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Effect of Variety and Environmental Factors on Gluten Proteins: An Analytical, Spectroscopic, and Rheological Study

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Four cultivars of winter wheat with contrasting qualities for breadmaking were selected to study the effects of environmental factors on grain protein composition and properties. They were grown in the field and under two controlled regimens designed to mimic typical "hot/dry" and "cold/wet" conditions experienced during grain development in the United Kingdom. The composition of the gluten proteins determined by SDS-PAGE and their size distribution determined by SE-HPLC were consistent with the presence of higher proportions of high M_r polymers in the two varieties with good breadmaking performance (Spark and Soissons) with limited environmental effects on these parameters. Gluten protein fractions from three of the cultivars were analyzed by Fourier transform infrared (FTIR) spectroscopy and this, combined with creep measurements using a texture analyzer, showed that a conversion from β-turns to β-sheets occurred during extension, irrespective of the growth conditions. However, the breadmaking varieties Soissons and Spark showed greater differences related to environmental conditions than the variety Rialto, which has poorer processing quality.

KEYWORDS: Wheat gluten; rheology; FTIR; cultivar; environment

INTRODUCTION

The functional properties of wheat gluten are known to be determined by both genetic and environmental factors and by the interactions between these. The genetic effects are now fairly well understood and relate particularly to allelic variation in the amount and composition of the gluten proteins, notably the high molecular mass subunits of glutenin [reviewed by Payne (*1*) and Shewry et al. (*2*)]. However, the impacts of environmental factors and their interactions with genotype are less well understood. A number of studies of the effects of increased temperature on wheat gluten protein composition have been reported. These show increases in the ratio of gliadin to glutenin and decreases in the proportion of high molecular mass glutenin polymers, resulting in weaker dough and poorer quality for breadmaking [reviewed by Edwards et al. (*3*)]. However, most studies have been carried out on plants grown at temperatures above 30 °C (*4–7*) when heat shock effects can be expected: these conditions are greatly different from those that occur in more temperate wheat-growing countries such as the United Kingdom. Similarly, little has been reported on the effects of drought on wheat grain composition and quality, although Daniel and Triboï (*8*) reported effects on gluten protein aggregation. Conversely, Guttieri et al. (*9*) reported genotype × environment effects on quality in wheat grown with and without irrigation but did not analyze gluten fractions. This lack of information is important as climate change is predicted to result in ever greater variation in weather from year-to-year (*10*) with consequent effects on crop quality (*11*). Belton (*12, 13*) has proposed that conformational transitions in gluten protein structure contribute to differences in dough functional properties, with changes from conformations rich in β-turns to conformations rich in β-sheets occurring during grain dehydration and during the application of mechanical stress resulting from dough mixing. Furthermore, the development of a combined FT-IR spectrometer/texture analyzer allows these transitions to be measured directly during the application of stress (*14*).

We have therefore carried out a study to determine the effects of environmental variation on the gluten fractions of four wheat varieties selected for their contrasting processing qualities. These were grown under two regimens corresponding to typical "hot dry" and "cool wet" summers in the United Kingdom, and the effects on gluten protein subunit and polymer compositions were determined by SDS-PAGE and SE-HPLC, respectively. Gluten fractions were also prepared from three of the cultivars and used to monitor changes in β-turn/β-sheet conformations during mechanical deformation. These studies provide new insights into the mechanisms of environmental impacts on wheat grain functionality.

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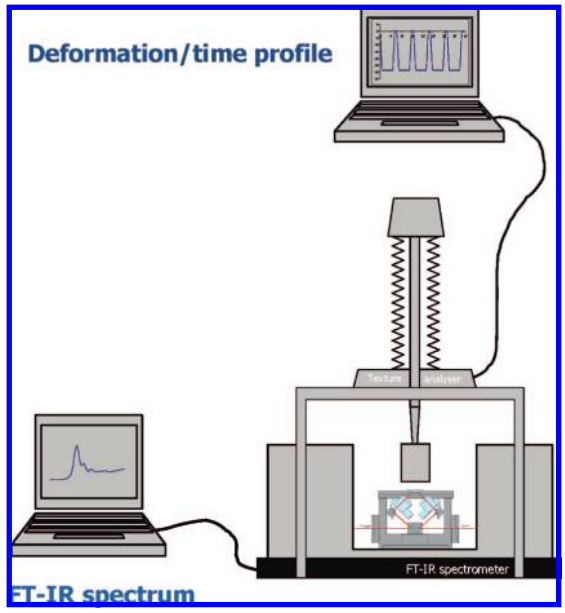

Figure 1. Setup for the Rheo-FT-IR measurement: the FTS 175 Bio-Rad spectrometer was combined with the TA XT2 texture analyzer.

MATERIALS AND METHODS

Wheat Varieties. Four varieties were selected on the basis of their contrasting end use properties. Spark, Rialto, and Soissons are all classified as strong breadmaking wheats, with Spark being in NABIM group 1 (best quality) and Soissons and Rialto in group 2 (*15*). In contrast, Beaver is a group 3 wheat with low gluten strength.

