# Contribution of the High Molecular Weight Glutenin Subunit 21* to Breadmaking Quality of Swedish Wheats

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ABSTRACT

Different technological tests were used to evaluate the functional value of the newly detected high molecular weight (HMW) subunit 21* of glutenin. Progenies of two different crosses, both comprising one parent with 21* and one without 21*, were used. This allows comparison of the functional value of subunit 21* with other HMW subunits of glutenin that share a similar genetic background. Progenies containing subunit 21* have higher sedimentation volumes than those containing allelic subunits 1 or 2*. The progenies containing subunit 21* also had higher loaf volumes than those containing subunit 1. In glutograph and mixograph tests, deformation and development times were no longer for progenies containing 21* than progenies containing subunits 1 or 2*; the times obtained for progenies containing the HMW subunits 5+10, however, were very long and did indicate a gluten strength too high for Swedish baking conditions. Since the development times were not too long, the glutenin subunit 21* seems to be of potential value for wheat improvement in Sweden.

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The endosperm storage proteins of hexaploid wheat (*Triticum aestivum* L.) are important primarily because of their influence on the baking characteristics of flour (Wall 1979). The major proteins are gliadin, a complex mixture of single polypeptides; and glutenin, consisting of polypeptides that are cross-linked by interpolypeptide disulfide bonds (Shewry and Tatham 1990). Reduced glutenin is subdivided into high molecular weight (HMW) and low molecular weight (LMW) subunits (Payne et al 1984). The HMW subunits of glutenin constitute only a small portion (10%) of the storage proteins (Payne et al 1984), but they exert a pronounced effect on gluten elasticity and breadmaking quality of the flour (Payne et al 1987). Correlations have been established between particular HMW subunits and breadmaking quality (Payne et al 1979, 1981, 1984, 1987; Burnouf and Bouriquet 1980; Moonen et al 1982; Branlard and Dardevet 1985; Campbell et al 1987; Cressey et al 1987; Ng and Bushuk 1988; Uhlen 1990; Johansson et al 1993, 1994). Each cultivar contains three to five HMW subunits that can be distinguished by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Payne et al 1979). In 1983, Payne and Lawrence established a numbering system for identification of the HMW glutenin subunits. Since then, additional HMW subunits of glutenin have been detected (Payne et al 1983, Johansson et al 1993, Margiotta et al 1993). There are indications that the newly detected subunit 21* correlates with a high gluten strength (Johansson et al 1993). In this study, we have further investigated the contribution of subunit 21* to different baking quality parameters.

## MATERIAL AND METHODS

### Plant Material

In a previous study (Johansson et al 1993), crosses were made to determine the chromosomal location of the locus encoding subunit 21*. From the progenies of two of these crosses, W 3879 (HMW subunit composition: 21*, 17+18, 5+10) × Kadett (HMW subunit composition: 1, 7+9, 5+10) and W 31169 (HMW subunit composition: 21*,7+9, 5+10) × Nemares (HMW subunit composition: 2*, 14+15, 2+12), seeds determined by electrophoresis to be homozygous for the three HMW glutenin-encoding loci, were selected and multiplied. The first generation was produced in the greenhouse and the second generation was produced under field conditions during the summer of 1993 at Svalöf Weibull AB, Landskrona, Sweden. Seeds from the cross W 3879 × Kadett were sown in the field at the normal sowing date; W 31169 × Nemares seeds were more sparsely sown one month later. The seeds from the multiplications were tested to evaluate the protein quality for the different combinations of HMW subunits of glutenin.

### Evaluation of Protein Characteristics

Kernel and flour protein concentrations were determined on a dry weight basis by near-infrared reflectance spectroscopy, with calibrations made by the Swedish Cereal Laboratory, Svalöf Weibull AB, Svalöv, Sweden. Kjeldahl analysis (ICC 1980) was used for calibration.

Zeleny sedimentation tests (ICC 1972) were performed on white flour of low extraction rate obtained by milling grains on a Brabender Sedimat mill (ICC 1972). Also, a modified sedimentation test was performed as above, but with 2.4 g of white flour. The flour was obtained by milling grains on a Brabender Quadrumat Senior mill using the micro-milling technique developed by the Grain Marketing Research Laboratory, Manhattan, KS. The micro mill is composed of a Brabender three-break milling head, sifters, and a Brabender three-reduction milling head.

