Triticum aestivum ssp. *vulgare* and ssp. *spelta* cultivars. 1. Functional evaluation

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Abstract

Over recent decades, several types of ancient grain varieties have been reintroduced through organic farming systems. In the past 20 years, there has been a renewed interest in growing spelt, and this species has a market niche in Europe and North America in natural, organic and healthy products. Are there any differences between commercial wheat varieties and spelt? The analysis of the bread-making quality of three bread wheat and two spelt cultivars has found no significant differences between the two types of wheat in protein, Fe or Zn, although differences were observed between cultivars. In mean quality parameters, a marked difference was observed between the bread wheat and spelt cultivars, the latter having poorer alveo-consistographic characteristics, indicative of weaker gluten and dough. With the exception of the variety ‘Craklin’, the gliadin/glutenin (Gli/Glu) ratio is negatively correlated with the technological quality of cultivars. Principal component analysis shows that the two commercial wheats ‘Bonpain’ and ‘Sensas’ lying close to gluten strength parameters; and, dough extensibility was associated with total gliadin content and the Gli/Glu ratio, both types of spelt being more closely related to these parameters, indicating that spelts are associated with greater dough extensibility.

Keywords Bread wheat · Spelt wheat · Quality wheat · RP-HPLC · Organic farming

Introduction

Spelt (*Triticum aestivum* ssp. *spelta* L., $2n=6x=42$, genome AABBDD) is an ancient type of hulled wheat and was the main grain used for making bread in Europe from the fifth to the early twentieth century [1]. Due to advances in bread wheat (*Triticum aestivum* ssp. *vulgare* L., $2n=6x=42$,

genome AABBDD) and the introduction of maize in Europe, spelt stopped being grown in many places. Currently, it is only grown in some areas in North America and in Europe, where it can be found in some isolated regions such as in Asturias (North of Spain) and some regions of Germany and Switzerland. Modern varieties of bread wheat have higher yields, lower production costs and better bread-making characteristics.

Nevertheless, over recent decades, several types of ancient wheat varieties have been reintroduced. In particular, there has been a renewed interest in growing spelt, and this species has a market niche in organic and healthy products. This is attributable to various factors: the fact it is a hulled wheat (i.e. the grains are not readily separated from the glumes) means that spelt is more resistant to pathogens and adverse conditions, making it particularly suitable for organic farming [2]. Further, in some genotypes of spelt, high concentrations of zinc, iron and protein have been detected, being highly stable under different environmental conditions [3]. In addition, spelts have a higher lipid content and lower insoluble and total fibre content than wheat [4].

In wheat flours, proteins contribute to the three main quality attributes that determine bread-making potential:

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protein content of the flour, rheological properties of the dough and grain hardness. The first two of these parameters relate to the importance of the quantity and quality of protein, while the third, hardness, is related to the interaction (or lack of thereof) between proteins surrounding the starch granules and the protein matrix of the endosperm.

Since the nineteen-seventies, research on dough quality has mainly focused on assessing the composition of wheat grain proteins and especially of the proteins in flour that form gluten. The proteins that form the viscoelastic complex known as gluten are prolamins: glutenins and gliadins. Glutenins, polymeric proteins, can be classified by their molecular weight, assessed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), into high and low molecular weight glutenin subunits (HMW-GS and LMW-GS, respectively). It is considered that HMW-GS are the gluten proteins with the greatest influence on the rheological properties of flour and are encoded by *Glu-1* loci, on the long arm of the homoeologous group 1 chromosomes [5]. On the other hand, gliadins are monomeric proteins and can be classified into ω 5-, ω 1.2-, α/β - and γ -gliadins on the basis of their primary structure and molecular weight, using reversed-phase high-performance liquid chromatography [6].

In allohexaploid wheat, grain hardness (or endosperm texture) is an important quality factor and, for this reason, wheat is generally traded by and classified into ‘hard’ and ‘soft’ classes being hardness related with puroindoline A and B proteins [7]. The genes that code for these proteins, *Pina-D1* and *Pinb-D1*, respectively, have been localised on the short arm of the 5D chromosome (the hardness [*Ha*] locus) [8]. When both genes are in their wild type, the grain texture is soft, while mutations in either of the genes result in a hard grain texture [9].

