



# Changes in bread-making quality attributes of bread wheat varieties cultivated in Spain during the 20th century



Miguel Sanchez-Garcia\*, Fanny Álvaro, Ariadna Peremarti, Juan A. Martín-Sánchez, Conxita Royo

IRTA (Institute for Food and Agricultural Research and Technology), Field Crops Program, Av. Rovira Roure, 191, E-25198 Lleida, Spain

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## ABSTRACT

Genetic gains in quality traits were assessed in grain samples from 4 field experiments involving 16 bread wheat varieties representative of those most widely cultivated in Spain during the 20th century. The allelic composition at three glutenin loci (*Glu-A1*, *Glu-B1*, and *Glu-D1*) was obtained by PCR-based DNA markers and published references. From 1930 to 2000 grain protein content decreased by  $-0.030\% \text{y}^{-1}$ , or in relative terms by  $-0.21\% \text{y}^{-1}$ , but the protein produced per hectare increased by  $0.39\% \text{y}^{-1}$ . Alveographic tests revealed significant changes in dough rheological properties. Dough strength (*W*) and tenacity (*P*) increased at relative rates of  $1.38\% \text{y}^{-1}$  and  $0.99\% \text{y}^{-1}$ , respectively, while dough extensibility (*L*) decreased by  $-0.46\% \text{y}^{-1}$ , resulting in an increase of  $1.45\% \text{y}^{-1}$  in dough equilibrium (*P/L*). The rise in protein quality could be related to the replacement of the null allele by subunits 1 or 2\* at *Glu-A1* and the prevalence of subunits 7+8 and 5+10 at *Glu-B1* and *Glu-D1* loci, respectively, in the most recent varieties. Dough extensibility was affected by water input during the crop cycle, this relationship being partially explained by the presence of the 5+10 HMW glutenin subunit. Fermentation tolerance was improved in the most modern varieties. Collapse during fermentation was avoided only in doughs with a  $W \geq 159 \text{ J} \times 10^{-4}$  and a  $P/L \geq 0.56 \text{ mm H}_2\text{O mm}^{-1}$ , levels achieved by most of the modern varieties. The over-strong and unbalanced rheological properties of some modern varieties resulted in highly porous doughs, and no clear advances in dough maximum height during fermentation were attained.

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## 1. Introduction

Bread wheat (*Triticum aestivum* L.) is the second most important staple crop in the world, providing 18% of the daily calorie intake worldwide in 2011 (FAOSTAT, 2011). Given its predominance in human diets, cultivated wheat has to meet the specific quality criteria for the manufacture of the wide range of food products derived from it.

**Abbreviations:** TW, test weight; PC, grain protein content; GPpHa, grain protein produced per hectare; P, tenacity; L, extensibility; W, strength; P/L, configuration ratio; DMH, dough maximum height; DFH, dough final height at the end of the 3 h fermentation; DHL, dough height lost between maximum and final height;  $T_{\text{DHM}}$ , time to DMH;  $VP_{\text{CO}_2}$ , volume of total  $\text{CO}_2$  produced;  $VR_{\text{CO}_2}$ , volume of  $\text{CO}_2$  retained by the dough;  $T_{\text{CO}_2}$ , the time to  $\text{CO}_2$  release; RQ, retention coefficient.

\* Corresponding author. Permanent address: International Center for Agricultural Research in the Dry Areas (ICARDA), PO Box 6299, Rabat Instituts, 10112 Rabat, Morocco. Tel.: +212 537682909; fax: +212 537675496.

E-mail address: [m.sanchez-garcia@cgiar.org](mailto:m.sanchez-garcia@cgiar.org) (M. Sanchez-Garcia).

During the last century, in many countries including Spain (Royo and Briceño-Félix, 2011), wheat breeding efforts concentrated on yield increases, with grain quality improvement being a secondary breeding objective. Furthermore, studies that assess genetic gains have frequently referred to yield and associated traits, while the enhancement of grain quality has received little attention. These studies, which usually involve historical series of genotypes, allow breeders to evaluate selection efficiency and to identify traits associated with genetic gains. Research into genetic gains in wheat has been conducted in several European countries, such as Italy (Canevara et al., 1994), France (Brancourt-Hulmelet et al., 2003), UK (Austin et al., 1989), and more recently Spain (Royo et al., 2007; Sanchez-Garcia et al., 2013; Subira et al., 2014). When addressed, wheat quality is tackled mainly through attributes such as test weight or protein content; however, the evaluation of key end-use quality traits, such as the rheological properties of dough or fermentation performance, are frequently neglected.

Wheat end-use quality is strongly related to the properties of the gluten matrix, which are determined primarily by the quantity and quality of gluten proteins (Finney and Barmore, 1948). High molecular weight glutenin subunits (HMW-GS) are of particular

interest in bread wheat because of their large influence on the rheological properties of dough (Branlard and Dardevet, 1985; Payne et al., 1987; Vawser and Cornish, 2004) and their involvement in the genotype x environment interaction, particularly regarding gluten strength and extensibility (Blumenthal et al., 1995; Johansson et al., 1999; Panozzo and Eagles, 2000; Hristov et al., 2010). Subunits 1 and 2\* encoded at *Glu-A1* loci, and subunit 5+10 at *Glu-D1* are considered suitable for promoting dough strength and have contributed to enhancing the quality of bread wheat in a number of countries, among them France and Italy (Mesdag and Donner, 2000). In contrast, other subunits, such as the null allele at *Glu-A1*, is considered to have a negative effect (Payne et al., 1987). Although the relationship between both dough strength (*W*) and Zeleny sedimentation volume and the allelic composition at *Glu-1* was examined in Spanish varieties (Payne et al., 1988), there is still no information regarding changes induced by breeding in the HMW-GS composition and their effect on quality traits.

In southwestern European countries, bread wheat is consumed mainly as baguette bread, and the dough properties during mixing and fermentation are included in the quality criteria addressed by breeding programs. The study of the response of dough to common constraints that occur during baking is of relevance in order to predict end-use quality (Dobraszczyk and Morgenstern, 2003) since the rheological properties of dough determine product functionality (Dobraszczyk and Morgenstern, 2003). Biaxial extension parameters, such as dough strength or extensibility, usually obtained through Chopin's alveograph procedures, are among the bread-making quality attributes used to characterize wheat samples by official quality classifications (Mesdag and Donner, 2000). All the rheological tests performed on dough seek to predict behavior in response to bread-making processes, such as mixing or fermentation. During the latter, gas cells in the dough expand, causing the final volume of the loaf, which is critical for bread appeal (Dobraszczyk et al., 2000). In spite of this, most fermentation tests are performed on yeast-free doughs at room temperatures, conditions in which the rheological properties of the dough may differ from the fermentation conditions found in commercial bread production lines (Dobraszczyk and Morgenstern, 2003). To reliably determine the fermentation behavior of wheat varieties, recent studies have proposed the use of fermentation monitoring

techniques, particularly the rheofermentometer F3 (Ktenoudaki et al., 2010, 2011).

