

# Characterization of Functional Properties of Gluten Proteins in Spelt Cultivars Using Rheological and Quality Factor Measurements<sup>1</sup>

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## ABSTRACT

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Wet glutens of 27 European spelt (*Triticum aestivum* ssp. *spelta* (L.) Thell.) cultivars were examined using fundamental rheological methods (oscillatory and creep tests) in conjunction with the determination of moisture contents of these glutens and the wet gluten contents of the flours. Furthermore, SDS sedimentation volumes were determined. A special baking test for spelt was developed that encompassed the characteristic elements used in the production of traditional German spelt speciality breads. Various significant correlations between gluten properties and baking results were found for three sets of spelt cultivars obtained from different

demographic locations and years of harvest. Furthermore, the relationship between baking results (response) and gluten properties (predictors) could be modeled quite well with the help of multiple linear regression analysis. Radar charts used to profile the gluten properties of a particular cultivar showed a great amount of diversity within the spelt material, but there were also similarities between several cultivars. The differences between spelt cultivars should be taken into account when characterizing spelt in general terms or when comparing spelt and modern wheat.

Recently, interest has increased in the ancient wheat species einkorn (*Triticum monococcum* L.), emmer (*T. dicoccon* Schrank), and spelt (*T. aestivum* ssp. *spelta* (L.) Thell.). This is reflected by numerous publications concerning technological properties or nutrient composition of these wheats (Abdel-Aal et al 1995, 1997, 1999; Ranhotra et al 1995, 1996a,b; Pirgiovanni et al 1996; Campbell 1997). In the southern parts of Germany and also in the adjacent areas, growing of spelt (German name *Dinkel*) has a long tradition. Therefore, much research work on spelt has been conducted at the University of Hohenheim, located in the south of Stuttgart: breeding programs (Kling 1993), examination of spelt technological properties (Gräber 1993; Schober et al 1998; Schober 2001) and composition (Dinkelacker Stiftung 1991), genetic studies (Harsch et al 1997; Radić et al 1997; Radić-Miehle et al 1998), and also symposia (Dinkelacker Stiftung 1989, 1991).

The studies described here were conducted within a breeding program of the State Plant Breeding Institute, University of Hohenheim, to predict technological quality of spelt cultivars and breeding lines. Continuing research will extend to a more thorough identification, comparison, and classification of different spelt cultivars or lines and to the detection of differences between spelt and modern wheat cultivars available in Germany. Several sets of spelt samples were included as part of the current study, representing an extremely broad range of old and new European spelt cultivars with different properties, grown under different conditions.

Often, the only way of detecting quality that is accepted by plant breeders, millers, and bakers alike is the performance of a baking test. On the other hand, for breeding programs, methods are needed that can be performed with small amounts of sample in a relatively short period of time. Most standard test baking procedures do not meet these demands. The Rapid Mix Test, the German standard procedure for wheat (Arbeitsgemeinschaft Getreideforschung e.V. 1994) requires 1 kg of flour, and no reliable results can be achieved without a lot of practical experience in baking. Furthermore, no insight can be gained as to the physicochemical structures responsible for

quality. The SDS sedimentation test (Axford et al 1978, 1979) meets the requirements for simplicity and small sample size. Correlations of SDS sedimentation volume to loaf volume are established, but this test is not very useful for understanding the theoretical basis of quality. Rheological tests, especially fundamental tests, on the other hand, provide data that can be interpreted with regard to physicochemical structure. Moreover, for fundamental rheometry, generally only small amounts of sample are required. Rheological tests are relatively quick in comparison to baking tests. In spite of all these advantages, fundamental rheological tests are often used for more basic studies (Janssen et al 1996a,b), but their use is not widespread for the characterization of a large number of cultivars, as found in breeding programs. This may be due to the fact that results are difficult to interpret and relationships between fundamental rheology and baking quality have not as yet been sufficiently examined. Therefore, one important objective of the present study is to examine these relationships for spelt. If significant correlations are found, fundamental rheometry could replace baking tests in the future. Another objective is to establish a means of presenting the results of fundamental rheometry that enable a quick interpretation.

On the basis of previous results (Gräber 1993, Schober 1995), a combination of two rheological tests was used for the current study: dynamic (oscillatory) test and creep test. These methods subject the sample to shear at different amounts and rates of deformation. Additionally, the SDS sedimentation volume and also the moisture content of the wet gluten were determined. The latter is another quick and simple method that has been successfully used for the differentiation of spelt cultivars (Schober 1995; Martini 1997).

When conducting rheological tests, in addition to the specific test method, the nature of the sample must also be considered. Generally, when examining wheat species, doughs or wet glutens of different cultivars can be compared. For dough, it is well established that different kinds of interactions contribute to the rheological behavior such as protein-protein, starch-protein, and starch-starch interactions (Amemiya and Menjivar 1992). According to these authors, short-range interactions such as starch-protein and starch-starch interactions should dominate at small deformations (<3%). Another factor governing the rheological behavior of dough is the quantity of added water. Smith et al (1970) pointed out that the distribution of water between the gluten and starch phases is unknown in doughs and that free water may also exist. Taking all these factors into account, it is evident that dough is an extremely complex system and it is therefore very difficult to interpret rheological data, especially when comparing many cultivars. Examining wet gluten can be a simplification. The starch granules are largely washed out; starch-starch and starch-protein interactions can only occur with remaining starch and are thus strongly reduced. If wet gluten is prepared

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according to ICC Standard No. 137/1 (ICC 1999) by machine washing and removing the residual water by centrifugation, the water content is an individual property of each cultivar's gluten, resulting from its swelling capacity. If the moisture content of the gluten is also determined, the influence of this parameter can be examined and included in the interpretation of the data.

Finally, the baking test has to be considered. It serves as a reference for the other methods. Therefore, it is of great importance, although the aim is to replace it in the future. Spelt shows a behavior different from modern wheat in baking. Above all, spelt doughs are very soft and sticky after kneading. Handling of spelt doughs is therefore more difficult and the loaf volume is generally lower in comparison with modern wheat cultivars. In accordance with these weak properties, Ranhotra et al (1995) found a much shorter dough development time and less mixing tolerance for spelt in comparison with hard red winter wheat. Furthermore, spelt bread had a lower specific volume and a rather open grain and coarse texture. These authors recommended the use of spelt for speciality breads. In southern Germany, such spelt speciality breads do exist traditionally (e.g., Oberschwäbische Seelen, a kind of longish roll, weighing ≈80 g, with an irregular shape and crumb structure and a crisp crust). Seelen are usually sprinkled with salt and caraway seeds. They are prepared from a very soft yeast dough and baked at high temperatures. After kneading, the dough is subjected to a long bulk fermentation time of several hours at low temperatures (20°C or below). Within this rest period, the dough becomes firmer and can be molded more easily afterward. Moreover, the loaf volume increases, if the rest period of a spelt dough is prolonged from 30 min to several hours, even if no ascorbic acid is used (Schober, unpublished data). The only condition is that the dough should be reshaped regularly within this time.

