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The Occurrence of Friabilins in Triticale and Their Relationship with Grain Hardness and Baking Quality

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Grain hardness is a quality parameter in wheat and other cereals. In wheat, a group of M_r 15 000 proteins called friabilins have been shown to be related to grain hardness. The objective of this study was to determine the presence of friabilins on starch granules of different triticale lines and their relationship with grain texture and baking quality. The triticale lines studied have a wide range of hardness, which presented correlation with baking quality parameters such as damaged starch and solvent retention capacity. All of the triticale lines contained friabilins on the starch granules. However, the correlation between hardness and friabilin content was not observed, suggesting that these proteins would not be directly involved in grain texture determination of triticale. Consequently, friabilin content did not have any relation with cookie quality in triticale flours, but it could be related to breadmaking quality because it has a positive correlation with the sodium dodecyl sulfate sedimentation index.

KEYWORDS: Friabilin; triticale; grain hardness; baking quality

INTRODUCTION

Grain texture, classified as hardness or softness, is a first discriminating characteristic for the end uses of wheat grain. Generally, hard wheats are used for breads and other yeast-leavened foods whereas soft wheats are used for cakes, cookies, and pastries. Texture is defined as (i) the resistance to deformation or fracture properties, (ii) the particle size distribution after grinding or milling, or (iii) the level of damaged starch after grinding or milling (*1*). Endosperm texture influences the particle distribution and damaged starch in wheat flours. Hard wheat is more difficult to reduce to flour-sized particles so hard wheat flour has a larger mean particle size than soft wheat flour. Hard wheat flour has a higher damaged starch produced during the milling process. Damaged starch produces a higher water absorption capacity and is more readily hydrolyzed by α-amylase. In formulas containing little or no added sugar, damage starch levels should be high enough for good yeast gas production to occur but not so high that dough handling problems are encountered (*2*). Endosperm texture is controlled by a major gene, referred to as Hardness (*Ha*) located on the short arm of chromosome 5D (*3*−*5*).

Greenwell and Schofield (*6*) reported the relationship between the grain softness and the presence of a group of M_r 15 000 proteins (friabilins) on the surface of starch granules and suggested that this component acted as a "nonstick" protein preventing the adhesion of the starch to the matrix protein. Bettge et al. (*7*) found that these proteins are abundant on the starch of soft wheat in contrast with their occurrence in hard wheat starch. Conversely, other authors showed that friabilin levels in native endosperm were similar in both hard and soft wheat; consequently, the suggested action of friabilin as nonstick protein appears less probable (*8*, *9*).

Friabilin is a mixture of two or more polypeptides including two major proteins called puroindolines A and B (Pin A and Pin B) (*10*, *11*). Puroindolines are basic cystein-rich proteins that contain tryptophan-rich domains that are involved in lipid recognition. In wheat, Pin A and Pin B exhibit 60% homology in their sequence, but the tryptophan-rich domain is truncated in Pin B. Gene coding for these two proteins, Pin A and Pin B, is tightly linked to the *Ha* locus on chromosome 5DS (*8*, *12*), and they probably function together as the *Ha* locus. Mutations in Pin A and Pin B are associated with the occurrence of hard texture. It has been suggested that the absence of Pin A or the presence of a mutation in Pin B is required for grain hardness in *Triticum aestivum*. However, this conclusion has not been confirmed for Australian wheat samples in which some hard cultivars have the soft Pin B (*Pinb-D1a* allele) while Pin A (*Pina-D1a* allele) is present (*13*). Up to now, seven hardness alleles have been identified for wheat. At the *Pina-D1* locus, allele *Pina-D1b* is a null form present in certain hard wheat. The other six alleles correspond at the *Pinb-D1* locus. These alleles differ from each other in a single nucleotide mutation that determines an amino acid change. *Pinb-D1b* changes Gly-46 of wild-type Pin B to serine; *Pinb-D1c* changes Leu-60 to Pro; *Pinb-D1d* changes Trp-44 to Arg; and *Pinb-D1e*, *Pinb-D1f*, and *Pinb-D1g* change Trp 39, Trp 44, or Cys 56 to the stop codon, respectively (*14*).