The cataloging of bread wheat varieties on the basis of quality standards is critical not only to establish their compliance with industrial requirements, but also to ascertain the success of breeding programs to address quality improvement. The first classification of bread wheat quality in Spain was implemented in 1945 (Mesdag and Donner, 2000), but it did not include the alveographic parameters already used for quality classification in other European countries (Mesdag and Donner, 2000). Alveographic parameters were first introduced in Spain for wheat quality characterization in the 1970s (Mesdag and Donner, 2000), and not until 2010 was an official classification—similar to those used in neighboring countries and mainly based on alveographic parameters—proposed. This lack of regulation may have contributed to traditionally large imports of bread wheat grain, mainly of high quality, destined to the local milling and baking industries, which in 2012 accounted for up to 51.6% of the total wheat consumption in the country (MAGRAMA, 2012). The enhancement of bread wheat quality in Spain is therefore, a major challenge for present and future breeding programs.

The objectives of the present study were: (i) to investigate the changes caused by bread wheat breeding in Spain during the 20th century on the quality attributes related to bread-making performance; (ii) to assess the relationship between quality improvement and changes in the HMW-GS composition, and (iii) to analyze whether breeders' selection for good rheological attributes led to an improvement in the variety performance during fermentation.

## 2. Materials and methods

### 2.1. Plant material

A collection of 16 bread wheat varieties representative of those most widely cultivated in Spain during the 20th century was gathered. These varieties were grouped into three breeding periods (BPs) on the basis of their year of release or cultivation period, as follows: (i) Spanish landraces grown before 1940; (ii) initial varieties

**Table 1**  
Description of the varieties used in the study and their allelic composition at the high molecular weight glutenin subunits (HMW-GS) loci.

Variety	Pedigree	Origin	Year of release in Spain	HMW-GS <sup>a</sup>			References
				Glu-A1	Glu-B1	Glu-D1	
<b>Landraces</b>							
Aragón 03	Selection of the landrace "Catalan de Monte"	Spain	<1940	<b>Null</b>	20	4+12	Carrillo et al., 1988
Barbilla	Landrace	Spain	<1940	? <sup>b</sup>	NI	NI+12	
Candeal	Landrace	Spain	<1940	<b>Null/1</b>	NI	5+12	
<b>Initial varieties</b>							
Impeto	Frassineto-405/Villa-glori	Italy	1950	<b>1</b>	7+8	2+12	Mesdag and Donner, 2000
Mara	Autonomia/Aquila	Italy	1947	<b>Null</b>	7	2+12	Dencic and Borojevic, 1991
Estrella	Mon-desir/Ardito//Mouton-a-epi-rouge/k-3/3/Mouton-epi-rouge	France	1952	<b>Null</b>	7+8	2+12	Branlard et al., 2003
Pané 247	L-4/Mentana	Spain	1955	<b>Null</b>	7+8	2+12	Peña, 2004
<b>Modern varieties</b>							
Yecora	Ciano-67//Sonora-64/Klein-rendidor/3/Siete-cerros-66	CIMMYT	1972	<b>1</b>	17+18	5+10	CRC, 1998
Cajeme	Ciano-67//Sonora-64/Klein-rendidor/3/Siete-cerros-66	CIMMYT	1972	<b>1</b>	17+18	5+10	CRC, 1998
Anza	Lerma-rojo-64//Norin-10/Brevor/3/3*Andes-enano	CIMMYT/USA	1974	<b>Null</b>	7*+8	2+12	León et al., 2009
Marius	Cadet//Thatcher/Vilmorin-27/3/Ariana/Fundulea	France	1980	<b>Null</b>	7+9	2+12	Branlard et al., 2003
Rinconada	Unknown	CIMMYT	1981	<b>1</b>	7+8	5+10	Peña, 2004
Soissons	Iena/hn-35	France	1990	<b>2*</b>	7+8	5+10	Branlard et al., 2003
Gazul	Unknown	Spain	1992	<b>2*</b>	7+8	5+10	Peña, 2004
Isengrain	Apollo/Soissons	France	1998	<b>Null</b>	7+8	5+10	Lemelin et al., 2005
Califa Sur	Unknown	Spain	2001	<b>1</b>	7+8	5+10	Lucas et al., 2010

NI: Not identified.

<sup>a</sup> In bold-type are identified the HMW-GS characterized in the present study through PCR-based DNA markers.

<sup>b</sup> It is neither the null, the 1 nor the 2\* subunit.

**Table 2**  
Experimental details of the study conducted in Gimenez (Lleida, northeastern Spain).

Year	2009		2010	
	Rainfed	Irrigated	Rainfed	Irrigated
Experiment identification	R09	I09	R10	I10
Water input (rainfall + irrigation, mm) from sowing to maturity	191	288 <sup>a</sup>	277	427
Environmental conditions during grain filling				
Average daily temperature (°C)	19.6	19.9	17.2	17.8
Water input (rainfall + irrigation, mm)	9	54 <sup>a</sup>	5	82
Fertilization (kg ha <sup>-1</sup> )				
N (top dressing)	30	40	50	94
P <sub>2</sub> O <sub>5</sub>	68	113	105	105
K <sub>2</sub> O	113	188	175	175
Means across varieties				
Grain protein content (%; mean ± S.D.)	16.5 ± 1.24	15.2 ± 1.56	14.1 ± 1.11	11.4 ± 0.96
Falling Number (s; mean ± S.D.)	479 ± 101	495 ± 106	428 ± 77	354 ± 55
Yield (kg ha <sup>-1</sup> )	5434	7201	4318	7120

<sup>a</sup> Sprinkler irrigation.

derived from crosses, including those improved and grown from the mid 1940s until the Green Revolution, and (iii) modern varieties introduced or released in Spain from 1970 to 2001 and mostly derived from semi-dwarf germplasm of CIMMYT and French origin (Table 1).

## 2.2. Field experimental setup

Four field experiments were conducted during two growing seasons, 2008–09 and 2009–10 in Lleida (north-eastern Spain: 41°40'N, 0°20'E; 200 m a.s.l.). Two water regimes were assayed, namely: rainfed and irrigated, the latter with additional water inputs of 96 mm and 150 mm in 2009 and 2010, respectively (Table 2). According to the FAO soil classification (FAO, 1998), the soil type was calcic cambisol with a fine loam texture.

