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Gluten proteins from spelt (*Triticum aestivum* ssp. *spelta*) cultivars: A rheological and size-exclusion high-performance liquid chromatography study ☆

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Abstract

The aim of this study was to understand the chemistry of spelt (*Triticum aestivum* ssp. *spelta*) gluten in relation to its quality, to classify European spelt cultivars based on gluten quality, and to compare their protein compositions with those of modern wheats. Gluten quality of two sets of 25 spelt cultivars was studied using dynamic oscillatory and creep tests, an SDS sedimentation test, moisture content of the wet gluten and wet gluten content. These data were compared with the results of size-exclusion HPLC analyses of the spelt proteins. Significant correlations indicated that insoluble polymeric proteins (IPP) contributed resistance to deformation in creep tests, elasticity in oscillatory and creep tests, and swelling capacity of the gluten. Gliadins had the opposite effects, whereas the contribution of soluble polymeric proteins (SPP) depended on the type of test. In creep tests (strain 0.3–1.5) SPP behaved similarly to gliadins, in oscillation (strain 0.001) they tended to increase elasticity. In comparison to hard red winter wheats, spelt was characterized by lower IPP, but higher gliadins and SPP, resulting in softer and less elastic glutens. A wide variation in gluten quality was found among the spelts. Three groups could be identified by cluster analysis (one closer to modern wheat, a second typical spelt group and a third a poor quality group).
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1. Introduction

Spelt (*Triticum aestivum* ssp. *spelta* L. Thell.) is an ancient subspecies of modern bread wheat (*Triticum aestivum* ssp. *aestivum*). Until the beginning of the 20th century, spelt was the predominant grain for bread production in many regions, for example in southwestern Germany, and parts of Switzerland and Austria. Since then, however, it has largely been displaced by modern wheat (Kling, 1993; Winzeler and Rüegger, 1990). This is due to spelt's lower yield and its long straw with a tendency to lodge, especially if levels of nitrogen fertilizer applied are too high. Furthermore, as spelt is a hulled grain, a dehulling step prior to milling is required (Campbell, 1997; Kling, 1993).

In the past few decades, however, spelt has undergone a renaissance as a niche product. This may be due to the perception that it is a 'healthier', more 'natural', or less 'over-bred' grain than modern wheat. Consequently, there are an increasing number of international publications on spelt food quality, spelt proteins, rheology of spelt dough or gluten, or comparisons of spelt and modern wheat from

Abbreviations: AG, albumins and globulins; db, dry basis; EP, extractable proteins; fp, flour protein; $|G^*|$, complex shear modulus (absolute value); Gli, gliadins; IPP, insoluble polymeric proteins; SE-HPLC, size-exclusion high-performance liquid chromatography; SPP, soluble polymeric proteins; wb, wet basis

☆Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Belgium (Legros and Castille, 1972; Ruibal-Mendieta et al., 2004), Canada (Abdel-Aal, 2003; Abdel-Aal et al., 1995, 1998), Czech Republic (Dvořáček et al., 2001, 2002; Moudrý and Dvořáček, 1999), Germany (Belitz et al., 1989; Gräber and Kuhn, 1992; Martini and Kuhn, 1999; Schober, 2001; Schober and Kuhn, 2003; Seibel et al., 1989), Italy (Marconi et al., 1999, 2002; Piergiovanni and Volpe, 2003; Piergiovanni et al., 1996), Poland (Grela, 1996), Slovak Republic (Bojňanská and Frančáková, 2002), Switzerland (Siedler et al., 1994; Winzeler and Rüegger, 1990), USA (Ranhotra et al., 1995, 1996), or international cooperations (Italy-Slovenia: Bonafaccia et al., 2000; Slovak Republic-Germany: Smolková et al., 2000; Slovenia-Sweden: Skrabanja et al., 2001). Results of this international research allows comparisons across a large variety of environments with different types of modern wheat as controls.

Unfortunately, very few differences between spelt and modern wheat have been confirmed experimentally. In respect to food quality, most of the studies found higher protein contents for spelt than for modern wheat (Bonafaccia et al., 2000; Campbell, 1997; Dvořáček et al., 2001, 2002; Marconi et al., 1999; Moudrý and Dvořáček, 1999; Piergiovanni et al., 1996; Ranhotra et al., 1996), although in some, the opposite was reported (Grela, 1996; Ranhotra et al., 1995) and in some cases differences were not significant. Overall, the values found for proximate compositions and minor components in spelt are within the range found among modern wheats (for an overview, see Campbell, 1997). Spelt gluten tends to be more extensible and less elastic than gluten from modern wheat, resulting in the typical, weaker spelt doughs (Abdel-Aal et al., 1997, 1998, 1999; Gräber et al., 1994; Marconi et al., 1999, 2002; Ranhotra et al., 1995). As a consequence, for bread, rolls or two-layer flat bread, high doses of ascorbic acid, shorter mixing times, reduced water addition or longer dough rest times have been proposed (Abdel-Aal et al., 1998, 1999; Gräber et al., 1994; Ranhotra et al., 1995; Seibel et al., 1989), and for pasta, high-temperature drying has been recommended (Marconi et al., 1999, 2002).

It is important to emphasize that spelt is as harmful for celiac patients as modern wheat (Forssell and Wieser, 1995; Kasarda and D'Ovidio, 1999). Furthermore, although many end-users seem to believe that spelt might be tolerated by people with allergic reactions to wheat (not celiac disease), there is no supporting scientific evidence. In fact, in a single study with only one subject with severe wheat allergy, this person was also allergic to spelt (Friedman et al., 1994). Clearly, until more research is conducted on the overall allergenicity of spelt, a wheat subspecies, spelt cannot be recommended for people with wheat allergy. Spelt has, however, been shown to contribute to genetic diversity within wheat (Abdel-Aal, 2003; Siedler et al., 1994). It may also have a role for speciality breads and other food products with characteristics different from regular wheat products or for organic food (Abdel-Aal, 2003; Abdel-Aal et al., 1998; Ranhotra et al., 1995). For example, in southern Germany, traditional spelt breads are produced (Schober et al., 2002) and breeding spelt cultivars with typical properties, different from modern wheat but specific for such speciality breads, might be desirable.

Agronomically, spelt may be more resistant to disease, and do better under less advantageous growing conditions, such as wet, cold soils and at high altitudes (Campbell, 1997; Kling, 1993; Winzeler and Rüegger, 1990). Because of the protection provided by the hulls, chemical treatment of hulled seeds used for sowing may not be required (Kling, 1993). Additionally, excess nitrogen fertilization is not possible due to the incidence of lodging of the long, less stable straw (Campbell, 1997; Kling, 1993; Winzeler and Rüegger, 1990). This low nitrogen tolerance may thus be seen as an advantage in providing a more environmentally friendly crop. For the same reason, it is not easy to compare spelt and modern wheat grown under identical conditions, because optimum growth conditions for modern wheat would include more nitrogen fertilization than for spelt. This was clear from Kling's (1993) agronomic comparison of 23 spelt cultivars and two modern German bread wheat cultivars under growth conditions optimized for spelt, where the yield of the modern wheats was most likely below their real potential.

Size-exclusion high performance liquid chromatography (SE-HPLC) has been widely used to characterize the proteins of modern wheat (Batey et al., 1991; Bean and Lookhart, 2001; Bean et al., 1998; Larroque et al., 1997; Singh et al., 1990a) and to study the functional effects of gluten components that differ in their degree of polymerization (Bean et al., 1998; Cornec et al., 1994; Dachkevitch and Autran, 1989; Gupta et al., 1993, 1995; Lundh and MacRitchie, 1989; Nightingale et al., 1999; Park et al., 2006; Rao et al., 2001; Singh et al., 1990b). An overview is given by Southan and MacRitchie (1999), whereas Hamer and van Vliet (2000) provide a general discussion on the structure-functionality relationships of gluten. Although there are comprehensive studies including SE-HPLC, empirical and fundamental rheological tests, and hearth and pan loaf breadmaking tests made on large sets of Norwegian spring and winter wheat cultivars (Tronsmo et al., 2002, 2003) similar information for spelt is not available. There is a wide range of gluten quality within spelt, so that glutens from different spelt cultivars grown under controlled conditions might be an interesting model system. Although old landraces of spelt may represent primitive quality, they may also have already been selected for improved quality, while modern spelt cultivars are often crosses between spelt and modern wheat which have been systematically bred to resemble modern wheat in quality.