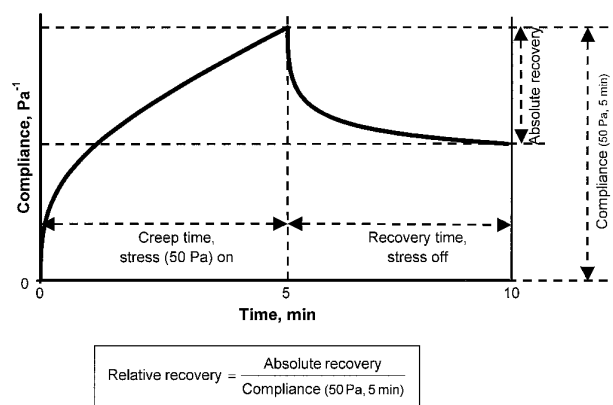
While developing a baking test for spelt, the aim of the current study was to encompass the main elements of the production of traditional speciality breads. However, the procedures required modification to obtain reproducible results. In this baking test, therefore, a high water addition of 67% (flour basis) is used, and the dough is subjected to a total bulk fermentation time of 2 hr and 30 min, with reshaping after 1 and 2 hr. Round rolls are produced by machine rounding instead of moulding by hand as traditionally done for the production of Seelen. Besides the loaf volume, the shape of the rolls

is characterized by calculating the ratio of the width to the height. This is an important parameter because spelt bread tends to become flat.

## MATERIALS AND METHODS

### Materials

Three sets of spelt cultivars were available: Set I contained 11 cultivars of the 1995-96 growing season, set II and set III contained the identical 25 cultivars, grown in the 1996-97 season, at two different locations in or near Stuttgart (set II: "Hohenheim", set III: "Oberer Lindenhof") with different levels of fertilizer applied. The nitrogen (N) levels of set II and set III were 30 kg/ha and 70 kg/ha, respectively. The 25 cultivars are listed in Table I, together with their abbreviated names as used in this article, their countries of origin, and the years of their first appearance or registration. The 11 cultivars included in set I are marked. These sets represent a broad range of old and new cultivars from different European countries



**Fig. 1.** Creep test on wet gluten. During creep time (5 min), sample is subjected to a constant shear stress of 50 Pa, during recovery time (another 5 min), no stress is applied. Compliance (50 Pa, 5 min) is directly obtained from the creep curve. Relative recovery is absolute recovery at the end of recovery time divided by compliance (50 Pa, 5 min).

**TABLE I**  
Description of Spelt Cultivars<sup>a</sup>

Abbreviation	Cultivar	Country of Origin	1st Appearance or Registration	Included in Set I
ALB	Albin	Belgium	1979	x
ARK	Altgold Rotkorn	Switzerland	1952	x
BAU	Bauländer Spelz	Germany	1924	x
FBH	Fuggers Babenhauser Zuchtvesen	Germany	1916	
FRA	Franckenkorn	Germany	1995	x
GOL	"Goldir"	?	?	
HER	Hercule	Belgium	1982	x
HUB	Hubel	Switzerland	1992	
LUE	Lueg	Switzerland	1990	
NEU	Neuegger Weißkorn	Switzerland	~1925	
OAR	Ostar	Switzerland	1995	
OKR	Oberkulmer Rotkorn	Switzerland	1948	
OST	Ostro	Switzerland	1978	
RBW	von Rechbergs Brauner Winterspelz	Germany	1945	x
RFD	von Rechbergs Früher Dinkel	Germany	1949	
RKD	Roter Kolbendinkel	Germany	<1904	
ROU	Rouquin	Belgium	1979	x
RSD	Roter Schlegeldinkel	Germany	<1904	
SKO	Schwabenkorn	Germany	1988	x
SRT	Steiners Roter Tiroler	Germany	1911	x
VOE	Vögeler's Dinkel	Germany	<1904	x
WKS	Weißer Kolbenspelz	Germany	<1904	
WWK	Waggershauser Hohenheimer Weißer Kolbendinkel	Germany	1923	
ZWS	Zeiners Weißer Schlegeldinkel	Germany	1914	x
ZZD	Zuzger (Dinkel)	Switzerland	~1925	

<sup>a</sup> Kling (1993), supplemented.

(Table I). Together with set II, two additional cultivars were grown (identical conditions as set II): ‘Balmegg’ (BEG) and ‘Sertel’ (SER), both registered in Switzerland in 1995. If BEG and SER are included in statistical analyses together with set II, this will be indicated clearly.

All samples (dehulled kernels) were obtained from the State Plant Breeding Institute, University of Hohenheim, Stuttgart-Hohenheim, Germany. They were milled into flour ( $\approx 0.6\%$  ash, dry basis) on a Brabender Quadrumat Jr. flour mill (Duisburg, Germany).

### Flour Moisture and Ash Content

Moisture was determined at  $130^\circ\text{C}$  with a moisture analyzer (MA 51, Sartorius AG, Göttingen, Germany). The ash content was determined by ICC Standard No. 104/1, with ashing at  $900^\circ\text{C}$  and calculation made on a dry basis (ICC 1999).

### SDS-Sedimentation Test

This test was conducted according to Axford et al (1979) and ICC Standard No. 151 (ICC 1999) with some modifications in accordance with Gräber (1993). The modifications included 1) use of 5 g of flour (14% moisture basis) in combination with a sedimentation time of 20 min; 2) use of the motor driven mixer rack described in the ICC Standard No. 116/1 (Zeleny test) (ICC 1999) for shaking. The flour-water suspension in the cylinder was manually shaken for 15 sec after the addition of the water to the flour, then machine shaken for the rest of the 4 min. At the end of this time period, the suspension was shaken manually four times, the SDS-lactic reagent was added, and the suspension was mixed by inverting the cylinder four times. Then the machine was shaken until the end of the 6 min. Afterward, the mixture was allowed to settle for 20 min.

### Wet Gluten Preparation and Rheometry

Wet gluten was prepared by machine washing using a Glutomatic 2202 and the centrifuge accessory (Perten Instruments AB, Huddinge, Sweden) as described in the ICC Standard No. 137/1 (ICC 1999) with small modifications. An amount of 5.5 mL of sodium chloride solution had to be added at the beginning for dough preparation. Glutens were washed from two identical samples simultaneously, centrifuged one after the other, weighed, and finally combined. The

combined glutens were used for rheometry and determination of the moisture content of the wet gluten. The wet gluten content was calculated on a 14% moisture basis.

The rheological tests were performed with a controlled-stress rheometer (Bohlin CS, Bohlin Instruments Vertriebs GmbH, Pforzheim, Germany) equipped with a parallel plate configuration (40 mm diameter, 2 mm gap). The temperature of the lower plate was maintained at  $30^\circ\text{C}$  by water circulation.

