

## Paper 23

# INTER-RELATIONSHIP BETWEEN SOME BAKING QUALITY PARAMETERS IN SPRING WHEAT

**K. Ringlund, A. K. Uhlen and E. Mosleth**

Department of Crop Science, Agricultural University of Norway  
Boks, Norway

### ABSTRACT

Spring wheat samples from yield trials in 1983 and 1984 were evaluated for gluten content, Zeleny's sedimentation, protein content, extensigram area, and loaf volume. On a set of 11 varieties tested in both years, significant varietal differences in protein and gluten content, and highly significant varietal differences in sedimentation and extensigram area were found. Varietal differences in loaf volume were not significant.

Protein content, gluten content, sedimentation, and extensigram area contribute to the loaf volume. All these tests should, therefore, be included in a selection programme for improved baking quality of bread wheats. An interaction term between gluten content and sedimentation is the main factor responsible for loaf volume variation.

Groups of varieties differing in their glutenin composition obtained by SDS-electrophoresis have significantly different sedimentation, extensigram area, gluten content, and protein content.

### KEYWORDS

Zeleny's sedimentation, gluten content, loaf volume, extensigram, glutenins.

### INTRODUCTION

Norway has imported most of its bread wheat for many years. Increased grain production has resulted in too much feed grain, and there has been a shift towards wheat production during the last 10 to 12 years. Increased domestic bread wheat production requires emphasis to be placed on quality aspects in breeding programmes. Many methods for evaluating baking quality have been developed (AACC, 1983), but very few in depth analyses of the relationships between the different methods can be found in the literature. A new method is often compared only to one or a few older methods by simple regression or correlation.

Important results have been reported on the separation of storage proteins and the influence of different glutenin subunits and gliadins on the technological properties of wheat in the baking process (Payne *et al.*, 1984). There has been some controversy on the relative importance of

glutenins and gliadins. The fact that different tests for baking quality have been used might explain that controversy.

In this paper an attempt is made to analyse the contribution of the quality factors measured by Zeleny's sedimentation, gluten content, protein content, and extensigram area on loaf volume. Different varieties of spring wheat are grouped according to their glutenin composition and differences in the previously mentioned quality parameters between these groups are evaluated.

### MATERIALS AND METHODS

The plant materials analysed were Norwegian spring wheat varieties grown in 1983 and 1984. Protein content was determined by NIRR (Ringlund, 1978), gluten content by Falling Number's Glutomatic, and sedimentation according to Zeleny (AACC, 1983). Extensigram area and loaf volume were determined by standard methods at the Swedish Cereal Laboratory, Svalof. HMW glutenin subunits were determined by SDS-PAGE electrophoresis (Payne *et al.*, 1981), and the electrophoretic bands were numbered according to the system described by Payne *et al.* (1984). The electrophoretic pattern was scanned on a Shimadzu scanning densitometer at 570 nm.

Data were analysed by analysis of variance, t-test, simple correlation, multiple linear regression (Nissen and Mosleth, 1985; Sokal and Rohlf, 1969), and a multivariate analysis called Partial Least Squares regression (PLS) (Wold *et al.*, 1983).

### RESULTS

Results are presented for the evaluation of the standard errors of treatment means for different testing methods. Since loaf volume is the most commonly accepted test for baking quality an evaluation is presented of other methods as predictors of loaf volume. The electrophoretic pattern of the HMW glutenin subunits is discussed in relation to baking quality

#### Standard error of treatment means

Results for 11 varieties that were included in the main yield trials in both years are presented in Table 1. Loaf volume, extensigram area, and gluten content were

**Table 1. Yield, protein content, and protein quality of 11 spring wheat varieties over two years.**

Variety	Kg grain per ha	% prot.	Zeleny's sediment.	% gluten	Extensigram	Loaf volume
Reno	4980	13.0	36	25.8	98	852
Runar	4750	13.4	35	26.6	77	878
T9054	5160	13.3	35	28.9	70	907
T10013	5320	12.7	26	27.0	23	826
T1028	5540	11.7	30	22.0	85	759
T2002	5490	12.9	31	23.7	75	866
T2015	5270	14.0	37	27.3	73	854
T2025	5820	11.8	28	22.8	56	800
T2038	5150	14.1	50	25.4	108	830
T2052	5120	13.3	42	23.9	98	777
T2054	5270	13.3	27	27.7	17	823
Standard error	180	0.4	1.5	1.1	6	36
Sig. level	*	*	***	*	***	NS

\*P<0.05, \*\*P<0.01, \*\*\*P<0.001.

analysed at only one location each year, whereas yield, protein content, and sedimentation were analysed at nine locations.

