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Effect of high molecular weight glutenins and rye translocations on soft wheat flour cookie quality

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ABSTRACT

The influence of high molecular weight glutenin subunits (HMW-GS) on wheat breadmaking quality has been extensively studied but the effect of different *Glu-1* alleles on cookie quality is still poorly understood. This study was conducted to analyze the effect of HMW-GS composition and wheat-rye translocations on physicochemical flour properties and cookie quality of soft wheat flours. Alleles encoded at *Glu-A1*, *Glu-B1* and *Glu-D1* locus had a significant effect over physicochemical flour properties and solvent retention capacity (SRC) profile. The null allele for *Glu-A1* locus presented the highest cookie factor observed (CF = 7.10), whereas 1BL/1RS and 1AL/1RS rye translocations had a negative influence on CF. The three cultivars that showed the highest CF (19, 44 and 47) had the following combination: *Glu-A1* = null, *Glu-B1* = 7 + 8, *Glu-D1* = 2 + 12 and no secalins. Two prediction equations were developed to estimate soft wheat CF: one using the HMW-GS composition and the other using physicochemical flour parameters, where SRCsuc, SRC carb, water-soluble pentosans, damaged starch and protein turned out to be better CF predictors. This data suggests that grain protein allelic composition and physicochemical flour properties can be useful tools in breeding programs to select soft wheat of good cookie making quality.

1. Introduction

The quality of soft wheat flours depends mainly on the composition and characteristics of starch, proteins, lipids and non-starch polysaccharides. Good cookie flours hold water poorly; if flour holds less water, more water is available for the sugar to form syrup, dough viscosity decreases during baking and the dough spreads further, producing larger diameter cookies (Slade and Levine, 1994). The use of flours with high water retention capacity in cookie making requires increased baking times with a higher energy cost.

High damaged starch and pentosan content increase flour water absorption and produce smaller cookie diameter. Since good cookie flours are low in protein, it is generally believed that protein composition has no importance in soft wheats. However, soft wheat flours are suitable also to produce bread; this suggests that protein composition would not play a minor role in soft wheat flour functionality and product quality. Besides, there is relatively little information about the relationship between soft wheat proteins and product quality, compared with the research done on proteins of hard wheat.

Protein content has been used as a predictor of soft wheat quality, with a negative correlation between protein content and cookie diameter but with a strong influence of protein quality in cookie quality. In previous works, Colombo et al. (2008) and Moiraghi et al. (2011) found no correlation between protein content and cookie quality.

Abbreviations: HMW-GS, high molecular weight glutenin subunit; LMW-GS, low molecular weight glutenin subunit; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; A-PAGE, acid polyacrylamide gel electrophoresis; PSA, particle size analysis; WSP, water-soluble pentosans; TP, total pentosans; DS, damaged starch; SRC, solvent retention capacity profile; SRCsuc, solvent retention capacity sucrose; SRCcarb, solvent retention capacity carbonate; SRCw, solvent retention capacity water; SRClac, solvent retention capacity lactic; CF, cookie factor; ML, maximum likelihood; LSD, least significant difference; MSPE, square predictive error; MANOVA, multivariance analysis of variance; BLUP, best linear unbiased predictor coefficient.

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It is generally agreed that storage proteins, such as high and low molecular weight glutenin subunits, (HMW-GS and LMW-GS) are important in determining dough properties of bread wheat flours. Compared to bread, the effect of HMW-GS on end-use quality of soft wheats has received little attention. Souza et al. (1994) found that sugar-snap cookie diameter was negatively affected only by 13 + 19 subunits of the *Glu-B1* locus, while allelic composition of *Glu-A1* and *Glu-D1* did not show any association. Higher biscuit values were observed in wheat lines carrying subunits 13 + 16 at the *Glu-B1* locus. This allelic variant also had a significant and positive effect on the *L* alveograph parameter (Igrejas et al., 2002). Genotypes containing *Glu-A1* null subunit from *Chinese Spring* had better cookie quality than those with *Glu-A1* subunits 1 and 2* from *T monococcum* in recombinant substitution lines (Tranquilli et al., 2002). However, Hou et al. (1996) found a positive correlation between subunit 1 from *Glu-A1* and cookie diameter, while subunit 2* presented a negative correlation. Contradictory results regarding allelic composition of glutenin and cookie quality may be indicating that there are a number of factors involved in soft wheat quality.