### Dynamic Oscillatory Measurements

Stress sweeps were performed initially with wet glutens from selected cultivars to determine the linear viscoelastic region. For the stress sweeps, stresses of 0.0625 to 100 Pa were applied at a frequency of 10 Hz.

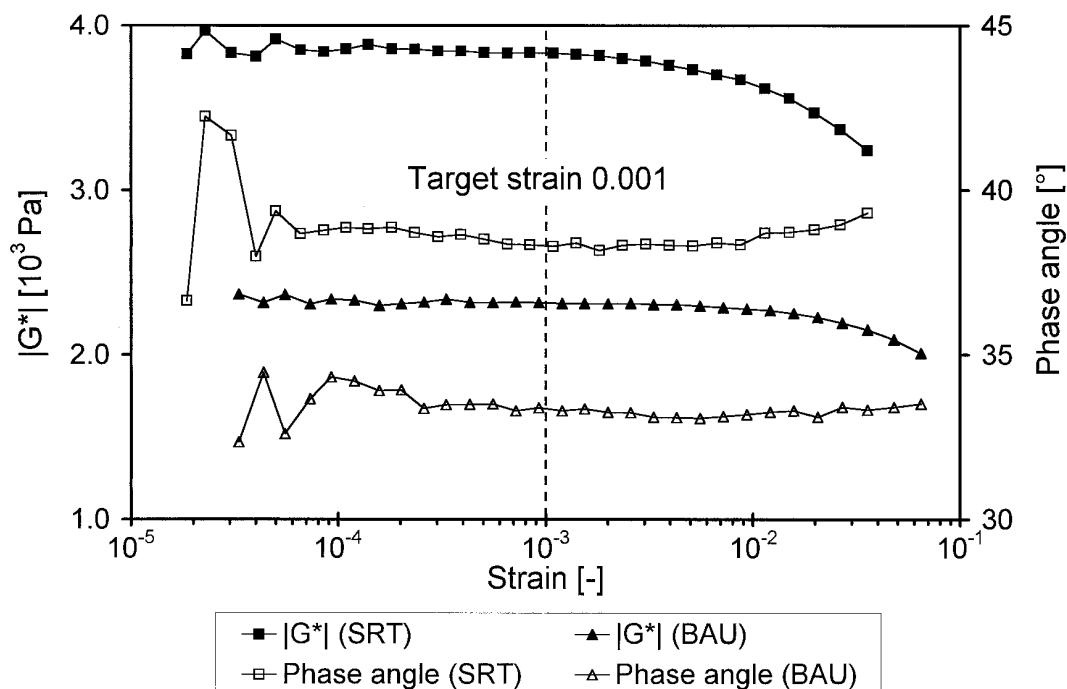
To compare all the different samples, dynamic oscillatory measurements with wet glutens from these samples were conducted within the linear viscoelastic region at a target strain of 0.001 (0.1%). For these tests, the frequency sweep mode was used with frequencies of 0.07 to 10 Hz. The absolute value of the complex dynamic modulus ( $|G^*|$ ) and the phase angle ( $\delta$ ), at 10 Hz, respectively, were used to characterize the rheological properties of the samples.

### Creep Tests

The wet glutens were subjected to a constant shear stress of 50 Pa for 5 min (creep time). The resultant compliance (strain/stress) was monitored as a function of time. Afterward, the stress was removed and the compliance monitored for another 5 min (recovery time). During the recovery time, the compliance was calculated by dividing by 50 Pa (the stress applied during the creep time). Two parameters were used to characterize each sample's rheological properties (Fig. 1): 1) compliance (50 Pa, 5 min), the compliance reached at the end of the creep time; 2) relative recovery after the recovery time. For the calculation of the relative recovery, the absolute recovery is divided by the compliance (50 Pa, 5 min).

### Procedure for Rheometry

A 4-g sample quantity of two centrifuged, combined glutens was weighed out, a ball was formed and placed exactly in the center



**Fig. 2.** Absolute value of the complex dynamic modulus ( $|G^*|$ ) and phase angle ( $\delta$ ) as a function of shear strain for wet gluten from two spelt cultivars (SRT and BAU) representing different extremes. Stress sweeps were performed applying stresses between 0.0625 Pa and 100 Pa at 10 Hz. A shear strain of 0.001 (0.1%) was chosen as target strain for dynamic oscillatory measurements with wet glutens from different cultivars.

of the lower plate of the rheometer. The gluten was fixed between the plates by lowering the upper plate. Normally, it was not necessary to trim off excess sample at the edge. If it was necessary, a plastic knife was used. The edge of the sample was covered with vegetable oil to prevent drying. Additionally, the measuring system was covered with a lid containing moist foam rubber to create a chamber saturated by water vapor. The sample was allowed to relax for 30 min, during which time temperature equilibration could occur between the sample and the 30°C plate.

After the 30-min relaxation period, dynamic oscillatory measurement (frequency sweep mode) was performed first, directly followed by creep test. The stress sweeps were conducted separately, the test piece preparation and the 30-min relaxation period were the same as for frequency test and creep test.

### Moisture Content of the Wet Gluten

From two centrifuged, combined glutes, 4 g were used for rheometry as described above. The remainder was stored in a tightly closed vessel to prevent moisture loss, until the preparations for rheometry were finished. It was then dried for 4 min at ≈150°C using the Glutork gluten dryer (Perten Instruments AB, Huddinge, Sweden).

### Baking Test

The formula was spelt flour (300.0 g, 14% moisture basis), deionized water (201.0 mL), fresh compressed yeast (3.0 g), analytically pure sodium chloride (6.0 g), sucrose (3.0 g).

The ingredients were mixed in the farinograph (300-g mixing chamber) until maximum consistency was reached. The dough was then put into a bowl that was tightly covered and allowed to rest for 2 hr and 30 min at 14°C (bulk fermentation time). During this time, at 1 and 2 hr, the dough was reshaped by spreading flatly on the table and folding four times, once from each side. After the bulk fermentation time, six portions, 65.0 g each, were weighed, the surface covered with a small quantity of wheat starch, and machine rounded for 10 sec in a small rounder (ball homogenizer of the Maturograph, Brabender OHG, Duisburg, Germany). A final proof followed (35 min at 30°C and 90% rh). Finally, the rolls were baked for 20 min at 220°C in a thermostatically controlled oven. At the beginning, steam was injected for 7 sec to provide an adequate level of oven moisture.

One hour after baking, the six rolls were weighed, and their volume was determined by rapeseed displacement. Afterward, the ratio of the width to the height was determined for each roll separately and the mean was calculated from the six values. The volume was used to calculate the volume-yield [mL/(100 g flour)] and from the weight of the rolls and the weight of the dough pieces (6 × 65 g), the baking loss (relative loss of weight, based on the weight of the six dough pieces) was calculated.

### Statistics

The rheological data, the SDS-sedimentation volume, and the moisture content of the wet gluten were determined at least in duplicate. If the correspondence was not satisfactory, up to five replicates were made. Because two wet glutes had to be combined for rheology, the wet gluten content was calculated from at least four replicates.