Sowing was done in late November at a density of 450 seeds m<sup>-2</sup> following a randomized complete block design with three replications, and crops were harvested in July. Plots consisted of eight 5 m-length rows, 0.15 m apart. Nitrogen fertilization was applied in a top-dressing as ammonium nitrate. Further experimental details and management practices are described in Table 2.

## 2.3. HMW-GS allelic composition

PCR-based DNA markers were used to determine the HMW-GS composition at two loci (*Glu-A1* and *Glu-D1*). The methodology proposed by De Bustos et al. (2000) was followed to discriminate the allele encoding for the 2\* subunit from those encoding for subunit 1 and the null allele at *Glu-A1* locus. PCR-based DNA markers were used at *Glu-D1* locus to detect the alleles encoding for the *Dx5*, *Dy10*, and *Dy12* glutenin subunits, following the methodology proposed by Ahmad (2000). All the fragments obtained were separated in agarose gels. The allelic composition at *Glu-B1* was obtained from published references, which were also used to verify and complete the results obtained (Table 1).

## 2.4. Grain quality

All the plots were mechanically harvested at grain ripening. Test weight (TW; kg hl<sup>-1</sup>) was measured from cleaned grain samples from each plot, and grain protein content (PC, %) was analyzed with a near-infrared transmittance spectrophotometer (Infratec® 1241-grain analyzer, Foss Tecator AB, Sweden). The total amount of protein produced per unit area (GPpHa, kg ha<sup>-1</sup>) was determined for each plot as the product of PC and grain yield.

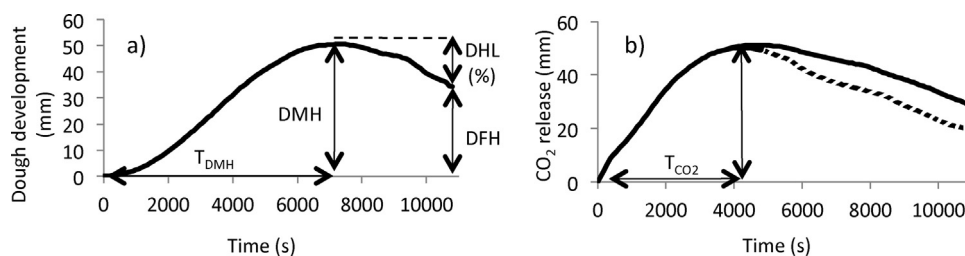
## 2.5. Flour attributes

White flour samples were obtained from milled grain from each plot. Before milling, grain samples were tempered overnight to 16% moisture content. The grain was ground in a moulin CD1 mill (Chopin S.A., Villeneuve la Garenne, France).

White flour samples were used to determine the following rheological properties of dough biaxial extension: tenacity (*P*, maximum overpressure), extensibility (*L*, length of the curve), strength (*W*, deformation energy), and the configuration ratio (*P/L*) with the alveograph (Chopin S.A., Villeneuve la Garenne, France) following the ICC standard method No. 122 (ICC, 1992). Other flour attributes determined were: ash content (%; AACC International Method 08.21.01; AACC, 2000); water absorption, using the near-infrared spectrophotometer Inframatic 8600 (Perten Instruments, Sweden); and α-amylase activity, using the Falling Number 1313 (Perten Instruments, Sweden) and the AACC International Method 56-81.03 (AACC, 1999). During the second crop season only two replicates per environment were analyzed for the aforementioned attributes.

## 2.6. Fermentation test

In the second year, dough samples were prepared for fermentation tests with 250 g of white flour from each plot of one block of the irrigated experiment, 3 g (1.2%) of instant active dry yeast (Pante, Puratos, Belgium) and 5 g of salt. The amount of water added was adjusted from the measured water absorption value. The fermentation of each dough sample was monitored using a Chopin rheofermentometer F3 (Chopin S.A., Villeneuve la Garenne, France). The dough was constrained with a piston with a 2 kg resistance within the fermentation basin over 3 h at a stable temperature of 28.5 °C. The following parameters were recorded: dough maximum height (DMH); dough final height at the end of the 3 h fermentation process (DFH); dough height lost between maximum and final height (DHL, %); time to DMH (*T*<sub>DHM</sub>) from the dough development curve and volume of total CO<sub>2</sub> produced (VP<sub>CO2</sub> or the area behind the gas production curve); volume of CO<sub>2</sub> retained by the dough (VR<sub>CO2</sub> or the area behind the gas retention curve); time to CO<sub>2</sub> release (*T*<sub>CO2</sub>); and the retention coefficient (RQ or the proportion of CO<sub>2</sub> volume produced, lost at the end of the process) from the gas production and retention curves. Curves and parameters are shown in Fig. 1.



**Fig. 1.** Pattern of changes in: a) dough development, and b) CO<sub>2</sub> production (solid line) and release (discontinuous line) of samples tested with a Chopin Rheofermentometer F3. DMH: dough maximum height; DFH: dough final height; DHL: dough height lost, %;  $T_{DMH}$ : time to attain DMH;  $T_{CO_2}$ : time to CO<sub>2</sub> release.

## 2.7. Statistical analysis

Analyses of variance were carried out for all the studied traits. All factors were considered fixed except for the block (nested to the year and water regime) that was considered random. In the model, the genotypic effect was partitioned into differences between periods and the residual genotypic variance retained within periods. The genotypic residual and its interactions were considered as the error for individual contrasts with the breeding period effect and its interactions with the environment. According to the results obtained from the ANOVA analyses, the variety by environment ( $V \times E$ ) interaction of the extensibility ( $L$ ) parameter of the alveogram was partitioned in an AMMI model (Gauch and Zobel, 1997). The number of bilinear terms retained in the analysis was determined on the basis of the proportion of the sum of squares (SS) of the  $V \times E$  interaction explained by each IPCA. The effect of the 5 + 10HMW-GS encoded at *Glu-D1* locus on the  $V \times E$  interaction was studied by partitioning the variety factor of the ANOVA in differences between varieties carrying or not this HMW-GS and the residual.

Absolute and relative genetic gains during the 20th century were computed as the slope of the linear regression between the absolute or relative values of the trait and the year of release. Relative values were calculated for each cultivar as a percentage with regard to the average value of the whole set.