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Table 1. Pedigree of the Eight Triticale Lines

name	pedigree
Tatú "S"[a]	BGL. DERIV. SEL BULK/3/MTZ TCL/TRIGO GOOD SED//BGL GOOD SEED/4/NUTRIA
383[b]	GIBON-1 * CTM86.1262
393[b]	YOGUI1/TARANCA 87–3//HARE 212/3/NIMIR3
390j95[b]	GIBON-2 * CTM86.1262
392j95[b]	GNU-1/ARDI-1//RHINO-3 * CTM86M.2019
397j95[b]	TATU2/3/MUS "S"/LYNX "S"//YOGUI "S"
Boaglio[c]	BUCK PAMPERO/INSAVE F. A.//CACHIRULO IN-CA
Remedios[c]	BUCK PAMPERO/INSAVE F. A.//CACHIRULO IN-CA

[a] CIMMYT variety (México). [b] Advanced lines of the Estación Experimental Agropecuaria Marcos Juárez. Instituto Nacional de Tecnología Agropecuaria (INTA) Argentina. [c] Hexaploid cultivars obtained by different selection from the same crossing. Facultad de Ciencias Agropecuarias de la Universidad Nacional de Córdoba, Argentina; RNPC numbers 1243 and 1244.

The physical interaction of friabilin with starch granules is poorly understood. The friabilin/puroindolin−starch interactions might be mediated by residual polar lipids found at the surface of purified starch granules (*9*). The glycine−serine sequence change produced in mutated Pin B may alter the structure−function relationship of this protein thus producing an increase in grain hardness (*15*).

Puroindolines have intrinsically good foaming properties, and their lipid binding properties confer puroindolines the capability of preventing the lipid-induced destabilization of protein foams (*16*−*19*). Dubreil et al. (*20*) reported that puroindolines improve the crumb texture resulting from the good foaming properties, and they are capable of inducing changes in dough tenacity and extensibility.

Triticale (x*Triticosecale* Wittmack) is a hybrid resulting from crossing wheat (*Triticum* sp.) and rye (*Secale* sp). Triticale flours have been found to be more suitable for the manufacture of products that may be prepared with gluten of lower tenacity than that needed in bread manufacture. These flours have been used in the experimental preparation of waffles and pancakes (*21*), crackers (*22*), cakes (*23*), and cookies (*24*). The quality of triticale is also influenced by the structure of the endosperm, but in this cereal, the role of hardness is less well-studied than in wheat. Most triticale varieties yield soft-texture grain, similar to that of rye, and flour from such grain is of fine particle size with relatively low starch damage (*25*).

Most hexaploid triticales (AABBRR genomes) are moderately soft, whereas the parent rye is very soft and the parent *Triticum durum* is very hard. The presence of chromosome 5R in triticale determines the grain softness and the presence of friabilin. However, it should be noted that in a study in which 280 triticale cultivars were tested, they exhibited the complete range of hardness from very hard to very soft (*26*). In wheat, puroindolines have been shown to be the key controlling elements in grain hardness and in every aspect of wheat quality and utilization.

The objective of this study was to determine the presence of friabilins on different triticale lines and to evaluate the relationship between friabilin content, grain texture, and baking quality.

MATERIAL AND METHODS

Plant Materials. Eight lines of triticale (Tatu, Remedios, Boaglio, 383, 393, 390j95, 392j95, and 397j95) and two wheat cultivars, a durum wheat (Topacio) and a soft wheat (LA6), were used. Crops were grown in middle level fertility soils at Campo Escuela of the Facultad de Ciencias Agropecuarias of the Universidad Nacional de Córdoba, Argentina. The lines were sowed in June 2001 with a density of 250 plants per m^2. No watering or fertilizing was used. The pedigree of triticale lines is shown in **Table 1**. The flours were produced with an Agromatic AG 109 mill (Laupen, Suiza).

Grain Hardness. Grain hardness was determined by the particle size index (PSI), following AACC 55-30 method (*27*), using the Agromatic AG 109 mill. The result was calculated as the relative weight of sieved flour × 100, and then, the data were compared with a table to obtain the relative hardness.