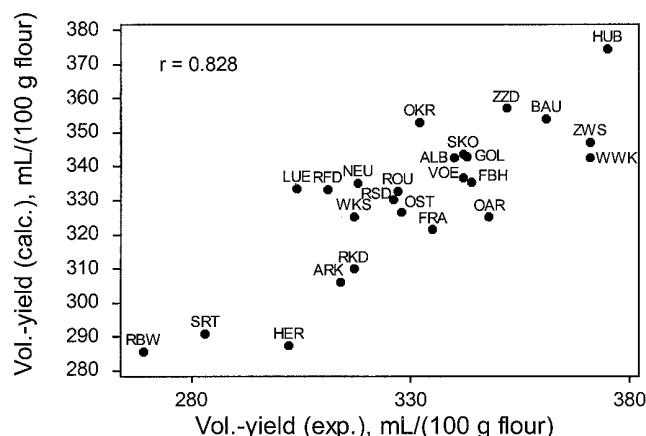
Assuming equal variances for all samples, the pooled sample standard deviation was calculated. Because the total sample size was very high (between 129 for the SDS test and 306 for the wet gluten content), the pooled sample standard deviation was regarded to equal the population standard deviation  $\sigma$ . Then,  $\sigma$  is a known parameter, and designated as  $\sigma_0$ .

To estimate the worst case, the 95% confidence interval for the population mean of any sample  $i$  was calculated assuming normal distribution and a minimum sample size of 4 for wet gluten content and 2 for all other results, respectively. The length of that confidence interval is:

$$L = \frac{2\sigma_0}{\sqrt{n_i}} z_{\frac{1+\gamma}{2}}$$

where  $\sigma_0$  is the known population standard deviation,  $n_i$  is the sample size of sample  $i$  assumed as 2 (or 4 for the wet gluten content only), and  $z_{(1+\gamma)/2}$  is the value from the normal table with  $\gamma$  being the confidence level (0.95).

Multiple linear regression using least squares regression was performed with statistical software (v. 12.21, Minitab Inc., State College,



**Fig. 3.** Calculated values of volume-yield vs. experimental values for 25 cultivars of set II. For multiple linear regression, response was experimental value of volume-yield; predictors were seven parameters characterizing functional gluten properties. Regression equation calculated volume-yield using the seven parameters.

**TABLE II**  
Parameters for Characterization of Functional Gluten Properties<sup>a</sup>

Parameter	Low Values	Minimum	Maximum	High Values
SDS sedimentation volume, mL	Low amount of swollen glutenin when flour is treated with SDS-lactic reagent	9	78	High amount of swollen glutenin when flour is treated with SDS-lactic reagent
Compliance (50 Pa, 5 min), 10 <sup>-3</sup> /Pa	Higher resistance to deformation (firm) at creep	6.06	33.0	Lower resistance to deformation (soft) at creep
Relative recovery, %	Lower percentage of deformation at creep is recoverable (gluten is less elastic)	36.6	67.0	Higher percentage of deformation at creep is recoverable (gluten is more elastic)
IG*  (10 Hz), 10 <sup>3</sup> Pa	Lower resistance to deformation (soft) in linear viscoelastic region	1.59	3.97	Higher resistance to deformation (firm) in linear viscoelastic region
Phase angle $\delta$ (10 Hz), °	More elastic components (0°: totally elastic material, Hookean solid)	30.9	40.4	Less elastic components (90°: totally viscous material, Newtonian liquid)
Moisture content of the wet gluten, %	Lower hydration capacity of gluten	60.3	66.0	Higher hydration capacity of gluten
Wet gluten content, %	Lower percentage of gluten proteins	21.4	57.1	Higher percentage of gluten proteins

<sup>a</sup> Range of values obtained for spelt cultivars (sets I, II, III) and meaning of low and high values.

PA). Either the volume-yield or the ratio of the width to the height from the baking test were used as the response while seven parameters characterizing functional gluten properties were used as predictors: SDS-sedimentation volume (1), compliance (50 Pa, 5 min) (2) and relative recovery (3) from the creep tests with wet gluten, absolute value of the complex dynamic modulus ( $|G^*|$ ) (4) and phase angle ( $\delta$ ) (5) at 10 Hz from the dynamic oscillatory measurements, moisture content of the wet gluten (6), and wet gluten content (7).

The resulting regression equation was used to calculate volume-yield or ratio of the width to the height from the seven predictors. These calculated values were plotted versus the experimental values (volume-yield or ratio of the width to the height from the baking test). Additionally, the correlation coefficients between corresponding calculated and experimental data were calculated.

## RESULTS AND DISCUSSION

### Linear Viscoelastic Region and General Dependence of Rheological Properties on Amount of Strain

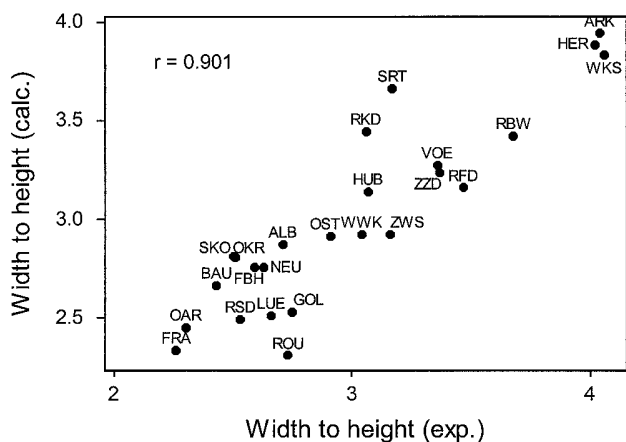
Dynamic oscillatory measurements should be performed within the linear viscoelastic region of a material. In that region, the amplitude of the shear strain is proportional to the amplitude of the shear stress applied. Then, the absolute value of the complex dynamic modulus,  $|G^*|$ , is independent of the stress or strain amplitude:

$$|G^*| = \sqrt{(G')^2 + (G'')^2} = \frac{\tau_0}{\gamma_0}$$

where  $G'$  is the storage modulus,  $G''$  is the loss modulus,  $\tau_0$  is the stress amplitude, and  $\gamma_0$  is the strain amplitude.

Furthermore, it is only when a specimen responds linearly to a sinusoidal stress that the resulting strain is also sinusoidal. When the response is nonlinear, a sinusoidal stress gives a nonsinusoidal strain, in which case  $G^*$  cannot be defined (Smith et al 1970).