Previously we compared quality characteristics of spelt cultivars with their pedigrees, taking into account whether they were crosses between spelt and modern wheat (Schober, 2001; Schober and Kuhn, 2003). Also we (Schober et al., 2002) established correlations of fundamental rheological

and other quality related parameters for spelt gluten with baking quality, which indicated wide variations of quality within spelt.

The aim of the present study was twofold. First, to use the large variation within spelt in order to establish correlations between fundamental rheological and quality parameters of gluten and the size distribution of gluten proteins as measured by SE-HPLC. Second, to classify 25 spelt cultivars from a German breeding program, grown in two environments and to compare them with modern American wheat cultivars grown under conditions typically used for wheat. This information may help to provide breeders with spelt cultivars of well-defined quality for use in further breeding or as references in breeding programs and may also facilitate an in depth understanding of the diversity in gluten quality within spelt.

2. Experimental

2.1. Materials

Twenty-five spelt cultivars were grown in two environments in the 1996–97 season. Location H was an experimental farm at University of Hohenheim, Stuttgart, Germany, while location OLi was an experimental farm (Oberer Lindenhof) south of Stuttgart. The altitudes, average rainfall, average temperature and nitrogen (N) levels at the locations H and OLi were 400 and 730 m, 685 and 960 mm, 8.5 and 6.6 °C, 30 and 70 kg/ha, respectively. The cultivars used and their abbreviations are: Albin (ALB), Altgold Rotkorn (ARK), Bauländer Spelz (BAU), Fuggers Babenhauser Zuchtvesen (FBH), Franckenkorn (FRA), "Goldir" (GOL, experimental line), Hercule (HER), Hubel (HUB), Lueg (LUE), Neuegger Weißkorn (NEU), Ostar (OAR), Oberkulmer Rotkorn (OKR), Ostro (OST), von Rechbergs Brauner Winterspelz (RBW), von Rechbergs Früher Dinkel (RFD), Roter Kolbendinkel (RKD), Rouquin (ROU), Roter Schlegeldinkel (RSD), Schwabenkorn (SKO), Steiners Roter Tiroler (SRT), Vögelers Dinkel (VOE), Weißer Kolbenspelz (WKS), Waggershauser Hohenheimer Weißer Kolbendinkel (WWK), Zeiners Weißer Schlegeldinkel (ZWS), Zuzger Dinkel (ZZD). Their countries of origin, years of first appearance or registration, pedigrees, similarities in gliadin patterns, and relatedness to modern wheat have been described previously (Schober et al., 2002; Schober and Kuhn, 2003), and their agronomic performance described by Kling (1993).

All samples (dehulled kernels) were obtained from the State Plant Breeding Institute, University of Hohenheim, Stuttgart, Germany. They were milled into flour (0.5–0.8%db ash, ICC Standard No. 104/1, ICC, 2000) on a Brabender Quadrumat Jr. flour mill (Duisburg, Germany).

2.2. Methods

2.2.1. SE-HPLC analysis

Flours were extracted with 50% aqueous 1-propanol as described by Bean et al. (1998) with the following modifications: 100 mg of flour were used per ml of extractant. After extraction the pellets were freeze-dried before nitrogen determination, instead of heat drying after application of acetone. Equal volumes of the first and second extracts were pooled and the third extract discarded. SE-HPLC was performed on a Hewlett-Packard 1090 HPLC system, using a Biosep SEC-4000 column (Phenomenex, Torrance, CA). Column temperature was maintained at 40 °C and the mobile phase was 50% acetonitrile and 0.1% (w/v) trifluoroacetic acid at a flow rate of 0.5 ml/min. Injection volume was 20 µl and UV-detection was done at 210 nm (adapted from Bean and Lookhart, 2001). The weight (mg) of insoluble polymeric protein (IPP) was calculated from the weight and protein content of the freeze dried pellet, extractable protein (EP) was calculated from the difference between flour protein (mg) and protein in the pellet (IPP, mg). In all cases, protein was calculated as $N \times 5.7$. The protein size groups (soluble polymeric proteins, SPP; gliadins, Gli; and albumins and globulins, AG) were quantified as the percentage of the respective areas relative to the total HPLC area multiplied by EP (more details on properties of IPP, SPP, Gli and AG, and the cutoff in the chromatograms are given later in this section). In all cases, the size groups were calculated as absolute values (mg protein), corresponding to percent protein on a flour weight basis, (100 mg of flour was used for extraction). In addition, the percentage of each size group was calculated on the basis of flour protein (%fp).

To test for correlations between protein properties and the fundamental rheological and quality parameters, additional relevant sums and ratios between the protein size classes were calculated. A complete description of all individual classes and sums and ratios is given as follows: IPP, insoluble glutenin polymers of the highest molecular weight (Bean and Lookhart, 2001; Lundh and MacRitchie, 1989) having a greater HMW/LMW subunit ratio than SPP (Gupta et al., 1993); EP, all proteins soluble in 50% 1-propanol (SPP, Gli, AG); SPP, soluble glutenin polymers with a continuous range of molecular sizes and a lower average molecular weight than IPP, having also a lower HMW/LMW subunit ratio (Bean and Lookhart, 2001; Dachkevitch and Autran, 1989; Gupta et al., 1993); Gli, monomers of lower molecular weight than SPP (Dachkevitch and Autran, 1989; Larroque et al., 1997); AG, metabolic proteins (non-gluten proteins) of lower molecular weight than Gli (Larroque et al., 1997; Tronsmo et al., 2002, 2003); IPP/EP, ratio insoluble (highest molecular weight polymers) to 50% 1-propanol soluble proteins (SPP, Gli, AG); IPP/SPP, ratio insoluble (large) to soluble (smaller) glutenin polymers; IPP/Gli and SPP/Gli, ratio large and smaller glutenin polymers, respectively, to monomers; (IPP + SPP)/Gli, ratio glutenin (polymers of all sizes) to gliadin; IPP/(SPP + Gli), ratio insoluble to soluble gluten proteins (AG excluded); IPP [%fp] + SPP [%fp], percentage glutenin in flour protein; SPP [%fp] + Gli [%fp], percentage soluble (lower molecular weight) gluten proteins in flour protein.

This slightly simplified scheme facilitates interpretation; e.g. not only the HMW and LMW glutenin subunits, but also small amounts of HMW albumin subunits (mostly *beta*-amylases) and globulin subunits (triticins) form disulphide bond mediated polymers (Gupta et al., 1995; MacRitchie, 1992). The exact cutoff points between the protein size classes in the SE-HPLC separations are also slightly ambiguous. Following Singh et al. (1990b) we selected characteristic local minima (valleys) for the cutoff SPP-Gli and Gli-AG, comparable to Larroque et al. (1997). The identity of the AG area was confirmed by running samples from sequential extractions with albumin-globulin-solvent (50 mM Tris–HCl buffer, pH 7.8, containing 100 mM KCl and 5 mM EDTA), water, and 50% aqueous 1-propanol, and comparing to the pattern from direct extraction of spelt flour with 50% aqueous 1-propanol.

2.2.2. Fundamental rheological properties of wet gluten and other quality data

The methods used are described in detail by Schober et al. (2002). Briefly, wet gluten was isolated by washing with a Glutomatic 2202 and the centrifuge accessory (Perten Instruments AB, Huddinge, Sweden) and calculated on a 14% moisture basis. Dynamic oscillatory measurements (0.07–10 Hz) in the linear viscoelastic region and creep tests (50 Pa, 5 min creep and recovery time, respectively) were conducted with a Bohlin CS rheometer (Bohlin Instruments Vertriebs GmbH, Pforzheim, Germany) equipped with a parallel plate configuration (40 mm diameter, 2 mm gap) at 30 °C. Moisture content of the wet gluten was determined with a Glutork gluten dryer (Perten Instruments AB, Huddinge, Sweden). The SDS-sedimentation test was a modification of ICC Standard No. 151 (ICC, 2000).

2.2.3. Statistics

Minitab statistical software (v. 12.21, Minitab Inc., State College, PA, USA) was used. Two-way analysis of variance (ANOVA) was done with the General Linear Model procedure, and cultivar, location and interactions between these two factors were incorporated in the model.

The least significant difference (LSD) for each individual parameter and location was calculated from the respective mean square error (MSE) from one-way ANOVA as

$$\mathrm{LSD} = t_{\alpha,df}\sqrt{\frac{2\,\mathrm{MSE}}{n}},$$

where $t_{\alpha,df}$ was the two-tailed t-value for the error probability $\alpha = 0.05$ and the degrees of freedom (df) of the MSE. The number of replicates (n) was 2 for SE-HPLC data, 2–5 for the seven quality parameters and flour protein, except for wet gluten content where it was 4–6 (2 wet glutens were combined for 1 rheological measurement). For LSD calculations, $n = 4$ was used for wet gluten content and $n = 2$ in all other cases. Thus the LSDs will tend to be conservative in case of the seven quality parameters and flour protein. The F-test showed highly significant ($P < 0.001$) sample effects at both locations for all parameters listed in Table 4 and in the supplementary Table A1.