The objective of this study was to compare the flour and bread-making potential of bread wheat against spelt wheat. Three bread wheat cultivars and two spelt wheat cultivars were selected for this study. To minimize the environment influence, all the samples were grown in the same year under the same soil and climate conditions.

Materials and methods

Plant materials

Three cultivars of bread wheat, ‘Bonpain’, ‘Craklin’ and ‘Sensas’, and two local cultivars of spelt wheat, ‘Espelta Álava’ and ‘Espelta Navarra’, were used for the present study. 25 kg of grain of each variety, grown organically in Vitoria (Álava, Spain) (42° 51' × 2"N, 2° 40' × 21"W) in 2010/2011, were kindly provided by the Diputación Foral de Álava (Spain).

Grain protein composition

Gluten proteins were extracted from wheats using a sequential procedure [10]. HMW-GS were separated by SDS–PAGE according to [11]. HMW-GS composition was identified using the nomenclature of [12]. Discrimination between HMW-GS 2* and 2•• subunits the *Glu-A1* locus and between the 7 and 7* subunits of the *Glu-B1* locus was based on PCR reactions with the specific primers as [13] and [14], respectively. A similar approach was used to identify the puroindolin alleles present in the varieties at the *Pina-D1* and *Pinb-D1* loci [15]. DNA was extracted following a standard CTAB protocol [16].

For quantification of glutenin and gliadin subfractions, prolamins were separated on the basis of hydrophobicity by reversed phase-high performance liquid chromatography (RP-HPLC) as described [17].

Quality analyses

The 1000 grain weight was determined by counting 1000 grains using a seed counter (Pfeuffer, Mess-und Prüfgeräte) and weighing them with digital scale. Grain hardness, protein, Fe and Zn content, and sodium dodecyl sulphate sedimentation volume (SDSS test) were determined using wholemeal flours, obtained with a Tecator mill with a 1-mm screen. The flours for measuring rheological properties, with an alveograph and consistograph, were milled using a Ceramic Heger GmbH and Co. HG, Kv10 mill. After milling with the same procedure, the fractions extracted from each one of the five cultivars were mixed for 20 min in a flour blender (Chopin Technologies) at 48 rpm.

Kernel hardness was estimated by near-infrared reflectance (NIR) using a PerCon Inframatic 8120 spectrometer (Pertin Instruments GmbH, Germany), according to Method 39-70.02 [18]. Whole protein content was measured by NIR using a Technicon InfraLyzor 300 spectrometer (Technicon Instrument Co. Ltd, Hants, U.K.).

To assess mineral iron (Fe) and zinc (Zn) content the [19] method was followed. Gluten strength was estimated with an SDSS test [20].

Viscoelastic behaviour of samples was determined using an NG-97 Alveograph (Chopin Technologies, Tripette et Renaud, France) (Method 54-30A.2010 [18]). The effect of wheat flour on dough rheology during mixing was determined using a Consistograph (Chopin Technologies, Tripette et Renaud, France) (Method 54–50.2000 [18]). All measurements were performed in two replications, except the SDSS test, in four replications.

Data analysis

Statistical analysis was performed using SAS statistical software [21]. Differences between the different wheats were assessed by one-way analysis of variance (ANOVA). Multiple comparisons were made using Tukey's post hoc test (significance $p < 0.05$). Pearson's correlation coefficients were calculated using the CORR procedure. Principal component analysis was applied for multivariate statistical analysis using InfoStat software [22].

Results and discussion

Characterization of HMW-GS and puroindolines

To assess heterogeneity, 100 randomly selected grains of each cultivar were analysed using SDS-PAGE. Table 1 lists the HMW-GS and puroindoline composition for each cultivar. In the case of 'Espelta Álava', we found different prolamin profiles, indicating that it is a heterogeneous cultivar (as is the case in the landrace of wheat), the other four cultivars having homogeneous electrophoretic profiles.

