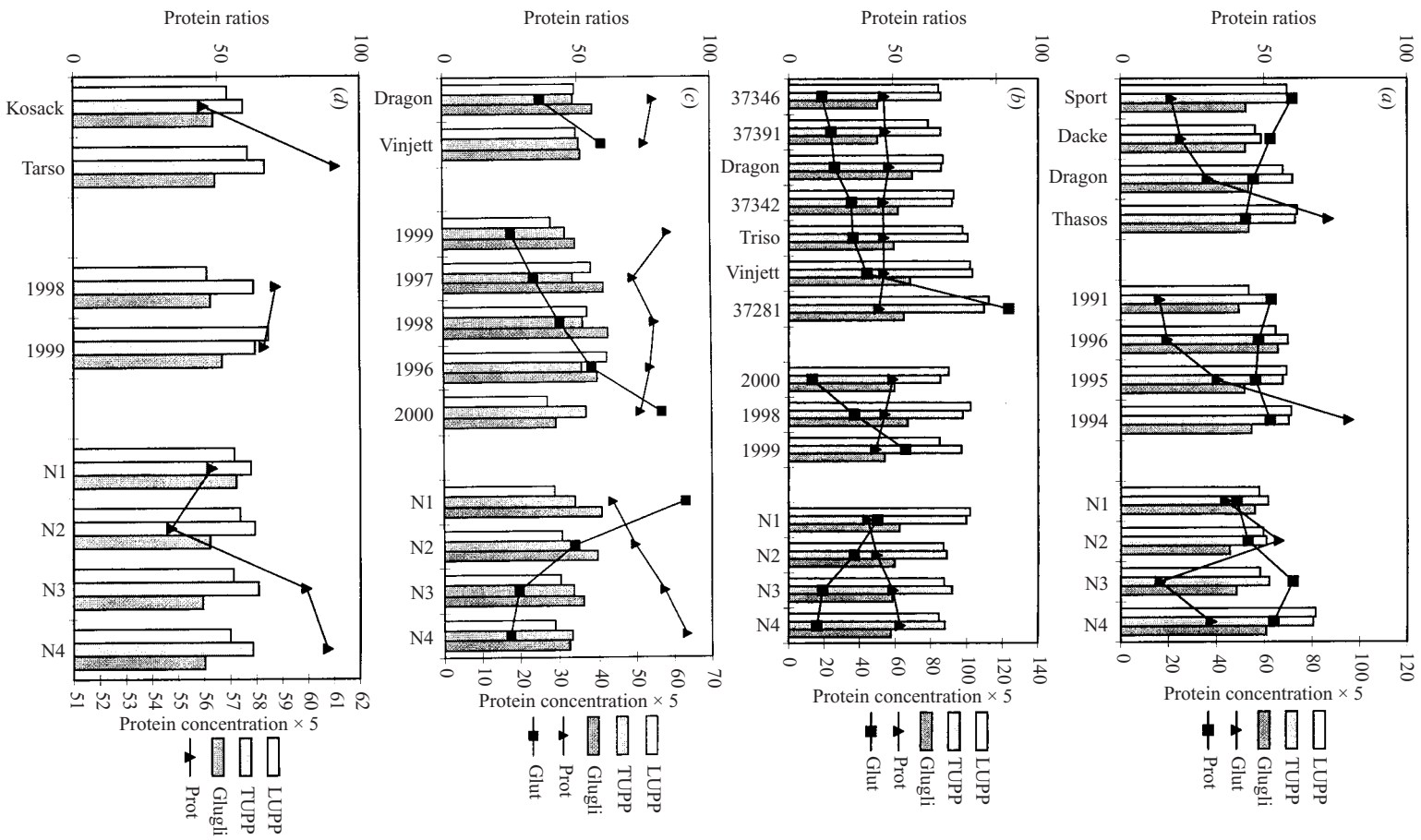
Variation in cultivar influenced the amount of most of the mono- and polymeric proteins ( $P < 0.05-0.005$ ), with the cultivar with the highest protein concentration having the highest amounts of SDS-insoluble LPP, and percentage of LUPP and TUPP ( $P < 0.05-0.005$ ; Figs 1*d* and 2*d*). The year also influenced the amount of mono- and polymeric proteins ( $P < 0.005$ ).