For cluster analysis the following variables from both locations (H, OLi) were included: the seven gluten quality parameters, flour protein (N × 5.7, %db), and IPP, SPP, Gli, in mg and %fp, respectively. The variables were standardized by subtracting the means of all cultivars and then dividing by the standard deviation. The Euclidean method was used as the distance measure, and average linkage was used as the linkage method.

3. Results

The broad ranges of values within the 25 cultivars for the seven fundamental rheological and quality parameters (Table 1) at locations H and OLi indicated considerable differences between cultivars at both locations. The average values for all cultivars, the quartiles and the ranges differed between both locations, except for moisture content of the wet gluten, which were virtually identical. Values of OLi relative to H were higher for SDS-sedimentation volume, creep compliance, phase angle, and wet gluten content, and lower for relative recovery and complex modulus ($|G^*|$). These findings were confirmed by two-way ANOVA that showed highly significant ($P < 0.001$) effects of cultivar and location for all parameters except wet gluten moisture content, where location effects were not significant. Interactions between cultivar and location were highly significant ($P < 0.001$) for all parameters, indicating that the individual cultivars responded differently to environmental changes. Similar results were found for the protein data (Table 2). Again, there was a wide range of values among the 25 cultivars for all parameters and both locations, and ranges, quartiles and average values differed between locations for all parameters, except for SPP [%fp]. Higher values at OLi relative to H were observed for flour protein and all absolute values [mg] of protein size classes, and additionally for Gli [%fp], whereas lower values were found for AG [%fp] and IPP [%fp]. In the latter case the maximum value at OLi was higher than at H, but average, quartiles and minimum were lower. Again, for all parameters, two-way ANOVA showed highly significant ($P < 0.001$) effects of cultivar, location (except for SPP [%fp]), and highly significant interactions ($P < 0.001$). Significant location effects for IPP [%fp], together with the lower average, minimum and quartiles at OLi indicate a global decrease at OLi relative to H, so that the high maximum value at OLi should be regarded as an outlier. The percentage increase of the average values (Av) at OLi relative to those at H ($[\mathrm{Av_{OLi}} - \mathrm{Av_H}]/\mathrm{Av_H}$) for flour protein, and absolute values (mg) of IPP, SPP, Gli, and AG were 35%, 30%, 37%, 48%, and 18%, respectively. In agreement with the changes of the relative values (%fp) at OLi relative to H, SPP increased about as much as flour protein, Gli increased

Table 1
Ranges for the original seven fundamental rheological and quality parameters for spelt gluten (Schober et al., 2002) at the two locations (H, OLi)[a,b]

	Location 'Hohenheim' (H)					Location 'Oberer Lindenhof' (OLi)				
	Min	Q1	Av	Q3	Max	Min	Q1	Av	Q3	Max
SDS sedimentation volume (ml)	9	18	33	45	58	14	27	42	56	68
Creep compliance (50 Pa, 5 min) (10^{-3} Pa^{-1})	6.1	8.6	12.6	15.8	22.3	10.8	12.9	18.4	22.3	30.5
Relative recovery (%)	47.7	53.8	58.2	64.3	67.0	41.7	46.5	51.3	55.0	59.5
Complex modulus $\|G^*\|$ (10 Hz) (10^3 Pa)	1.97	2.64	2.88	3.15	3.85	1.59	2.34	2.72	3.08	3.71
Phase angle δ (10 Hz) (°)	30.9	34.3	35.5	37.4	39.2	33.1	35.0	36.4	37.8	40.4
Moisture content of the wet gluten (%)	61.7	62.9	64.0	65.1	65.9	61.8	62.4	63.9	65.2	66.0
Wet gluten content (%)[c]	21.4	25.0	29.1	30.9	41.7	34.3	40.3	44.0	47.6	57.1

[a]Min, Minimum; Q1, first quartile; Av, average; Q3, third quartile; Max, maximum out of the 25 cultivars.
[b]Two-way ANOVA showed highly significant ($P<0.001$) effects of cultivar, location, and interactions for all parameters except moisture content of the wet gluten, in case of which location effects were not significant ($P>0.05$).
[c]14% moisture basis.

Table 2
Ranges for flour protein and protein size classes from SE-HPLC at the two locations (H, OLi)[a,b]

Parameter[c]	H					OLi				
	Min	Q1	Av	Q3	Max	Min	Q1	Av	Q3	Max
Flour protein (N × 5.7) (%db)[d]	8.8	10.4	11.0	11.2	14.5	12.2	13.8	14.9	15.6	18.0
IPP (%fp)	22.9	25.7	29.3	32.5	36.7	21.1	24.2	27.9	31.3	38.7
IPP (mg)	2.05	2.49	2.83	3.16	3.79	2.79	3.09	3.69	4.21	4.99
SPP (%fp)	10.9	14.9	16.7	18.2	21.2	11.1	14.6	16.7	18.5	21.4
SPP (mg)	0.96	1.44	1.63	1.76	2.39	1.33	1.87	2.23	2.54	3.28
Gli (%fp)	35.2	38.7	40.8	43.1	46.2	38.0	41.5	44.1	46.6	49.9
Gli (mg)	2.72	3.72	3.96	4.31	5.24	4.53	5.17	5.87	6.40	7.22
AG (%fp)	11.4	12.1	13.2	14.0	15.8	9.9	10.7	11.4	11.9	13.6
AG (mg)	1.08	1.19	1.27	1.35	1.47	1.21	1.41	1.50	1.60	1.71

[a]Min, minimum; Q1, first quartile; Av, average; Q3, third quartile; Max, maximum out of the 25 cultivars.
[b]Two-way ANOVA showed highly significant ($P<0.001$) effects of cultivar, location, and interactions for all parameters except SPP (%fp), in case of which location effects were not significant ($P>0.05$).
[c]IPP, insoluble polymeric proteins (in 50% aqueous 1-propanol); SPP, soluble polymeric proteins; Gli, gliadins; AG, albumins and globulins; (mg), mg protein of the respective class in 100 mg flour; (%fp), % protein of the respective class based on total flour protein (fp).
[d]Flour protein (N × 5.7) (%wb) in the same order as in the table was 7.7, 9.2, 9.7, 10.0, 12.8 (H), and 10.9, 12.3, 13.3, 14.0, 16.1 (OLi).

much more, whereas IPP and especially AG increased less than flour protein.

Correlation coefficients between the seven rheological and gluten quality parameters (vertical) and flour protein, individual protein size classes and sums and ratios of those (horizontal) are shown in Table 3. Significance levels ($P<0.05$, 0.01 and 0.001) are indicated for each of the seven parameters. SDS sedimentation volume was clearly correlated to the absolute amount of IPP (mg), where higher IPP amounts were associated with higher sedimentation volumes. It is noteworthy that there is a negative correlation with the relative amount of Gli (%fp), while at the same time, there was no correlation with the absolute amount of Gli (mg). There was also no correlation with SPP.

Creep compliance clearly increased as the sum of the relative amounts of SPP and Gli (SPP [%fp] + Gli [%fp]) increased. The amount of Gli alone was also significantly positively correlated with creep compliance, whereas SPP alone showed a strong positive relationship only at H. Highly significant negative correlations were found with the relative amount of IPP, and ratios of IPP to other classes.

Overall, relative recovery showed similar absolute values for the correlation coefficients as did creep compliance, but the relationships were inverse. Additionally, an unexpectedly clear negative correlation with flour protein was found at H. More protein was associated with less relative recovery, i.e. less elasticity. The significant correlations at H between the relative amount of AG and creep compliance and relative recovery are noteworthy and discussed in Section 4.