The varieties under study showed clearly distinct HMW glutenin profiles. The first studies that related the presence of specific HMW-GS with bread-making quality [23] identified HMW-GS 1 (observed in 'Sensas', 'Espelta Navarra' and the heterogeneous spelt variety 'Espelta Álava') and 2* (observed in 'Bonpain'), coded by the *Glu-A1* locus, as being associated with high dough strength and hence "good" bread-making quality, while the null subunit (observed in 'Craklin') is related to "poor" bread-making quality. There is much less studies about the influence of HMW-GS 2•• (observed in 'Espelta Álava') on dough strength but a beneficial effect similar to that of subunit 2* has been reported for this subunit in a double haploid wheat population [24] and in Spanish bread wheat landraces [13].

The subunit pairs coded by the *Glu-B1* locus found in the three bread wheat varieties (7 + 8, 7* + 9 and 17 + 18) have already been described in commercial bread wheat, all being related to high dough strength [25]. In 'Espelta Álava', we found subunit pairs 13 + 16 and 13 + 18. The latter has only been described previously in another spelt cultivar of Spanish origin [26] and its influence on quality remains unknown. In the review of [27], the subunit pair 13 + 16 was described as having a good influence on bread-making quality.

The subunit pair 6* + 8* found in 'Espelta Navarra' was identified by [28] in Canadian bread wheats. This pair has not been described in Spanish [26] or Central European [29, 30] spelt varieties although it has been observed analysing the HMW-GS of the 'Obelkurmer' and 'Bättig Rothen' Central European spelt varieties and as in 'Dawbull' (the standard for this subunit pair) (unpublished data).

Table 1 Composition of HMW glutenin subunits (HMW-GS) coded by *Glu-1* loci, puroindoline proteins and quantities ($\pm$ standard deviation) of gluten subfractions (gliadin and glutenins) of the cultivars studied

Cultivars	Loci, subunits and alleles of HMW glutenin subunits			Loci and alleles of puroindolines			RP-HPLC gliadins				RP-HPLC glutenins			Gli/Glu ratio	
	Glu-A1	Glu-B1	Glu-D1	Pina-D1	Pinb-D1	Pina-D1	$\omega 5$ g/100 g	$\omega 1.2$ g/100 g	α/β g/100 g	γ g/100 g	Total	HMW-GS g/100 g	LMW-GS g/100 g	Total	
'Bonpain'	2* (b)	17 + 18 (i)	5 + 10 (d)	a	b		0.262 $\pm$ 0.02	0.701 $\pm$ 0.05	2.175 $\pm$ 0.09	2.054 $\pm$ 0.02	5.192 $\pm$ 0.17	0.870 $\pm$ 0.04	2.347 $\pm$ 0.10	3.216 $\pm$ 0.15	1.614
'Craklin'	Null (c)	7 + 8 (b)	2 + 12 (a)	a	a		0.236 $\pm$ 0.02	0.395 $\pm$ 0.02	1.153 $\pm$ 0.03	0.790 $\pm$ 0.03	2.574 $\pm$ 0.10	0.521 $\pm$ 0.02	1.647 $\pm$ 0.08	2.168 $\pm$ 0.06	1.187
'Sensas'	1 (a)	7* + 9	5 + 10 (d)	a	b		0.346 $\pm$ 0.02	1.208 $\pm$ 0.08	2.628 $\pm$ 0.07	2.011 $\pm$ 0.05	6.192 $\pm$ 0.22	0.919 $\pm$ 0.02	2.399 $\pm$ 0.11	3.318 $\pm$ 0.09	1.866
'Espelta Navarra'	1 (a)	6* + 8* (w)	2 + 12 (a)	a	a		0.450 $\pm$ 0.03	0.873 $\pm$ 0.06	4.570 $\pm$ 0.10	1.725 $\pm$ 0.05	7.617 $\pm$ 0.24	0.623 $\pm$ 0.04	1.848 $\pm$ 0.07	2.471 $\pm$ 0.10	3.082
'Espelta Álava'	1 (a)/ 2* (y)	13 + 16 (j)	2 + 12 (a)/ 2 + 12* (j)	a	a		0.443 $\pm$ 0.04	0.738 $\pm$ 0.03	2.140 $\pm$ 0.08	1.915 $\pm$ 0.06	5.236 $\pm$ 0.14	0.831 $\pm$ 0.04	2.181 $\pm$ 0.08	3.013 $\pm$ 0.13	1.737