To estimate the linear viscoelastic region of wet gluten from split in general, two cultivars from set I were chosen representing very different extremes: SRT has a very soft gluten, BAU a very firm one, when compared manually. Stress sweeps were performed with wet gluten from these cultivars. Because the breakdown of any structure depends on strain or strain rate (and only indirectly on stress),  $|G^*|$  was plotted versus the strain (amplitude) resulting from the stress (amplitude) (Fig. 2). The  $|G^*|$  of the gluten from BAU is horizontal in the diagram over a wide range, up to a strain of  $\approx 5 \times 10^{-3}$ . The horizontal  $|G^*|$  indicates that it is independent of the strain amplitude. Therefore, this is the linear viscoelastic region. The gluten from SRT has a shorter linear viscoelastic region, up to  $\approx 1 \times 10^{-3}$ . Based on these results,  $1 \times 10^{-3}$  (0.001) was chosen as target



**Fig. 4.** Calculated values for ratio of width to height vs. experimental values for 25 cultivars of set II. For multiple linear regression, response was experimental value for width-to-height ratio; predictors were the seven parameters characterizing functional gluten properties. Regression equation calculated width-to-height ratio using the seven parameters.

strain for the dynamic oscillatory measurements with different samples. With regard to SRT and similar cultivars, even smaller strain amplitudes could be considered. On the other hand, at strain amplitudes of  $10^{-4}$  or smaller, close to the limit of the instrument, some noise in the measurements is visible. Therefore, too small amplitudes will probably produce less reproducible results.

When comparing the results of the two gluten, the SRT gluten has much higher  $|G^*|$  values within the strain range examined, in spite of the fact that for the SRT gluten,  $|G^*|$  starts to decrease earlier. Higher values of  $|G^*|$  indicate that a certain stress amplitude will cause a smaller strain amplitude (resistance of the gluten to the very small stresses in the oscillatory measurement is higher, i.e., the gluten is firmer in the linear viscoelastic region). This seems to be a contradiction to what was said earlier: the SRT gluten is very soft when compared with the BAU gluten manually. It should be emphasized, therefore, that the results obtained from the oscillatory measurements are valid only at very small strains. Deformations of 0.001 (0.1%) are much smaller than those deformations that occur when touching a wet gluten with one's fingers. (When one pulls a wet gluten with one's fingers, it can be extended to more than twice its original length easily.) Concerning the former case, at strains  $< 3\%$ , short-range interactions dominate, like van der Waals and hydrogen bond interactions (Amemiya and Menjivar 1992). Concerning the latter case, according to Amemiya and Menjivar, at strains of 25–1,300%, longer range (protein-protein) interactions are important. Amemiya and Menjivar were examining doughs, while wet gluten were examined in the present study. In doughs, starch-starch and starch-protein interactions are short-range. In wet gluten, starch granules are largely washed out. The gluten proteins (gliadin and glutenin) must therefore be the focus of attention, of which glutenin contains intermolecular disulfide bonds unlike gliadin. Therefore, a higher percentage of glutenin would mean more longer range disulfide bonds. This could explain why BAU gluten also shows

**TABLE III**  
Pearson Correlation Coefficients Between Parameters Characterizing Functional Gluten Properties with Volume-Yield and Width-to-Height Ratio of Rolls<sup>a,b</sup>

Parameter	Volume-Yield	Width-to-Height Ratio
SDS sedimentation volume		
Set I	0.777**	-0.944***
Set II	0.458*	-0.801***
Set III	0.749***	-0.822***
Compliance (50 Pa, 5 min)		
Set I	-0.398	0.860***
Set II	-0.379	0.656***
Set III	-0.505**	0.846***
Relative recovery		
Set I	0.504	-0.849***
Set II	0.337	-0.521**
Set III	0.608**	-0.742***
$ G^* $ (10 Hz)		
Set I	-0.855***	0.649*
Set II	-0.625***	0.494*
Set III	-0.568**	0.308
Phase angle $\delta$ (10 Hz)		
Set I	-0.408	0.815**
Set II	-0.525**	0.701***
Set III	-0.347	0.663***
Moisture content of the wet gluten		
Set I	0.507	-0.815**
Set II	0.645***	-0.536**
Set III	0.501*	-0.570**
Wet gluten content		
Set I	-0.126	0.215
Set II	-0.006	0.070
Set III	-0.123	0.104

<sup>a</sup> \*, \*\*, \*\*\* = significantly different from zero  $P = 0.05, 0.01, \text{ and } 0.001$ , respectively. Two-tailed test ( $H_0 \rho = 0$  vs.  $H_1 \rho \neq 0$ ).

<sup>b</sup> Sets II and III ( $n = 25$ ), set I ( $n = 11$ ).

linear behavior at higher strains in comparison to SRT. The higher values of  $|G^*|$  of the SRT gluten could be caused by short-range interactions between gliadin molecules, probably in combination with a lower moisture content of that gluten and with a greater amount of particles dispersed in the gluten matrix (e.g., residual starch granules and bran particles). Indeed the moisture contents of the SRT and BAU glutes were 62.0 and 63.4%, respectively.

Besides  $|G^*|$ , the phase angles of the two glutes should be compared. For any given material the phase angle is between  $0^\circ$  (totally elastic material, Hookean solid) and  $90^\circ$  (totally viscous material, Newtonian liquid). Both glutes show viscoelastic behavior. Nevertheless, in contrast to BAU, the SRT gluten has higher phase angles throughout the complete strain range, indicating a more viscous, fluid-like behavior. It is a well-established fact that gliadin is the viscous and glutenin the elastic component of gluten (Kim et al 1988). Finally, while the phase angle of the BAU gluten is more or less constant within the strain range examined, the phase angle of the SRT gluten starts to increase at a strain of  $\approx 10^{-2}$ , indicating a decrease of elasticity, probably caused by the destruction of the short-range interactions (Fig. 2). The hypotheses concerning the differences

between BAU and SRT are in keeping with data reported by Kieffer et al (1997), although the results of these authors are not entirely comparable to the results of the present study because the material used originated from a different breeding program. Using a combined extraction and RP-HPLC procedure, Kieffer et al (1997) determined the gluten protein types of spelt cultivars quantitatively and calculated an almost twofold higher gliadin-to-glutenin ratio for SRT than for BAU.

One last aspect has to be discussed here. The hypothesis that gliadin is responsible for higher values of  $|G^*|$  seems to be in contradiction to the results of Janssen et al (1996b), who describe that the dynamic moduli increased with increasing glutenin content of reconstituted glutes. Nevertheless, several facts have to be taken into account when comparing the findings of Janssen et al (1996b) with those of the current study.

Janssen et al (1996b) used freeze-dried gluten fractions and a constant amount of water was added (50%). In the present study however, the amount of water in the gluten was variable, depending

**TABLE IV**  
Relationship Between Parameters Characterizing Functional Gluten Properties and High Volume-Yield or Round Shape of Rolls<sup>a</sup>

	High Volume-Yield	Round Shape <sup>b</sup>
SDS sedimentation volume	High	High
Compliance (50 Pa, 5 min)	ns <sup>c</sup>	Low
Relative recovery	ns	High
$ G^* $ (10 Hz)	Low	ns
Phase angle $\delta$ (10 Hz)	ns	Low
Moisture content of the wet gluten	ns	High
Wet gluten content	ns	ns

<sup>a</sup> Based on correlation coefficients in Table III.