Grain texture classification is based primarily on either the resistance of kernels to crushing or the particle size distribution of ground grain or flour. Puroindoline genes *Pina-D1* and *Pinb-D1*, encoding small lipid-binding proteins located on chromosome 5D of common wheat, are considered to be key genes in the determination of grain texture, the soft texture being wild-type and the hard texture being determined by either *Pina-D1* gene deletion or a number of separate point mutations in the *Pinb-D1* gene. Another aspect that has a strong influence on flour quality is the presence of a rye translocation. Wheat-rye translocation has been used to enhance the agronomic performance of wheat through gene transfer from rye to wheat. The 1AL/1RS and 1BL/1RS are the most common ones, and both have been detrimental to wheat quality (Graybosch, 2001). Doughs made from cultivars carrying 1RS translocations are sticky, have low overmixing tolerance and produce low bread loaf. The negative impact on breadmaking quality is attributed to the substitution of genes encoding LMW-GS for genes producing rye secalins (Amiour et al., 2002). Additionally, bread quality deficiency associated with 1RS can be affected by the genetic background (Lee et al., 1995). However, the effect of 1RS translocation on cookie quality has not been considered. In addition to genotype, growth conditions have an influence on physicochemical properties of wheat flours. Flour composition (protein and ash content) and rheological properties (mixograph water absorption and mixing time) were significantly influenced by year, cultivar and environment.

This study was conducted to analyze the effect of HMW-GS composition and wheat-rye translocations on physicochemical flour properties and cookie quality in soft wheat germplasm.

2. Experimental

2.1. Plant material

Forty four soft wheat genotypes including advanced lines and cultivars of diverse origins adapted to Argentina's central wheat region were grown for two consecutive years, 2006 and 2007, in Marcos Juarez (32°42′ S, 62°07′ W, 114 m.a.s.l.) under rainfed conditions.

The pedigree and the origin of genotypes are shown in Table 1. Genotypes were sown on recommended dates using a 7 × 8 alpha lattice design with three replicates (plot size 5.0 m long × 7 rows wide). Seeds harvested in 2006 and 2007 trials were milled on a four-roller laboratory mill (Agromatic AG AQC 109, Laupen, Switzerland). Moisture content was determined using Approved Method 44-19.01 (AACC International, 2010).

2.2. High molecular weight glutenins and secalins scoring

The glutenin fraction was extracted and separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to determine HMW-GS composition of the genotypes studied, using the procedure designed by Pflüger et al. (2001). Monomeric proteins (such as secalins and gliadins) were extracted with 1.5 M dimethylformamide from wholemeal flour (single seed). The pellet of polymeric proteins (glutenins) was then solubilized with 0.08 M Tris–HCl buffer (pH 8.5) and alkylated with 1.4% 4-vinylpyridine. Reduced and alkylated glutenin fractions were analyzed by SDS-PAGE in 8% acrylamide. Vertical gels were run at 30 mA/gel for approximately 12 h, then stained overnight with 0.2% (w/v) Coomassie Blue R-250 in 5% (v/v) ethanol and 12% (w/v) trichloroacetic acid, and a final destaining step with tap water for 24 h. Wheat-rye translocations were easily observed through electrophoresis at low pH. Extracted secalins (and gliadins) were separated by acid polyacrylamide gel electrophoresis (A-PAGE, aluminum lactate buffer, pH 3.1), according to Khan et al. (1985). Electrophoresis was performed at a constant current of 20 mA/gel for 3 h with inverted polarity. After running, the gels were stained as before.

2.3. Detection of puroindolines alleles

The presence of wild type "soft" alleles in puroindoline genes was determined using allele specific primers developed by Gaultier et al. (1994). PCR reactions were performed in an MJ Research PTC 100 thermocycler, in a 25 μl reaction mixture. Each reaction included 1.0 U of Taq DNA polymerase, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.2 μM of each primer, and 100–150 ng of wheat genomic DNA as template. PCR amplifications were as follows: 3 min at 94 °C and 39 cycles of 45 s at 94 °C, 40 s at 50 °C and 50 s at 72 °C. In the case of the *Pind-D1* marker, 10 μl of PCR products were directly digested with restriction enzyme Bsr BI, by adding 5 units of enzyme to the PCR products and incubating for 90 min at 37 °C. Direct PCR fragments and digested products were separated by electrophoresis on 2% agarose gels in 1X SB Buffer (Brody and Kern, 2004), stained with ethidium bromide (0.5 g/l) and visualized by UV exposure.

2.4. Flour analysis

Flour particle size distribution (PSA) was determined with a laser diffraction particle size analyzer (Helos, Sympatec, Germany) combined with a Rodos dry particle disperser. The diameter was measured based on volume distribution and the average value with standard deviation was established.

Protein flour content was determined by a combustion type N autoanalyzer (FP-2000, Leco, St. Joseph, MI) (AOAC, 1998). Crude protein was calculated as N × 5.7.

For water-soluble pentosan (WSP) determination, a flour sample (100 mg) and water (10 mL) were shaken at 30 °C for 120 min. After centrifugation, 1.0 mL of supernatant was mixed with the same 4 N hydrochloric acid volume and heated at 100 °C for 120 min in a sealed tube. After cooling, an equal volume of water was added to a portion of the hydrolyzed sample and 1.0 mL of the resulting mixture was analyzed using the orcinol-hydrochloric acid method (Hashimoto et al., 1987).