Regression models were fitted in order to assess the relationship between the variables studied. Bi-linear regression models [ $Y = BX + A(XC) + B \times C (X \geq / \leq C)$ ; with the slope of the second segment restricted to 0] were selected when they performed significantly better than the linear one. All analyses were carried out with the SAS-STAT (SAS Institute Inc., 2009) and GENSTAT (Payne

et al., 2006) statistical packages, and Tablecurve 2D v2.03 software (Jandel, 1991) was used for fitting the bi-linear models.

## 3. Results

### 3.1. HMW-GS allelic composition

The identification of the HMW-GS composition of the varieties included in the historical series revealed differences between breeding periods. The null allele at *Glu-A1* predominated in landraces and initial varieties, with frequencies greater than 57%, while it was present in only 33% of the modern varieties (Table 1). The *Glu-B1* locus showed the highest number of allelic variants in the historical series. Nevertheless, four out of the six alleles detected at this locus were present in only one variety. Allele 7 + 8, which was absent in landraces, was the most frequent in initial and modern varieties (75% and 55%, respectively). Two of the alleles present at *Glu-D1* (4 + 12 and 5 + 12) were exclusive to landraces. For the landrace ‘Barbilla’, the  $Dx$  subunit encoded at *Glu-D1* could not be identified, although our results showed that it was not the  $Dx5$  subunit. All the initial varieties were monomorphic for the 2 + 12 allele at *Glu-D1*, while in the most recent cultivars allele 5 + 10 predominated (77%, Table 1).

### 3.2. Grain quality

The analyses of variance showed that water regime was the only environmental factor significantly affecting TW, which depended mostly on the variety effect (Table 3). However, despite the tendency of modern varieties to show a greater TW (Table 4), differences between breeding periods were not statistically

**Table 3**  
Percentage of the sum of squares of the ANOVA for grain and bread-making quality attributes. Variety effect and its interactions are partitioned in differences between breeding periods (BP) and within them (residual not shown).

	df	Test weight	Grain protein content	Grain protein per ha	Water absorption	Ash content	Biaxial extension parameters			
							W	P	L	P/L
Year	1	<0.01	47.3**	37.7**	13.7**	33.6**	2.74**	0.58*	1.87**	0.07
Water regime	1	21.3**	20.9**	18.6**	2.53*	14.1**	4.05**	2.87**	0.99	1.53**
Year $\times$ Water regime	1	0.56	2.43**	0.01	1.04**	1.97*	3.24**	1.08*	6.76**	0.1
Block (Year $\times$ Water regime)	8	1.51	0.63*	1.79**	1.04	1.22	0.31	0.65	1.36	0.78
Variety	15	44.7**	18.6**	18.2**	73.8**	22.5**	80.3**	85.5**	44.9**	76.2**
Between BP	2	19.1	77.2**	75.0**	42.6*	27.2	43.6*	30.4	16.9	30.7
Variety $\times$ Year	15	6.34**	3.83**	10.7**	1.49**	7.96**	1.77**	3.18**	9.93**	5.86**
Between BP $\times$ Year	2	2.59	28.5	55.57**	1.69	3.23	25.2	10.8	15.1	15.29
Variety $\times$ Water regime	15	6.06**	0.76	3.16**	0.36	2.72	1.41**	1.28**	13.4**	3.04**
Between BP $\times$ Water regime	2	18.96	0.7	47.1*	22.4	9.64	3.95	8.82	64.4*	49.62*
Variety $\times$ Year $\times$ Water regime	15	6.54**	1.26**	1.76*	1.04	4.81**	3.26**	1.50**	4.59*	4.01**
Between BP $\times$ Year $\times$ Water Regime	2	24.63	51.38**	5.84	40.1*	44.5*	45.99*	56.5**	18.3	8.96
Error	120	12.9	4.31	8	2.1	11	2.91	3.33	16.3	8.37
Total	191									

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

\*\*\*  $P < 0.001$ .



**Table 4**

Mean values of yield and grain and bread-making quality attributes of 16 bread wheat varieties from 3 breeding periods across 4 environments. The varieties were grouped according to the breeding period they belong. Numbers in parentheses indicate the percentage of change with respect to the previous period.

		Landraces (2df)	Initial (3df)		Modern (8df)		SED (df <sub>e</sub> :13)
Test weight	(kg hl <sup>-1</sup> )	76.7	78.7	(2.61)	79.1	(0.51)	1.37
Grain protein content	(%)	16.0	14.2	(-11.3)	13.8	(-2.8)	0.34
Grain protein per ha	(kg ha <sup>-1</sup> )	692	815	(17.8)	908	(11.4)	35
Grain yield	(kg ha <sup>-1</sup> )	4325	5739	(32.7)	6579	(14.6)	192
Water absorption	(%)	51.5	50.4	(-2.14)	53.1	(5.36)	0.86
Ash content	(%)	0.654	0.65	(-0.76)	0.63	(-3.39)	0.013
Biaxial extension parameters							
<i>W</i>	(J × 10 <sup>-4</sup> )	83.1	164	(97.4)	249	(51.8)	51.9
<i>P</i>	(mm H <sub>2</sub> O)	36.2	53.4	(47.5)	70.8	(32.6)	14.51
<i>L</i>	(mm)	134	119	(-11.2)	108	(-9.24)	13.7
<i>P/L</i>	(mm H <sub>2</sub> O mm <sup>-1</sup> )	0.28	0.49	(75.0)	0.74	(51.0)	0.181

df<sub>e</sub>: degrees of freedom of the error.

significant for this trait. Grain protein content and GPpHa were greatly affected by the environmental conditions, but differences between breeding periods accounted for most of the variability induced by the genotypic effect (Table 3). With the introduction of initial varieties PC decreased by 11.3%, but no significant additional reductions occurred with the introduction of modern cultivars (Table 4). In spite of the reductions in PC, significant increases in GPpHa were observed across breeding periods. Thus, although the relative genetic gain was negative for PC (-0.21% y<sup>-1</sup>), GPpHa increased at a rate of 0.39% y<sup>-1</sup> (Table 5).

### 3.3. Flour and dough attributes

The environmental factors 'year' and 'water regime' explained about half of the variance for flour ash content (Table 3). The relative genetic gain in ash content was statistically significant ( $P < 0.05$ ), but the resulting rate of change was extremely low (-0.078% y<sup>-1</sup>, Table 5), and no significant differences were detected between breeding periods. Water absorption (WA) was strongly genotype-dependent, since the variety effect accounted for 73.8% of total variation (Table 3). Changes in WA between breeding periods were statistically significant due to differences between initial and modern varieties (Table 4). However, the estimated rate of change across periods was very low (0.08% y<sup>-1</sup>, Table 5).

