

Relationship between common wheat (*Triticum aestivum* L.) gluten proteins and dough rheological properties

Gluten proteins and rheological properties in wheat

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Summary

This paper reports the correlation between the rheological properties of bread wheat dough and the types and quantities of endosperm proteins in 28 common wheat cultivars. Different methods were used to analyse the allelic composition of these cultivars and the relative quantities of the different proteins contributing to the gluten structure. Neither dough strength (*W*) nor tenacity/extensibility (*P/L*) correlated with allelic composition. Different wheats with the same allelic composition (i.e., with respect to glutenins) showed different rheological properties. The glutenins were the most influential components of *W* and *P/L*, especially the high molecular weight (HMW) glutenin subunits and in particular the type x form. These proteins seem to increase *W* and are the main constituents of the gluten network. The gliadins and low molecular weight (LMW) glutenin subunits appear to act as a “solvent”, and thus modify the rheological properties of the dough by either interfering with the polymerisation of the HMW glutenin subunits, or by altering the relative amounts of the different types of glutenin available. Thus, the protein subunits coded for by the alleles *Glu-B1x7* and *Glu-D1x5* stabilised the gluten network, whereas those coded for by *Glu-B1x17* and *Glu-D1x2* had the opposite effect. Dough properties therefore appear to depend on the glutenin/gliadins balance, and on the ratio of the type x and type y HMW proteins. The influence of external factors seems to depend on the allelic composition of each cultivar.

Introduction

Wheat is currently the most important crop in the world. It is unique because of the special properties of its flour, which forms a cohesive mass – gluten or dough – which is useful in baking. The properties of wheat flour reside primarily in the types and quantities of gluten proteins it contains, the glutenins and gliadins being the most important in the formation of the gluten network. Glutenins are composed of two types of subunit, one of high molecular weight (HMW), the other of low molecular weight (LMW), coded for by different genes. Many authors have focused their analyses on the different HMW subunits because of the influence

they appear to have on the rheological properties of the dough (Payne, 1987; Shewry et al., 2001).

It has been shown, however, that LMW glutenin subunits influence the quality of durum wheats, and the question arises as to whether they may do the same in bread-making flour. Gupta et al. (1991) produced a list of the LMW alleles in bread wheats according to the extensographic behaviour of their corresponding doughs. D’Ovidio (1993) and Masci et al. (2000) characterised the LMW glutenin subunits associated with quality in durum wheats that showed similarity to subunits present in bread wheats. However, the influence of the relative amount of LMW subunits on the rheological properties of bread wheat doughs has not been

analysed. The gliadins would appear to be less important in determining bread quality, yet the addition of gliadins or the over-expression of certain gliadins reduces dough strength (Fido et al., 1997; Metakovsky et al., 1990). Their relationship with dough extensibility has also been speculated (Branlard & Dardevet, 1985).

The influence of the different gluten protein fractions on the rheological properties of the dough, and consequently on bread quality, is influenced greatly not only by cultivar genotype but also by the environmental conditions faced by the plants during grain ripening. The aim of the present work was to characterise the allelic composition of a number of Spanish wheat cultivars cultivated under the same environmental conditions, and to quantify the proteins of their corresponding glutes. The types of protein found plus their absolute and relative quantities were correlated with the main variables thought to determine the rheological properties of dough.

Materials and methods

Flours

Flour was obtained from the grain of 12 selected wheat lines (all in an advanced state of improvement) and from 16 varieties (the most commonly cultivated in Spain). The plant material was grown following a randomised complete block design with three replications at the *La Canaleja*, experimental field station (National Institute of Agricultural Research [I.N.I.A.], Alcalá de Henares, Madrid, Spain). The milling was carried out on 500 g of grain of wheat cleaned and conditioned for humidification or for desiccation. The grain was cleaned, scoured and tempered overnight to optimum moisture of 16.5%. A laboratory mill (Chopin CD1) for the milling was used. Five hundred grams of grain were added in the chute of the mill. Two parts of milling were obtained, the flour and the "rest". If it was necessary, the rest was returned to the mill to obtain a minimal quantity of flour of 255 g: 250 g for the assay and 5 g to determine the dampness of the flour.