The *Glu-D1* locus codes for the HMW-GS pairs 5 + 10 in the ‘Bonpain’ and ‘Sensas’ and the pair 2 + 12 in ‘Craklin’ and in the two spelt varieties ‘Espelta Navarra’ and ‘Espelta Álava’. Some grains of this latter cultivar presented the subunit pair 2 + 12*, considered rare though described in Italian cultivars [31] and in Spanish landraces of common and spelt wheat (e. g., [26]). Based on SDSS test, its influence on dough strength seems similar to that of the subunit pair 2 + 12 (Dr Rodriguez-Quijano, personal communication), that is, “poor” compared to the allelic pair 5 + 10 [25, 27].

Considering puroindoline allelic composition (Table 1), varieties ‘Bonpain’ and ‘Sensas’ have moderate endosperm hardness while ‘Craklin’ and the two spelts, having the wild-type *a* allele at both puroindoline loci, have a low endosperm hardness [32].

Quantification of prolamins subfractions

To complement the electrophoresis used to identify different prolamins proteins (HMW-GS, LMW-GS and gliadins), analysis based on RP-HPLC enabled us to perform quantitative analysis of these proteins [6] and the results are summarized in Table 1.

Regarding gliadins, either total gliadin and the proportions of the different gliadin types varied considerably among the wheat cultivars analysed, being this diversity in agreement with previous studies [6, 15]. Within the gliadin types (Table 1), the α/β -type gliadins were generally the most abundant, followed by the γ -type gliadins. The ω -type gliadins were present at lower levels but, among them, the $\omega 1.2$ -type was dominant. The highest levels of $\omega 5$ -gliadins and $\omega 1.2$ -gliadins were found in the two spelt varieties and in ‘Sensas’. The highest levels of α/β -gliadins were observed in ‘Espelta Navarra’ followed by ‘Sensas’. With regard to γ -gliadins, ‘Bonpain’ and ‘Sensas’ showed the highest levels, followed by ‘Espelta Álava,’ whereas ‘Craklin’ had the lowest levels of all gliadin fractions. Notably, γ -type gliadins have been shown to be positively correlated with dough strength [33] and also with loaf volume, suggesting a positive contribution to bread-making [34]. The $\omega 1.2$ -type produces the least positive effects on mixing tolerance and loaf volume [35], suggesting that it is less important for bread-making than the other gliadin types.

The lowest levels of HMW- and LMW-GS were found in ‘Espelta Navarra’ and ‘Craklin’. In the case of ‘Espelta Navarra’ it was associated with the highest Gli/Glu ratio, a parameter that has been inversely related with good dough strength properties. If ‘Craklin’ (in which the low protein content could have influenced the analysis) is excluded, the Gli/Glu ratio would predict the following bread-making quality: ‘Bonpain’ > ‘Espelta Álava’ > ‘Sensas’ > ‘Espelta Navarra’.

Several other authors have described some key differences between spelt and bread wheats in terms of the quantity of the different glutenin and gliadin fractions (see [36]). Spelt is characterized by a much higher total gliadin and lower total glutenin content. The Gli/Glu ratio is significantly higher in spelt, and α/β -gliadins are predominant, followed by γ -gliadins while LMW-GS, ω -gliadins and HMW-GS are generally minor components [37]. Noteworthy, when the cultivars are individually considered, the amounts of all subfractions in ‘Espelta Álava’ are more similar to the values in bread wheat ‘Bonpain’ and ‘Sensas’ than to those found in ‘Espelta Navarra’.