Gluten content was measured according to the standard method (ICC 1982). The resulting gluten was then analyzed on a Brabender Glutograph to obtain deformation time (Sietz 1987). Mixograph tests were performed according to standard methods (AACC 1983). Alveograph tests were performed according to the standard method (ICC 1972), and the baking tests were according to Thorén (1981) and Johansson (1989).

All tests were applied to the progenies of the cross W 3879 × Kadett. Protein concentration, Zeleny sedimentation volume, the modified sedimentation volume, and in most cases gluten content, glutograph, and mixograph analyses were performed on the limited amount of grain obtained from the progenies of the cross W 31169 × Nemares.

### Statistics

Analyses of variance were performed where lines with one electrophoretic pattern in common were grouped into one class, and the *F*-test was used to evaluate the differences between the two classes. The GLM procedure (SAS Institute, Cary, NC) was used.

## RESULTS

### Protein Concentration

Grain protein concentration range was 15.2–16.3%, with the

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highest values in the progenies of the cross W 31169 × Nemares. In the flour, the values were somewhat lower, 14.4–15.5%, but the highest values were still obtained from the progenies of the cross W 31169 × Nemares (Table I). These protein concentrations are higher than that of commercial wheat grown in Sweden.

### Sedimentation Tests

The Zeleny sedimentation values were very high and similar for all the progenies. All the progenies, irrespective of HMW subunit combination, had a value of 70–71 in this test. The highest possible value in Zeleny sedimentation test is 75. Samples with high protein content and quality can reach a "ceiling", therefore a slightly modified sedimentation test was also used. In the modified sedimentation test, some differences were obtained between progenies with different HMW glutenin subunit patterns (Table I). Among the progenies from the cross W 3879 × Kadett, the ones containing subunit 21* had statistically significant ($P < 0.05$) higher values than those containing subunit 1. The progenies containing the combination 21* and 17+18 had the highest value. Among the progenies from the cross W 31169 × Nemares, those containing 21* gave significantly higher ($P < 0.05$) modified sedimentation values than those containing 2*.

### Gluten Content

The wet gluten content range was 32.3–39.1% in the wheat progenies (Table I). The progenies of the cross W 3879 × Kadett (34.2–35.5%) showed limited variation, but presence of the HMW glutenin subunits 21* and 17+18 seems to influence the gluten content toward a higher value when compared to subunits 1 and 7+9. In the progenies from the cross W 31169 × Nemares, indications of a positive influence of subunits 14+15 and 2+12 on the gluten content were found in comparison with subunits 7+9 and 5+10. The dry gluten content varied from 11.5 to 13.6%, and the ranking was similar to that based on wet gluten content.

### Glutograph Tests

The glutograph deformation time varied from 17.4 to 51.4 sec (Table I). Generally, the glutograph time was longer for progenies of cross W 3879 × Kadett than for those of cross W 31169 × Nemares. Subunit 1 gave significantly ($P < 0.05$) longer glutograph times than did subunit 21* for progenies of cross W 3879 × Kadett. Subunits 5+10 gave significantly ($P < 0.05$) longer glutograph times than 2+12 for progenies of cross W 31169 × Nemares. There were also some indications that subunit 2* is correlated to longer glutograph times than is 21*, although these differences were not significant.

### Mixograph Tests

The dough development time varied from 3.8 to 7.4 min (Table I). Significantly ($P < 0.01$) longer development times were found in progenies of W 31169 × Nemares containing subunits 5+10 than in those containing subunits 2+12. Indications of longer development times were found in progenies from the cross W 3879 × Kadett containing subunit 1 compared to those containing 21*, although these differences were not significant.

### Alveograph Tests

Alveograph tests were only made on the progenies from the cross W 3879 × Kadett (Table I). The $P/L$ value varied from 0.97 to 1.13 and the $W$ value from 417 to 447. A lower $P/L$ value (not significant) was ascribed to subunit 21* but not to subunit 1. The $W$ value was lowest for the subunit combination 21*, 7+9 and 5+10, and highest for the subunit combination 21*, 17+18, and 5+10. Progenies with subunit combinations containing subunit 1 were intermediate.