Alveographic determinations

The rheological properties of the different doughs (strength [*W*], tenacity [*P*], extensibility [*L*] and the ratio *P/L*) were determined following the method of Faridi and Raspe (1987) (800 g of flour, 2.5% (w/v) NaCl [water constant]).

Protein extraction

Samples of seeds from each line were crushed and the proteins extracted in accordance with the method of Sing and Shepherd (1991). The HMW glutenin subunits were obtained using the method of Melas et al. (1994).

SDS-PAGE analysis

SDS-PAGE was used to identify the alleles involved in glutenin production in each cultivar. An amount of 20 ml of the above extracts were loaded onto 12% acrylamide gels for analysis. All gels were stained overnight with Coomassie Brilliant Blue R-250.

DNA extraction and AS-PCR

Genomic DNA was isolated using the method of Benito et al. (1993). AS-PCR molecular markers were used to identify any alleles that presented difficulties in SDS-PAGE analysis. The PCR conditions and primers used for the HMW subunits were those described by De Bustos et al. (2000, 2001) and De Bustos and Jouve (2003); those used for the LMW subunits were previously described by D'Ovidio (1993) and D'Ovidio and Porceddu (1996).

RP-HPLC

RP-HPLC was used to quantify the different protein fractions in each type of flour, and to establish the alleles of the main genes involved in glutenin synthesis. All analyses were repeated three times. The proteins were extracted according to the method of Singh and Shepherd (1991) with the variation of using 70% ethanol instead of propanol-1. For the analysis of the gliadins, after centrifugation of the first step, the supernatant was injected directly into the HPLC after passing through a 0.45 μ m PVDF filter. The glutenins were separated using acetone as a solvent according to the method of Melas et al. (1994). Aliquots of the different extracts were analysed using two different systems: a Hewlett Packard 1100 apparatus with a Vydac C18 column, and a Beckman System Gold machine with a Zorbax 300JB-C18 column. The proteins were separated on a linear acetonitrile gradient containing 0.05% trifluoroacetic and Milli-Q water with 0.07% TFA at a flow rate of 0.8 ml/min. The time lapse between injection and recording of the result was 35 min. The column temperature was 60 °C. The eluent was monitored at a detection wavelength of 210 nm.

Statistical analysis

Statgraphic 5.0 plus software was used for all calculations.

Results and discussion

Table 1 shows the plant material used, the rheological properties of the dough produced from their grains, the classification of the doughs according to their *P/L* values, and the allelic composition of the plants with respect to HMW and LMW glutenin subunits.

Payne (1987) reported that good bread-making quality is related to the possession of *Glu-A1x1*,

Glu-D1x5 and *Glu-D1y10*, and that poor quality, in contrast, is related to the presence of *Glu-A1xNull*, *Glu-D1x2* and *Glu-D1y12*. However, comparisons of the genetic compositions of the lines analysed in the present work using values allocated by Payne to predict bread-making quality indicated a poor relationship. Cultivars with the same allelic composition produced flours of different *W* and different *P/L* (AST and PANE), while others with different alleles showed similar values for these variables. However, the majority of lines (86.67%) with balanced flours ($0.5 < P/L < 1$) possessed alleles associated with good quality according to Payne (1987).

No direct relationship appears to exist between *W* and *P/L*, nor does there appear to be any antagonism

Table 1. Types of alleles coding for the HMW and LMW glutenins and the rheological properties of 28 wheats varieties