Three growth conditions were used. Samples were sown at 400 seeds/m^2 in the field into deep sandy loam at the Crops Research Unit, Sonning, in October 2002, with two applications of 100 kg of N/ha and applications of crop protection chemicals as required. Samples were also grown at four plants/pot in 18 cm diameter pots containing 2:1:2:0.5 of vermiculite/sand/gravel/compost mixed with Osmocote slow-release fertilizer granules. The pots were placed in two polythene tunnels arranged in four plots of each cultivar per tunnel, each plot comprising 125 pots. The tunnels were maintained with a natural photoperiod, fan-assisted air ventilation, and full drip irrigation for up to 14 days after flowering of the main head. At this stage one tunnel was allowed to continue at ambient temperature with full irrigation (cool/wet) while the temperature in the second tunnel was maintained at about 5 °C above ambient (but below 28 °C) and the soil moisture content allowed to fall to about 15% (hot/dry). These conditions were maintained until grain maturity. Grain from the replicate blocks was bulked and milled in a Buhler test mill with a target extraction rate of 75%, with the field-grown grain being milled in the same way. This white flour was used for all analyses.

Two parameters were measured to provide information on the glutenin composition of samples representing the 12 combinations of variety and growth conditions. These were the proportions of the high molecular weight (HMW) subunits of glutenin and the protein size distribution measured by SE-HPLC.

The HMW subunits of glutenin are major determinants of dough strength with both quantitative effects (related to subunit amount) and qualitative effects (related to composition) contributing to differences in dough strength between cultivars. The distribution of proteins is illustrated by **Figure 2**, which shows SDS-PAGE patterns of total grain protein fractions from the samples.

It should be noted that Soissons, Spark, and Rialto all contain an "allelic pair" of subunits encoded by chromosome 1D, which is associated with strong (i.e., highly elastic) gluten (1Dx5 + 1Dy10), whereas Beaver has an allelic pair of subunits (1Dx2 + 1Dy12) associated with weak gluten (*16*). In addition, the three strong varieties contain subunit pairs encoded by subunit 1B which are associated with strong gluten (1Bx7 + 1By8 and 1Bx17 + 1By18), whereas Beaver has the "weak" subunit pair 1Bx6 + 1By8 (*16*). Finally, Rialto and Soissons both contain additional "quality associated" subunits encoded by chromosome 1A (1Ax1 or 1Ax2*), whereas this locus is null (i.e., silent) in Spark and Beaver. On the basis of these compositions, Rialto would be predicted to have stronger gluten than Spark. However, this is not the case as both Rialto and Beaver have a chromosome translocation with the short arm of the 1R rye chromosome replacing the corresponding short arm of chromosome 1B of wheat (1BL/1RS) (*17*). This translocation is associated with weaker gluten properties and dough stickiness, which limit processing quality. In the case of Beaver, the combination of this translocation with the absence of "quality associated" subunits means that the grain is not preferred for use in food processing.

Protein Extraction and Analysis. Total proteins were extracted from white flour and separated by SDS-PAGE (indicated in **Figure 2**) as described by Shewry et al. (*2*). Four replicate samples of each of the 12 treatments (four varieties by three growth conditions) were run using a statistically randomized design on four separate gels, these forming four (statistical) blocks. The combined proportions of the HMW subunits were quantified by gel scanning using *phoretix* software (Nonlinear Dynamics, Newcastle, U.K.).

SE-HPLC was carried out by Campden & Chorleywood Research Association (Chipping Campden, U.K.) as described by Morel et al. (*18*). Two replicate samples were used for each analysis, being prepared and run separately and forming two (statistical) blocks.

Statistical Analysis. The residual maximum likelihood (REML) (*19*) method was used to fit a mixed model to the data (percentage areas) from the SDS-PAGE and SE-HPLC separations, with each band or peak being analyzed using a separate model. The modeling allowed the significance of the design factors of gels and lanes within gels to be assessed for the SDS-PAGE analyses, such that any overall differences between gels and also any systematic variation across lanes within gels could be accounted for. Similarly, for SE-HPLC, the significance of differences between the two replicates was assessed. Then the significance of the treatment factors could be considered. For this part of the model, the structure of the treatments was taken into account by having a factor for "environment" (field or polytunnel) and a factor nested within this for "condition" (field; "hot/dry"; "cool/wet"). This allowed the significance of the overall environment to be assessed and then the conditions within environment. The significance of design factors in the model was assessed using the change in model deviance on the corresponding degrees of freedom for the change, whereas the treatment factors (and their interactions) were assessed using the Wald test (*20*).

Using the model deviance, there was some evidence of differences in variability between gels ($p < 0.05$) for SDS-PAGE and between replicates for SE-HPLC. However, there were no significant effects ($p > 0.05$) due to positions of lanes or systematic variation within gels as a whole, so the full model for any variate, Y_{ijkl}, of SDS-PAGE data was

$$Y_{ijkl} = \text{variety}_i + \text{environment}_j + (\text{variety} \times \text{environment})_{ij} + (\text{environment} \times \text{condition})_{jk} + (\text{variety} \times \text{environment} \times \text{condition})_{ijk} + \text{gel}_l + r_{ijkl} \quad (1)$$

for variety_i, $i = 1, 2, 3, 4$; for environment_j, $j = 1, 2$; for condition k, $k = 1, 2, 3$; for gel_l, $l = 1, 2, 3, 4$. For SE-HPLC, the gel factor in this model is replaced by replicate_l, $l = 1, 2$. The error term in the model is given by r_{ijkl}, and the times sign between terms indicates their interaction. The method of forward selection of significant ($p < 0.05$) terms was employed to obtain a parsimonious model for each variate. Analysis of residuals revealed that no data transformations were required for the fitting of any of the models.

Tables of means together with the standard errors of differences between means (SED) (on the corresponding model residual degrees of freedom) were used to compare varietal performance in the different environments and conditions. All modeling was performed using the GenStat statistical system (GenStat (2006) 9th ed., GenStat Procedure Library Release PL17.1, Lawes Agricultural Trust (Rothamsted Experimental Station, U.K.).