$|G^*|$ was unusual insofar as correlation coefficients at H were low, and not significant at all at OLi. Attempts to correlate $|G^*|$ with other indicators of rheological properties showed significant correlations with flour ash, %db (0.428* and 0.579** for H and OLi, respectively), whereas ash was not significantly correlated with creep compliance

Table 3
Correlation coefficients of fundamental rheological and quality parameters of spelt gluten with protein classes from SE-HPLC[a,b]

Parameter	Loc	Flour protein, protein class or sums and ratios of protein classes from SE-HPLC																	
		Flour protein (%db)	IPP (mg)	IPP (%fp)	EP (mg)	SPP (mg)	SPP (%fp)	Gli (mg)	Gli (%fp)	AG (mg)	AG (%fp)	IPP/ EP	IPP/ SPP	IPP/ Gli	SPP/ Gli	(IPP + SPP)/ Gli	IPP/ (SPP + Gli)	IPP (%fp) + SPP (%fp)	SPP (%fp) + Gli (%fp)
SDS sedimentation volume	H	0.424*	0.792***	0.559**	0.106	0.154	−0.087	−0.016	−0.661***	0.462*	−0.042	0.533**	0.293	0.586**	0.225	0.666***	0.507**	0.705***	−0.494*
	OLi	0.223	0.643***	0.525**	−0.087	−0.066	−0.253	−0.152	−0.602**	0.288	0.046	0.517**	0.419*	0.553**	0.007	0.584**	0.521**	0.557**	−0.527**
Creep compliance (50 Pa, 5 min)	H	0.488*	−0.347	−0.784***	0.734***	0.654***	0.624***	0.770***	0.750***	−0.025	−0.549**	−0.775***	−0.714***	−0.802***	0.275	−0.668***	−0.813***	−0.532**	0.869***
	OLi	0.121	−0.584**	−0.646***	0.403*	0.355	0.425*	0.466*	0.707***	−0.117	−0.281	−0.641***	−0.566**	−0.673***	0.144	−0.655***	−0.658***	−0.592**	0.689***
Relative recovery	H	−0.642***	0.091	0.628***	−0.792***	−0.714***	−0.613**	−0.820***	−0.612**	0.000	0.694***	0.624***	0.658***	0.654***	−0.323	0.502*	0.684***	0.323	−0.770***
	OLi	−0.268	0.454*	0.593**	−0.489*	−0.438*	−0.456*	−0.544**	−0.636***	0.053	0.406*	0.579**	0.534**	0.614**	−0.194	0.575**	0.602**	0.490*	−0.661***
Complex modulus $\|G^*\|$ (10 Hz)	H	0.037	−0.388	−0.491*	0.232	0.248	0.378	0.260	0.448*	−0.250	−0.269	−0.474*	−0.491*	−0.476*	0.162	−0.396*	−0.490*	−0.344	0.522**
	OLi	0.025	−0.100	−0.126	0.081	0.124	0.186	0.093	0.128	−0.241	−0.325	−0.111	−0.177	−0.122	0.118	−0.085	−0.129	−0.032	0.185
Phase angle δ (10 Hz)	H	0.099	−0.594**	−0.765***	0.400*	0.259	0.319	0.520**	0.872***	−0.188	−0.272	−0.753***	−0.518**	−0.828***	−0.093	−0.848***	−0.761***	−0.783***	0.771***
	OLi	−0.290	−0.559**	−0.404*	−0.023	−0.109	0.024	0.081	0.583**	−0.280	0.058	−0.417*	−0.268	−0.467*	−0.226	−0.574**	−0.419*	−0.568**	0.388
Moisture content of the wet gluten	H	−0.164	0.562**	0.753***	−0.455*	−0.522**	−0.670***	−0.444*	−0.609**	0.278	0.414*	0.734***	0.754***	0.711***	−0.381	0.534**	0.753***	0.446*	−0.802***
	OLi	−0.344	0.546**	0.722***	−0.609**	−0.714***	−0.805***	−0.556**	−0.539**	0.021	0.424*	0.704***	0.797***	0.677***	−0.631***	0.482*	0.729***	0.393	−0.791***
Wet gluten content	H	0.958***	0.377	−0.348	0.931***	0.836***	0.549**	0.912***	0.318	0.304	−0.761***	−0.362	−0.477*	−0.382	0.400*	−0.204	−0.439*	0.012	0.536**
	OLi	0.776***	0.064	−0.347	0.753***	0.752***	0.566**	0.718***	0.227	0.317	−0.620***	−0.332	−0.440*	−0.321	0.501*	−0.156	−0.365	−0.044	0.459*

[a]*, **, ***: $P<0.05$, 0.01 and 0.001, respectively ($\rho = 0$ vs. $\rho \neq 0$).

[b]Loc, Location; IPP, insoluble polymeric proteins; EP, extractable proteins; SPP, soluble polymeric proteins; Gli, gliadins; AG, albumins and globulins; (mg), mg protein of the respective class in 100 mg flour; (%fp), % protein of the respective class based on total flour protein (fp).

(data not shown). In addition, we observed that weak gluten was generally browner due to the presence of bran particles, than stronger gluten. Furthermore, $|G^*|$ was significantly correlated with the moisture content of the wet gluten at H (−0.647***), but not at OLi (data not shown). These observations indicate that as well as protein properties, the effect of trapped particles and water in the gluten matrix must be considered. The weak significant correlations ($|r| \leqslant 0.522$) at H (Table 3) indicated that $|G^*|$ tended to increase with the sum of the relative amounts of SPP and Gli (SPP [%fp] + Gli [%fp]) and with the relative amount of Gli alone, whereas $|G^*|$ tended to decrease if the relative amount of IPP and all ratios of IPP to other classes increased.

The phase angle increased as the relative amount of Gli increased and tended to decrease when IPP and the ratios of IPP to other protein classes increased. These trends were much more pronounced at H than at OLi. It is also noteworthy that SPP alone was not correlated with phase angle, in contrast to creep compliance and relative recovery, where the effect of SPP was similar to that of Gli. Furthermore, (IPP + SPP)/Gli, the ratio of glutenin to gliadin, was clearly negatively correlated with the phase angle. At both locations, this correlation coefficient was (in absolute terms) higher than those with IPP/SPP, IPP/Gli, IPP/(SPP + Gli) and SPP [%fp] + Gli [%fp]. Additionally, at both locations IPP [%fp] + SPP [%fp] was, in absolute terms, more highly correlated with phase angle than SPP [%fp] + Gli [%fp], and IPP [%fp] alone.

Moisture content of the wet gluten clearly increased together with IPP and decreased when SPP, or less so Gli, increased. In agreement with these findings, IPP/EP, IPP/SPP, IPP/Gli, IPP/(SPP + Gli) were positively, and SPP [%fp] + Gli [%fp] negatively correlated with moisture content of wet gluten.

Wet gluten content was highly positively correlated with flour protein and absolute values of EP, SPP and Gli, but not with IPP. A significant negative correlation was found with the percentage of AG, whereas absolute amounts of AG showed no significant correlation with wet gluten content.

Finally, it has to be pointed out that flour protein showed little overall effect on the rheological and quality parameters except gluten content. Significant correlations with SDS sedimentation volume, creep compliance and relative recovery existed only at H, the latter being unexpectedly high in absolute terms, while the former two correlations were low and only significant at $P < 0.05$. Generally the correlations at OLi tended to be lower than at H. Nevertheless, the correlation coefficient between flour protein and wet gluten content at OLi (0.776) was unexpectedly low and this is discussed in Section 4.

Cluster analysis was used to group the spelt cultivars based on all available rheological, quality and protein data. The resulting dendrogram is shown in Fig. 1. Apart from the experimental line 'Goldir' (GOL) which was well separated from the remaining 24 cultivars, three clearly distinct groups were formed (Fig. 1). For a detailed characterization of the groups, two representatives of each group were selected as follows: 'Franckenkorn' (FRA) and 'Rouquin' (ROU) from group 1, 'Oberkulmer Rotkorn' (OKR) and 'Waggershauser Hohenheimer Weißer Kolbendinkel' (WWK) from group 2, and 'von Rechbergs Brauner Winterspelz' (RBW) and 'Steiners Roter Tiroler' (SRT) from group 3. These cultivars were selected because they were grouped together at different distances, thus allowing an estimation of the practical relevance of the finer structures at lower distances in the dendrogram. The complete data and least significant differences (LSDs) for all cultivars, are shown in Supplementary Table A1, separately for the two locations and in the same order as in Fig. 1. Table 4 shows these data for the selected six cultivars only, together with the LSDs. A comparison of the two most closely grouped cultivars RBW and SRT