### Baking Tests

Baking tests were limited to progenies from the cross W3879 × Kadett. The appearance of the breads produced in these tests was good and very similar, irrespective of HMW glutenin subunit combination. However, the bread volume differed somewhat (Table I). The progenies containing subunit 21* gave significantly ($P < 0.05$) higher volumes than those containing subunit 1. The highest bread volume was recorded for the progenies containing subunit combination 21*, 17+18, and 5+10.

## DISCUSSION

The most common HMW glutenin subunit pattern in Swedish wheat cultivars and breeding lines is the combination of 2*, 6+8, and 2+12 (Johansson et al, *in press*). This pattern is correlated with a relatively weak gluten strength, which is often undesirably weak for the Swedish baking industry. The breeders can predict a weak gluten in a breeding line by its low Zeleny sedimentation volume, short glutograph deformation, short mixograph development times, or low alveograph $W$-value. One possible way to overcome the problems with a too weak gluten is to select for other HMW subunit combinations that promote a stronger gluten. However, the introduction of subunits 5+10, correlated with high gluten strength (Payne et al 1987; Uhlen 1990; Johansson et al 1993, 1994), often leads to a too strong gluten, which is also a disadvantage for the Swedish baking industry. An overstrong gluten can be recognized by long glutograph and mixograph times or by too high $P/L$ values on the alveograph.

Recently, the novel HMW subunit of glutenin 21* was detected in Swedish wheat material (Johansson et al 1993). Some preliminary results show a positive correlation between the presence of subunit 21* and high Zeleny sedimentation volumes (Johansson et al 1993). Thus, introducing subunit 21* might be of interest to Swedish wheat growers.

Irrespective of HMW subunit composition and type of technological test employed, almost all the progenies obtained from the two crosses (W 3879 × Kadett and W 31169 × Nemares) showed test values that indicated a high gluten strength. One explanation of this could be the high protein concentration in the studied wheat material caused by the weather conditions of

**TABLE I**
**Technological Test Results**

| Cross | High Molecular Weight Glutenin Subunit Combination | Protein Concentration (%) | | Modified Sedimentation Test Score | Gluten Content (%) | | Glutograph Value (sec) | Mixograph Value (min) | Alveograph Value | | Baking Test Volume (ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Kernel | Flour | | Wet | Dry | | | $P/L$ | $W$ | |
| W 3879 × Kadett | 1, 17+18, 5+10 | 15.7 | 14.5 | 62 | 34.9 | 12.5 | 51.4 | 7.4 | 1.05 | 431 | 955 |
| | 1, 7+9, 5+10 | 15.4 | 14.5 | 62 | 34.2 | 12.1 | 47.7 | 6.6 | 1.13 | 427 | 964 |
| | 21*, 17+18, 5+10 | 15.2 | 14.4 | 65 | 35.5 | 12.6 | 31.2 | 4.8 | 1.05 | 447 | 94 |
| | 21*, 7+9, 5+10 | 15.5 | 14.6 | 64 | 35.4 | 12.5 | 33.6 | 5.6 | 0.97 | 417 | 1,003 |
| W 31169 × Nemares | 2*, 7+9, 2+12 | 16.3 | 14.9 | 60 | 36.9 | 12.9 | 21.2 | 4.8 | | | |
| | 2*, 7+9, 5+10 | 15.5 | 14.4 | 56 | 32.3 | 11.5 | 33.4 | 6.6 | | | |
| | 2*, 14+15, 2+12 | 15.5 | 15.0 | 53 | | | | | | | |
| | 2*, 14+15, 5+10 | 15.9 | 15.1 | 53 | | | | | | | |
| | 21*, 7+9, 2+12 | 16.0 | 15.2 | 63 | 36.6 | 12.7 | 17.9 | 4.6 | | | |
| | 21*, 7+9, 5+10 | 15.6 | 15.0 | 59 | 34.8 | 12.3 | 25.1 | 6.0 | | | |
| | 21*, 14+15, 2+12 | 15.8 | 15.2 | 63 | 39.1 | 13.6 | 17.4 | 3.8 | | | |
| | 21*, 14+15, 5+10 | 16.2 | 15.5 | 64 | 36.2 | 12.8 | 42.2 | 6.4 | | | |

the summer of 1993 (high protein concentration in the kernels, accompanied by a low grain yield obtained in 1993, as compared to years with average weather conditions). Another explanation could be the late sowing and sparsity of the W 31169 × Nemares progenies, since more nitrogen would be available per plant.