<sup>b</sup> Low width-to-height ratio.

<sup>c</sup> Not significantly different from zero in at least one of the three sets.

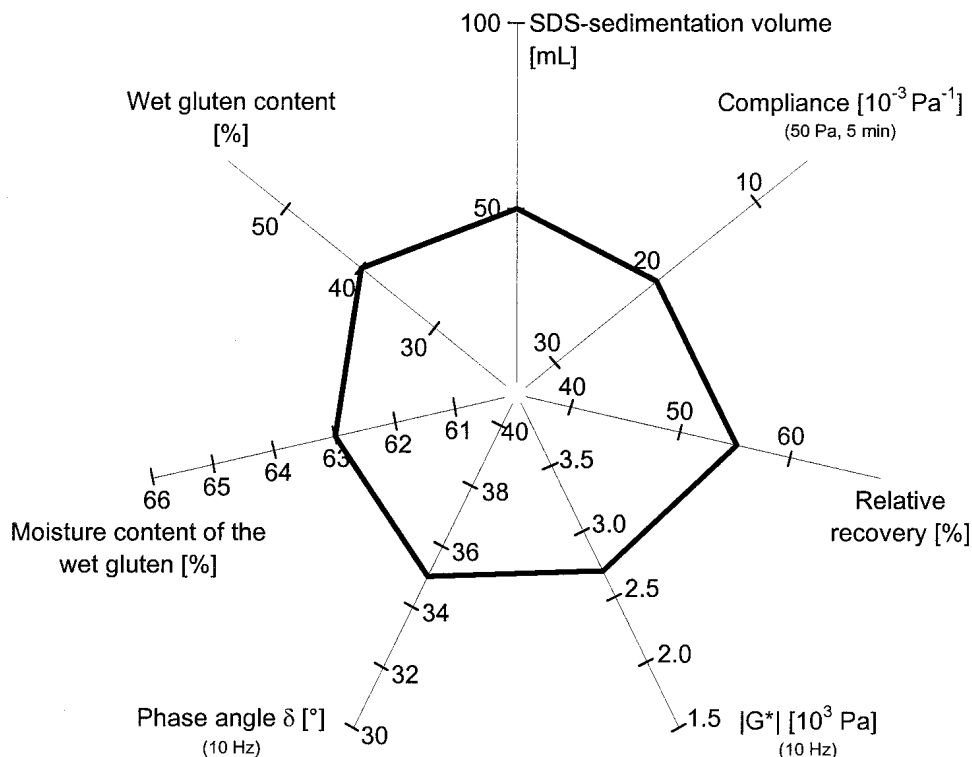
**TABLE V**  
Reproducibility of Parameters<sup>a</sup>

Parameter	$s^b$	$L^c$
SDS sedimentation volume, mL	1.23	<3.5
Compliance (50 Pa, 5 min), $10^{-3}/Pa$	1.82	<5.1
Relative recovery, %	2.18	<6.1
$ G^* $ (10 Hz), $10^3 Pa$	0.165	<0.46
Phase angle $\delta$ (10 Hz), $^\circ$	0.403	<1.2
Moisture content of the wet gluten, %	0.396	<1.1
Wet gluten content, %	0.512	<1.1

<sup>a</sup> All results of Sets I, II, and III were used; BEG and SER were included.

<sup>b</sup> Pooled sample standard deviation. Because total sample size was very high,  $s$  was regarded as equal to the population standard deviation ( $\sigma$ ). Thus,  $\sigma$  is a known parameter and designated as  $\sigma_0$ .

<sup>c</sup> Length of 95% Z-confidence interval based on pooled sample standard deviation ( $s \approx \sigma$ ). Minimum sample assumed ( $n = 4$  for wet gluten content;  $n = 2$  for all other results); results always rounded up (data are maximum values).



**Fig. 5.** Radar chart profile of a hypothetical cultivar with seven axes for the seven parameters characterizing functional gluten properties. On each axis, values correlated with a high volume-yield or a round shape (Tables III and IV) are located on the periphery. High values of wet gluten content were arbitrarily assigned to the periphery.

on the swelling capacity of the gluten. Furthermore, in the present study, the correlation coefficients between the moisture content of the wet gluten and the SDS-sedimentation volume were 0.838\*\*, 0.620\*\*, and 0.548\*\* for sets I, II, and III, respectively, indicating that a higher amount of swollen glutenin could be responsible for a higher moisture content of the wet gluten. Moreover, |G\*| was correlated with SDS-sedimentation volume (-0.798\*\*, -0.648\*\*, and -0.565\*\*) and with moisture content of the wet gluten (-0.536, -0.647\*\*, and -0.267). This indicates that in the present study, swollen glutenin decreased |G\*| due to its higher water content.

The amount of residual starch in the gluten seems to be another factor. Martini (1997) found higher amounts of residual starch in softer, more sticky glutes prepared by machine washing according to ICC Standard No. 137/1 (ICC 1999). Gliadin is a very sticky substance that will probably prevent the starch granules from being completely washed out. In contrast, the gliadin fraction obtained by

extraction with 70% ethanol by Janssen et al (1996) contained <1% starch, while the glutenin fraction (the residue) contained up to 15% starch. Therefore, in the study of Janssen et al (1996), there were more starch granules in the gluten when more glutenin was present, in contrast to the current study where there were probably more starch granules in the gluten when the gluten contained more gliadin. In view of the fact that short-range starch-protein interactions (Amemiya and Menjivar 1992) could increase the moduli, this difference in the amount of starch associated with the gliadin and glutenin fractions could be an additional explanation for the different effect of the gliadin and of the glutenin fraction on the moduli in the two studies.

### Characterization of Functional Gluten Properties

Seven parameters, obtained from various methods, were chosen to characterize the functional gluten properties: 1) SDS-sedimen-