For total pentosan (TP) determination a flour sample (10 mg) was mixed with 2 mL of 2 N hydrochloric acid. The mixture was then hydrolyzed at 100 °C for 150 min. After cooling, neutralization was achieved by addition of 2 mL of 2 N sodium carbonate. Fermentable sugars were removed through fermentation, where 2 mL of a 25 mg/mL of 0.2 M sodium phosphate buffer (pH 7) were added in a suspension of fresh compressed yeast (*Saccharomyces*

Table 1
Pedigree, origin and HMW-GS composition of 44 wheat experimental lines.

Entry	Genotype name	Origin	*Glu-A1*	*Glu-B1*	*Glu-D1*	Secalin
1	CADOUX (Australia)	Australia	2*	17 + 18	2 + 12	No
2	BRS 177	BRAZIL	2*	7 + 9	5 + 10	No
3	94309-7-2	Georgia USA	2*	7 + 8	2 + 10*	No
4	LA95177BUB26-1-3-B	Louisiana USA	2*	17 + 18	5 + 10	1BL/1RS
5	941673-3-1	Georgia USA	2*	13 + 16	2 + 12	1AL/1RS
6	T84-331/COKER9134	AWD97-6961R Louisiana-USA	2*	7	2 + 10*	1BL/1RS
7	95468-9-3	Georgia USA	2*	7 + 9	2 + 12	1BL/1RS
8	FLLA95134-A7-B11	Georgia USA	2*	13 + 16	2 + 12	No
9	95151-10-8	Georgia USA	1	13 + 16	5 + 10	No
10	AW-M94*1549-1	Louisiana-USA	2*	7	2 + 10*	1BL/1RS
11	MASON/3/FREEDOM//N8675/CATBIRD	97-1078-7-2 Arkansas-USA	2*	7 + 9	2 + 12	No
13	95341-5-2	Georgia USA	2*	7 + 8	2 + 12	1BL/1RS
14	95154-16-1	Georgia USA	2*	6 + 8	2 + 12	No
18	931257-1-3	Georgia USA	1	7 + 8	2 + 12	1BL/1RS
19	92485 E15	Georgia USA	Null	7 + 8	2 + 12	No
20	LA 9585 D 17-2	Louisiana USA	2*	7 + 8	5 + 10	1BL/1RS
21	94776-1-1	Georgia USA	1	7 + 8	2 + 12	1BL/1RS
22	901146 E 15	Georgia USA	2*	7 + 8	2 + 12	No
23	93435-1-10	Georgia USA	1	7 + 8	5 + 10	1AL/1RS
24	GA 932911 E 38	Georgia USA	2*	7 + 9	2 + 12	1BL/1RS
25	FL 93024-6-1	Georgia USA	2*	7 + 9	2 + 12	1BL/1RS
26	94261-22-2	Georgia USA	2*	7 + 8	2 + 12	No
27	YACO//ALTAR84/AE.SQR(191)/3/2*YACO	CIMMYT	1	7 + 8	2 + 12	No
29	CROC_1/AE.SQR(205)//BORL95	CIMMYT	1	7 + 8	2 + 12	1BL/1RS
30	LA 422 (soft red winter wheat)	Louisiana USA	Null	7 + 9	2 + 12	No
31	CROC_1/AE.SQR(205)//BORL95	CIMMYT	1	7 + 8	2 + 12	1BL/1RS
32	CROC_1/AE.SQR(205)//BORL95	CIMMYT	1	7 + 8	2 + 12	1BL/1RS
33	ABT/BPAT/3/VI/SNB'S'//PAZUL/5/DONATA/3/FLN/ACC//ANA/4/ALD	INTA EEA M JUAREZ	2*	7 + 8	2 + 12	1BL/1RS
34	951181-17-2	Georgia USA	2*	7 + 9	5 + 10	No
35	AR 839-25-8-2	Arkansas USA	1	7 + 9	2 + 12	No
36	NC 98-26192	North Carolina USA	1	7 + 8	2 + 12	No
37	GA 932911 E38	Georgia USA	2*	7 + 9	2 + 12	1BL/1RS
38	YACO//ALTAR84/AE.SQR(191)/3/2*YACO	CIMMYT	1	7 + 8	2 + 12	No
39	SS 520	Louisiana USA	2*	7 + 9	2 + 12	No
41	FL 93024-6-1	Georgia USA	2*	7 + 8	2 + 12	No
42	951216-2-2	Georgia USA	1	6 + 8	2 + 12	No
43	FFR502W//8576A53-2-1	LA9397D5-3-3 Louisiana-USA	1	7 + 9	2 + 12	No
44	931257-1-3	Georgia USA	Null	7 + 8	2 + 12	No
45	951255-17-2	Georgia USA	2*	7 + 8	2 + 10*	No
47	TERRAL LA422 (soft red winter wheat)	Louisiana USA	Null	7 + 8	2 + 12	No
48	951300-7-1	Georgia USA	2*	7 + 8	2 + 12	No
49	931257-1-5	Georgia USA	2*	7 + 8	2 + 12	No
50	94665-2-4	Georgia USA	2*	6 + 8	2 + 10*	No
51	MAYOOR//TK SN1081/AE. SQR. (222)	CIMMYT	1	17 + 18	2 + 12	1BL/1RS