Variety	Code	<i>W</i> ^a	<i>P/L</i>	<i>P/L</i> ^b	Glu-A1	Glu-B1	Glu-D1	Glu-B3 ^c allele
AGSA 10	S10	179	0.3	a	Null	7 + 8	5 + 10	2
AGSA 11	S11	181	0.3	a	2*	7 + 8	2 + 12	2
AGSA 26	S26	230	0.5	a	1	17 + 18	5 + 10	1
AGSA 44	S44	150	0.4	a	1	7 + 8	2 + 12	1
Astral	AST	100	0.4	a	Null	7 + 8	2 + 12	1
Bavaro	BAV	200	0.3	a	1	7 + 9	5 + 10	1
Bolero	BOL	160	0.4	a	2*	7 + 9	2 + 12	1
Fiel	FIEL	90	0.4	a	Null	7 + 8	5 + 10	1
Marius	MAR	80	0.3	a	Null	7 + 9	4 + 12	1
AGSA 12	S12	283	0.8	b	2*	7 + 9	5 + 10	2
AGSA 28	S28	268	0.9	b	1	7 + 9	5 + 10	1
AGSA 47	S47	240	0.8	b	2*	17 + 18	2 + 12	2
AGSA 580	S5801	350	0.9	b	1	7 + 8	5 + 10	2
AGSA 9	S9	253	0.8	b	1	17 + 18	5 + 10	1
Anza	ANZ	100	0.8	b	Null	7 + 8	2 + 12	1
Gazul	GAZ	330	0.9	b	2*	7 + 8	5 + 10	2
Horzal	HOR	350	0.8	b	1	7 + 8	5 + 10	1
Livio	LIV	260	0.6	b	1	7 + 8	5 + 10	1
Pata negra	PN	280	0.6	b	1	17 + 18	5 + 10	1
Pompeyo	POM	160	0.8	b	1	7 + 8	5 + 10	1
Rinconada	RIN	300	0.7	b	1	7 + 8	5 + 10	2
Soissons	SOI	220	0.8	b	2*	7 + 8	5 + 10	1
Yecora	YEC	280	0.9	b	1	17 + 18	5 + 10	2
Zentos	ZEN	277	0.6	b	Null	7 + 9	5 + 10	1
AGSA 30	S30	269	1.3	c	Null	7 + 9	2 + 12	1
AGSA 34	S34	140	1	c	1	7 + 9	5 + 10	2
AGSA 8	S8	324	1.6	c	1	17 + 18	5 + 10	1
Pané 247	PANE	110	1.3	c	Null	7 + 8	2 + 12	1

^a*W*: strength.

^bGroups according the value of *P/L*: $a = P/L < 0.5$; $b = 0.5 < P/L < 1$; $c = P/L > 1$.

^cAllele 1 (=45K) and allele 2 (=42K).

between both these variables. Cultivars were seen with similar *W* and low *P/L* values (PANE and S34), whereas others were seen with similar *W* and high (AST, SEA) *P/L* values.

Pogna et al. (1982, 1990), D'Ovidio (1993), D'Ovidio et al. (1992) and Masci et al. (1998) reported proteins in bread wheats very similar to the LMW 42K and 45K protein subunits associated with quality in durum wheats. However, the present analysis of the LMW proteins confirmed no relationship between the presence of proteins similar to the 42K and 45K elements of durum wheat and the rheological properties of the bread cultivars analysed. Thus, *P/L* and *W* seem to be independent of the types of HMW and LMW glutenin subunits present.

Quantification of gluten proteins

Given the apparent lack of any direct relationship between the presence or absence of any particular alleles and the rheological behaviour of the dough, RP-HPLC was used to study the possible relationship between the latter and the quantity of each type of protein present. Marchylo et al. (1992) classified the different protein

fractions detected by RP-HPLC and recognized new glutenin alleles. Some of the new subunits reported by these authors were observed in the RP-HPLC profiles produced in the present work. However, the classification of Payne and Lawrence (1983) was maintained since these observations may have been the result of variations in protein quantity rather than mobility.

Figure 1 shows the chromatograms for the glutenins and gliadins. The different fractions were classified using the criteria of Wieser et al. (1998). Quantitative analyses were performed for each HMW glutenin detected, and for the LMW glutenin and gliadin fractions as a whole. Table 2 shows the results (standardised with respect to the weight of flour used for each line). The nomenclature of Marchylo et al. (1992) was used to identify the HMW subunits in the chromatograms. Differences due to genetic determinants were seen in the quantities of proteins forming the gluten.

Correlations between protein quantities and rheological properties

Statistical tests were performed to look for relationships between the quantities of the different protein fractions found in each cultivar and the rheological