Cultivar quality

About grain quality, in all cases, the ANOVA tests conducted have demonstrated a highly significant effect of the cultivar in the variation found for grain quality as shown in Table 2. In this sense, Table 3 shows the mean values of grain hardness, rheological parameters and others related to the nutritional value, such as the Fe and Zn content, found for each of the cultivars studied.

‘Bonpain’ and ‘Sensas’ bread wheat varieties had higher endosperm hardness than the spelt and ‘Craklin’ varieties. This result is the expected according to the allelic composition observed for puroindoline loci (Table 1). Among the three soft endosperm cultivars, the spelt varieties had significantly higher hardness values than ‘Craklin’. This may be related to the low protein content in ‘Craklin’ (Table 3) since a positive correlation has been found between grain hardness and protein content [38].

The nutritional value of a wheat variety is largely dependent on the grain protein, Fe and Zn content. Protein content is known to be very sensitive to environmental conditions and has a very low heritability. Some authors [2] found a high protein content in spelt genotypes; however, the two spelt varieties had protein content values in the middle of the overall range for the five cultivars analysed (Table 3). Fe and Zn content varied significantly between cultivars but again the kind of wheat cultivar, either common or spelt, was not a defining criterion. So ‘Espelta Navarra’ had the highest content of both micronutrient elements among all varieties under study while ‘Espelta Álava’ showed the lowest mean values.

Flour and bread-making quality

According to the genetically determined quality, bread-making quality can be expected to differ between the cultivars analysed. The ANOVA tests confirmed the highly significant influence of the variety in the variation found for all parameters (Table 2).

Table 2 Analysis of variance of grain and bread quality parameters. Values are given for the mean squares and significance levels. R(%) indicates the amount of variance explained by the model

Source of variation	Hard	Protein (%)	SDSS test (mm)	Fe mg/Kg	Zn mg/Kg	ALVEOGRAPH				CONSISTOGRAPH					
						W (10 ⁻⁴ J)	P (mm)	L (mm)	P/L Ratio	W'(10 ⁻⁴ J)	HYD (%)	TprMax (s)	Tol (s)	D ₂₅₀ (mb)	D ₄₅₀ (mb)
Cultivar	25.2**	5.1**	2396.9**	31.7**	224.7**	13289.0**	7751.2**	930.4**	37.7**	13367.8**	14.6**	8628.3**	6966.8*	583421.8**	208446.8*
Error	0.3	0.0	0.52	0.18	0.15	30.6	10.3	16.3	0.04	95.0	0.35	353.8	483.8	18322.5	16083.3
R (%)	98.6	99.9	99.9	99.3	99.9	99.7	99.8	97.9	99.8	99.12	96.9	94.8	91.5	96.0	90.7

Hard hardness, W dough strength, P tenacity, L extensibility, W' (W degradation, HYD water absorption capacity, TprMax time to reach maximum pressure, Tol kneading tolerance, D₂₅₀ dough weakening at 250 s, D₄₅₀ dough weakening at 450 s

**And *significant at $P < 0.01$ and $P < 0.05$, respectively

Tukey's test revealed significant differences in SDSS test means across the five cultivars. The largest mean was recorded for 'Bonpain' and 'Sensas', which agrees with the presence of the beneficial HMW-GS pair 5 + 10 coded by the *Glu-D1* locus, although 'Bonpain' had a significantly larger SDSS test than 'Sensas'; considering the HMW-GS composition of these cultivars, the difference might be due to the pair 17 + 18 [39]. The two spelt varieties had intermediate SDSS test though 'Espelta Álava' was significantly higher than 'Espelta Navarra'; considering that the main difference in HMW-GS composition between these varieties lies in the *Glu-B1* locus, a more positive effect of the pair 13 + 16 over the pair 6* + 8* can be suggested. These results can also be partly explained by the quantity of glutenins and gliadins and particularly the (Gli/Glu) ratio (Table 1). 'Bonpain' and 'Sensas' showed indeed very similar amounts of glutenins, but 'Sensas' had a higher Gli/Glu ratio, known to negatively affect gluten properties. Similarly, 'Espelta Navarra' showed a markedly higher Gli/Glu ratio than 'Espelta Álava'. The smallest SDSS test for 'Craklin' is likely due to a prolamin profile associated with low gluten strength at both *Glu-A1* and *Glu-D1* loci. Some negative influence of the low protein content in this variety, and specifically of the very low amount of γ -gliadins, must also be taken into account.