cerevisiae) incubated for 1.5 h at 30 °C. The mixture was centrifuged at 1000 × g for 10 min and an aliquot of supernatant was analyzed by the orcinol-hydrochloric acid method (Hashimoto et al., 1987).

The content of damaged starch (DS) was determined according to Approved Method 76-30.02 (AACC International, 2010). Fungal enzyme from *Aspergillus oryzae* (A6211, Sigma Chemical, St. Louis, MO) was used.

The solvent retention capacity profile (SRC) was obtained according to Approved Method 56-11.01 (AACC International, 2010). White flour samples (5 g) were suspended with 25 g of water, 50% sucrose, 5% sodium carbonate, and 5% lactic acid. Samples were hydrated for 20 min and centrifuged at 1000 × g for 15 min. Each precipitate obtained was weighed and the SRC was calculated for each sample according to Approved Methods (AACC International, 2010).

2.5. Preparation of cookies

Cookies were prepared according to León et al. (1996). Ingredients used were flour (43.4%), caster sugar (26.0%), shortening (19.3%), powdered milk (2.2%), NaHCO3 (0.5%), NaCl (0.4%), and 8.2% of water. Cookies were baked at 200 °C for 10 min. The term "cookie factor" was introduced to determine cookie quality as the ratio between the width and height of four cookies picked at random. The higher value was correlated with better quality.

2.6. Statistical analysis

Analyses were carried out on the data for 44 wheat experimental lines grown during 2 consecutive crop years. Triplicate analysis for different parameters was conducted on each wheat genotype drawn from the same batch of flour. Allelic frequency was calculated, considering HMW-GS and secalin composition (1AL/1RS, 1BL/1RS and no secalin). A general linear mixed model was fitted to the experimental data. Crop year as well as *Glu-A1, Glu-B1, Glu-D1* and secalin composition were considered fixed effects. Genotypes were considered random effects. Variance components were estimated by maximum likelihood (ML) to compare the relative magnitude of sources of variation. The mean values were compared according to Fisher's least significant difference (LSD) procedure.

Pearson's linear correlation coefficients among different quality parameters were calculated by genotype means.

Multiple linear regressions were conducted with cookie factor as the dependent variable. The best-fit linear regression model was determined using backward variable elimination. Medium square

predictive error (MSPE) was calculated using the computer intensive method (jackknife) as a measure of the capacity of the model to predict cookie factor. Data were subjected to a multivariate analysis of variance (MANOVA), considering glutenins and secalins as classification variables. Hotelling test at a significance level of 0.05 was used in order to compare samples.

All analyses were performed using the INFOSTAT statistical software (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina).

3. Results and discussion

Experimental lines with softer grain texture (carrying *Pina-D1a y Pinb-D1a* alleles), low protein content and bad breadmaking performance were selected to be used in cookie making. The HMW glutenin subunit composition, rye translocation and flour properties in tested genotypes are displayed in Table 1. Additionally, the average of chemical flour parameters (protein, total and water-soluble pentosan, damaged starch and SRC profile) and cookie quality (measured as CF) from the 44 experimental lines harvested in two consecutive years are shown in Table 2. In each parameter wide ranges were found, indicating the variability among wheat genotypes.

3.1. Flour characterization

Protein content varied according to crop year, all genotypes presented the lowest protein content during 2007. It is known that protein content depends on environmental conditions and varies between harvest years in the same cultivars.

Flour particle size distribution showed a wide range (41.61–100.86 μm); it varied significantly ($p < 0.01$) with the crop year, the PSA is related to hardness degree in the wheat grain, and it may be used as grain texture indicator. Although grain hardness is genetically determined, environment has such a strong influence on it that grain hardness varies between crop years. Damaged starch also presented a wide range of values (2.47–10.02%) and the average was higher in 2007 than in 2006, probably because of the highest grain hardness of the 2007 harvest.

Total and water-soluble pentosan values obtained agree with values previously reported (Dornez et al., 2008). Water-soluble pentosans did not show significant differences between crop years; however, in the case of TP 2006, values were significantly lower than the 2007 ones. Zhang et al. (2005) examined 17 soft wheat genotypes and reported that both genotype and environment were important sources of variation on pentosan content but genotype was the most important for water-soluble pentosan.