Gluten Preparation. A Glutomatic at the University of Reading was used as follows (ICC standard 137): approximately 10 g of flour

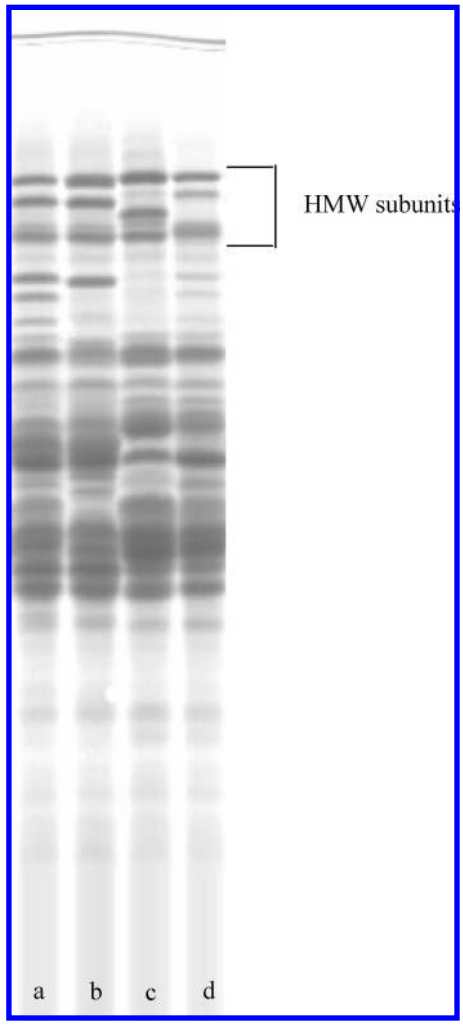

Figure 2. SDS-PAGE profiles of total grain proteins from the four cultivars: lane a, Spark; lane b, Soissons; lane c, Rialto; lane d, Beaver.

was weighed and transferred into a Perspex chamber. Five milliliters of deionized water was gently poured onto the surface of the flour sample. Once the wet gluten residue was produced, it was then deep frozen using dry ice and stored at −80 °C. Using this method we were unable to prepare suitably cohesive samples from Beaver, and thus no Rheo-FTIR results are reported for this cultivar.

Rheo-FTIR Setup. Spectra were recorded on a Bio-Rad FTS 175 FT-IR spectrometer with a mercury−cadmium−telluride detector (BioRad) and a single-reflection diamond attenuated total reflection (ATR) accessory (Graseby SPECAC, Orpington, U.K.). The system was combined with a texture analyzer as shown in **Figure 1**. First the background spectrum of the ATR cell covered with water from the sample supernatant was recorded (32 scans at 8 cm^{-1} resolution). Then the thawed gluten sample (in excess liquid) was placed on the ATR crystal and covered with a piece of Teflon foil and a microscope slide. This both prevented evaporation and allowed an even application of pressure on the sample.

Biaxial extension of the sample was achieved with a TA-XT2 texture analyzer (Stable Microsystems, Godalming, Surrey, U.K.) attached to the spectrometer (**Figure 1**). The texture analyzer was programmed to apply cycles that consisted of a 5 min period during which a force of 4 N was applied, followed by a 3 min period during which no force was applied. Five periods of force application were used in a complete compression−relaxation cycle. Applied force and sample deformation were recorded every 10 s. Infrared spectra were recorded continuously with a time resolution of 0.5 s^{-1} and a spectral resolution of 8 cm^{-1} (averaging 10 scans per spectrum). The whole experiment was repeated five times.

To determine the changes in the secondary structure of the gluten proteins, the infrared spectra were processed using Win-pro. Because the optical contact of the sample with the ATR crystal is likely to be variable, the overall intensity of the spectrum may vary from sample to sample. It is necessary therefore to use internal ratioing of intensities to obtain quantitative data. Inevitably these indicate changes in relative proportions intensities rather than absolute intensity. Because previous work (*14*) has indicated that under compression the major changes are in sheet and turn structures, we have chosen to measure their relative intensities by taking the ratio of the signal from the α-helical region. This does not necessarily imply that there is no change in compression in this region but does give some indication of the relative intensities of sheet and turn. Thus, the peak intensities at 1620 and 1668 cm^{-1} were ratioed to the 1650 cm^{-1} peak to give an estimate of the changes in the proportions of β-sheets and β-turns, respectively (*14*). These assignments follow closely those given in ref *21*, where there is an extensive discussion of the assignment problem. Error bars shown in the diagrams in this study are the standard error of the mean for five replicates.

Fitting of the relative strain data from biaxial extension cycles was carried out using Table Curve 2D v5.01 (Systat Software U.K. Limited, Hounslow, London, U.K.). ANOVA was carried out using Excel.

RESULTS AND DISCUSSION

Determination of Gluten Protein Subunit and Polymer Compositions. The proportions of HMW subunits in total protein extracts of the flours were determined by quantitative scanning of SDS-PAGE separations (as shown in ref *2*). This showed that Rialto and Soissons contained higher proportions of HMW subunits (14.83 and 14.63%, respectively) than Beaver and Spark (10.72 and 11.32%, respectively) with no significant differences ($p > 0.05$) between these pairs (the SED between pairs of cultivars being 0.34).