With regard to the alveograph parameters (Table 3), 'Bonpain' and 'Sensas' had significantly higher mean W values than either of the spelt varieties and 'Craklin', while the highest W' values were found for 'Bonpain' and the lowest for the two spelt varieties and 'Craklin'. Regarding tenacity, 'Sensas' had a significantly higher P value than other varieties, followed by 'Bonpain', and differences between the two spelt varieties were not significant. Taking into consideration that 'Espelta Álava' had significantly higher L than 'Espelta Navarra', no difference was observed between 'Bonpain' and both spelt cultivars. Analysing the alveograph curve, the two spelt varieties obtained the lowest mean P/L ratios.

Consistographic parameters could not be measured for the 'Craklin' cultivar at constant hydration as the dough did not reach a consistency equivalent to the target pressure of 2200 mb. Analysing the consistograms (Table 3) for the other cultivars, the dough behaviour differed between the commercial wheats and the spelt varieties. The spelts obtained lower mean values for HYD, TprMax and Tol and higher mean values for dough weakening (D₂₅₀ and D₄₅₀), although the mean D₄₅₀ of 'Bonpain' was not significantly different to those of the two spelt varieties.

The alveo-consistographic analyses indicate that the wheats with the best rheological properties were 'Bonpain' and 'Sensas'. This finding is related to their greater endosperm hardness, and stronger gluten, which is indicated by the significantly higher values for most parameters associated with good bread-making behaviour (i. e., SDSS test, W, W', HYD, TprMax and Tol; see Table 3). Filipče et al. [40]

Table 3 Multiple comparison (Tukey's test) of mean ^(*) ($\pm$ standard deviation) quality parameters describing the nutritional and rheological properties of cultivars

Cultivar	1000gw (g)	Hard	Protein (%)	Fe (mg/Kg)	Zn (mg/Kg)	SDSS (mm)	ALVEOGRAPH				
							W (10 ⁻⁴ J)	P (mm)	L (mm)	P/L Ratio	W' (10 ⁻⁴ J)
'Bompain'	50.1 ± 0.14e	41.0 ± 0.61a	10.0 ± 0.03a	26.8 ± 0.30d	25.9 ± 0.77c	111.5 ± 0.71a	200.0 ± 7.94a	103.5 ± 3.54b	55.0 ± 2.83ab	1.9b	220.0 ± 12.98a
'Craklin'	59.1 ± 0.07a	33.0 ± 0.51c	6.0 ± 0.13d	30.0 ± 0.81c	36.1 ± 0.39b	31.5 ± 0.71e	33.5 ± 4.95b	82.5 ± 4.95c	9.0 ± 0.03c	9.4a	40.5 ± 2.12c
'Sensas'	51.5 ± 0.07d	41.5 ± 0.71a	9.0 ± 0.04c	31.8 ± 0.11b	26.1 ± 0.07c	106.5 ± 0.71b	187.5 ± 10.61a	191.0 ± 2.83a	21.5 ± 0.71c	8.8a	175.0 ± 21.21b
'E. Navarra'	52.3 ± 0.35c	36.5 ± 0.71b	9.6 ± 0.44b	36.6 ± 0.40a	50.0 ± 0.04a	51.0 ± 0.65d	49.0 ± ± 2.83b	43.0 ± 1.41d	39.5 ± 7.78b	1.1bc	54.5 ± 3.54c
'E. Álava'	55.3 ± 0.35b	36.5 ± 0.71b	8.8 ± 0.02c	27.2 ± 0.08d	25.7 ± 0.15c	76.3 ± 1.06c	55.0 ± 2.83b	36.5 ± 2.12d	59.5 ± 3.94a	0.6c	60.0 ± 2.83c
Cultivar	Consistograph										
	HYD (%)			TprMax (s)		Tol (s)		D ₂₅₀ (mb)		D ₄₅₀ (mb)	
	55.3 ± 1.06ab			166.5 ± 26.16a		195.0 ± 38.18a		310 ± 212.130b		851.5 ± 198.13ab	
	–			–		–		–		–	
	57.2 ± 0.14a			208.0 ± 2.83a		196.5 ± 3.54a		92 ± 19.80b		716.0 ± 73.54b	
	53.0 ± 0.49bc			71.0 ± 15.56b		89.0 ± 14.14b		1110 ± 115.97a		1311.5 ± 116.67a	
51.0 ± 0.09c			84.5 ± 21.92b		98.5 ± 16.26b		1137 ± 120.21a		1356.0 ± 76.37a		