Regarding SRC, the ranges obtained for water (47.92–68.04%), carbonate (62.23–98.68%), sucrose (82.67–122.59%) and lactic SRC (65.85–131.69%) were similar to the values reported by Zhang et al. (2007).

Table 2
Mean and standard deviation for flour parameters and cookie factors[a,b].

	Mean ± SD		
	2006	2007	2006 and 2007
PSA	50.16[b] ± 7.91	70.29[a] ± 9.08	60.24 ± 13.20
Protein	10.27[a] ± 1.24	9.00[b] ± 0.88	9.69 ± 1.25
DS	4.75[b] ± 1.15	5.71[a] ± 1.40	5.07 ± 1.36
WSP	0.57[a] ± 0.17	0.54[a] ± 0.12	0.55 ± 0.15
TP	4.53[b] ± 1.68	6.06[a] ± 0.60	5.22 ± 1.47
SRCsuc	97.73[a] ± 8.20	98.39[a] ± 7.39	96.93 ± 7.77
SRClac	106.29[a] ± 11.96	94.94[b] ± 13.63	99.22 ± 13.77
SRCcarb	75.03[a] ± 6.02	72.88[a] ± 4.85	73.09 ± 5.54
SRCw	57.77[a] ± 3.75	56.22[a] ± 3.15	56.84 ± 3.45
CF	6.10[b] ± 0.63	6.44[a] ± 0.63	6.34 ± 0.65

[a] Particle size analyzer (PSA), damaged starch (DS), water-soluble pentosan (WSP), total pentosan (TP), sucrose SRC (SRCsuc), lactic SRC (SRClac), carbonate SRC (SRCcarb), water SRC (SRCw) and cookie factor (CF).
[b] Values followed by different letters are significantly different ($p < 0.05$).

3.2. Flour correlations

Pearson's correlation coefficients were calculated among flour composition parameters, SRC profile and cookie quality. SRC parameters correlated with their corresponding flour components (Table 3). WSP and TP correlated positively with SRCsuc, in agreement with Zhang et al. (2007). DS showed significant correlation with SRCsuc, SRCcarb and SRCw, as was reported in hard wheat by Colombo et al. (2008), but no correlation was found between protein and SRClac, in agreement with Guttieri et al. (2001).

Flour particle size distribution correlated positively with proteins, DS, SRCcarb and SRCw. Flours with larger particle size are related to higher damaged starch content and harder wheat endosperm, which are generally associated with high protein content and higher water retention capacity (Zhang et al., 2007).

Significant negative correlations were detected between cookie factor, with PSA, WSP, TP, DS and SRC parameters, whereas no correlation was found with protein content. Correlations between SRCsuc, WSP, TP, SRCcarb, DS and SRCw with CF suggest that pentosans, DS and their capacity to retain aqueous solutions have a strong influence in flour cookie performance, whereas protein content did not play a very important role. This lack of correlation between protein and CF has been previously reported by Nemeth et al. (1994) for a group of soft wheat flours from different countries.

3.3. Variation in HMW-GS and secalins

A wide variation in the HMW-GS was observed between the different wheats studied (Table 1). The allelic frequencies at each *Glu-1* and wheat-rye translocations are shown in Table 4. For the *Glu-A1* locus, three allelic variants were found, among which subunit 2* was the most frequent (59.1%). The second most common was subunit 1 (31.8%), in agreement with the frequencies reported by Lerner et al. (2009), who analyzed 119 Argentine grown bread wheat cultivars. At the *Glu-B1* locus, six allelic variants were found, among which, subunits 7 + 8 occurred in 50.0% and 7 + 9 in 25.0% of the accessions. Lerner et al. (2009) reported six alleles at *Glu-B1* and 7 + 8, 7 + 8* and subunits 7 + 9 represented 80.7%. Three allelic variants were found in *Glu-D1* locus, where subunits 2 + 12 were the most frequent ones (75.0%), contrasting with the findings of Lerner et al. (2009), where subunits 5 + 10 were the most frequent ones (94.1%). A possible explanation could be that the selection of lines tested in this study was done based on poor breadmaking performance. Three different genotypes were found, according to the presence of 1RS translocations: 59.1% had no rye translocation, 36.4% had 1BL/1RS and 4.5% had 1AL/1RS.

3.4. Quality implications of different HMW-GS and secalin rye translocation

Physicochemical flour properties and cookie quality parameters were analyzed, according to the *Glu-1* allelic variation and secalin presence. A general linear mixed model was used to determine the significance of allelic variations at *Glu-1* and secalin (Table 5).

Alleles encoded at the *Glu-A1* locus affected protein content significantly; subunit 2* was associated with the lowest values, whereas subunit 1 showed the highest value. On the other hand,

Table 3
Phenotypic correlations[a,b].