The differences between the proportions of HMW subunits in the four cultivars are consistent with the expression of five HMW subunit genes in Soissons (encoding subunits 1Ax2*, 1Dx5, 1Dy10, 1Bx7, and 1By8) and Rialto (1Ax1, 1Dx5, 1Dy10, 1Bx17, and 1By18) and only four genes in Spark (1Dx5, 1Dy10, 1Bx7, and 1By8) and Beaver (1Dx2, 1Dy12, 1Bx6, and 1By8). No significant differences ($p > 0.05$) were found between the proportions of HMW subunits in the individual lines grown under the three different environments. However, when data for the four lines were combined, a significant difference ($p = 0.012$) in the proportions of HMW subunits was observed between the hot/dry conditions (12.39%) and the field or the cool/wet conditions (13.14 and 13.11%, respectively; SED = 0.29).

Thus, there were significant differences between varieties and between environments but no significant variety × environment interactions.

SDS-PAGE measures the proportion of total HMW subunits but not their incorporation into high molecular mass glutenin polymers. The proportion of these polymers is known to be a major determinant of dough strength and is influenced by the allelic composition of the HMW subunits, being greater in lines with the "quality associated" subunits 1Dx5 + 1Dy10 (*22–27*). High temperatures have also been reported to decrease the proportion of large glutenin polymers, although these studies used temperatures above 30 °C, which result in a heat shock response (*6, 7, 28*).

We determined the size distribution of gluten proteins in the flours by SE-HPLC essentially as described by Morel et al. (*18*). This separates proteins extracted by sonication with SDS into five fractions corresponding broadly to high molecular mass glutenin polymers (F1), lower molecular mass glutenin polymers (F2), ω-gliadins (F3), α-type and γ-type gliadins (F4), and

Table 1. SE-HPLC Analysis of the 12 Flour Samples[a]

		% total area (TA)							
		F1	F2	F3	F4	F5	F1/F2	(F3 + F4)/F1	TA
Beaver	field	10.7b	20.1cd	6.9bc	47.7g	14.6d	0.53a	5.11h	25.7a
	cool/wet	10.2a	19.9bc	6.8b	48.9j	14.2c	0.51a	5.47i	28.2c
	hot/dry	10.0a	19.2a	7.1de	48.6i	15.1f	0.52a	5.57i	29.7e
Rialto	field	11.9c	19.9bc	6.4a	48.2h	13.5b	0.60b	4.58g	32.1h
	cool/wet	12.8d	19.7b	7.0cd	45.3f	15.3f	0.65cd	4.08e	25.9a
	hot/dry	12.2c	19.3a	7.2e	45.1f	16.2g	0.63c	4.30f	27.4b
Spark	field	14.6e	22.9fg	8.2g	41.2e	13.1a	0.64c	3.39d	30.4fg
	cool/wet	14.9ef	23.2g	8.3g	40.5d	13.1a	0.64c	3.29cd	29.2d
	hot/dry	14.5e	23.1g	7.6f	40.7d	14.1c	0.63c	3.34cd	30.2fg
Soissons	field	15.1f	22.4de	8.7i	40.2c	13.5b	0.67de	3.23bc	30.0ef
	cool/wet	15.6g	22.7ef	8.5h	38.7a	14.5d	0.69e	3.02a	27.8bc
	hot/dry	15.2fg	22.4de	8.5h	39.0b	14.9e	0.68e	3.13ab	30.6g
SED (10 df)		0.18	0.14	0.05	0.12	0.06	0.010	0.049	0.21

[a] Means (of two replicates) and standard error of differences between means are shown. Different letters within fractions, ratios, and total area indicate significant differences between means at the 5% level. Total area (TA) is given in arbitrary units.

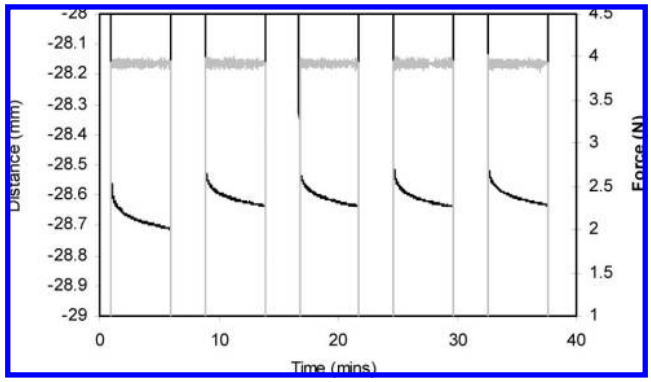

Figure 3. Texture analyzer distance and force profiles from gluten prepared from Rialto flour, field grown, undergoing five biaxial compression cycles: black line, displacement; gray line, force.

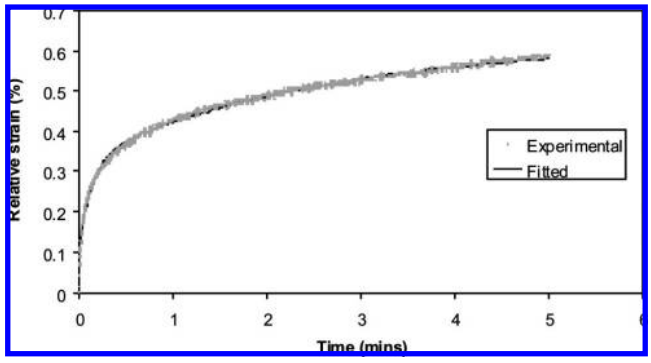

Figure 4. Relative strain plot for Rialto gluten, field grown, fitted with a two Voigt elements creep curve.

albumin and globulin proteins (F5). The total area (F1−F5) therefore provides an estimate of total grain protein, whereas Millar (*29*) has shown that F1%/F2% and (F3% + F4%)/F1% (i.e., the fractions all calculated in percentage terms of TA) correlate well with other measures of dough strength.