The alveographic parameters *W* and *P* showed a strong variety effect and, accordingly, the environmental contribution to their total variance was very low (Table 3). However, differences between breeding periods were only significant for *W*, despite the large increases observed in *P* and *P/L* across periods (Table 4), which resulted in significant relative genetic gains of 0.99% y<sup>-1</sup> and 1.45% y<sup>-1</sup>, respectively, while *L* decreased at a rate of -0.46% y<sup>-1</sup> (Table 5).

**Table 5**

Absolute and relative genetic changes for grain and bread-making quality attributes of 16 bread wheat varieties from 3 breeding periods across 4 experiments.

	Absolute changes	Relative changes (% y <sup>-1</sup> )	R <sup>2</sup>	Absolute changes units
Test weight	-	-	0.14	kg hl <sup>-1</sup> y <sup>-1</sup>
Grain protein content	-0.03	-0.21	0.51**	% y <sup>-1</sup>
Grain protein per ha	0.003	0.39	0.64**	kg ha <sup>-1</sup> y <sup>-1</sup>
Water absorption	0.042	0.080	0.30 <sup>†</sup>	% y <sup>-1</sup>
Ash content	-0.0005	-0.078	0.27 <sup>†</sup>	% y <sup>-1</sup>
Biaxial extension parameters				
<i>W</i>	2.74	1.39	0.42 <sup>†</sup>	J × 10 <sup>-4</sup> y <sup>-1</sup>
<i>P</i>	0.59	0.99	0.32 <sup>†</sup>	mm H <sub>2</sub> O y <sup>-1</sup>
<i>L</i>	-0.53	-0.46	0.29 <sup>†</sup>	mm y <sup>-1</sup>
<i>P/L</i>	0.009	1.45	0.38 <sup>†</sup>	mm H <sub>2</sub> O mm <sup>-1</sup> y <sup>-1</sup>

<sup>†</sup>  $P < 0.05$ .

\*\*  $P < 0.01$ .

Dough extensibility (*L*) was the alveographic parameter that showed the lowest stability across environments, with a V × E interaction that explained 27.9% of total variance (deduced from Table 3). The component that most contributed to *L* was the variety × water regime interaction, which accounted for 13.4% of the total variance, while the variety × year and the variety × year × water regime interactions accounted for lower percentages (Table 3). In order to understand the V × E interaction for *L*, we performed an AMMI model analysis. The first two IPCAs explained 88.6% of the V × E variation (Fig. 2). The first IPCA, which accounted for 53.0% of the total variance, separated the rain-fed environments, located on the negative side of the axis, from the irrigated ones, which were placed on the positive side, thus, arranging them on the basis of the water input they received. Moreover, the varieties carrying the 5 + 10 subunit at *Glu-D1* were situated near the irrigated environments. In the ANOVA, when the variety effect and its interactions were partitioned in differences between varieties carrying or not the 5 + 10 subunit, the presence or absence of this allele explained 30.4% of the total variance of the V × E interaction and 44.1% of the total variance of the variety × water regime interaction (data not shown). These results suggest that the response of *L* to water input depended to a great extent on the genotype at *Glu-D1*, particularly the presence/absence of the 5 + 10 subunit. Moreover, the lowest PC was recorded in the environment with the highest water input, while the highest PC corresponded to the driest environment (Table 2).

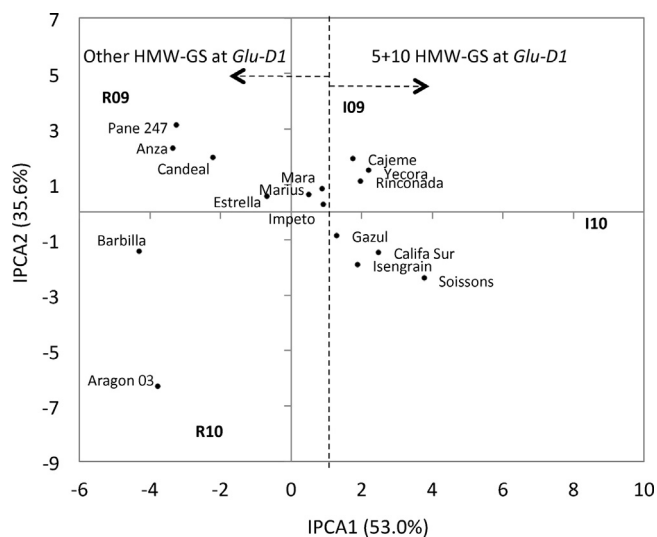
### 3.4. Fermentation test

None of the coefficients of determination of the linear regression models used to calculate absolute and relative genetic gains in dough fermentation parameters were statistically significant.

**Table 6**  
Dough development and characteristics of CO<sub>2</sub> release during fermentation of an historical series of 16 bread wheat varieties from 3 breeding periods determined from samples obtained in the experiment conducted in 2009 under irrigated conditions.

Variety	DMH (mm)	DFH (mm)	DHL (%)	T <sub>DMH</sub> (s)	T <sub>CO<sub>2</sub></sub> (s)	VP <sub>CO<sub>2</sub></sub> (ml)	VR <sub>CO<sub>2</sub></sub> (ml)	RQ (%)
<b>Landraces</b>								
Aragon 03	48.2	24.7	48.8	7830	4860	1098	1010	91.9
Barbilla	39.3	35.3	10.2	8550	4680	1078	977	90.7
Candeal	50.6	34.3	32.2	7470	4950	1089	1014	93.1
<b>Initial</b>								
Mara	45.8	30.3	33.8	5940	NC	895	870	97.2
Impeto	41.9	41.8	0.24	10710	3600	1135	1012	89.1
Estrella	39	31.8	18.4	7290	NC	908	858	94.6
Pane 247	43.4	39.6	8.76	9000	5490	987	927	93.9
<b>Modern</b>								
Cajeme	47.3	46.2	2.33	9630	4050	1234	1087	88.1
Yecora	44	41.9	4.77	8190	3150	1317	1112	84.4
Anza	41.6	39.4	5.29	9090	4950	1212	1075	88.7
Marius	42	19.3	54.1	6210	NC	862	843	97.8
Rinconada	42.1	41.4	1.66	8820	3870	1313	1115	85
Soissons	44.2	42.5	3.85	9420	4320	1105	1010	91.4
Gazul	32.7	32.7	0	10800	3870	1091	961	88.1
Isengrain	45.5	45	1.1	9360	4230	1150	1033	89.9
Califa Sur	31.8	31.8	0	10800	2880	1218	1032	84.7
<b>Means of the breeding periods (BP)</b>								
Landraces	46	31.4	30.4	7950	4830	1088	1000	91.9
Initial	42.5	35.9	15.3	8235	4545	981	917	93.7
Modern	41.2	37.8	8.1	9147	3915	1167	1030	88.7
SEM (df <sub>e</sub> : 13)	3.34	5.05	11.3	967	464	81.1	50.6	2.13

NC: these varieties did not released significant amounts of CO<sub>2</sub> within the 3 h the test was planned to last; df<sub>e</sub>: degrees of freedom of the error; DMH: dough maximum height; DFH: dough final height; DHL: dough height lost, %; T<sub>DMH</sub>: time to DMH; T<sub>CO<sub>2</sub></sub>: time to CO<sub>2</sub> release; VP<sub>CO<sub>2</sub></sub>: total CO<sub>2</sub> volume produced; VR<sub>CO<sub>2</sub></sub>: total CO<sub>2</sub> volume retained; RQ: retention coefficient.