	CF	PSA	PROT	DS	WSP	TP	SRCsuc	SRClac	SRCcarb	SRCw
CF	1									
PSA	−0.39*	1								
PROT		0.63**	1							
DS	−0.51**	0.58**	0.34*	1						
WSP	−0.64**				1					
TP	−0.39*		−0.37**		0.54**	1				
SRCsuc	−0.73**			0.32*	0.72**	0.44**	1			
SRClac								1		
SRCcarb	−0.69**	0.29*		0.62**	0.62**	0.36*	0.80**		1	
SRCw	−0.57**	0.36*		0.63**	0.52**	0.32	0.68**		0.91**	1

[a] Cookie factor (CF), particle size analyzer (PSA), protein (Prot), damaged starch (DS), water-soluble pentosan (WSP), total pentosan (TP), sucrose SRC (SRCsuc), lactic SRC (SRClac), carbonate SRC (SRCcarb) and water SRC (SRCw).
[b] **,* Indicate significance at $P < 0.01$ and $P < 0.05$, respectively.

the null allele presented the highest CF observed (7.10, $p < 0.01$). Jondiko et al. (2012) reported a larger diameter on wheat flour tortilla on cultivars with null *Glu-A1*. They found that deletion at *Glu-D1* results in decreased percentage of insoluble polymeric proteins. MacRitchie and Lafiandra (2001) reported that deletion of HMW-GS subunits at any *Glu-1* locus reduced dough mixing strength.

Glu-B1 alleles had effects on DS, SRCcarb and SRCw ($p < 0.005$), and protein ($p < 0.05$). Subunits 13 + 16 and 17 + 18 showed the highest DS, SRCcarb, and, SRCw, although *Glu-B1* alleles have no significant effects on cookie factor ($p > 0.05$); subunits 13 + 16 and 17 + 18 presented the lowest values.

The variation in the *Glu-D1* locus only affected SRClac ($p < 0.001$); subunit 5 + 10 was associated with higher SRClactic. It is well known that the HMW-GS pairs 5 + 10 are associated with good breadmaking quality; likewise, a positive correlation was found between lactic acid SRC and glutenin content (Colombo et al., 2008; Gaines, 2000), as well as with bread volume (Colombo et al., 2008). *Glu-D1* locus did not show a significant effect on cookie quality.

1BL/1RS and 1AL/1RS rye translocations had a strong influence on DS, TP, WSP, SRCcarb, SRCsuc, SRCwater and cookie factor ($p < 0.001$). Genotypes with no rye translocations presented flours with low DS, pentosan and solvent retention values and higher CF. Secalins are detrimental to cookie (and breadmaking) quality, probably as a result of a change in protein composition, an increment in damaged starch, total and WSP and an increase in the solvent retention capacity of their flours. 1RS replaces the short arm of at least one wheat chromosome pair, leading to a permanent loss of some potentially important wheat genes, such as those encoding the LMW-GS (Graybosch, 2001). 1BL/1RS contribute to higher flour water absorption due to the replacement of LMW-GS by rye secalin genes on 1RS (Lee et al., 1995) and to other changes in the chemical composition, such as higher pentosan content (Selanere and Andersson, 2002).

Table 4
Individual allelic frequencies observed at glutenin and secalin loci studied.

Locus	Subunits	Frequency
Glu-A1	2*	59.1
	1	31.8
	Null	9.1
Glu-B1	13 + 16	6.8
	17 + 18	6.8
	6 + 8	6.8
	7 + 8	50.0
	7 + 9	25.0
	7	4.5
Glu-D1	2 + 10*	11.4
	5 + 10	13.6
	2 + 12	75.0
Secalin	1AL/1RS	4.5
	1BL/1RS	36.4
	No	59.1

According to these results, *Glu-A1* allele variation ($p < 0.001$) and rye translocation presence ($p < 0.001$) had an influence on cookie quality. Fig. 1 shows cookies made with two wheat flours having different *Glu-A1* alleles and rye translocation (A: null, 7 + 8, 2 + 12 and no rye translocation; B: 2*, 7 + 9, 5 + 10 and no rye translocation; and C: 1, 7 + 8, 2 + 12 and 1BL/1RS rye translocation).