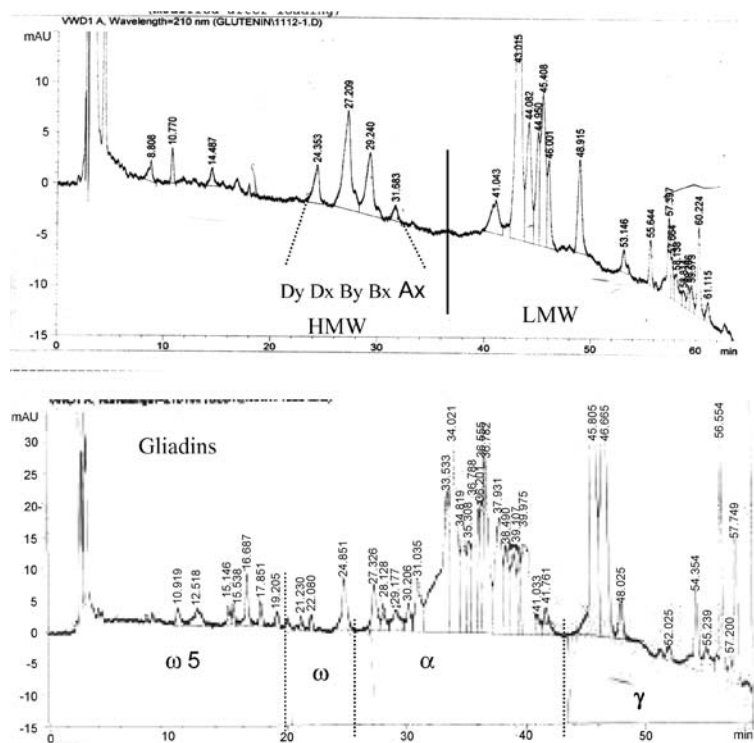


Figure 1. Chromatograms of glutenins and gliadins in common wheat following RP-HPLC separation.

Table 2. Quantities and relations of the types of proteins present in the flour

Wheat	mAU ^a															
	Gluten total	Gliadins (GLI)					Glutenins					Ratios				
		Total	ω 5	ω 1.2	α	γ	Total	HMW	x	y	LMW	GLI/GLU	GLI/HMW	GLI/LMW	LMW/HMW	x/y
ANZ	942	631	13	48	309	261	311	94	16	7	217	2.03	6.71	2.91	2.31	2.28
AST	651	414	9	31	202	172	237	56	14	11	181	1.75	7.39	2.28	3.23	1.23
BAV	1791	1191	19	84	705	383	600	156	56	19	444	1.99	7.63	2.68	2.84	3
BOL	811	430	7	43	218	162	381	101	30	10	280	1.13	4.25	1.54	2.77	3
FIEL	912	519	17	21	204	277	393	100	37	12	293	1.32	5.19	1.77	2.93	3.08
GAZ	1320	741	10	75	329	327	579	216	56	13	363	1.28	3.43	2.04	1.68	4.3
HOR	1321	514	16	61	256	181	807	288	61	7	519	0.64	1.78	0.99	1.8	8.7
LIV	1268	654	3	152	305	194	614	195	122	29	419	1.06	3.35	1.57	2.15	4.2
MAR	1124	767	28	35	432	272	357	114	31	12	243	2.15	6.73	3.15	2.13	2.58
PANE	1773	1063	16	109	517	421	710	223	30	8	487	1.49	4.77	2.18	2.18	3.75
PN	1124	696	5	120	328	243	428	142	60	24	286	1.62	4.9	2.43	2.01	2.5
POM	1728	961	32	36	552	341	767	322	134	18	445	1.25	2.98	2.16	1.38	7.44
RIN	2804	1513	12	100	771	630	1291	509	128	28	782	1.17	2.97	1.93	1.53	4.6
S10	2953	2103	109	145	1090	759	850	249	16	10	601	2.47	8.45	3.5	2.41	1.6
S11	1493	983	33	68	505	377	510	98	67	10	412	1.93	10	2.38	4.2	6.7
S12	2081	1363	19	74	873	397	718	262	52	7	456	1.9	5.2	2.99	1.74	7.4
S26	1278	744	3	162	332	247	534	236	102	51	298	1.39	3.15	2.5	1.26	2
S28	1805	1195	9	21	745	420	610	252	53	12	358	1.96	4.74	3.34	1.42	4.42
S30	1397	750	3	151	350	246	647	282	143	21	365	1.16	2.66	2.05	1.29	7
S34	1316	883	8	139	437	299	433	182	107	6	251	2.04	4.85	3.52	1.38	3.16
S44	1503	1002	9	235	567	191	501	198	6	4	303	2	5.06	3.3	1.53	1.5
S47	1654	690	37	61	355	236	964	324	45	6	640	0.72	2.13	1.08	1.97	7.5
S5801	1811	982	47	89	503	343	829	397	239	18	432	1.18	2.47	2.27	1.08	13.27
S8	1517	905	15	59	485	346	612	240	157	40	372	1.47	3.77	2.43	1.55	4
S9	941	450	6	74	209	161	491	149	24	4	342	0.92	3.02	1.32	2.29	6
SOI	1296	680	26	72	331	251	616	127	31	18	489	1.1	5.35	1.39	3.85	1.7
YEC	1471	726	44	85	351	246	745	266	34	13	479	0.97	2.73	1.51	1.8	2.61
ZEN	995	390	3	27	266	94	605	141	52	18	464	0.64	2.77	0.84	3.29	3