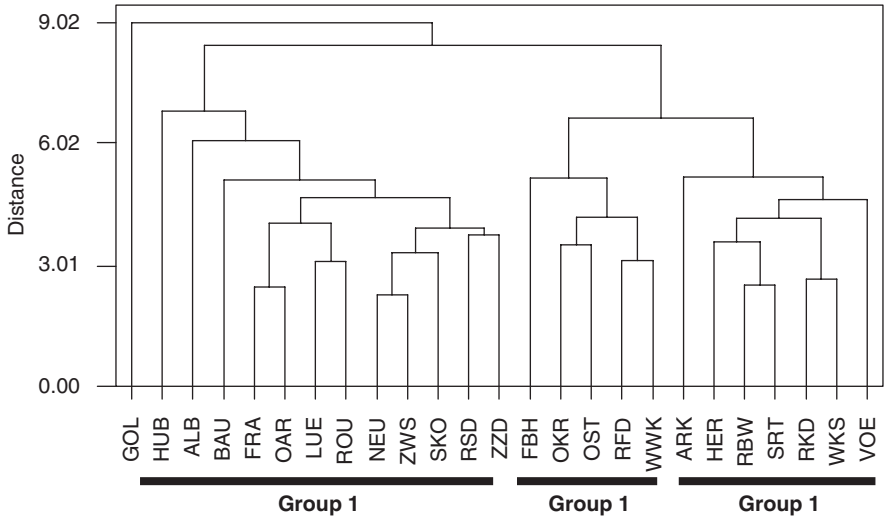

Fig. 1. Cluster analysis (standardized variables, Euclidean distance, average linkage), based on the seven fundamental rheological and quality parameters for spelt gluten (Table 1); flour protein; and IPP, SPP, Gli, in mg and %fp, respectively. The data from both locations (H, OLi) were used.

Table 4
Fundamental rheological data, quality parameters and protein classes from SE-HPLC for six selected cultivars and both locations[a]

Parameter[b]		Group 1		Group 2		Group 3		
	Location	FRA	ROU	OKR	WWK	RBW	SRT	LSD[c]
SDS sedimentation volume (ml)	H	43	43	36	34	12	12	2
	OLi	63	59	49	37	15	17	3
Creep compliance (50 Pa, 5 min) (10^{-3} Pa^{-1})	H	7.7	6.1	13.6	12.2	14.8	15.6	2.7
	OLi	16.9	12.3	20.0	21.5	20.5	23.4	4.6
Relative recovery (%)	H	64.4	67.0	58.0	58.7	56.2	54.3	4.3
	OLi	54.6	59.0	48.8	46.3	48.1	46.4	4.8
Complex modulus $\|G^*\|$ (10 Hz) (10^3 Pa)	H	2.61	2.77	2.84	2.68	3.80	3.85	0.35
	OLi	2.02	2.30	2.67	2.62	3.71	3.39	0.30
Phase angle δ (10 Hz) (deg.)	H	34.6	33.2	35.6	34.6	38.0	38.1	0.7
	OLi	37.2	35.7	35.1	34.0	38.4	38.5	0.9
Moisture content of the wet gluten (%)	H	63.8	64.2	63.5	64.0	61.7	62.6	0.9
	OLi	64.6	64.2	62.0	62.2	62.3	62.5	0.6
Wet gluten content (%)	H	24.9	24.1	34.1	28.7	28.2	29.2	0.6
	OLi	42.9	37.8	52.6	52.1	39.8	39.8	0.8
Flour protein (N × 5.7) (%db)	H	10.4	10.1	12.1	10.8	10.7	10.7	0.5
	OLi	15.7	13.9	17.3	14.8	14.9	13.3	0.5
IPP (%fp)	H	28.8	35.4	25.9	27.0	25.5	23.3	3.3
	OLi	27.3	31.3	22.4	21.1	21.6	24.6	2.1
SPP (%fp)	H	17.4	13.7	21.2	18.4	18.2	18.2	1.2
	OLi	17.4	15.1	21.2	21.4	18.6	17.5	0.7
Gli (%fp)	H	38.0	36.8	40.9	41.3	43.8	45.4	1.8
	OLi	43.5	41.3	45.6	46.0	48.3	46.6	1.4

[a]Extract from supplementary Table A1, which shows data for all 25 cultivars.
[b]IPP, insoluble polymeric proteins; SPP, soluble polymeric proteins; Gli, gliadins; (%fp), % protein of the respective class based on total flour protein (fp).
[c]Least significant difference, calculated with the mean square error from all 25 cultivars.

shows that they were identical, or different only within the LSDs for all parameters except for wet gluten content at H, flour protein, IPP and SPP at OLi, and additionally, just outside the LSDs, for $|G^*|$ (OLi), moisture content of the wet gluten (H) and Gli (OLi). All these differences were small relative to the differences between the representatives from the three groups.

Large significant differences were found for SDS sedimentation volume between all three groups and OKR-WWK (OLi). Creep compliance at H was significantly lower for FRA and ROU than for the four other cultivars, whereas at OLi, FRA took a position between ROU (low) and the four other cultivars (high). Relative recovery was significantly higher for FRA and ROU than for the four other cultivars, and ROU tended to be higher than FRA. $|G^*|$ was clearly higher for RBW and SRT than for the other four cultivars. At OLi, also OKR and WWK were significantly higher than FRA and ROU. Phase angle was characteristically high for RBW and SRT. Further significant differences indicated that FRA had a higher value than ROU. At H, ROU was also significantly lower than OKR and WWK. For moisture content of the wet gluten, RBW and SRT were low. At H, the remaining four cultivars were significantly higher, at OLi OKR and WWK were low as well, and only FRA and ROU were significantly higher. Wet gluten content was characteristically high for OKR and at OLi also for WWK, and although much lower than OKR, FRA was still significantly higher than ROU. Flour protein overall confirmed the results of wet gluten content, except that WWK was lower than OKR at both locations. IPP was characteristically high for ROU, significantly lower for FRA, and for the other four cultivars more or less distinctly lower than for FRA. SPP was opposite to IPP in that it was characteristically low for ROU, significantly higher for FRA, and for the other four cultivars more or less higher than for FRA. Gliadins showed similar tendencies for SPP, but were overall higher, and at both locations OKR, WWK, RBW and SRT were significantly higher than FRA.

4. Discussion

4.1. Variation within spelt

Effects of cultivar, location and their interactions were found with respect to gluten rheology and quality parameters, and protein size classes. Among the spelt cultivars the values for all parameters covered a wide range indicating that there is a high variability within spelt, as is known for modern bread wheat. The heterogeneity within the two subspecies accounts for some contradictions between studies comparing 'spelt' and 'wheat', e.g. with respect to crude protein content.

The two locations H and OLi differed in several aspects; in particular total nitrogen (N) fertilization level and, consequently, flour protein and wet gluten content at OLi were significantly higher. For bread wheat, it is widely accepted that increasing nitrogen fertilization increases gliadins more than glutenins, thus increasing the gliadin/glutenin ratio, and that absolute amounts of AG are scarcely affected by N fertilization (Doekes and Wennekes, 1982; Gupta et al., 1992; Jia et al., 1996; Wieser and Seilmeier, 1998). The spelt cultivars showed the typical reaction of a wheat species to the increased N fertilization provided at OLi relative to H.

4.2. Spelt and modern wheat

Bean et al. (1998) compared flours from 28 hard red winter wheats applying comparable extractions and SE-HPLC conditions to those used in the present study. The averages and standard deviations for these 28 wheats were 41.2±2.6, 12.1±1.9, and 34.8±4.3%fp for IPP, SPP, and Gli (including AG), respectively. The corresponding flour protein content (N × 5.7, %wet basis, wb) was 12.5±1.4, with a range between 9.7 and 15.2 (Bean S.R and Lookhart G.L., unpublished results). This latter flour protein range agrees reasonably with the flour protein ranges of the spelts on a wb when results from the two locations are pooled (see footnote d, Table 2). The spelt cultivars from both locations compared to the hard red winter wheats were characterized by much lower relative amounts of IPP, whereas relative amounts of SPP were higher overall, and Gli much higher, taking into account that for the wheats, that AG was included with Gli.

4.3. Effect of protein size classes on rheological and quality parameters

To understand the significance of the seven fundamental rheological and quality parameters for spelt gluten, their correlations with protein data need to be considered. The SDS sedimentation volume increased with absolute amounts of IPP, but not Gli or SPP. In agreement, Eckert et al. (1993) reported that swelling of glutenin was responsible for the sedimentation effect, whereas gliadin quickly dissolved in the SDS-lactic acid solution and thus could not contribute. SPP is soluble in 50% 1-propanol and would almost certainly dissolve in the SDS-lactic acid solution, which combines detergent and acidity and thus is a very potent protein solvent. In fact, most SE-HPLC studies use SDS solutions at neutral pH to extract SPP. The negative correlation with the relative amount of Gli indicates that for different cultivars in the same environment, glutens with a higher proportion of Gli originate from flours with lower absolute amounts of IPP, which consequently yield lower sedimentation volumes This is exemplified by cultivars like RBW and SRT from group 3, where low flour protein and low relative IPP, together equivalent to low absolute IPP, and low sedimentation volumes are combined with high Gli percentages. ROU (group 1) shows opposite tendencies: high IPP and sedimentation volume and low proportion of Gli in the gluten.