**Fig. 2.** Biplot of the first two axes of the AMMI model for extensibility (*L*). Environments are represented in bold (see Table 2 for environment identification). The discontinue line separates the varieties carrying the 5 + 10HMW-GS at *Glu-D1* locus from the remainder.

However, a tendency was detected toward a reduction of DMH across periods, as well as an increase in the time needed to attain it ( $T_{DMH}$ ) (Table 6). Changes in DMH were due mostly to the low values attained by the modern varieties ‘Gazul’ and ‘Califa Sur’ during the 3 h fermentation. These two varieties seemed to need more than 3 h to reach their DMH. Although differences between breeding periods were not statistically significant, dough final height (DFH) tended to increase across time (Table 6). The decrease in DMH and increase in DFH may explain of the 73% drop in the relative dough height loss (DHL) at the end of the 3 h fermentation. No changes were detected in the total CO<sub>2</sub> volume produced and retained. However, the time of release ( $T_{CO_2}$ ) decreased by more than 15 min in

modern varieties when compared with landraces (Table 6). In addition, the introduction of modern varieties caused a significant reduction in the retention coefficient (RQ).

In order to ascertain whether the genetic improvement of the rheological properties resulted in changes in dough fermentation behavior, we calculated correlation coefficients between dough deformation and fermentation parameters, obtained, respectively, from the alveograph and the rheofermentometer. Significant and negative relationships were found between both *W* and *P* with DHL and DMH (Table 7). The negative relationship between *W* and DMH was due to the high *W* and extremely low DMH values of the varieties ‘Gazul’ and specially ‘Califa Sur’, which showed the highest *W* and *P* ( $491 \times 10^{-4} J$  and 131 mm H<sub>2</sub>O, respectively), resulting in the highest *P/L* value (1.45 mm H<sub>2</sub>O mm<sup>-1</sup>). Indeed, when these two cultivars were removed from the calculations, the correlation coefficient decreased to  $r=0.05$ . Similarly, the correlation coefficient between *P* and DMH was  $r=0.02$  when the data of

**Table 7**

Pearson's correlation coefficients for the relationships between the rheofermentometer F3 results and the biaxial extension parameters of 16 bread wheat varieties of three breeding periods. Samples were obtained from one replication of the experiment conducted in 2009 under irrigated conditions.

	<i>W</i>	<i>P</i>	<i>L</i>	<i>P/L</i>
DMH	-0.52*	-0.53*	0.20	-0.47
DFH	0.40	0.35	-0.27	0.29
DHL	-0.68*	-0.66*	0.38	-0.56*
T <sub>DMH</sub>	-0.68**	-0.67**	-0.30	-0.55*
T <sub>CO<sub>2</sub></sub>	-0.83***	-0.86***	0.69**	-0.80***
VP <sub>CO<sub>2</sub></sub>	0.61*	0.69**	-0.63*	0.69**
VR <sub>CO<sub>2</sub></sub>	0.49	0.57	-0.56*	0.57*
RQ	-0.76***	-0.84***	0.65**	-0.81***

DMH: dough maximum height; DFH: dough final height; DHL: dough height lost (%); T<sub>DMH</sub>: time to DMH; T<sub>CO<sub>2</sub></sub>: time to CO<sub>2</sub> release; VP<sub>CO<sub>2</sub></sub>: total CO<sub>2</sub> volume produced; VR<sub>CO<sub>2</sub></sub>: total CO<sub>2</sub> volume retained; RQ: retention coefficient.

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

\*\*\*  $P < 0.001$ .

these two varieties were excluded. Although the correlation coefficient between  $W$  and DFH was not significant, a bilinear model fitted properly to the relationship between these two variables (Fig. 3a). This relationship also improved ( $R^2 = 0.73$ ,  $P < 0.01$ ) when the varieties ‘Gazul’ and ‘Califa Sur’ were removed from the model. Accordingly, the relationship between  $W$  and DHL was negative for  $W$  values lower than  $159 \times 10^{-4} J$  (Fig. 3b) and  $P/L$  values lower than  $0.56 \text{ mm H}_2\text{O mm}^{-1}$  (data not shown). For  $W$  values above  $159 \times 10^{-4} J$ , and  $P/L$  values greater than  $0.56 \text{ mm H}_2\text{O mm}^{-1}$ , the DHL was close to zero (Fig. 3b), indicating that DMH and DFH took similar values (Table 6).

The varieties showing higher  $W$ ,  $P$ , and  $P/L$  values produced and retained more  $\text{CO}_2$  ( $\text{VP}_{\text{CO}_2}$  and  $\text{VR}_{\text{CO}_2}$  values, respectively, Table 7). However, negative associations were also found between  $W$ ,  $P$ , and  $P/L$  with  $T_{\text{CO}_2}$  and  $\text{RQ}$  (Table 7). Indeed, a significant bi-linear regression model properly fitted the relationship between  $P/L$  and  $\text{RQ}$ , which showed a negative association between these two variables for  $P/L$  values below  $0.83 \text{ mm H}_2\text{O mm}^{-1}$  (Fig. 3c). The correlation coefficients between  $T_{\text{CO}_2}$  and  $\text{RQ}$  with  $L$  were positive and significant (Table 7). Neither PC nor ash content was significantly related to any fermentation parameter (data not shown).