^aMilli-units of absorbance (mAU) of the HPLC which correspond to 1 mg of flour. Three observations were made for all the values registering an average change of ± 1.5 and 4.1%.

properties of their doughs (Table 3). In agreement with that observed by Wieser and Kieffer (2001), the quantity of gliadins was not correlated with either *W* or *P/L*. This contrasts with that reported by Ahmad et al. (2000), who did find correlations between the gliadins and certain rheological variables. *W* was, however, strongly correlated with the quantity of glutenins, especially with the HMW glutenin subunits and in particular with the quantity of type x HMW glutenin subunits (Figure 2). This correlation between *W* and the total quantity of HMW glutenin subunits might be explained by the presence of active HMW type x glutenin alleles, coded for by the gene *Glu-A1*, and the over-expression

of some alleles of the gene *Glu-B1x* (S8, HOR, S5801). *W* might also be influenced by the presence of LMW glutenin subunits, though any such influence would be minor. The influence of the active alleles of *Glu-A1* has been analysed by other authors who indicate that the lines of wheat that carry them produce higher strength gluten (Khan et al., 1989; Halford et al., 1992).

Some authors report that the presence of *Glu-B1x7* and the over-production of the glutenin subunit *x7* render the corresponding dough a higher *W* with no loss of *L* (Butow et al., 2003; Gianibelli et al., 2002). The effect would be to increase the quantity of proteins capable of forming aggregates via disulphide bonds.

Table 3. Correlations coefficients (r)^a between the different fractions of glutenins and gliadins and the rheological properties

	Glutenins						Ratio							
	Gluten	Gliadins	Total	HMW	LMW	x	y	GLI/GLU	GLI/HMW	GLI/LMW	GLI/x	GLI/y	LMW/HMW	x/y
P/L ^b	0.11	-0.01	0.27	0.40*	0.15	0.50**	0.44*	-0.26	-0.45*	-0.11	-0.32	-0.25	-0.42*	0.25
W ^b	0.26	0.06	0.54**	0.57**	0.45*	0.49**	0.17	-0.51**	-0.51**	-0.4	-0.31	-0.15	-0.28	0.53**

^aLevel of significance: $r = 0.35-0.45$, $p = 0.05$ (*); $r = 0.49-0.57$, $p = 0.01$ (**).

^b P/L : tenacity/extensibility.