If IPP is responsible for swelling in SDS solution, the increase in the absolute amount of IPP at OLi relative to H also explains the higher sedimentation volumes at OLi.

Creep compliance and relative recovery of the gluten were affected by IPP, SPP and Gli: the largest glutenin polymers (IPP) decreased the compliance, i.e. the gluten was more resistant to deformation, and IPP increased the relative recovery, i.e. the degree of elasticity was higher. IPP or the comparable fractions of unextractable polymeric protein (UPP) and glutenin macropolymer (GMP) are widely regarded as responsible for gluten or dough elasticity and dough strength (Gupta et al., 1995; Hamer and van Vliet, 2000; Southan and MacRitchie, 1999). Small monomers (Gli) had the opposite effect, acting as a lubricant and allowing easier deformation of the gluten and less recovery due to a higher impact of viscous flow. Evidence obtained using different techniques has shown that gliadin is the viscous component of gluten (e.g. Janssen et al., 1996b; Kim et al., 1988; Tronsmo et al., 2003). SPP, the soluble glutenin polymers with a lower average molecular weight than IPP, had a similar effect to Gli, i.e. they contributed viscosity rather than elasticity under the conditions of the creep test, in agreement with what Hamer and van Vliet (2000) reported for the large deformations that occur in extension tests. The typical properties of spelt gluten—high extensibility, low elasticity—and correspondingly weak doughs can thus be interpreted as a result of the lack of IPP and abundance in Gli and SPP. Breeding for improved breadmaking quality may have resulted in higher IPP and lower SPP and Gli contents of modern wheat cultivars, and consequently stronger gluten and dough.

The correlations between creep compliance and relative recovery of gluten with AG at H were unexpected. Metabolic proteins (AG) should not affect gluten properties. Unexpected correlations were also found between flour protein and relative recovery and also creep compliance. Most likely, an indirect relationship can explain these effects. The absolute amount of AG in flour did not notably increase as wet gluten content increased. Consequently, the relative amount of AG in total flour protein was lower when flour protein and wet gluten were higher. Cultivars with high protein and gluten however tended to be those with more SPP and Gli and less IPP. Conversely, increased relative amounts of AG may be associated with less flour protein and wet gluten and more IPP, but less SPP and Gli. Such glutens could be expected to be firmer and more elastic, i.e. lower compliance and higher recovery. Cultivars like OKR with more gluten, that is softer and less elastic, and with increased SPP and Gli, represent one direction. While in the other direction are cultivars like ROU with less protein and gluten but high levels of IPP, whose gluten is at the same time firmer and more elastic.

The lower overall proportion of IPP in the cultivars at OLi relative to H together with the increased proportion of Gli are in agreement with the overall higher compliances and lower recoveries at OLi. At a higher N fertilization level, spelt gluten becomes generally softer and less elastic.

In interpreting the dynamic oscillatory measurements, it is most important to consider that in the creep test, strains (γ) of 0.3–1.5 were reached at a constant stress of 50 Pa (Table 1, creep compliance = strain/stress), whereas the dynamic oscillatory measurements were conducted at much lower strains of 0.001. Nevertheless, the phase angle measured under these conditions showed similar relationships to the protein size classes as did creep compliance and recovery, i.e. lower phase angles (equivalent to a higher degree of elasticity) were associated with a higher amount of IPP and a lower amount of Gli. However, in contrast to the results from creep test, SPP was not associated with a less elastic and more viscous behavior, but tended to supplement the IPP and add elasticity. This is shown by the fact that SPP was alone not significantly correlated with phase angle, but that (IPP + SPP)/Gli was more highly correlated to phase angle than other protein parameters, e.g. IPP/(SPP + Gli), and that IPP [%fp] + SPP [%fp] was higher correlated to phase angle than IPP [%fp] alone. Due to the small magnitude of the deformation and its dynamic nature, smaller polymers can still contribute stability by interacting with other polymers, whereas at larger, constant deformations as in the creep test, these interactions may be too weak.

In agreement with the lower relative IPP and higher amounts of Gli at OLi, the phase angles of the samples from this location were higher than those from H, again indicating less elasticity. This is in agreement with reports for Norwegian spring wheats (Tronsmo et al., 2003). Also, Janssen et al. (1996b) using fractionation and reconstitution techniques with gluten, found that the ratio of total glutenin to gliadin reduces the tangent of the phase angle, i.e. the percentage of gliadins in the gluten increases the phase angle.

That flour ash was correlated with $|G^*|$ from oscillatory measurements needs consideration. A previous study on spelt whole meal (Schober, T.J., unpublished results) indicated that complete removal of bran during gluten isolation is difficult. This is true even when using the meal procedure (AACC Method 38-12A, AACC, 2000) that following initial gluten washing uses a large (840 μm) screen through which bran particles should easily pass. However, the soft sticky spelt gluten tends to trap and bind these particles strongly, so that they do not pass through the screen. Consequently, more ash in the flour, indicative of more bran in the flour, results in more bran trapped in the glutan. (Differences in gluten stickiness might be an additional factor influencing the amount of trapped particles). The fact that flour ash was correlated with $|G^*|$ but not creep compliance implies that impurities in the gluten, e.g. bran particles, or possibly remaining starch granules, interfere with firmness measurements more strongly at small deformations in the oscillatory measurement than at the larger deformations in the creep test. As a consequence, correlations between protein results and $|G^*|$ were low or non-significant. Amemiya and Menjivar (1992) have suggested starch-starch and starch-protein interactions to be a major problem in small deformation oscillatory measurements on dough if the aim is to evaluate differences in the protein phase. Thus, factors which may affect $|G^*|$ of wet gluten are protein composition as indicated by the weak correlations found at H, particles (bran or starch granules and their interactions with gluten), and water, as indicated by the strong correlation to gluten moisture at H. Water content is known to strongly decrease the moduli of gluten, whereas the phase angle or its tangent are scarcely affected (Dreese et al., 1988; Janssen et al., 1996a). The protein classes that tended to increase $|G^*|$ and thus resistance to deformation at small deformations were Gli and SPP, whereas IPP had the opposite effect. Monomers and smaller polymers may act as a highly viscous lubricant: allowing better creep, once the system starts moving, but may increase the resistance, as long as there is only oscillation at small deformation. Additionally, it is likely that Gli and SPP prevent particles from being washed out due to their stickiness, and the particles tend to increase resistance to deformation. This hypothesis is in contrast to that suggested by Tronsmo et al. (2003) for Norwegian spring wheats. However, it should be kept in mind that spelt has much higher Gli and SPP contents than modern wheats. Thus, particle binding due to gluten stickiness might be an issue in spelt, whereas in modern wheat, particle trapping in the glutenin network might be dominant. Our hypothesis for spelt is supported by the comparatively low correlation between wet gluten and flour protein content at OLi (0.776), suggesting that some of the glutens from OLi contain more non-protein impurities than others. The hypothesis would also explain why the correlations at OLi were mostly lower than at H. Because of the higher Gli content at OLi, more particles may be bound in the gluten phase and mask some of the properties of the gluten protein. IPP, on the other hand, tended to reduce $|G^*|$ since it could bind more water than the other protein size classes, and thus increased gluten moisture.

The high molecular weight of IPP and its ability to form networks might be a factor in its higher water binding capacity. This is in agreement with Tronsmo et al. (2002) whose PLS plots showed that the highest molecular weight polymers (sonicated extract F1*) were relatively close to hydration and oil absorption capacity whereas lower molecular weight glutenin polymers (F2) were on the opposite side. Also in the present study, SPP clearly reduced the moisture content of the wet gluten.

4.4. Classification of spelt cultivars

When considering the classification by cluster analysis, the first question to be addressed is how far down into the

finer structures are the differences relevant. In the present sample set, RBW and SRT were found to be identical due to mislabeling of SRT in the breeding program (Schober and Kuhn, 2003). A limited number of significant differences between RBW and SRT were found in the present study, but overall they were similar, i.e. they were grouped together with a distance below 3. Such small differences may originate from small random differences in growing, milling, or measuring the data. ROU and FRA, with a distance of about 4, although still not significantly different in some important parameters such as relative recovery, were characterized by important differences that were clearly outside the LSDs, such as IPP and SPP [%fp], and also phase angle. Thus a distance of 3 appears to be a sensible cut-off point.