## DISCUSSION

In earlier investigations it has been shown that (1) the quantity of specific glutenin protein fractions measured by RP-HPLC correlates with baking performance in wheat cultivars from New Zealand and overseas (Sutton *et al.* 1990); (2) the quantity of total HMW and LMW glutenin subunits measured by RP-HPLC correlate with dough properties (Andrews *et al.* 1994); (3) the quantity of HMW protein amounts determined by ELISA correlate with rheological parameters, such as strength and extensibility (Skerritt 1991); and (4) the proteins that are most difficult to extract are of high importance for determining the gluten strength (Huebner & Wall 1976).

Fig. 1. Variation of amount of SDS-soluble large polymeric proteins (sLPP), SDS-insoluble large polymeric proteins (iLPP), glutenins (Glu), gliadins (Gli), glutograph dough development time (Glut) and  $5 \times$  protein concentration (Prot) in different cultivars, years and with different fertilizer rates (N1–N4 = increasing N). (a) wheat set A; (b) wheat set B; (c) wheat set C; (d) wheat set D.





The influences of genotype and environment on bread-making quality have also been reported (e.g. Peterson *et al.* 1992). However, only few investigations (e.g. Graybosch *et al.* 1995, 1996; Zhu & Khan 2001), have tried to understand the biochemical background on a protein level for the variation in bread-making quality determined by genotype and different types of environments. The influences of different sources on different protein factors leading to different types of variations are discussed below.

### Cultivar

Both variation in gluten strength and protein concentration were found in the investigated wheat material, especially in sets A and B, in which the wheat cultivars were chosen in order to get as much variation as possible. In set A, the protein concentration ranged from 8.0, in the cultivar Thasos grown with the lowest fertilizer rate in the year 1995, to 17.8, in the cultivar Sport grown with the highest fertilizer rate in the year 1991. In set B, the gluten strength measured by glutograph dough deformation time ranged from 6.9, in SW 37346 grown with the highest fertilizer rate in the year 2000, to 124.2, in 37281 with low fertilizer rates in all 3 years. The ranking of cultivars according to protein concentration and gluten strength in the present study was in accordance with earlier findings of protein concentration and gluten strength in these cultivars (Johansson *et al.* 2001, 2002).

Variation in total amounts of protein groups and amount and size distribution of polymeric proteins were found due to variation in cultivar. A significant positive correlation was found between the amount of most protein groups as well as of amounts of mono- and polymeric proteins and wheat cultivars ranged in order of increasing protein concentration. This is in accordance with findings by Wieser & Seilmeier (1998), showing increases of amounts of gliadins and glutenins when the protein concentration is increased. Variation in protein concentration between cultivars is thereby explained by differences in all protein parameters containing glutenins and gliadins. The background for variation in protein concentration giving rise to differences in bread-making quality (Finney & Barmore 1948) might thus be due to variation in amounts of all protein parameters containing glutenins and gliadins.

Increased gluten strength in the cultivars was positively correlated to an increase in total amounts of glutenins and a decrease in gliadins, leading to an

increase in the glutenin/gliadin ratio. Similar findings have been reported by other authors (e.g. Andrews *et al.* 1994). Also an increase in SDS-insoluble LPP and SPP and a decrease in SDS-soluble LPP and SPP, leading to an increase in LUPP and TUPP, were found. This is in accordance with the findings in Johansson *et al.* (2001) and also with similar findings by other authors (Gupta *et al.* 1993). Variation in gluten strength between cultivars has mainly been reported to be due to differences in specific proteins and protein subunits (Payne *et al.* 1987), and the amount and size distribution of protein polymers (Gupta *et al.* 1993). The results in the present investigation agree with the findings that the amount and size distribution of polymeric proteins play an important role in determining the gluten strength in wheat cultivars.