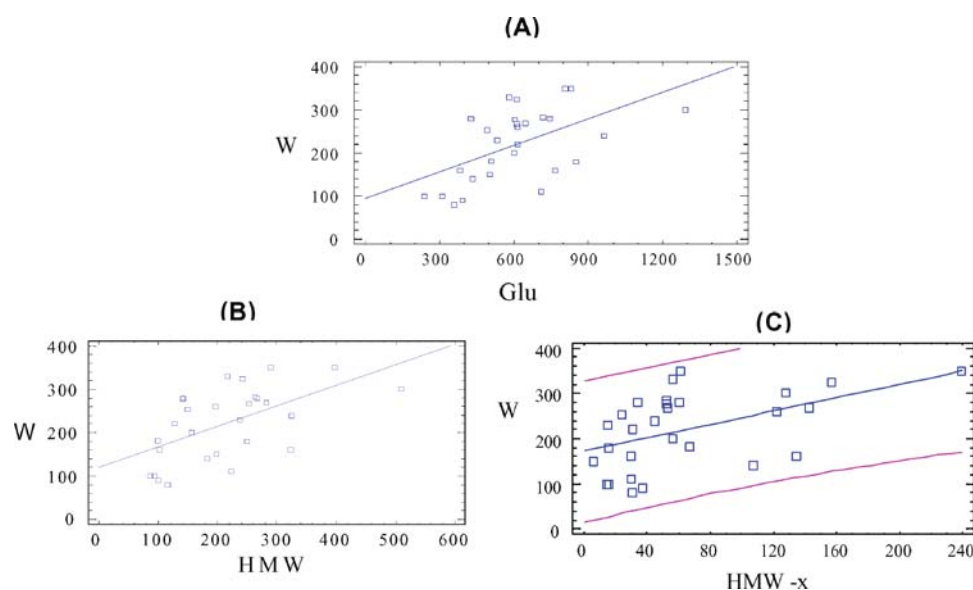


Figure 2. Correlation between the strength of the dough (W) and: (A) the total amount of glutenins (HMW + LMW) ($r = 0.54$ **); (B) the quantity of HMW glutenins ($r = 0.57$ **); and (C) the quantity of HMW type x glutenins ($r = 0.49$ **).

Strength, however, was negatively correlated with the ratio between the total amounts of gliadins and glutenins. Moreover, W showed a positive correlation with the ratio between the amounts of type x and type y HMW glutenin subunits. Thus, for a fixed quantity of HMW glutenin subunits, an increase in the quantity of gliadins leads to a decrease in W . Clearly, for the formation of the gluten network there must be a balance between the different types of proteins present. While the gliadins provide a “solvent” effect, the type y and especially the type x HMW glutenin subunits help form the gluten network. The relative effect of the HMW glutenin subunits of both types was analysed by Butow et al. (2003).

In agreement with Gupta et al. (1991), *Glu-B1x7* influenced W and contributed to the stabilization of the gluten network. In contrast, *Glu-B1x17* had no influence on the strength and appears to exert no effect on extensibility either. Therefore, besides the expression

of *Glu-B1x*, the type of *Glu-D1* allele present influences the strength of the gluten network.

The ratio P/L correlated only with the total amount of HMW glutenin subunits, and with the quantities of type x and type y HMW glutenin subunits. As with W , P/L showed a negative correlation with the ratio between the gliadins and the HMW glutenin subunits, and with the ratio between the LMW and HMW glutenin subunits.

The positive correlation between P/L and the different types of glutenin was strongest with the type x elements. This might be explained in that type x glutenin subunits play a major role in the polymerisation of the gluten.

The cultivars with the same allelic composition for gliadins but which produce greater quantities of these proteins showed a lower P/L value, and, consequently, a greater L value. For example, lines ‘S11’ and ‘S44’, with the allelic composition *Glu-A1x1*

[=x2*], *Glu-B1x7*, *Glu-B1y8*, *Glu-D1x2* and *Glu-D1y12*, showed similarly high quantities of gliadins (absorbance approximately 1000 mAU), but had *P/L* values of 0.3 and 0.4, respectively. The increase in *L* caused by the increase in gliadins also explains the decrease in *W*. The gliadins have a solvent effect that interferes with the bonding between the HMW glutenin subunits. Uthayakumaran et al. (1999, 2003) also found relationship between extensibility and gliadin quantity.

As with *W*, the wheat lines with the highest *P/L* ratios (high tenacity or low extensibility) were those with a high quantity of proteins coded for by the gene *Glu-B1x* (Figure 3). In agreement with the present results, Butow et al. (2003) reported a relationship between a high content of glutenins coded for by the gene *Glu-B1x7* and an increase in *W* plus a decrease in *L*.

No correlation was found between the quantity of LMW glutenin subunits in the gluten and *P/L*. However, a negative correlation was found between the former and the ratio LMW glutenin subunits/HMW glutenin subunits. For a fixed quantity of HMW glutenin subunits, an increase in LMW glutenin subunits produces a

decrease in *P/L*, and consequently favours extensibility. This decrease in *P/L* might be explained by the solvent effect. This implies that both types of protein – gliadins and LMW glutenin subunits – interfere in the establishment of the gluten network, which is mainly formed by HMW glutenin subunits. In the context of the formation of a network of proteins, the longest subunits (those of higher molecular weight) might encourage bonding, contributing to the formation of aggregates. To explain the variation in the quantities of gluten proteins (especially the glutenins), some authors suggest the presence of multiple copies of the active genes involved, or the existence in some wheats of very effective transcription and translation mechanisms for these genes.