Taking into account that *Glu-B1* locus did not show a significant effect on CF, using a linear mixed model, but presented wide differences between CF averages of different alleles, a MANOVA was carried out using all parameters measured as variables. These analyses showed significant differences for *Glu-A1*, *Glu-B1*, *Glu-D1* and secalin composition ($p < 0.05$). In the case of *Glu-A1* and secalins, the best CF was for null allele and no rye translocation, respectively ($p < 0.05$), as was estimated by linear mixed model; for *Glu-B1,* the best subunits were 6 + 8, 7 + 9 and 7 + 8 and the worst one was 17 + 18 ($p < 0.001$); for *Glu-D1,* 2 + 10* and 2 + 12 had the highest CF ($p < 0.001$). Igrejas et al. (2002) found significant influences of the *Glu-A1* locus on cookie density and *Glu-B1* locus on cookie length. On durum wheat genotype HMW-GS 6 + 8 were characterized by significantly greater dough extensibility (Ammar et al., 2000). Null *Glu-A1* allele from Chinese Spring wheat had been associated to good cookie quality (Tranquilli et al., 2002).

According to the results obtained, the optimal HMW-GS allelic combination (including the secalins in the analysis) to obtain the highest cookie quality was: *Glu-A1* = null, *Glu-B1* = 6 + 8, *Glu-D1* = 2 + 10*or 2 + 12, without wheat-rye translocation; however no experimental lines analyzed presented these combinations. Wheat lines with higher cookie factor were 19, 44 and 47 with the combination *Glu-A1* = null, *Glu-B1* = 7 + 8, *Glu-D1* = 2 + 12 and no secalin.

HMW-GS composition can be useful information to estimate the cookie factor according to the equation. CF = CF mean + (CF *Glu-A1* − CF mean) + (CF *Glu-B1*- CF mean) + (CF *Glu-D1*- CF mean) + (CF secalin − CF mean), so for each genotype the CF can be calculated according to HMW composition and to secalins ± the best linear unbiased predictor coefficient (BLUP) of the genotype. For example, the estimated CF of entry number 44 (pedigree in Table 1) with *Glu-A1* null, *Glu-B1* 7 + 8, *Glu-D1* 2 + 12 and no secalin was CF = 6.34 + (7.10−6.34) + (6.33−6.34) + (6.38−6.34) + (6.54−6.34) ± BLUP; CF = 7.33 + 0.24. In fact, the CF obtained from the 2006 harvest was 7.56 and from the 2007 harvest was 7.86. In order to test the CF estimator equation we used it with two soft wheat cultivars, Alpowa and Penawawa, which were not included in the analysis.

Table 5
Effects of different HMW-G subunits and rye translocations on flour parameters and cookie factor[a,b].

	PSA	Prot	DS	TP	WSP	SRCw	SRClact	SRCcarb	SRCsuc	CF
Glu A1										
Null	53.33 a	9.53ab	4.12 a	4.54 a	0.49 a	55.58 a	95.81 a	71.11 a	93.02 a	7.10 a
1	64.44 a	10.03 a	5.40 a	5.17 a	0.51 a	56.99 a	101.17 a	72.70 a	95.80 a	6.28b
2*	59.11 a	9.45b	5.30 a	5.46 a	0.58 a	58.02 a	101.84 a	74.99 a	99.92 a	6.15 b
Glu B1										
6 + 8	59.78 a	10.59 a	5.33b	4.81 a	0.50 a	55.28 cb	97.01 a	70.04 c	94.94 a	6.54 a
7	53.80 a	8.99 cd	4.75 bc	5.14 a	0.55 a	56.74 bcd	90.82 a	73.26 bc	97.17 a	6.29 a
7 + 8	61.07 a	9.68 bc	4.99 b	5.44 a	0.54 a	56.89 bc	100.57 a	73.16 bc	96.50 a	6.33 a
7 + 9	55.97 a	9.34 c	4.28 c	4.93 a	0.53 a	55.24 d	100.52 a	70.62 c	94.19 a	6.54 a
13 + 16	58.06 a	8.45 d	5.71 b	5.53 a	0.55 a	59.14 b	104.80 a	75.05 b	102.22 a	5.99 a
17 + 18	69.83 a	10.46 ab	7.00 a	5.80 a	0.66 a	63.69 a	107.40 a	84.68 a	107.96 a	5.66 a
Glu D1										
2 + 10*	58.23 a	9.92 a	4.75 a	4.87 a	0.49 a	55.82 a	103.45 b	71.67 a	96.30 a	6.40 a
5 + 10	58.60 a	9.07 a	5.29 a	6.03 a	0.59 a	59.39 a	113.74 a	76.68 a	102.07 a	5.85 a
2 + 12	61.17 a	9.77 a	5.30 a	5.13 a	0.55 a	57.2 a	96.44 b	73.50 a	97.08 a	6.38 a
Secalins										
No	58.29 a	9.69 a	4.86 b	4.85 b	0.50 b	55.96 b	102.82 a	71.40 b	95.22 b	6.54 a
1AL/1RS	63.26 a	8.26 a	5.49 ab	6.56 a	0.60 ab	60.39 a	98.59 a	76.71 a	99.09 ab	5.87 b
1BL/1RS	63.24 a	9.93 a	5.88 a	5.82 a	0.65 a	59.73 a	98.41 a	78.27 a	103.45 a	5.84 b

[a] Particle size analyzer (PSA), proteins (Prot), damaged starch (DS), total pentosan (TP), water-soluble pentosan (WSP), water SRC (SRCw), lactic SRC (SRClac), carbonate SRC (SRCcarb), sucrose SRC (SRCsuc) and cookie factor (CF).
[b] Values followed by different letters are significantly different ($P < 0.05$).