### Cultivation year

Variation in cultivation year was found to influence protein concentration and gluten strength in all the investigated wheat materials. Other studies have shown the importance of yearly weather variations to protein concentration and gluten strength (Johansson & Svensson 1998, 1999). In Sweden, a warm and sunny grain-filling period was correlated with increased gluten strength (Johansson & Svensson 1998).

Variation in weather conditions due to variation in cultivation year had more influence on the amount and size distribution of polymeric proteins compared with total amount of protein groups in Set A. This was also indicated in the study of Johansson *et al.* (2002). For the other investigated sets of wheat, in which years giving rise to low falling numbers were included, the years also influenced the total amounts of protein groups. Low falling numbers have been found to correlate with low amounts of gliadins and glutenins (Hwang & Bushuk 1973).

Years implying higher protein concentrations were positively correlated with increased amounts of gliadins and glutenins as well as many mono- and polymeric proteins. The increase in protein concentration due to variation in year often led to a higher increase in gliadins and SDS-soluble proteins compared with glutenins and SDS-insoluble proteins, which led to a decreased glutenin/gliadin ratio and/or a decrease in LUPP and TUPP. Other studies have shown the relationship between the amounts of gliadins and glutenins and the protein concentration (e.g. Wieser & Seilmeier 1998; Johansson *et al.* 2001). The protein parameter background for yearly variation in protein concentration has not been much studied. The results

Fig. 2. Variation of the percentage of large unextractable polymeric protein in the total large polymeric protein (LUPP), total unextractable polymeric protein in the total polymeric protein (TUPP), glutenin/gliadin ratio (Glugli), glutograph dough development time (Glut) and  $5 \times$  protein concentration (Prot) in different wheat, years and with different fertilizer rates. (a) wheat set A; (b) wheat set B; (c) wheat set C; (d) wheat set D.