The clustering of all the data suggested three major groups. Group 1, represented by ROU and FRA is closest in characteristics to modern wheat. FRA in many aspects was less similar to modern wheat than ROU and tended in the direction of OKR, although overall it was still distinctly closer to ROU than to OKR. Group 2 contained OKR as a representative of typical spelt, selected from a Swiss land variety in 1948 (Siedler et al., 1994), and was characterized by high protein and gluten contents relative to other cultivars at a given environment, and by otherwise generally intermediate properties. WWK on the other hand, although with overall similarities to OKR, lacked its high protein content. Thus it is important to check for individual, desired traits, even if clustering suggests similarity. Group 3 contained weak cultivars, to be avoided for most applications due to their poor quality. This cluster analysis cannot claim to provide the last word on the cultivar classification. Additional cultivars might be incorporated, and data on the existing cultivars from more environments might change the grouping to some extent due to the interactions between cultivar and environment. Data from additional environments might be important to further stabilize the classification. So far, however, most studies on spelt quality only compared comparatively small numbers of spelt cultivars, and thus here for the first time a more complete picture on available European spelt is provided. This might prove valuable when selecting cultivars for future studies on quality, protein composition, nutritional aspects or allergenicity, and in breeding programs.

4.5. Summary

Spelt is very heterogeneous with regard to gluten properties and protein size classes. Nevertheless, overall, the European spelt cultivars were characterized by similar protein levels, lower relative amounts of IPP, and higher relative amounts of Gli and SPP in comparison to US hard red winter wheats. Typical spelt cultivars had intermediate gluten quality and high protein and wet gluten contents. Increased protein due to environmental differences were associated with higher percentages of Gli in the total flour protein, and lower percentages of IPP and AG as found in modern wheat. SDS sedimentation volume increased as protein increased due to environmental differences, but was not notably correlated with flour protein when environmental conditions were identical and variation came only from differences between cultivars. The combination of dynamic oscillatory and creep tests provided useful information on protein properties. In creep tests, IPP caused increased resistance to deformation and increased elasticity, whereas Gli had the opposite effects, and SPP had similar effects as Gli. The phase angle from oscillatory tests decreased (i.e. elasticity increased) if IPP increased. Phase angle increased along with Gli, however, in this case SPP tended to act more like IPP. Particles trapped in the gluten matrix interfere with the accuracy of rheological measurements, and are not easily removed. The grouping of the 25 old and new spelt cultivars based on gluten quality is novel and may help in the selection of suitable cultivars for future studies.

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jcs.2006.05.007

References

AACC, 2000. Approved Methods of the American Association of Cereal Chemists, 10th ed., AACC Method 38-12A, approved: 2000. AACC International, St. Paul, Minnesota, USA.

Abdel-Aal, E., 2003. Alte Weizenarten: Nährwert und Verwendungsmöglichkeiten. Getreide Mehl und Brot 57, 323–328.

Abdel-Aal, E.-S.M., Hucl, P., Sosulski, F.W., 1995. Compositional and nutritional characteristics of spring einkorn and spelt wheats. Cereal Chemistry 72, 621–624.

Abdel-Aal, E.-S.M., Hucl, P., Sosulski, F.W., Bhirud, P.R., 1997. Kernel, milling and baking properties of spring-type spelt and einkorn wheats. Journal of Cereal Science 26, 363–370.

Abdel-Aal, E.-S.M., Hucl, P., Sosulski, F.W., 1998. Food uses for ancient wheats. Cereal Foods World 43, 763–766.

Abdel-Aal, E.-S.M., Hucl, P., Sosulski, F.W., 1999. Optimizing the bread formulation for soft spelt wheat. Cereal Foods World 44, 480–483.

Amemiya, J.I., Menjivar, J.A., 1992. Comparison of small and large deformation measurements to characterize the rheology of wheat flour doughs. Journal of Food Engineering 16, 91–108.

Batey, I.L., Gupta, R.B., MacRitchie, F., 1991. Use of size-exclusion high-performance liquid chromatography in the study of wheat flour proteins: An improved chromatographic procedure. Cereal Chemistry 68, 207–209.

Bean, S.R., Lookhart, G.L., 2001. Factors influencing the characterization of gluten proteins by size-exclusion chromatography and multiangle laser light scattering (SEC-MALLS). Cereal Chemistry 78, 608–618.

Bean, S.R., Lyne, R.K., Tilley, K.A., Chung, O.K., Lookhart, G.L., 1998. A rapid method for quantitation of insoluble polymeric proteins in flour. Cereal Chemistry 75, 374–379.

Belitz, H.-D., Seilmeier, W., Wieser, H., 1989. Die Proteine des Dinkels (*Triticum spelta*). Zeitschrift für Lebensmittel-Untersuchung und -Forschung 189, 1–5.

Bojňanská, T., Frančáková, H., 2002. The use of spelt wheat (*Triticum spelta* L.) for baking applications. Rostlinná Výroba 48, 141–147.

Bonafaccia, G., Galli, V., Francisci, R., Mair, V., Skrabanja, V., Kreft, I., 2000. Characteristics of spelt wheat products and nutritional value of spelt wheat-based bread. Food Chemistry 68, 437–441.

Campbell, K.G., 1997. Spelt: agronomy, genetics, and breeding. Plant Breeding Reviews 15, 187–213.

Cornec, M., Popineau, Y., Lefebvre, J., 1994. Characterisation of gluten subfractions by SE-HPLC and dynamic rheological analysis in shear. Journal of Cereal Science 19, 131–139.

Dachkevitch, T., Autran, J.-C., 1989. Prediction of baking quality of bread wheats in breeding programs by size-exclusion high-performance liquid chromatography. Cereal Chemistry 66, 448–456.

Doekes, G.J., Wennekes, L.M.J., 1982. Effect of nitrogen fertilization on quantity and composition of wheat flour protein. Cereal Chemistry 59, 276–278.

Dreese, P.C., Faubion, J.M., Hoseney, R.C., 1988. Dynamic rheological properties of flour, gluten, and gluten-starch doughs. II. Effect of various processing and ingredient changes. Cereal Chemistry 65, 354–359.

Dvořáček, V., Moudrý, J., Čurn, V., 2001. Studies of protein fraction in grain of spelt wheat (*Triticum spelta* L.) and common wheat (*Triticum aestivum* L.). Scientia Agriculturae Bohemica 32, 287–305.

Dvořáček, V., Čurn, V., Moudrý, J., 2002. Evaluation of amino acid content and composition in spelt wheat varieties. Cereal Research Communications 30, 187–193.

Eckert, B., Amend, Th., Belitz, H.-D., 1993. The course of the SDS and Zeleny sedimentation tests for gluten quality and related phenomena studied using the light microscope. Zeitschrift für Lebensmittel-Untersuchung und -Forschung 196, 122–125.

Forssell, F., Wieser, H., 1995. Dinkel und Zöliakie. Zeitschrift für Lebensmittel-Untersuchung und -Forschung 201, 35–39.

Friedman, H.M., Tortolani, R.E., Glick, J., Burtis, R.T., 1994. Spelt is wheat. Allergy Proceedings 15, 217–218.

Gräber, S., Kuhn, M., 1992. Beurteilung der Backqualität verschiedener Dinkelsorten. Getreide Mehl und Brot 46, 102–108.

Gräber, S., Lang, K., Noll, B., Kuhn, M., 1994. Einflüsse von Rezepturbestandteilen und Führungsbedingungen auf das Backergebnis bei Dinkelvollkornkleingebäck. Getreide Mehl und Brot 48, 36–41.

Grela, E.R., 1996. Nutrient composition and content of antinutritional factors in spelt (*Triticum spelta* L) cultivars. Journal of the Science of Food and Agriculture 71, 399–404.

Gupta, R.B., Batey, I.L., MacRitchie, F., 1992. Relationships between protein composition and functional properties of wheat flours. Cereal Chemistry 69, 125–131.

Gupta, R.B., Khan, K., MacRitchie, F., 1993. Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quantity and size distribution of polymeric protein. Journal of Cereal Science 18, 23–41.

Gupta, R.B., Popineau, Y., Lefebvre, J., Cornec, M., Lawrence, G.J., MacRitchie, F., 1995. Biochemical basis of flour properties in bread wheats. II. Changes in polymeric protein formation and dough/gluten properties associated with the loss of low M_r or high M_r glutenin subunits. Journal of Cereal Science 21, 103–116.