Regulatory genes that influence the expression of the glutenins have been identified (Wanous et al., 2003). The importance of environmental factors in the expression of every type of gluten protein was also confirmed (Wanous et al., 2003; Triboni et al., 2000; Dupont & Altenbach, 2003). It is likely that both the genetic composition of these wheat lines and external factors influence the quality of their dough. The extent of this effect

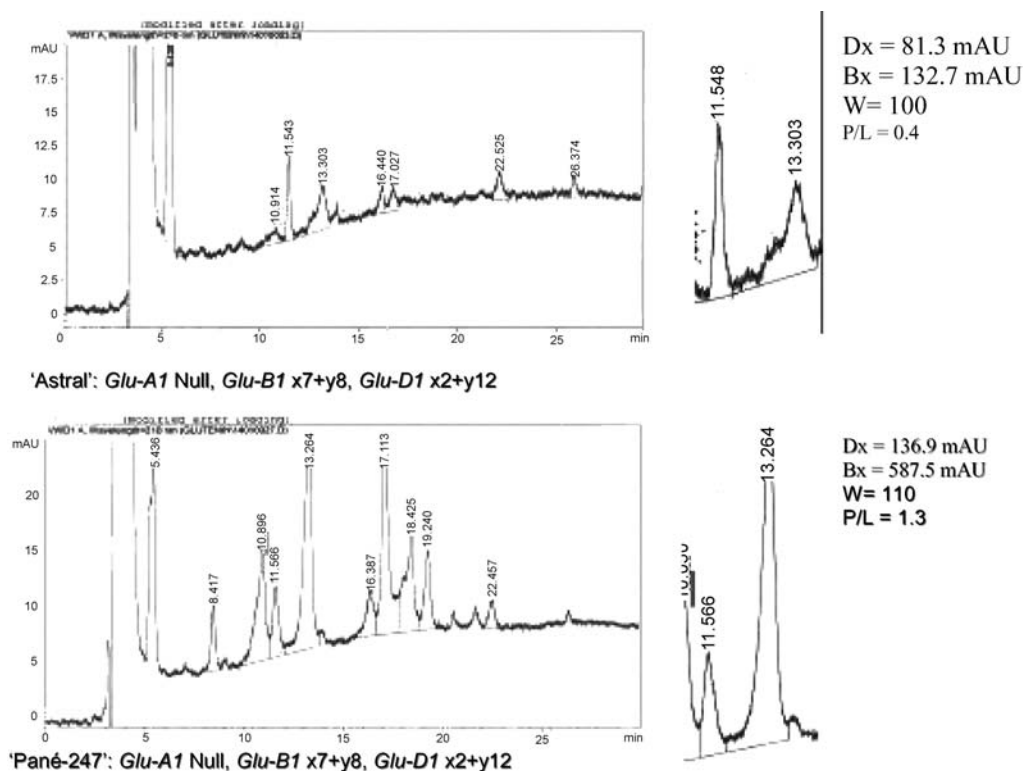


Figure 3. Chromatograms for comparison between an extensible and a tenacious dough made from 'Astral' and 'Pané-247' wheats (these have the same allelic composition with respect to the glutenin genes but express different amounts of type x subunits). At the right, detail of the chromatogram corresponding to the Bx and Dx glutenin subunits.

will depend on their allelic composition with respect to the glutenins, especially the HMW alleles.

This study shows that combinations of methods can be useful for determining the types and quantities of gluten protein in the evaluation of wheat flour quality. The results suggest that the quantities of the different gluten protein fractions are more important than the types of alleles a wheat variety may have. Moreover, the HMW glutenin subunits are the main component of the gluten network, while the gliadins and LMW glutenin subunits seem to act as solvents for the HMW glutenin subunits, interfering with the formation of intermolecular linkages between them. This interference could lead to a reduction in the number of disulphide bonds established between the cysteine residues of different HMW glutenin subunits. To improve the quality of segregant material, the following should be taken into account: if an extensible dough is desired, it would be preferable to select (among lines with similar amounts of HMW glutenin subunits) genotypes with greater quantities of gliadins and LMW glutenin subunits. Since an increase in the quantity of gliadins can reduce the strength of the dough, it would be better to select material that produces larger quantities of LMW glutenin subunits if this attribute is required.

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