There were no significant differences between varieties for loaf volume. This does not mean that genetic differences do not influence loaf volume, but the influence of other factors masks varietal differences. Varietal differences in sedimentation and extensigram values are very consistent from year to year. These two methods are, therefore, very efficient for selection of genetic differences. Gluten content and protein content show significant varietal differences, but at a relatively low significance level.

#### Prediction of loaf volume

Loaf volume is the final and most conclusive test for baking quality of a given sample. Due to high experimental error in genetic comparisons, it is important to identify parameters with a higher heritability and define their relationship to loaf volume.

#### Simple correlations

In the data presented in Table 1, only gluten content is significantly correlated with loaf volume,  $r=0.71$  ( $P<0.01$ ). On data from a preliminary yield trial with 64 varieties in 1984, significant correlations were found between loaf volume on one side and protein content,

$r=0.41$  ( $P<0.01$ ), gluten content,  $r=0.54$  ( $P<0.01$ ), and Zeleny's sedimentation test,  $r=0.33$  ( $P<0.01$ ), on the other. Extensigram areas were not obtained.

These data were divided into four groups according to the gluten content. For each group the correlation between sedimentation (Z) and loaf volume (LV) was calculated. The correlation coefficients were,  $r=-0.46$  (non significant (NS)),  $r=-0.01$  (NS),  $r=0.59$  ( $P<0.05$ ), and  $r=0.59$  ( $P<0.01$ ) for gluten content less than 25.5%, between 25.5% and 28.0%, between 28.0% and 30.0%, and higher than 30.0%, respectively. This interaction can also be presented as the product  $G \times Z$ , and the correlation coefficient between this product and LV was 0.56 ( $P<0.01$ ).

Simple correlation coefficients on data from the main yield trials in 1983 and 1984 are given in Table 2.

Gluten content and the interaction  $G \times Z$  are the two measures of quality which have the greatest influence on loaf volume when evaluated by the simple correlation coefficient.

In addition to the correlations presented above, there are intercorrelations between the different parameters. To eliminate the correlation between protein and gluten, and between protein and sedimentation, the gluten and sedimentation values were adjusted for their linear relationships with protein. The data obtained are termed specific gluten (SG) and specific sedimentation (SZ). Data

**Table 2. Simple correlation coefficients between loaf volume and other quality determinations on data from the main yield trials in 1983 and 1984.**

Number of varieties	Protein	r between loaf volume and:			
		Gluten	Sedimentation	Extensigram	GLU $\times$ ZEL
50 (1983/84)	0.44**	0.49**	0.28*	—	0.45**
25 (1983)	0.52**	0.66**	—	—	0.66**
25 (1984)	—	0.41*	0.42*	—	0.50*

\*P<0.05, \*\*P<0.01

from the preliminary trials in 1984, show that these two variables were also correlated,  $r = -0.47$  ( $P < 0.01$ ). By adjusting SZ with the linear regression to SG, a new variable termed specific sedimentation (SSZ) was obtained. SG and SSZ are thus independent of protein and SSZ is independent of SG. The correlations with LV were  $r = 0.37$  ( $P < 0.01$ ) and  $r = 0.32$  ( $P < 0.01$ ) for SG and SSZ, respectively. The three parameters protein content, gluten content, and Zeleny's sedimentation contain different types of information that contributes to the determination of loaf volume.

#### Linear multiple regression

Data from the preliminary yield trial show that protein content (P), specific gluten (SG), and specific sedimentation (SZ) all contributed significantly to the determination of loaf volume (LV) when analysed by the linear multiple regression technique.

$$LV = -199 + 0.318 \times P + 0.614 \times SG + 4.59 \times SZ$$

The overall  $R^2$  was 0.41. When the interaction term,  $SG \times SZ$ , was added as an independent variable, the  $R^2$  increased to 0.50 and the estimated equation changed to

$$LV = 164 + 0.30 \times P + 0.06 \times SG - 31.6 \times SZ + 12.5 \times SG \times SZ$$

When data from the main yield trials were evaluated using the multiple linear regression technique, the four variable gluten content,  $G \times Z$ , protein content, and extensigram area contributed significantly to loaf volume in 1983. Only the interaction term  $G \times Z$  and extensigram area (Ex) were significant contributors to loaf volume in 1984.

#### Multivariate analysis

In the PLS multivariate analysis the information from several independent variables are concentrated into a small number of regression factors. These factors describe the variation in the independent variables (P, G, Z,  $G \times Z$ , and Ex) which explain the variation in the dependent variable (LV).