1000gw 1000 grains weight, Hard hardness, W dough strength, P tenacity, L extensibility, W' W degradation, HYD water absorption capacity, TprMax time to reach maximum pressure, Tol kneading tolerance, D₂₅₀ dough weakening at 250 s, D₄₅₀ dough weakening at 450 s

*Values with the same letter were not significantly different ($P \leq 0.05$)

Table 4 Matrices of Pearson (r) correlation coefficients between the quality parameters of the cultivars and the subfractions of prolamins using RP-HPLC

Parameters	Gliadins (Gli)			Total gliadins		Glutenins (Glu)		Total glutenins		Gli/Glu ratio
	$\omega 5$ g/100 g	$\omega 1.2$ g/100 g	α/β g/100 g	γ g/100 g		HMW g/100 g	LMW g/100 g			
Hard	-0.00	0.74	0.20	0.85	0.50	0.89*	0.93*	0.92*		0.10
SDSS test	-0.03	0.70	0.36	0.79	0.34	0.96**	0.98**	0.98**		-0.09
Alveógraph										
W	-0.33	0.55	v0.02	0.65	0.22	0.80	0.86	0.84		-0.16
P	-0.42	0.57	-0.24	0.24	0.01	0.52	0.55	0.54		-0.27
L	0.49	0.19	0.70	0.62	0.41	0.50	0.49	0.49		0.24
P/L	-0.61	-0.01	-0.54	-0.53	-0.52	-0.19	-0.18	-0.19		-0.51
W'	-0.38	0.45	-0.04	0.63	0.18	0.77	0.83	0.81		-0.18
Consistógraph										
HYD	-0.68	0.61	-0.59	0.83	0.02	0.52	0.62	0.59		-0.23
TprMax	-0.79	0.61	-0.34	0.73	-0.34	0.81	0.87	0.85		-0.58
Tol	-0.91	0.40	-0.42	0.80	-0.43	0.79	0.87	0.84		-0.62
D250	0.85	-0.51	0.46	-0.82	0.26	-0.72	-0.81	-0.78		0.49
D450	0.85	-0.51	0.46	-0.82	0.29	-0.75	-0.83	-0.80		0.52

Hard hardness, W dough strength, P tenacity, L extensibility, W' W degradation, HYD water absorption capacity, TprMax time to reach maximum pressure, Tol kneading tolerance, D₂₅₀ dough weakening at 250 s, D₄₅₀ dough weakening at 450 s

**And *significant at $P < 0.01$ and $P < 0.05$, respectively

Fig. 1 Representation of the variables and the samples on the first and second principal component axes. *W* strength, *P* tenacity, *L* extensibility, *P/L* tenacity/extensibility ratio, *Totalglu* Total glutenin, *Totalgli* Total gliadins, *Gli/Glu* gliadin/glutenin ratio

analysed three spelt genotypes by a Brabender farinograph and found the doughs to have poor to moderate rheological properties and poor stability. In our study, we found lower TprMax for spelt doughs, this also indicating a weak gluten.

McRitchie [41] reported that the addition of gliadins shortened mixing time and reduced dough stability, and other studies have found the quantity of HMW- and LMW-GS to be correlated positively with bread-making quality and negatively with the Gli/Glu ratio [42].