High protein concentrations often lead to high Zeleny sedimentation values. In the studied wheat material, differences were only detectable when modified sedimentation values were determined. In the present investigation, as well as in an earlier one (Johansson et al 1993), subunit 21* was more positively correlated with high sedimentation volumes than were subunits 1 and 2*. However, in disagreement with earlier findings (Payne et al 1987, Uhlen 1990, Johansson et al 1993), progenies containing 5+10 did not generally have higher sedimentation values than those containing 2+12 in this study. The highest value obtained from the cross W 31169 × Nemares was in progenies containing the subunit combination 21*, 14+15, and 5+10 (i.e., the subunits from each chromosome giving the highest sedimentation values) (Johansson et al 1993).

The gluten content varied in the studied wheat material. However, the gluten content was influenced mainly by the cultivation method and protein concentration and not by the HMW subunits of glutenin (Branlard et al 1991). Since the protein concentration is similar to that of the studied wheat material, and since the cultivation method used is the same, there might be other explanations for the variations in gluten content. For example, one explanation of the positive effects of 2+12 in comparison with those of 5+10 could be that 2+12 gives a more elastic gluten, which probably contains increased amounts of other substances such as starch, and thus leads to a higher gluten content.

Several of the progenies used in this study showed very long glutograph deformation times, indicating the overstrong nature of their gluten for the Swedish baking industry. Normal values for Swedish spring wheats are ~17–18 sec; times longer than 25 sec are normally considered too long. The results from the mixograph tests were quite similar to those from the glutograph tests. In general, progenies containing subunits 5+10 have very long glutograph deformation and mixograph development times.

In alveograph tests of the progenies from the cross W 3879 × Kadett, all four HMW subunit combinations (Table I) showed very high $W$ values. The $P/L$ values were balanced. In Swedish wheats, a good cultivar should have a $P/L$ value around 1.00. A higher value is indicative of a dough that was too strong. The $P/L$ values obtained in this study are not as high as might be expected by the very high $W$ values. Thus, the glutenin-to-gliadin ratio and subunit composition must be good in the studied wheat material. A similar conclusion may be drawn from the baking tests. All the HMW subunit combinations resulted in bread with good and similar appearance. The bread volumes were high, despite the very high values of some of the technological tests that would be indicative of a too strong dough. Higher bread volumes were obtained for progenies that contained subunit 21* instead of 1, and the highest volumes were obtained for the subunit combination 21* and 17+18.

Both this study and an earlier one (Johansson et al 1993) indicated that subunit 21* gives higher sedimentation values than the other 1A-encoded HMW subunits. This study also showed that subunit 21* confers shorter glutograph and mixograph times than did subunit 1 and probably subunit 2*. The newly detected 21* seems to have somewhat different properties compared to other 1A-encoded HMW subunits. The sedimentation values indicate that 21* influences the dough toward a higher gluten strength, just as subunits 5+10 and 14+15 do. However, 21* does not seem to have those properties that make the dough overstrong, as 5+10 does. The unique properties of wheat lines with the HMW glutenin subunit 21* should make them especially useful as parents for breeding new wheat varieties with improved baking quality for the Swedish baking industry.

## CONCLUSION

Progenies containing 21* correlate with high sedimentation values and high bread volumes. These progenies also result in good alveograph values, but they do not give the very long glutograph deformation and mixograph development times, as do progenies containing subunits 5+10 but lacking subunit 21*. All this indicated positive influences on the baking quality by the subunit 21*, which generates a strong but not overstrong dough. However, this study covers a relatively small number of samples; further analysis would improve the knowledge of influences of subunit 21* on breadmaking quality.


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