The SE-HPLC results for the 12 treatment combinations are given in **Table 1**, which also shows significant differences between means at the 5% level. Statistical modeling allowed significant interactions between treatment factors to be determined.

For TA there was a significant interaction ($p < 0.001$) between variety, environment (field or polytunnel), and condition (field, hot/dry, or cool/wet). Means were considerably different for all comparisons between cultivars and environments/conditions except for Spark and Soissons grown in the field or in the hot/dry polytunnel. For all varieties, TA was greater in the samples grown under hot/dry conditions than those grown under cool/wet conditions.

For F1% there were significant interactions between variety and environment ($p < 0.001$) and between environment and condition ($p < 0.05$) (i.e., when combining over all varieties), but no significant three-way interaction ($p > 0.05$). Considering the first of these interactions, means were significantly different ($p < 0.05$) between the four varieties when considering the samples from the field or the polytunnels, but no overall significant differences ($p > 0.05$) were present between the material grown under the hot/dry and cool/wet conditions. However, significant differences ($p < 0.05$) between all three environments/conditions were observed when the data for all four cultivars were combined, that is, considering the environment × condition interaction, with mean values for F1% of 13.1, 13.4, and 12.9 (SED for pairs = 0.08) for the material grown in the field, cool/wet, and hot/dry conditions, respectively. Thus, it can be concluded that the amount of high molecular mass polymers (F1%) was higher in Spark and Soissons than in Rialto and Beaver. Similarly, this fraction was lower in samples grown under hot/dry conditions than in those grown under cool/wet conditions.

The variety × environment and the environment × condition interactions were also significant ($p < 0.05$) for F2%, F1%/F2%, and (F3% + F4%)/F1%, without a significant three-way interaction ($p > 0.05$). The mean values for F1%/F2% were significantly different ($p < 0.05$) between the four varieties grown in the field, demonstrating that the proportion of high M_r polymers was greatest in Soissons, followed by Spark and Rialto, and lowest in Beaver. However, no overall significant differences ($p > 0.05$) were present between the pairs of samples of these varieties grown under cool/wet and hot/dry conditions. When the data for all varieties were combined, in the environment × condition interaction the differences between environments/conditions were significant ($p < 0.05$), with mean values of 0.62 for the cool/wet and 0.61 for the field and hot/dry samples (SED for pairs = 0.005).

The mean values for (F3% + F4%)/F1% were greatest in Beaver followed by Rialto, Spark, and Soissons, which is

Table 2. Fast and Slow Relaxation (Retardation) Times τ_1 and τ_2 (Minutes) for Soissons, Rialto, and Spark (Standard Error of the Mean Given in Parentheses)

	τ_1			τ_2		
cycle	Soissons	Rialto	Spark	Soissons	Rialto	Spark
			(A) Field Grown			
1	0.12 (0.011)	0.11 (0.006)	0.10 (0.007)	2.17 (0.116)	2.00 (0.091)	2.25 (0.108)
2	0.14 (0.007)	0.11 (0.009)	0.15 (0.008)	2.39 (0.120)	2.17 (0.178)	2.60 (0.102)
3	0.12 (0.009)	0.15 (0.028)	0.14 (0.008)	2.74 (0.311)	2.60 (0.507)	2.53 (0.065)
4	0.11 (0.006)	0.09 (0.020)	0.13 (0.013)	2.63 (0.216)	1.76 (0.293)	2.58 (0.183)
5	0.10 (0.006)	0.10 (0.008)	0.12 (0.010)	2.49 (0.131)	2.06 (0.212)	2.45 (0.066)
			(B) Hot and Dry Grown			
1	0.10 (0.006)	0.12 (0.006)	0.12 (0.005)	2.06 (0.104)	2.19 (0.079)	2.06 (0.058)
2	0.12 (0.004)	0.15 (0.038)	0.13 (0.003)	2.62 (0.181)	2.54 (0.292)	2.65 (0.061)
3	0.11 (0.013)	0.15 (0.036)	0.11 (0.007)	2.70 (0.303)	2.90 (0.464)	2.84 (0.066)
4	0.10 (0.005)	0.12 (0.012)	0.11 (0.006)	2.72 (0.164)	2.40 (0.218)	2.72 (0.107)
5	0.14 (0.026)	0.14 (0.020)	0.12 (0.005)	2.81 (0.256)	2.68 (0.251)	2.50 (0.109)
			(C) Cool and Wet Grown			
1	0.13 (0.005)	0.10 (0.004)	0.14 (0.015)	2.03 (0.083)	2.10 (0.124)	2.27 (0.098)
2	0.14 (0.004)	0.12 (0.006)	0.14 (0.007)	2.30 (0.091)	2.21 (0.162)	2.61 (0.120)
3	0.12 (0.007)	0.12 (0.026)	0.15 (0.013)	2.50 (0.128)	2.08 (0.195)	2.63 (0.149)
4	0.11 (0.01)	0.17 (0.046)	0.13 (0.004)	2.50 (0.158)	2.92 (0.326)	2.69 (0.111)
5	0.10 (0.015)	0.09 (0.017)	0.14 (0.012)	2.78 (0.189)	2.13 (0.147)	2.66 (0.113)