In all the data analysed the interreaction  $G \times Z$  was the most important variable to explain the variation in LV. Further, the analysis revealed that the protein quality measured by Ex is different from the protein quality measured by the other methods.

In an analysis of the data from the preliminary trial the

adjusted values for G and Z were used. Still the interreaction, here  $SG \times SZ$ , was most important to explain the variation in LV. This means that in these variety tests variation in loaf volume was due more to variation in protein quality than in protein content. The analysis also indicated that SG and SZ are two variables measuring different protein properties.

#### Electrophoretic pattern

The varieties were grouped according to their HMW glutenin subunits, and the means of different technological tests were calculated for each group (Table 3).

Varieties with subunits 5 and 10 had higher SZ and larger Ex area than varieties with their allelic counterparts 2 and 12. These results agree with Payne *et al.* (1984) and Moonen *et al.* (1982). Significantly higher SZ and Ex area were also obtained with subunits 13+16 instead of their allelic counterparts 7+8 or 7+9 in addition to 5+10. Varieties with subunits 2 and 12 had a higher G and a slightly higher P value than varieties with subunits 5 and 10. The G value was also higher for varieties with subunits 7+8 or 7+9 compared to varieties with 13+16. No significant differences were found for LV between the groups.

The quantitative measurements of the HMW glutenin subunits obtained by the densitometric scanning were analysed by PLS which confirmed the influence of the different HMW glutenin subunits on baking quality. Further, the analysis clearly pointed out subunits 5+10 v. 2+12 were most important in the explanation of variation in baking quality.

## DISCUSSION AND CONCLUSION

Loaf volume, which is the final test for baking quality, is influenced by many different properties of the wheat kernel. In addition, there is a relatively large environmental influence. The kernel properties are partly determined by the genotype and partly by the conditions under which the wheat was grown. The data presented show that the sum of these properties, or loaf volume itself, is too complex to be used for varietal selection. In different sets of data it was shown that Zeleny's sedimentation, gluten content, protein content, extensigram area, and an interaction term  $G \times Z$ , all contribute to final loaf volume, and that all these measures contribute different information.

**Table 3. Differences in technological properties between varieties with different HMW glutenin composition.**

	HMW-glutenin subunits compared		Technological tests mean
Specific Zeleny Sedimentation	5 + 10	v. 2 + 12	34.1 v. 24.2***
Extensigram area (cm <sup>2</sup> )	13 + 16, 5 + 10	v. 7 + 8 or 9, 5 + 10	40.1 v. 32.6**
Gluten-amount	5 + 10	v. 2 + 12	78.9 v. 46.0**
	13 + 16, 5 + 10	v. 7 + 8 or 9, 5 + 10	110.2 v. 73.8**
Protein content (%)	5 + 10	v. 2 + 12	25.8 v. 31.0***
	13 + 16, 5 + 10	v. 7 + 8 or 9, 5 + 10	23.0 v. 26.3**
	5 + 10	v. 2 + 12	12.9 v. 13.8**

\*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

The electrophoretic data show that groups of varieties with different HMW glutenin subunits had different levels of sedimentation, extensigram area, and gluten content. The major differences were found between varieties with subunits 5+10 and 2+12. Varieties with subunits 2+12 have higher gluten content and lower sedimentation than varieties with subunits 5+10. Correlation, multiple regression, and multivariate analyses show that there is an important interaction between gluten and sedimentation. Since glutenin subunit 2+12 and 5+10 are allelic and cannot exist together in the same variety, the interaction  $G \times Z$  cannot be explained by the variation due to these subunits. It must, therefore, be other proteins, possibly gliadins, that contribute to the variation in gluten content or sedimentation or both of these measures. Since the different tests measure different properties that are important for loaf volume, selection for baking quality must be based on several test methods.