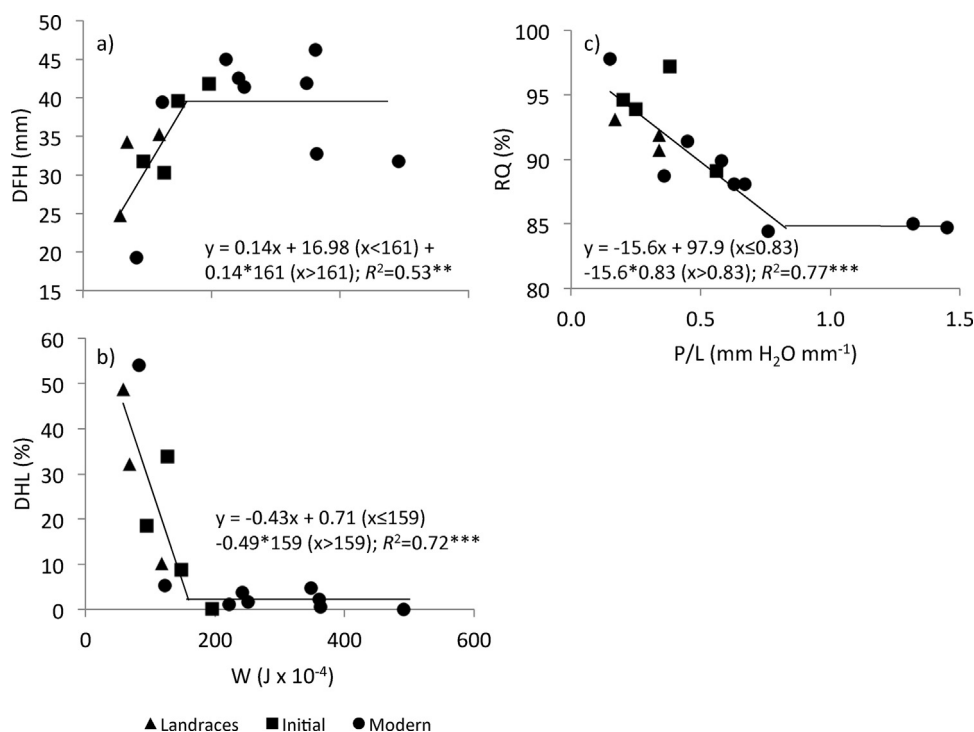
#### 4. Discussion

Our results reveal that the introduction of improved bread wheat varieties in Spain during the 20th century enhanced bread-making quality, while causing a general reduction of grain PC. The rate at which PC decreased ( $-0.21\% \text{ y}^{-1}$ ) was lower than that reported for bread wheat in France from 1946 to 1992 ( $-0.35\% \text{ y}^{-1}$ ; Brancourt-Hulmelet et al., 2003) and that recorded in CIMMYT-derived varieties after 1950 (ca.  $-0.40\% \text{ y}^{-1}$ ; Ortiz-Monasterio et al., 1997). However, the rate found in our study was greater than that described for Italian ( $-0.11\% \text{ y}^{-1}$  and  $-0.14\% \text{ y}^{-1}$ ; De Vita et al., 2007; Subira et al., 2014) and Spanish ( $-0.19\% \text{ y}^{-1}$ , Subira et al., 2014) durum wheat during the last century. Reductions in grain

PC have generally been associated with yield gains as a result of a dilution of nitrogen compounds when carbohydrate deposition increases during photosynthesis (Jenner et al., 1991). Accordingly, the decrease in PC in the wheat varieties grown in Spain during the 20th century may have been caused by the yield increase during this period, which has recently been estimated at  $35.1 \text{ kg ha}^{-1} \text{ y}^{-1}$  (Sanchez-Garcia et al., 2013). However, changes in the protein content did not follow a linear trend, since 82% of the reductions were due to the introduction of the initial varieties, as reported for Italy (Canevara et al., 1994). The cultivation of these first improved varieties—most of Italian origin—in Spain from the late 1940s resulted in yield increases of ca. 30% over that of landraces, also reported in previous studies (Sanchez-Garcia et al., 2013). However, the 11.3% decrease in grain PC limited the improvement in protein production per hectare to 18%, a much lesser proportion than that achieved by yield gains (52% as shown in Table 4).

With the introduction of modern varieties from the 1970s, no statistically significant decrease in PC accompanied the rises in yield. Modern varieties not only showed increased grain yield as shown here and in previous studies (Sanchez-Garcia et al., 2013), but also improved the capacity of protein allocation in grains. These results are consistent with the improvements in the nitrogen harvest index reported in France (Brancourt-Hulmelet et al., 2003) and Mexico (Ortiz-Monasterio et al., 1997), countries of origin of most modern varieties of wheat cultivated in Spain. Our results indicate that the previously reported negative relationship between yield and protein (Simmonds, 1995) ended with the introduction of the modern varieties, which stabilized grain PC, but significantly increased the protein produced per unit area.

Flour attributes also underwent changes during the last century. The ANOVA indicated that although the 3% decrease in ash content was not statistically significant, the coefficient of determination of the linear regression line fitted to calculate the genetic gain was. The divergence between these two results can be attributed to computation differences between the two statistical tests.



**Fig. 3.** Relationship between dough deformation energy ( $W$ ) of an historical series of 16 bread wheat varieties from three breeding periods and: a) dough final height (DFH), and b) dough height loss (DHL); and c) relationship between the retention coefficient (RQ) and the configuration ratio ( $P/L$ ). Samples were obtained from one replication of the experiment conducted in 2009 under irrigated conditions.

Nevertheless, the discrepancy was minimal since the rate of genetic gain estimated for ash content was extremely low ( $-0.078\%y^{-1}$ ). In contrast, changes in water absorption capacity were statistically significant, but the rate of increase was also very small ( $0.080\%y^{-1}$ ). The improvement in water absorption capacity was attributable to the modern varieties, probably because the importance of this trait for the formulation of different products, and its relationship with higher economic returns for the milling industry, were only evident in the last decades of the century (Wrigley et al., 2009).

Although the changes in grain and flour attributes were significant in the 20th century, the largest impact of breeding programs on wheat quality corresponded to the rheological properties of dough, particularly those related to protein composition. Dough strength ( $W$ ) increased during the 20th century at a rate of  $2.74 \times 10^{-4} J y^{-1}$  ( $1.39\%y^{-1}$  in relative terms), and tenacity ( $P$ ) augmented  $0.59 \text{ mm H}_2\text{O} y^{-1}$  ( $0.99\%y^{-1}$ ). Changes in these two traits were quite evenly distributed over time, since initial and modern varieties showed about twice and thrice the dough  $W$  values of the landraces, and about a 50% and 100% greater  $P$  than them, respectively. This timing differs from that reported for Italian varieties, in which improvements in gluten strength were attained only during the last three decades of the 20th century (Canevara et al., 1994). Given the Italian origin of many initial varieties grown in Spain, the lack of progress in Italy can be attributed solely to the high quality of Italian landraces, which according to data reported by Canevara et al. (1994) have  $W$  and  $P$  values that exceed by 58% and a 39%, respectively, those obtained in this study for the Spanish landraces. However, the pattern of  $W$  improvement in Spain was similar to that reported in France for the same period (Bonjean et al., 2001). This finding is consistent with the French origin of most initial and modern Spanish varieties.