According to Alpowa and Penawawa HMW-GS composition (*Glu-A1* null, *Glu-B1* 7 + 9, *Glu-D1* 5 + 10), the cookie factor calculated was 6.85 and the CF obtained making cookies were 6.65 and 6.78, respectively. When CF from four experimental soft wheat lines, with different HMW-GS composition, was calculated using the equation, the average percentage of variation with the real CF was 4.2%.

The CF can also be predicted from physicochemical flour parameters. The best-fit linear regression model was determined using a stepwise multiple-regression: CF = 12.85−0.03 SRCcarb − 0.03 SRC suc − 0.71 WSP − 0.10 Prot. The multiple linear regression model had a coefficient of determination R^2 = 0.52 and MSPE = 0.24 (3.78% of mean cookie factor of 44 experimental lines studied). A CF of 6.49 was estimated for Alpowa using the prediction equation. The percentage of variation between obtained and estimated CF was 2.41%. The comparison between real CF and calculated CF obtained from four experimental soft wheat lines (not included in the regression model) presented a range of variation from 0.1 to 4.9%. These data suggest that soft wheat flours can be selected using SRCsuc, SRC carb, WSP and Prot; which predict 55% of the variation in cookie diameter. In a previous work we found that SRCsuc and DS are the strongest predictors of cookie factor along with particle size of flour, according to the best fit equation with R^2 = 0.68 (Moiraghi et al., 2011). In this work, 51 genotypes from the 2006 cropping season were included in the analyses, so environment influences were not taken into account in the prediction equation. Zhang et al. (2007) obtained an optimum multiple regression models for cookie diameter including SRCsuc and flour particle size in the prediction equation (R^2 = 0.82). The study was carried out with seventeen genotypes that were grown in irrigated field trials in three Chinese locations, in the 2000–2001 and 2001–2002 cropping season. In addition, Gaines (2004) found that sugar-snap cookie diameter can be predicted by a simple regression equation with independent variable sucrose SRC, flour protein content, and a milling estimate of kernel softness.

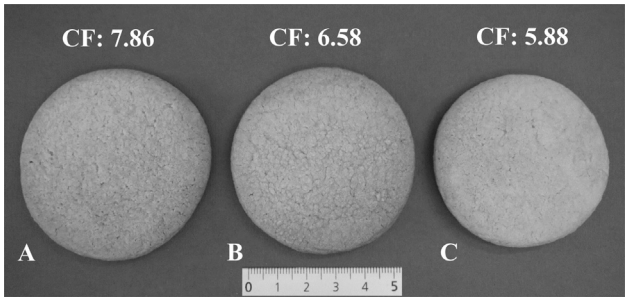

Fig. 1. Cookies made with two wheat flours with different *Glu-A1* subunits and rye translocation (A (genotype 931257-1-3): null, 7 + 8, 2 + 12 and no rye translocation; B (genotype 951181-17-2): 2*, 7 + 9, 5 + 10 and no rye translocation; and C (CROC_1/AE.SQR(205)//BORL95): 1, 7 + 8, 2 + 12 and 1BL/1RS rye translocation).

4. Conclusion

According to our results it is not possible to use a unique analytical procedure to assess the quality of soft wheat flour for cookie breadmaking. *Glu-A1* alleles variation and wheat-rye translocation presence had a significant influence on cookie factor. Null *Glu-A1* and the absence of a rye translocation showed a positive effect on cookie quality. Rye translocation has been associated with DS and pentosan content, and thus related to high values of sucrose and carbonate SRC. This data strongly support the hypothesis that the presence of 1BL/1RS and 1AL/1RS exerts a negative effect on CF.

Glu-A1 alleles influenced neither flour composition nor solvent retention SRC parameters; therefore the influence on cookie quality would be related to a decrease of dough strength due to a reduction in polymeric protein percentage.

Among soft grain texture genotypes, flour PSA difference is not a good parameter to estimate cookie making performance, probably because size diameter differences below certain values cannot produce differences in CF. Hydrophilic components (WSP and DS) and flour absorption capacity -SRCsuc and SRCcarb- are the strongest predictors of CF, according to multiple regression analysis and Pearson's correlation.

All this data suggest that grain protein allelic composition at *Glu-A1* and rye translocation presence, SRC parameters, WSP and

protein content can be useful tools to accurately predict functionality of soft wheat cultivars for cookie formulations in wheat breeding programs.

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