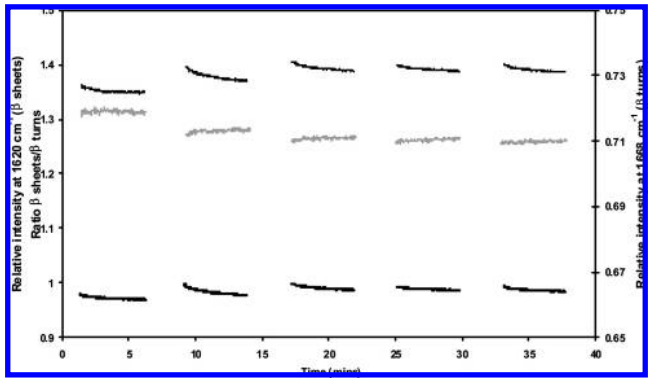

Figure 5. Changes in relative content in β-turns and β-sheets for Rialto, field grown: thick black line, β-sheets; gray line, β-turns; thin black line, sheet to turn ratio. Note differences between the left- and right-hand scales.

consistent with their differences in quality. However, Beaver also differed in that this ratio was lower in the field-grown material than in the material from the polytunnels; the converse being the case for the other varieties. This ratio also tended to be higher for the hot/dry than for the cool/wet conditions, although this was statistically significant only for Rialto when considering the full means table (**Table 1**). However, across all varieties using the environment × condition interaction, the (F3% + F4%)/F1% ratio was the same in the hot/dry and field samples (4.08), which was greater and significantly different ($p < 0.05$) from the cool/wet samples (3.96) (SED for pairs = 0.027).

These results are therefore consistent with the classification of Spark as group 1 (breadmaking) varieties and Rialto, Soissons, and Beaver as group 2 and 3 varieties, respectively, with the amounts and proportions of high molecular weight glutenin polymers being greater in the better quality varieties. However, no consistent effects of the environment (field or polytunnel) or the growth conditions within the polytunnels on these parameters were observed.

Rheo-FTIR Spectroscopy. Flours from the four varieties were used to prepare gluten fractions using a standard Glutomatic method. However, this method failed to give cohesive gluten fractions for any of the three samples of the cultivar

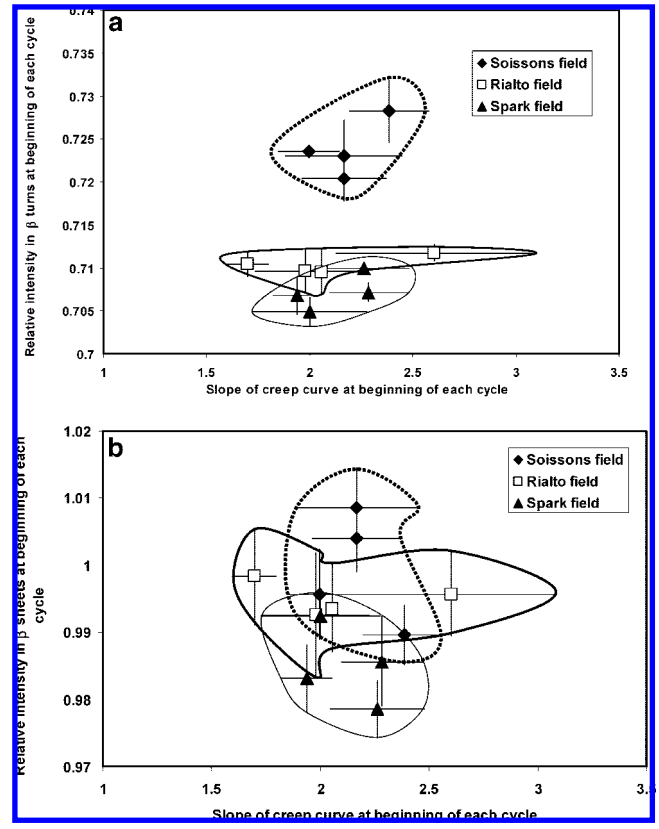

Figure 6. Changes in β-turns (**a**) and β-sheets (**b**) versus slope of creep at the beginning of the last four cycles for the three gluten samples from Soissons, Spark, and Rialto, field grown.

Beaver, reflecting its poor breadmaking performance and the presence of the 1BL/1RS translocation. Hence, detailed analyses of gluten properties could be carried out only on fractions from the cultivars Soissons, Spark, and Rialto. Conventional ATR spectroscopy showed very little significant difference between the spectra.

Typical Compression Curves. Figure 3 shows the five-cycle compression plot for gluten extracted from Rialto grown under field conditions and is typical of the plots obtained for all three cultivars. The plot shows the deformation of

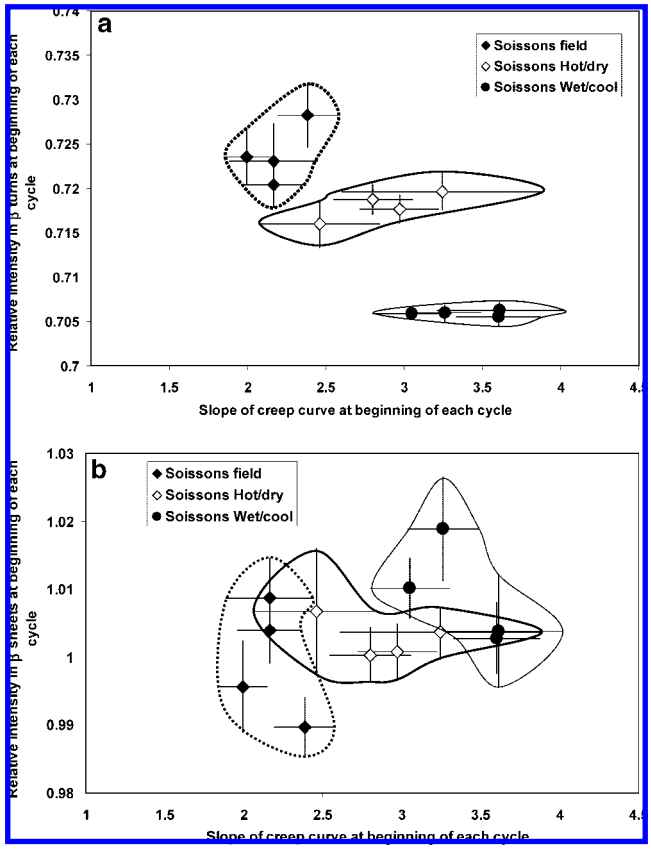

Figure 7. Changes in β-turns (**a**) and β-sheets (**b**) versus slope of creep at the beginning of the last four cycles for gluten samples from Soissons, variously grown.