The lack of complete information about the allelic composition of HMW-GS for the landraces examined did not allow us to associate changes in allelic variants with the improvement in the dough properties of initial varieties. However, some general trends were observed along the century. Changes in the frequency of the null allele at *Glu-A1*, reported to have a negative effect on  $W$  (Branlard et al., 1992; Cornish et al., 2001), could partially explain the quality improvement of modern varieties, but not the large  $W$  enhancement of the initial cultivars, in which this allele was predominant. Similarly, the presence of GS 2 + 12 at *Glu-D1* in initial varieties did not contribute to improved  $W$ , since its effect on this parameter has been reported to be similar to that of the 4 + 12 subunit (Wrigley et al., 2009), which was already present in the landrace 'Aragon 03' and is probably also present in 'Barbilla'. Therefore, the quality improvement of the initial varieties can attributed mostly to the replacement of the undesirable HMW-GS 20 at *Glu-B1*—present in the landrace 'Aragon 03' and according to Giraldo et al. (2010) predominant in Spanish landraces—by the 7 + 8 subunit, which has a demonstrated positive effect on dough properties (Branlard et al., 2003; Tohver 2007). We attribute the improved  $W$  of modern varieties not only to the prevalence of the 7 + 8 subunit, but also to the introduction of GS 5 + 10 at *Glu-D1* the subunit most determinant at this locus for promoting gluten strength (Branlard et al., 2003; Tohver, 2007), and to the replacement of the null allele at *Glu-A1* by subunits 1 or 2\*, which have a large positive effect on dough strength (Branlard et al., 2003; Tohver 2007; Wrigley et al., 2009).

The well-known large effect of HMW-GS composition on  $W$  (Payne et al., 1987; Branlard et al., 1992) is in agreement with the great genotypic effect found in this study on both  $W$  and  $P$ , and it explains the success of breeding programs to improve these traits. On the other hand,  $L$  appeared to be much more environmentally regulated—the  $V \times E$  interaction accounted for ca. 28% of total variance—and therefore, less prone to genetic manipulation. Although LMW glutenin alleles make a significant genetic contribution to  $L$  (Cornish et al., 2001), we also found a genetic effect

of HMW-GS on this parameter, particularly the allelic composition at *Glu-D1*. Our results showed that the presence of the 5 + 10 subunit explained 30.4% of the variance for the  $V \times E$  interaction and 44.1% of the variety  $\times$  water input interaction for  $L$ . The varieties carrying the 5 + 10 subunit seemed to be more adapted than the others to environments with high water input, in which the grains had a low PC and the resulting dough had a low  $L$ . Environmental conditions and the flour PC are recognized to have a considerable effect on  $L$  (Cornish et al., 2001), and the traits associated with PC have been reported to be more affected by the environment and the  $V \times E$  interaction than those related to protein quality and dough rheology (Williams et al., 2008).

The study of dough development during fermentation allowed us to ascertain the effect of breeding for rheological properties on dough fermentation behavior. The results of the rheofermentometer tests revealed that in baking procedures with long-fermentation  $W$  and  $P/L$  should reach minimum values to ensure that the bubble structure developed during mixing does not collapse during fermentation. Our results indicated that doughs with  $W \geq 159$  and  $P/L \geq 0.56$  were required in order to withstand 3 h fermentation. Doughs with  $W$  and  $P/L$  values below this minimum showed progressive alveolus collapse, resulting in differences between DMH and DFH of up to 54%, which was the DHL value obtained for the modern variety 'Marius', which had the lowest  $P/L$  value. Our results reveal that most of the modern bread wheat varieties grown in Spain meet these requirements. This finding is in agreement with the reported improvements in mixing time and fermentation tolerance, measured by means of a mixograph, in several countries (Khalil et al., 2002; Fufa et al., 2005). On the basis of our findings, we conclude that improvements in gluten quality through breeding allowed modern Spanish bread wheat varieties to meet industrial requirements in terms of fermentation tolerance and stability.

We found that, for satisfactory fermentation behavior, a balance between  $P$  and  $L$  values is required, given that the higher the  $P/L$  ratio, the greater the dough porosity and its faster start. The strength of the gluten matrix of varieties with high  $W$  and  $P$  values would prevent alveolus collapse. However, when  $P$  was not compensated by adequate extensibility, dough became too tenacious and too porous, so it could not achieve a high DMH, a trait correlated with final loaf volume (Ktenioudaki et al., 2010, 2011). This finding is in agreement with previous studies that reported the relevance of both,  $L$  and  $W$ , for an appropriate stability of gas cells during expansion (Sroan et al., 2009; Ktenioudaki et al., 2010).

The combination of subunit 7 + 8HMW-GS—encoded at *Glu-B1*, which over-expresses the Bx7 HMW-GS, promoting  $W$  (Wrigley et al., 2009) and whose effect on  $W$  and  $P$  is additive to that of the 5 + 10 subunit at *Glu-D1* (Vawser and Cornish, 2004)—resulted in over-strong and unbalanced doughs, which are unsuitable for attaining acceptable loaf volumes (Butow et al., 2003; León et al., 2009). Moreover, in spite of their positive effect on fermentation tolerance, the high  $W$  and  $P/L$  values of modern varieties probably enhanced the porosity of doughs. However, the interest of the milling and baking industries in varieties with high  $W$  and  $P$  depends on their suitability for mixtures with weaker flours, since varieties with  $W > 250$ –300 are usually classified by governments and millers' associations in France, Italy and Spain as "improver wheats" (Mesdag and Donner 2000; BOE, 2010).

Modern bread wheat varieties showed great variability in bread-making quality attributes and DMH values. Such variability could be explained by the farmers' choice of the most productive cultivars, the lack of economic incentives for growing high quality varieties, and industrial demand for wheat varieties with different characteristics for the manufacture of a wide range of products. The rising importance of frozen dough may promote interest in varieties with over-expressed HMW-GS, since dough storage under freezing



conditions depolymerizes HMW-GS, thus, affecting bread end-use quality (Ribotta et al., 2001).

On the basis of our results, we conclude that large rates of improvement for *W*, *P* and *P/L* occurred in the varieties of bread wheat grown in Spain during the 20th century, while dough *L* and grain PC were significantly reduced. Furthermore, the enhancement of rheological properties was related to changes in fermentation tolerance and stability, thus, complying with industrial requirements.

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