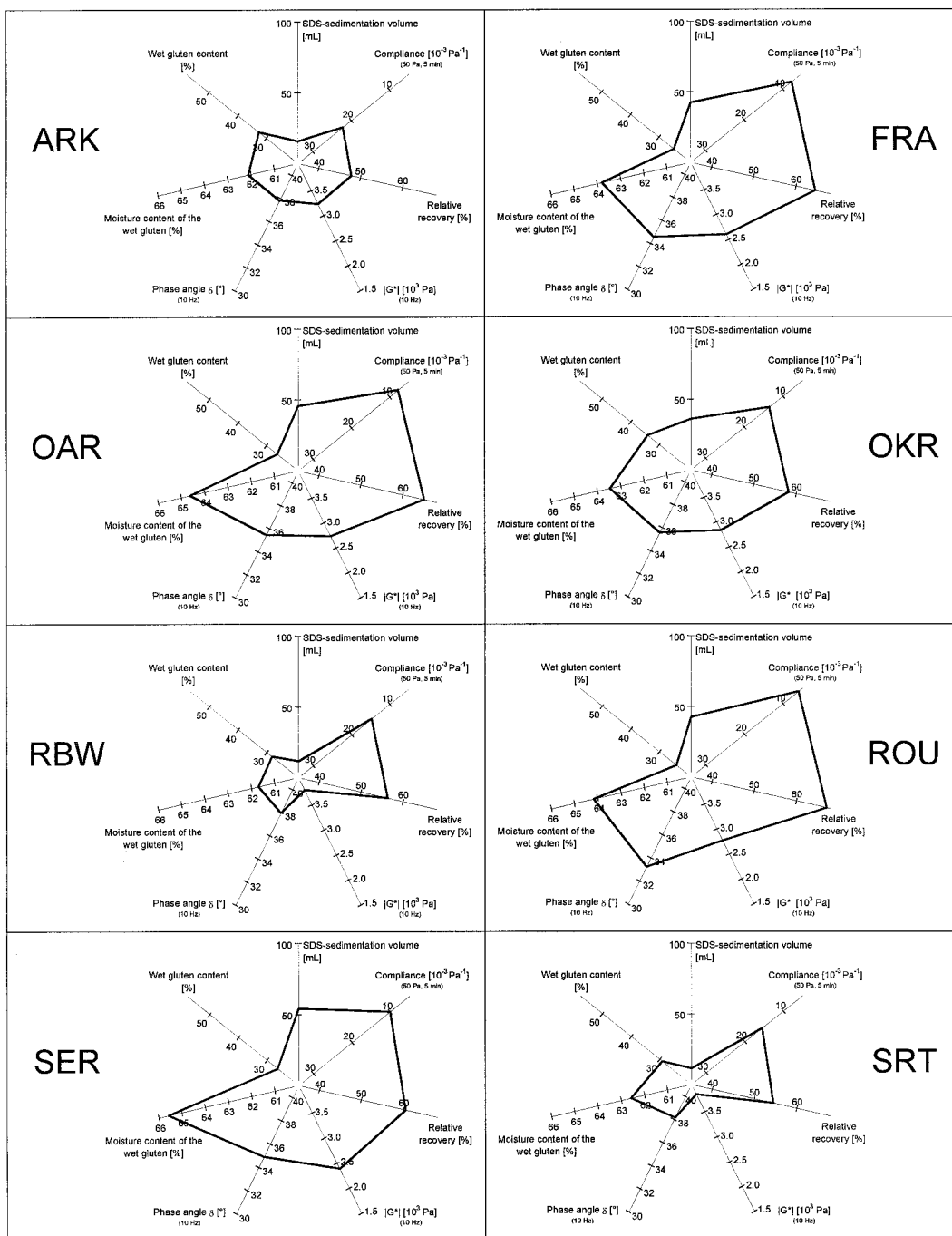


Fig. 6. Radar chart profiles of gluten properties for eight spelt cultivars (Table I, SER = Sertel). All cultivars were from set II.

tation volume; 2) compliance (50 Pa, 5 min) and 3) relative recovery from the creep tests with wet gluten; 4) absolute value of the complex dynamic modulus ( $|G^*|$ ) and 5) phase angle ( $\delta$ ) at 10 Hz from the dynamic oscillatory measurements; 6) moisture content of the wet gluten; and 7) wet gluten content. The range of values obtained for these parameters from all samples examined (sets I, II, and III) are presented in Table II. Some additional information concerning the meaning of low and high values is also given.

With regard to the SDS-sedimentation volume, Eckert et al (1993) showed that gliadin dissolves completely in the SDS-lactic reagent, whereas glutenin swells. These authors concluded that the swelling of glutenin was responsible for the sedimentation effect without any contribution from gliadin. Therefore, higher SDS-sedimentation volumes should indicate a greater amount of swollen glutenin, either because of a higher percentage of glutenin in the flour or because of a higher hydration capacity of the glutenin in the SDS-lactic reagent.

In contrast to the SDS-sedimentation volume, the moisture content of the wet gluten indicates the hydration capacity of the gluten as a whole. This includes the swelling of the gluten in dilute salt (NaCl) solution at pH 5.95 during washing and the capability of the gluten to bind the water during centrifugation. Wet gluten from spelt contains roughly two-thirds water (Table II).

The wet gluten content of spelt can vary a great deal between cultivars, as indicated by the minimum and maximum values given in Table II. Differences may result from the individual properties of the cultivars as well as from the level of nitrogen fertilizer added. Extraordinary high percentages of wet gluten can be found in some spelt cultivars (note the maximum of 57.1%).

The results of the fundamental rheological test methods confirm that wet gluten from spelt is a viscoelastic substance, the relative recoveries in the creep tests were between 0 and 100%, the phase angles in the dynamic oscillatory measurements were between 0 and 90°. Each of the two rheological test methods provides information about firmness (compliance,  $|G^*|$ ) and elasticity (relative recovery, phase angle). The differences in the amount of deformation occurring during the application of the two methods should be noted. Whereas the oscillatory measurements are performed at strains of 0.1%, the compliance values between  $6.06 \times 10^{-3}/\text{Pa}$  and  $33.0 \times 10^{-3}/\text{Pa}$  in the creep tests correspond to maximum strains of 30.3–165%.

### Functional Gluten Properties and Baking Quality

Seven parameters characterize functional gluten properties. To examine whether there are significant relationships between these parameters and baking quality, Pearson's correlation coefficients were calculated between the seven parameters and the volume-yield and ratio of the width to the height of the rolls. These correlation coefficients were calculated for the three sets of samples separately. A two-tailed test was performed to test whether the correlations are significantly different from zero (Table III).

Concerning the volume-yield, significant correlations in all three sets only exist with the SDS-sedimentation volume and  $|G^*|$ . Despite statistical significance, two of these six correlation coefficients are  $<0.6$ , indicating only a low degree of linear relationship. The ratio of the width to the height is significantly correlated with more parameters (all parameters except for  $|G^*|$  and wet gluten content). Furthermore, the correlation coefficients with the ratio of the width to the height are higher in general. Table IV summarizes these results. Based on the correlation coefficients in Table III, it is listed for each parameter whether high or low values indicate a high volume-yield or a round shape (low ratio of the width to the height). Only the correlations significant in all three sets were used for that prediction (Table IV).

In general, a round shape of the rolls is obtained 1) if the gluten is firm at the larger deformations of the creep test, 2) if more elastic components contribute to the rheological behavior at small deformations as well as at larger deformations (the gluten is "more

elastic"), 3) if the swelling of glutenin (SDS-sedimentation volume) and of the gluten as a whole (moisture content of the wet gluten) are more marked.