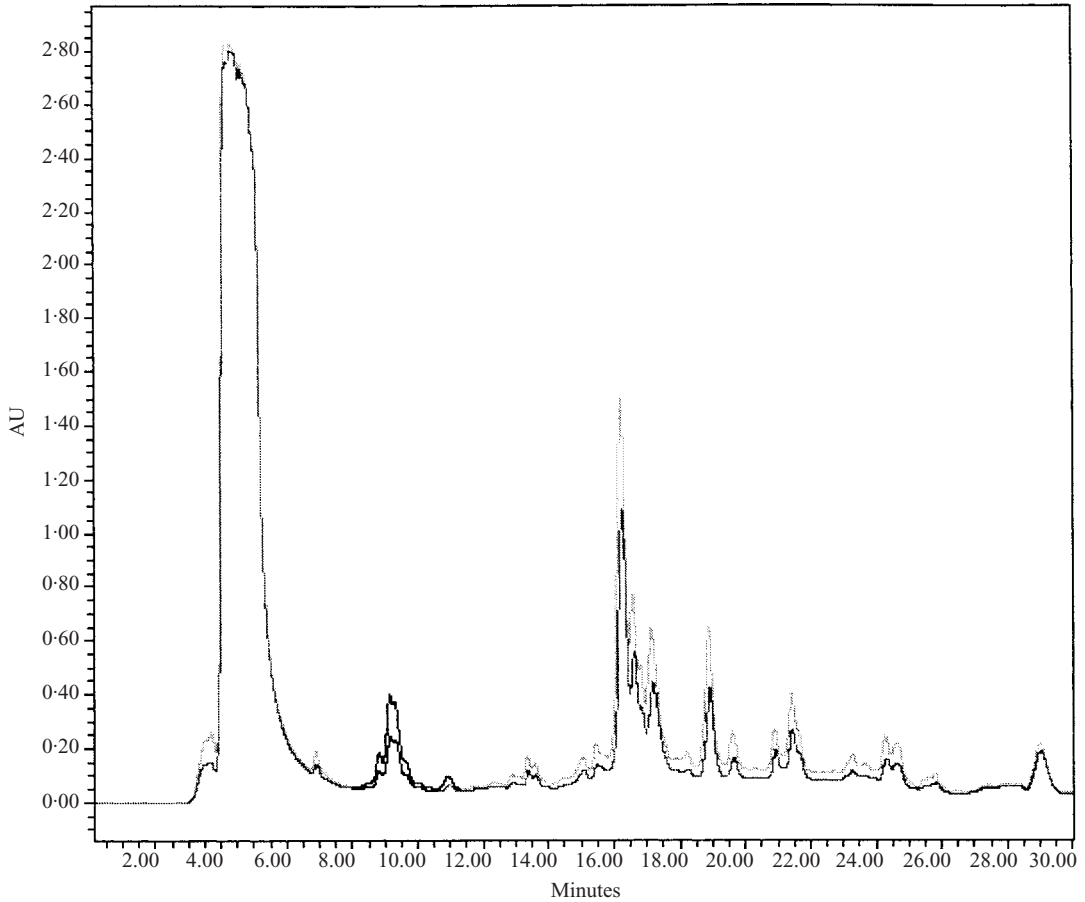


Fig. 3. RP-HPLC chromatograms of glutenin subunits from the cultivar Vinjett (Set B) grown in 1999 with two fertilizer rates, 55 and 155 kg N per hectare. The higher fertilizer level gives a higher amount of all glutenin subunits.

from the present investigation show that the background for yearly variation in protein concentration is similar to the background for cultivar or fertilizer rate variation in protein concentration.

Variation in cultivation year giving higher gluten strength to the cultivars, were related to an increase in SDS-insoluble proteins and a decrease in SDS-soluble proteins. This in turn increased the percentage of TUPP and/or LUPP. Few studies have investigated the relationships between protein variations and variation in gluten strength, due to the cultivation year when temperatures are clearly below 30 °C (Johansson *et al.* 2002). The results in the present study strengthen the findings of Johansson *et al.* (2002), i.e. that the yearly variation in gluten strength is caused by variations in LUPP and/or TUPP. Cultivar differences in variation in TUPP and LUPP between different years was found in the present study, and further work is required in order to better evaluate this. The cultivar Sport, for example, did not

increase the percentage of TUPP and LUPP in years implying higher gluten strength to the cultivars (results not shown). Earlier investigations have shown that cultivars differ in their gluten strength reaction to variation in weather between different years (Johansson & Svensson 1999). Differences in stability of years in relation to gluten strength have been demonstrated (Johansson *et al.* 2000).

#### *Fertilizer rate*

Variation in fertilizer rate influenced the protein concentration and the gluten strength. The influences of fertilizer rate on both protein concentration and gluten strength have been indicated by many previous studies (Johansson & Svensson 1999; Johansson *et al.* 2001). In the present investigation, both high and low protein cultivar increased protein concentration similarly due to increased fertilizer rate.



As in earlier investigations (e.g. Wieser & Seilmeier 1998; Johansson *et al.* 2001), the fertilizer rate influenced the total amounts of protein groups and the amount and size distribution of polymeric proteins. Generally, significant positive correlations were found between increased fertilizer rates, increased protein concentration and amounts of gliadins and glutenins and LPP, LMP and SPP. These results are in accordance with Johansson *et al.* (2001). The increase in protein concentration due to an increase in fertilizer rate often led to a higher increase in gliadins and SDS-soluble proteins compared with glutenins and SDS-insoluble proteins, which led to a decreased glutenin/gliadin ratio and/or a decrease in LUPP and TUPP.

### CONCLUSIONS

Both the genotype and the environment influence the protein concentration and gluten strength and thereby the bread-making quality of wheat.

Influences of genotype and/or environment leading to increased protein concentration also increase the total amounts of glutenins and gliadins and the amounts of most mono- and polymeric proteins. The glutenin/gliadin ratio and/or the percentage of LUPP and TUPP might be decreased due to the increase in protein concentration because of a higher increase in gliadins and SDS-soluble proteins compared with glutenins and SDS-insoluble proteins.

Influences of genotype leading to increased gluten strength increase the percentage of LUPP and TUPP.

Influences of the environment leading to an increased gluten strength increase the SDS-insoluble proteins and decrease the SDS-soluble proteins. Thereby the percentage of TUPP and/or LUPP is increased.

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