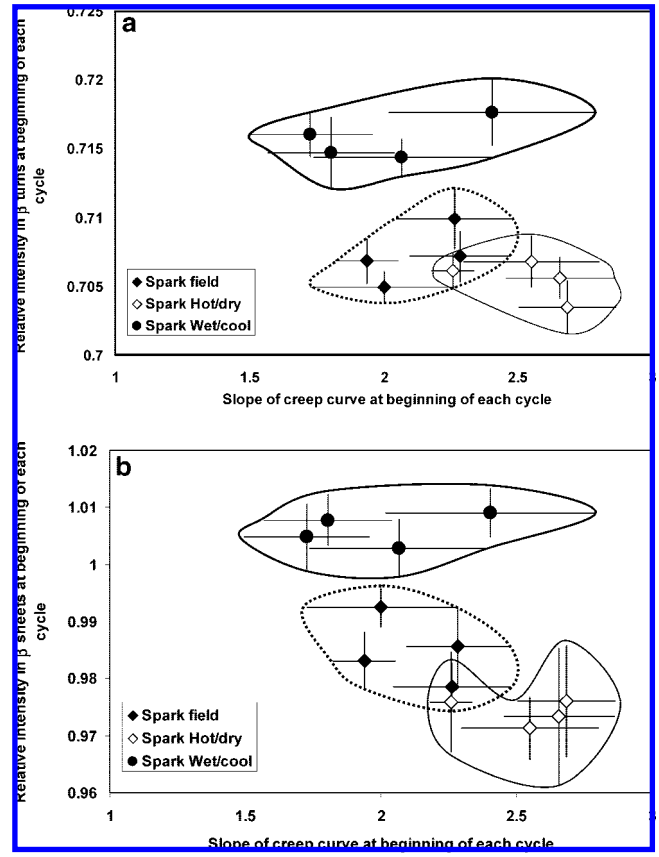

Figure 8. Changes in β-turns (**a**) and β-sheets (**b**) versus slope of creep at the beginning of the last four cycles for gluten samples from Spark, variously grown.

the sample represented as the distance the piston moves at constant applied force. During each compression at constant force there is a fast deformation followed by a slower deformation. The curve represents a creep versus time plot and shows that the biaxial extension caused by the compression contains two components. This phenomenon has been previously reported in strong and soft dough (*30–32*). The fast and slow components have been ascribed to slow and fast relaxation occurring in the biopolymer network, and this may arise from two major macromolecular interactions, the first one originating from weak bonds and the second being due to more entangled polymers. Belton (*13*) suggested a similar explanation based on combined FTIR and rheological results. To obtain comparable results, the change in the displacement (**Figure 3**) has been converted into a relative engineering strain defined as

$$\epsilon = \frac{\Delta h}{h_0} \tag{2}$$

where ϵ is the relative engineering strain and Δh is the difference between the displacement at time t and the initial displacement h_0. This is not an engineering strain, as the initial piston position was not the initial sample height because of the size of the sample and the ill-defined geometry. The initial displacement was taken as the value of the position of the probe when the applied force reached 4 N. Although the values calculated are not useful for rheological comparison outside this experimental setup, they do provide a way to quantitatively compare materials grown under different conditions within in the present experiment. A curve-fitting procedure has been adopted using a generalized form

$$\epsilon(t) = K_0 + \sum_{i=1}^{n} K_i\left[1 - \exp\left(\frac{-t}{\tau_i}\right)\right] \tag{3}$$

where $\epsilon(t)$ is the relative engineering strain at time t; K_0 and K_i are constants and τ is a relaxation time characterizing the creep rate. For gluten undergoing biaxial extension, a two-element model ($n = 2$) was found to fit the experimental data very well. An example is given in **Figure 4**. The two times, τ_1 and τ_2, were calculated using the fitting process. The data are represented in **Table 2**.

To characterize the parameters as a single weighted average process, we take the slope of the curve defined as

$$\frac{d\epsilon(t)}{dt} = \frac{K_1}{\tau_1}\exp\left(\frac{-t}{\tau_1}\right) + \frac{K_2}{\tau_2}\exp\left(\frac{-t}{\tau_2}\right) \tag{4}$$

at $t = 0$ to obtain

$$\frac{d\epsilon(0)}{dt} = \frac{K_1}{\tau_1} + \frac{K_2}{\tau_2} \tag{5}$$

This value is easily extracted from the curve-fitting process.