## REFERENCES

- American Association of Cereal Chemists 1983. Approved methods vol. 1 and 2, AACCC, Inc., St Paul Minn. ISBN 612-454-7250.
- Moonen, J.H.E., Scheepstra, A.C., Graveland, A. 1982. Use of the SDS-sedimentation test and SDS-polyacrylamide gel electrophoresis for screening breeder's samples of wheat for bread-making quality. *Euphytica* 31 (3): 677-690.
- Nissen, O., Mosleth, E. 1985. Brukerveiledning for MSTAT. Landbruksbokhandelen As-NLH. ISBN 82-557-0225-3.
- Payne, P.I., Corfield, K.G., Holt, L.M., Blackman, J.A. 1981. Correlations between the inheritance of certain high-molecular-weight subunits of glutenin and bread-making quality in progenies of six crosses of bread wheat. *Journal Science Food Agriculture* 32: 51-60.
- Payne, P.I., Holt, L.M., Jackson, E.A., Law, C.N. 1984. Wheat storage proteins: their genetics and their potential for manipulation by plant breeding. Philosophical Transactions Royal Society London, *Biological Sciences* 304: 359-371.
- Ringlund, K. 1978. Proteinanalyser pa Neotec GQA31. Nordisk Cerealistforbunds 20:e Kongress, Malmo 24.-26. Maj 1978. pp. 137-142.
- Sokal, R.R., Rohlf, F.J. 1969. Biometry. W.H. Freeman and Co. ISBN 0-7167-0663-6. pp. 1-776.
- Wold, S., Martens, H., Wold, H. 1983. The Multivariate Calibration Problem. In: Chemistry solved by the PLS Method. Proc. Conf. Matrix Pencils, A. Ruhe and B. Kagstrom (eds). March 1982, Lecture Notes in Mathematics, Springer Verlag, Heidelberg, pp. 286-293.

## SYMPOSIUM DISCUSSION

Dr W. Bushuk, University of Manitoba  
To comment and raise a question in relation to the use

## SYMPOSIUM DISCUSSION

Dr W. Bushuk, University of Manitoba

To comment and raise a question in relation to the use of the Zeleny's sedimentation as a measure of gluten quality for bread making. Sedimentation tests also depend strongly on the level of gamma starch in the flour. This in turn depends on the hardness of the grain from which the flour is milled, which in turn depends on  $\alpha$  amylase. So there are obviously non-protein factors that can affect the Zeleny's sedimentation value. These effects can be quite large, occasionally large enough to completely mask the effects of things like protein content, and certainly the effects of protein form. It is possible to avoid these problems if the samples you are applying the tests to are of similar hardness and contain about the same level of  $\alpha$  amylase activity. Do you know if your samples were of this type or did you take any precautions to grind to a constant starch damage value?

Ringlund

No we didn't grind to a constant starch damage value. In all these tests we had very high falling numbers, so at least there was no sprout damage. Even though we had several factors, we only explained 50% of the variation in low falling number, although the correlations are significant, they are very low. A correlation of 0.3 means there is only 10% common variation — this allows for a lot of variation due to other factors. We did not check into any of those.

Dr I.L. Gordon, Massey University

Do you have any plans or experiments to investigate the other 50% of variation that you have not explained.

Ringlund

Yes, to list two. We may proceed to look into the breakdown of starch and protein by these methods. We have also made some crosses between varieties with different bands and different properties to continue to look into these inter-relationships.

Mr G.R. Tempest, Northern Roller Mill Co.

For the baking tests, what size loaf and base temperature do you use.

Ringlund

We don't have equipment or facilities for baking tests, so we bought these in Sweden. I think they use half a kilo, but I don't know much more about the baking test — its used at the Cereal Laboratory, Svalöf, Sweden.

Professor D. von Wettstein, Carlsberg Laboratory

What is the evidence that some of these high molecular weight glutenin proteins are really responsible for the gluten of the baking? Have you isolated these proteins and put them into the dough to show that they really improve the quality of the bread?

Ringlund

The way we have approached it is to separate out two groups of varieties, and look at the mean of those

varieties. There may be a lot more to it than just these two bands.

Dr J. Bingham, Plant Breeding Institute

We are not claiming that these high molecular weight glutenins account for more than about 50% of the quality. The two approaches we have used are, firstly, to look at the correlations with large numbers of varieties. The correlation can be very close, although there are a couple of varieties that do not have any good high molecular weight glutenins yet have good bread-making qualities. The second approach which I think you have to take is to look at random lines within crosses to see how the background effects the glutenin. Peter Payne is doing this.

Ringlund

We could produce isogenic lines to do this, but that is a difficult job.

Bushuk

A comment on this point. The first evidence on the possible link between the large, heavy molecular weight glutenin sub-units and bread making quality came from two lines of work. One was in the comparison of bread wheats with durums which do not have some of these glutenin sub-units and which do not have the type of bread making quality that we are looking for. The other line of work was done 15 years ago with various aneuploid lines and substitution lines in which the long arm of the 1D chromosome was eliminated, thereby eliminating these high molecular weight glutenin sub-units. These also did not show the bread making quality of the original hexaploid wheats. So there are 2 types of evidence available that there is a relationship. What is in question still, is whether this is a functional relationship or whether the high molecular weight glutenin sub-units are linked to other factors which altogether make up the quality advantage.