Hamer, R.J., van Vliet, T., 2000. Understanding the structure and properties of gluten: an overview. In: Shewry, P.R., Tatham, A.S. (Eds.), Wheat Gluten. The Royal Society of Chemistry, Cambridge, UK, pp. 125–131.

ICC, 2000. Standard Methods of the International Association for Cereal Science and Technology (ICC), ICC Standard No. 104/1, approved: 1960, revised: 1990; ICC Standard No. 151, approved: 1990. International Association for Cereal Science and Technology, Vienna, Austria.

Janssen, A.M., van Vliet, T., Vereijken, J.M., 1996a. Rheological behaviour of wheat glutens at small and large deformations. Comparison of two glutens differing in bread making potential. Journal of Cereal Science 23, 19–31.

Janssen, A.M., van Vliet, T., Vereijken, J.M., 1996b. Rheological behaviour of wheat glutens at small and large deformations. Effect of gluten composition. Journal of Cereal Science 23, 33–42.

Jia, Y.-Q., Fabre, J.-L., Aussenac, T., 1996. Effects of growing location on response of protein polymerization to increased nitrogen fertilization for the common wheat cultivar Soissons: Relationship with some aspects of the breadmaking quality. Cereal Chemistry 73, 526–532.

Kasarda, D.D., D'Ovidio, R., 1999. Deduced amino acid sequence of an α-gliadin gene from spelt wheat (spelta) includes sequences active in celiac disease. Cereal Chemistry 76, 548–551.

Kim, J.-J., Kieffer, R., Belitz, H.-D., 1988. Rheologische Eigenschaften von rekonstituierten Weizenklebern mit variierenden Anteilen an Prolaminfraktionen verschiedener Getreidearten. Zeitschrift für Lebensmittel-Untersuchung und -Forschung 186, 16–21.

Kling, C.I., 1993. Dinkel—eine Alternative auf dem Getreidemarkt? Getreide Mehl und Brot 47, 3–6.

Larroque, O.R., Gianibelli, M.C., Batey, I.L., MacRitchie, F., 1997. Electrophoretic characterisation of fractions collected from gluten protein extracts subjected to size-exclusion high-performance liquid chromatography. Electrophoresis 18, 1064–1067.

Legros, R., Castille, J.-P., 1972. Utilisation de l'épeautre en panification. Revue de l'Agriculture 5, 725–752.

Lundh, G., MacRitchie, F., 1989. Size exclusion HPLC characterisation of gluten protein fractions varying in breadmaking potential. Journal of Cereal Science 10, 247–253.

MacRitchie, F., 1992. Physicochemical properties of wheat proteins in relation to functionality. Advances in Food and Nutrition Research 36, 1–87.

Marconi, E., Carcea, M., Graziano, M., Cubadda, R., 1999. Kernel properties and pasta-making quality of five European spelt wheat (*Triticum spelta* L.) cultivars. Cereal Chemistry 76, 25–29.

Marconi, E., Carcea, M., Schiavone, M., Cubadda, R., 2002. Spelt (*Triticum spelta* L.) pasta quality: Combined effect of flour properties and drying conditions. Cereal Chemistry 79, 634–639.

Martini, A., Kuhn, M., 1999. Einfluß der Stickstoffdüngung auf die Zusammensetzung und die Eigenschaften von Dinkelproteinen. Getreide Mehl und Brot 53, 282–290.

Moudrý, J., Dvořáček, V., 1999. Chemical composition of grain of different spelt (*Triticum spelta* L.) varieties. Rostlinná Výroba 45, 533–538.

Nightingale, M.J., Marchylo, B.A., Clear, R.M., Dexter, J.E., Preston, K.R., 1999. Fusarium head blight: Effect of fungal proteases on wheat storage proteins. Cereal Chemistry 76, 150–158.

Park, S.H., Bean, S.R., Chung, O.K., Seib, P.A., 2006. Levels of protein and protein composition in hard winter wheat flours and the relationship to breadmaking. Cereal Chemistry 83, 418–423.

Piergiovanni, A.R., Volpe, N., 2003. Capillary electrophoresis of gliadins as a tool in the discrimination and characterization of hulled wheats (*Triticum dicoccon* Schrank and *T. spelta* L.). Cereal Chemistry 80, 269–273.

Piergiovanni, A.R., Laghetti, G., Perrino, P., 1996. Characteristics of meal from hulled wheats (*Triticum dicoccon* Schrank and *T. spelta* L.): An evaluation of selected accessions. Cereal Chemistry 73, 732–735.

Ranhotra, G.S., Gelroth, J.A., Glaser, B.K., Lorenz, K.J., 1995. Baking and nutritional qualities of a spelt wheat sample. Lebensmittel-Wissenschaft und-Technologie 28, 118–122.

Ranhotra, G.S., Gelroth, J.A., Glaser, B.K., Stallknecht, G.F., 1996. Nutritional profile of three spelt wheat cultivars grown at five different locations. Cereal Chemistry 73, 533–535.

Rao, V.K., Mulvaney, S.J., Dexter, J.E., Edwards, N.M., Peressini, D., 2001. Stress-relaxation properties of mixograph semolina-water doughs from durum wheat cultivars of variable strength in relation to mixing characteristics, bread- and pasta-making performance. Journal of Cereal Science 34, 215–232.

Ruibal-Mendieta, N.L., Dekeyser, A., Delacroix, D.L., Mignolet, E., Larondelle, Y., Meurens, M., 2004. The oleate/palmitate ratio allows the distinction between wholemeals of spelt (*Triticum spelta* L.) and winter wheat (*T. aestivum* L.). Journal of Cereal Science 39, 413–415.

Schober, T., 2001. Eigenschaften der Kleberproteine des Dinkels. Entscheidungshilfen für Züchtungsprogramme aus technologischer Sicht. PhD thesis, University of Hohenheim, Stuttgart, Germany.

Schober, T.J., Kuhn, M., 2003. Capillary zone electrophoresis for gliadin separation: Applications in a spelt breeding program. European Food Research and Technology 217, 350–359.

Schober, T.J., Clarke, C.I., Kuhn, M., 2002. Characterization of functional properties of gluten proteins in spelt cultivars using rheological and quality factor measurements. Cereal Chemistry 79, 408–417.

Seibel, W., Brümmer, J.-M., Neumann, H., 1989. Backverhalten von Dinkelmahlerzeugnissen. Getreide Mehl und Brot 43, 183–188.

Siedler, H., Messmer, M.M., Schachermayr, G.M., Winzeler, H., Winzeler, M., Keller, B., 1994. Genetic diversity in European wheat and spelt breeding material based on RFLP data. Theoretical and Applied Genetics 88, 994–1003.

Singh, N.K., Donovan, G.R., Batey, I.L., MacRitchie, F., 1990a. Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. I. Dissolution of total proteins in the absence of reducing agents. Cereal Chemistry 67, 150–161.

Singh, N.K., Donovan, R., MacRitchie, F., 1990b. Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. II. Relative quantity of glutenin as a measure of breadmaking quality. Cereal Chemistry 67, 161–170.

Skrabanja, V., Kovac, B., Golob, T., Liljeberg Elmståhl, H.G.M., Björck, I.M.E., Kreft, I., 2001. Effect of spelt wheat flour and kernel on bread composition and nutritional characteristics. Journal of Agricultural and Food Chemistry 49, 497–500.

Smolková, H., Gálová, Z., Lacko-Bartošová, M., Scherer, R., 2000. Aminosäuren, Enzyme und Speicherproteine in 3 Dinkelsorten (*Triticum spelta* L.). Lebensmittelchemie 54, 2–5.

Southan, M., MacRitchie, F., 1999. Molecular weight distribution of wheat proteins. Cereal Chemistry 76, 827–836.

Tronsmo, K.M., Færgestad, E.M., Longva, Å., Schofield, J.D., Magnus, E.M., 2002. A study of how size distribution of gluten proteins, surface properties of gluten and dough mixing properties relate to baking properties of wheat flours. Journal of Cereal Science 35, 201–214.

Tronsmo, K.M., Magnus, E.M., Færgestad, E.M., Schofield, J.D., 2003. Relationships between gluten rheological properties and hearth loaf characteristics. Cereal Chemistry 80, 575–586.

Wieser, H., Seilmeier, W., 1998. The influence of nitrogen fertilisation on quantities and proportions of different protein types in wheat flour. Journal of the Science of Food and Agriculture 76, 49–55.

Winzeler, H., Rüegger, A., 1990. Dinkel: Renaissance einer alten Getreideart. Landwirtschaft Schweiz 3, 503–511.