Determination of the Ratios of β-Sheets to β-Turns. Figure 5 shows the variation in the ratio of β-sheets to β-turns for field-grown material of Rialto undergoing five biaxial extension periods. There is a distinct increase in β-sheet content with most of the increase occurring during the first four periods, after which the content stabilizes. In general, the β-sheet content at the beginning of a period is higher than that at the end of a period, and there is a tendency for the total β-sheet content to increase with each compression period. There is also a reciprocal change in the β-turn content, which is consistent with the direct

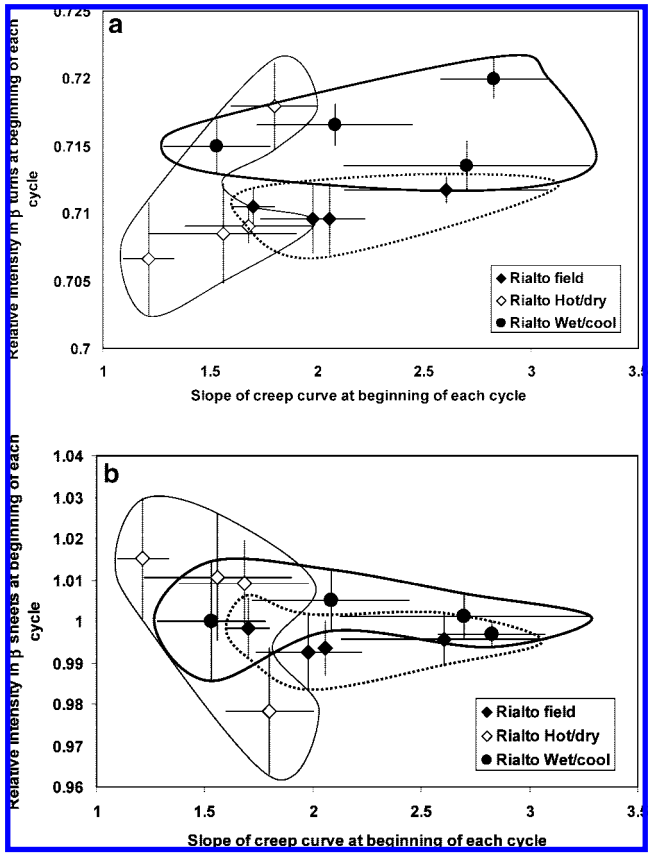

Figure 9. Changes in β-turns (**a**) and β-sheets (**b**) versus slope of creep at the beginning of the last four cycles for gluten samples from Rialto, variously grown.

conversion of β-turns into β-sheets. These results agree very well with data presented by Wellner et al. (*14*). Their work reported a similar effect for gluten prepared from dough of wheat cv. Hereward. They are also supported by the work of van Velzen (*33*), who reported that kneading and stretching of wheat dough resulted in an increase in β-sheet content. The behavior of the infrared spectra of gluten under compression has been discussed in detail elsewhere (*13*) and can be attributed to the formation of β-sheets from β-turns by elongation of HMW subunits immediately on compression followed by relaxation by creep during the hold period. The gradual buildup of sheet from compression to compression is due to the formation of a more stable sheet form that persists from compression to compression.

All values of τ_1 and τ_2 fall in a similar range for the field-grown cultivars. τ_1 has a mean value of 0.122 ($\pm$ 0.016) min and τ_2 is 2.53 ($\pm$ 0.27) min. These values are consistent with those reviewed by Dobraszczyk and Morgenstern (*34*). Li et al. (*35*) reported that the rapid relaxation (0.1−10 s) was associated with small polymer molecules which relax rapidly, whereas the slow phase (10−10000 s) was linked to the HMW polymers. When a one-way ANOVA was performed for τ_2 values, there was a significant ($p < 0.05$) difference between varieties [consistent with the results of Edwards et al. (*3*)], but not for τ_1, suggesting that the three varieties have only different slow phases. This is consistent with the results from the SE-HPLC experiments, which suggested that there are significant ($p < 0.05$) differences in the HMW fraction of the proteins. The rheological measurements are incomplete and do not represent a full characterization of the rheological properties of the system. However, it is possible to combine these data with the FTIR data to combine information about rheology and the state of the protein in the system. The initial slope $(K_1/\tau_1) + (K_2/\tau_2)$ has been plotted against the content of β-turns or β-sheets at the start of each compression period. As we have shown previously that the initial state of the protein is very dependent on sample history (*21*). We therefore discard the data from the first cycle and focus on the last four cycles. In **Figure 6** are shown the relative contents of β-turn and β-sheet versus the initial slope for the three field-grown varieties. The discrimination by β-sheet content is poor, but good separation is obtained when the contents of β-turn are plotted. Soissons is most clearly discriminated from Spark and Rialto. The standard deviations of the spectral data arise because of a combination of sample to sample variation in measurements using replicate samples.

Section **B** and **C** of **Table 2** show the retardation values for the hot/dry and cool/wet treatments, respectively. No significant effect of treatment was found on τ_1 or τ_2. In **Figures 7**, **8**, and **9** are shown the β-turn and β-sheet contents versus the slope of the creep curve for Soissons, Spark, and Rialto, respectively. For Soissons and Spark the β-turn data show a degree of discrimination between environmental treatments, but no such discrimination is apparent for Rialto.

In summary, analysis by SE-HPLC showed that the two varieties with good breadmaking performance (Spark and Soissons) contained higher proportions of high molecular mass glutenin polymers, but little difference was observed between the flour from samples grown under the different environmental conditions in the polytunnels. Similarly, combined analysis of the creep behavior together with the change in the secondary structure of gluten proteins showed that the field material of Soissons, Spark, and Rialto was comparable in terms of structural and rheological characteristics, although in this case effects of the hot/dry or wet/cool treatments were detected. These were easily separated for Soissons and Spark. This demonstrates that the combination of FTIR and TA is a powerful tool to detect small differences in the processing properties of wheat flour samples. This sensitivity may relate to the fact that it measures parameters which directly determine processing performance.

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