Table 4 shows the coefficients for the correlation between the quantity of gliadin and glutenin fractions and the Gli/Glu ratio in the cultivar analysed and the quality parameters. The quantities of ω 1.2- and γ -gliadins showed positive but non-significant correlations with endosperm hardness, strength parameters (SDSS test, *W* and *W'*), coinciding with [33] and the consistograph parameters HYD, TprMax and Tol. Furthermore, it is to be noted that D_{250} and D_{450} were negatively correlated with the quantities of these gliadins and positively correlated with the quantities of ω 5-gliadins. Extensibility (*L*) was positively correlated with all gliadin fractions though the correlation coefficients were not significant. We have found significant positive correlations between the quantities of glutenins (HMW-GS, LMW-GS and total) and endosperm hardness and SDSS test. Strong but non-significantly positive correlations were found with strength parameters (*W* and *W'*) and consistographic parameters (TprMax and Tol), while the correlation with D_{250} and D_{450} was negative but not significant. Correlations between most quality parameters and the Gli/Glu ratio were negative.

Pruska-Kedzior et al. [37] indicated that the rheological properties of spelt gluten are influenced by gliadins that act as a viscous monomeric plasticiser, while those of gluten in bread wheat varieties are influenced by the glutenins that act as polymeric factor promoting networking. In our study, 'Espelta Álava', with the typical genetic characteristics of an

Asturian spelt, is notably different from 'Espelta Navarra', that is more typical of a Central European origin. Regarding gliadin and glutenin subfraction composition 'Espelta Navarra' was found to be more similar to the commercial varieties analysed in our study. That is, although our results do indicate differences in rheological properties between spelt and bread wheat varieties, we also found differences between the spelts themselves (protein content and SDSS test).

In order to extract the major sources of variation in the content of the different gluten proteins and the quality parameters of the flours analysed, a principal component analysis (PCA) was performed (Fig. 1). The PCA score plot of the PC1 versus PC2 explains 86.6% of the original variation in the data, accounting for 58.6% and 28.0% the first and the second components, respectively.

Our findings can be summarised by the principal component analysis (Fig. 1). This type of analysis, based on a correlation matrix, was performed with the values of the glutenin and gliadin fractions and the quality parameters of the flours obtained from the five cultivars. The first two components explain 86.6% of the variation in the data, the first and second components accounting for 58.6% and 28.0%, respectively. It can be inferred that these two principal axes account for a high percentage of the total variation, in turn, allowing appropriate interpretation of the data (Fig. 1). The parameters associated with gluten strength (SDSS test, *W* and hardness) were grouped in the positive part of the first component together with the HMW- and LMW-GS content, the two strong cultivars, 'Sensas' and 'Bonpain', lying close to these parameters. Dough tenacity and the *P/L* ratio, in the positive part of the second component, were negatively correlated with extensibility, total gliadin content and the Gli/Glu ratio, both spelts lying closer to these parameters. Additionally, it can be observed that the cultivar 'Craklin' lies apart from the other cultivars.

Conclusions

Differences in rheological properties have been found comparing bread and spelt wheat cultivars. In the alveographic analysis, the flour from two spelt produced dough with lower values for strength, tenacity and degradation, than that from strong bread wheat varieties ('Bonpain' and 'Sensas'), indicating a weaker gluten in these cultivars, though the values were higher than for the bread wheat 'Craklin'.

In addition, the behaviour of the spelt doughs was markedly different from that of dough from 'Bonpain' and 'Sensas' during the kneading process, in terms of hydration, time to reach maximum pressure tolerance to kneading and dough weakening at 250 s and at 450 s the behaviour corresponding to doughs with weaker gluten.

In the principal component analysis, the wheat varieties 'Bonpain' and 'Sensas' lie close to gluten strength parameters (SDSS test, strength and hardness) and to HMW- and LMW-GS content, while the two spelts are closer to extensibility, total gliadin content and Gli/Glu ratio, implying that they both yield a more extensible dough.

In general, we can conclude that the differences observed in this study between the cultivars of bread and spelt wheat varieties are not so much due to differences between subspecies as to differences in the genetic composition of cultivars.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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