A high volume-yield is obtained if the SDS-sedimentation volume is high. This indicates once more the dominant role of quantity and quality of glutenin. Furthermore, low values of  $|G^*|$  are quite distinctly associated with high volume-yields. Low values of  $|G^*|$  indicate that the gluten is softer at the small deformations within the linear viscoelastic region. Thus, softness at small deformations is important for a high volume in contrast to firmness at larger deformations, which is important in achieving a round shape. During the final proof, the growth of the gas cells in the dough will cause an expansion of the membranes between the gas cells. This growth of the gas cells, and therefore also the expansion of the gluten network in the membranes, is a relatively slow process. A low resistance to small deformations should therefore enable easier membrane expansion. Furthermore, it was demonstrated by the response of the SRT gluten to stress sweeps that high firmness at small strains of  $\approx 0.1\%$  can be associated with a quick breakdown of structure at increasing deformations. Breakdown of structure was attributed to destruction of (short-range) interactions between gliadin molecules. If firmness at small deformations is caused by such short-range interactions, this could explain not only a high resistance to the expansion of the membranes between the gas cells, but also a quick rupture of these membranes because of the breakdown of the short-range interactions between the gliadin molecules.

It must be pointed out that there were no correlations between wet gluten content and baking quality when comparing different cultivars with the same level of nitrogen fertilization. This has to be distinguished from studies in which cultivars were grown at different nitrogen levels.

A lot of significant correlations do exist (Table III). Nevertheless, the correlation coefficients often are not too high, and the correlations are significantly different from zero only because of the relatively high number of samples. To better predict baking properties based on gluten properties, multiple linear regression was performed using the seven parameters as predictors and the volume-yield and the ratio of the width to the height as responses. For set II, Fig. 3 shows a plot of the calculated values of the volume-yield versus the experimental values, while Fig. 4 shows the same for the ratio of the width to the height. Similar results were obtained for sets I and III. The correlation coefficients between the calculated and experimental values of the volume-yield were 0.913, 0.828, and 0.787 for sets I, II, and III, respectively, while the correlation coefficients between the calculated and experimental values of the ratio of the width to the height were 0.994, 0.901, and 0.927. Multiple linear regression did improve the correlation between functional gluten properties and baking quality (volume-yield and ratio of the width to the height) in comparison with the single Pearson's correlation coefficients (Table III). This was the case for all three sets. The correlation coefficients between the calculated and the experimental values for the ratio of the width to the height were higher than those for the volume-yield in all sets. In principle, the experimental values of baking results never can be predicted exactly, as indicated by correlation coefficients  $<1$ . Reasons for this may include errors in the determination of the values of the seven parameters as well as in the determination of the baking results. Furthermore, other factors, besides the gluten properties characterized by the seven parameters, will probably influence baking results. Examples are rheological properties of the gluten proteins in ranges of strain or strain rate different from those applied here, as in extension tests, but also completely different variables, such as the  $\alpha$ -amylase-activity that influences gas formation by the yeast or pentosan content (water-binding capacity) of the flours. Moreover, interactions like those between the amount of water in the dough, starch, and pentosan content of the flour and gluten quantity and quality, may also influence the baking results. All these unknown factors seem to affect the volume more than the shape of rolls. Nevertheless, the seven parameters that characterize

functional gluten properties are important factors for the baking quality of spelt cultivars. The importance of the gluten properties in determining the baking properties may be explained by the results of Kieffer and Stein (1999). According to these authors, during periods of rest (relaxation periods), gluten and starch separate within a wheat dough. At first, the gluten contracts and forms more dense protein bodies (demixing). If the dough is reshaped after the rest period, these protein bodies reaggregate (fuse together) and form a more coherent network with thick gluten strands, whereas starch granules accumulate in the meshes of this network. This could explain why the intrinsic properties of the wet gluten reflect baking properties. The thick gluten strands could predominate with regard to baking quality while starch-gluten interactions could be of minor importance because the starch is accumulated in the network and largely separated from the gluten.

### Comparing Different Cultivars

Comparison of several cultivars on the basis of seven parameters is a problem. To enable such comparisons and to further allow a rapid assessment of a cultivar's gluten properties, we designed a special diagram. This is a radar chart with seven axes, one for each parameter (Fig. 5). On each axis, values correlated with a high volume-yield or a round shape (Tables III and IV) are located on the periphery, whereas values correlated with a low volume or flat shape are located in the center. The results listed in Tables III and IV are unequivocal for all except for the wet gluten content. On the axis for the wet gluten content, high values were arbitrarily assigned to the periphery, low values to the center. When all seven parameters of a cultivar are displayed in the radar chart, a profile of that cultivar's gluten properties is generated. Figure 6 shows the profiles of eight selected cultivars from set II.

In rheology, high standard deviations are a common problem. For example, Janssen et al (1996a) found coefficients of variation, depending on cultivar and frequency, of 0.7–14.5% for  $G'$  and 2.2–14.8% for  $G''$ , and 2.3–8.3% for  $\tan \delta$  in oscillatory measurements with glutes. To estimate these errors in the present work, the pooled sample standard deviations were calculated for all seven parameters, assuming equal variances for all samples. These calculations were based on all data available (i.e., all samples of set I, set II [BEG and SER included], and set III). Additionally, the lengths of the 95% confidence intervals for the population mean of any sample were calculated, assuming the minimum sample size of 4 for the wet gluten content and 2 for all other parameters. The results are presented in Table V. The relatively high errors preclude the differentiation of cultivars with similar gluten properties. However, a great range of different properties exists within European spelt cultivars as illustrated by a comparison of ROU, OKR, SRT, and ARK in Fig. 6. ROU has a low quantity of firm (based on the compliance) and elastic (based on phase angle and relative recovery) gluten, OKR a higher quantity of a more soft and less elastic gluten, whereas SRT and ARK, although somewhat different, seem to have altogether weak properties, especially with regard to the very low sedimentation volumes. On the other hand, several cultivars in Fig. 6 seem to be relatively similar, such as the four cultivars ROU, FRA, OAR, and SER, or the two cultivars SRT and RBW. Finally, it must be emphasized that all cultivars compared in Fig. 6 were grown in the same year, at the same location, and with the same quantity of applied nitrogen. Further work is in progress to classify the spelt cultivars by groups on the basis of gluten properties using cluster analysis. Due to the high standard deviations, analysis of variance (ANOVA) procedures will be performed on cultivars representative of each group. In addition, the influence of the nitrogen fertilization on the gluten properties will be examined.

### CONCLUSIONS

Gluten properties are the main factor determining the baking quality of spelt cultivars. The fundamental rheological methods described

here are well suited for the characterization of such functional gluten properties. The use of these methods in combination with some further simple tests (SDS-sedimentation test, determination of the moisture content of the wet gluten, and of the wet gluten content) is recommended. European spelt cultivars can be very different with regard to such gluten properties. Further investigations are necessary to classify all the cultivars available into groups with similar properties and to examine the influence of nitrogen fertilization and other environmental factors on gluten properties. Nevertheless, it is already obvious from the results presented here, that the great amount of diversity within the European spelt cultivars must be taken into consideration when characterizing spelt in general terms or when comparing spelt and modern wheat. Surely, simple statements about spelt in general are impossible because of that diversity.

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