Protein. The nitrogen content was determined by the AACC 46-13 Micro Kjeldhal method modified with boric acid (*27*). The sample was digested in a digestor Technicon II (Dublin, Irlanda) for 4 h, and then, the distillation was done in a unit of distillation VELP Scientifica UDK126A (Milan, Italy), the nitrogen was collected in a boric acid solution, and the crude protein was calculated $N \times 5.7$. Moisture was determined by the AACC 44-19 standard method (*27*).

Damaged Starch. The content of damaged starch was determined according to AACC 76-30A method (*27*). A fungal enzyme from *Aspergillus oryzae* (A6211, Sigma Chemical Co., St. Louis, MO) was used.

Determination of Flour Quality. Alkaline water retention capacity (AWRC) was determined according to AACC 56-10 method (*27*). Flour (1 g) was suspended in 5 mL of 0.1 N $NaHCO_3$, hydrated for 20 min, and centrifuged at 1000*g* for 15 min at room temperature. The precipitate obtained was weighed, and AWRC was calculated.

Sodium dodecyl sulfate sedimentation index (SDS-SI) values were determined using 1 g of flour moistened in a 25 mL cylinder with 8 mL of Coomassie Blue solution. The sample was left to stand for 3 min and 40 s; vortexed for 5 s; then left to stand for 1 min and 55 s; and vortexed again. SDS and lactic acid (12 mL) were added immediately and agitated for 1 min in a horizontal agitator. The resulting suspension was left to stand for 14 min, and the volume of moistened flour was measured. Results were expressed in cm^3 (*28*).

Solvent retention capacity profile (SRC) was obtained according to AACC 56-11 method (*29*). White flour samples (5 g) were suspended with 25 mL of water, 50% sucrose, 5% sodium carbonate, and 5% lactic acid. The samples were hydrated for 20 min and centrifuged at 1000*g* for 15 min. Each precipitate obtained was weighed, and the SRC for each sample was calculated according to AACC (*29*).

Preparation of Cookies. Cookies were prepared according to León et al. (*24*). The ingredients used were as follows: flour (45 g), caster sugar (27 g), vegetable fat (20 g), powdered milk (2.25 g), $NaHCO_3$ (0.50 g), NaCl (0.42 g), and 8.5 mL 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 the height of four cookies taken at random. The higher value was correlated to the better quality.

Purification of Starch Granules. The purification of starch granules was done according to the AACC 38-10 standard method for gluten obtention (*27*). It was modified for recovery of starch: 5 g of flour was weighted into a porcelain dish and hydrated with 2.3 mL of water to form a dough ball, and this was covered with a mesh and kneaded in a water stream over a sieve (63 μm). The starch and all soluble matter removed were collected and centrifuged at 800*g* for 3 min, and the precipitate was suspended with 10 mL of water and filtered in a vacuum over a filter (3.5 μm Schleicher and Schull, Dessel, Germany). Finally, the paper with the starch granules was recovered and dried for their conservation. The total protein in purified starch granules samples was 0.22 ± 0.08%.

Separation of Proteins from Starch Granules. The starch (50 mg) was suspended with 1.5 mL of a SDS solution (1%), the suspensions were shaken for 60 min at room temperature and centrifuged at 2000*g* for 10 min, the proteins were precipitated with 4.5 mL of acetone at −20 °C for 18 h, then the tubes were centrifuged at 12300*g* for 10 min, and the precipitates were suspended in 500 μL of sample buffer (0.063 M Tris-HCl, pH 6.8, 10% glycerol, 5% β-mercaptoetanol, 0.01% Bromophenol Blue). The total protein extracted from starch samples did not present significant differences between them ($p > 0.05$).

Electrophoresis. The proteins from the starch granules were separated on a linear gradient SDS−polyacrylamide gel electrophoresis (PAGE). The gels were performed mixing a light acrylamide solution (4% T and 2.6% C) and a dense acrylamide solution (15% T and 2.6% C) using a gradient former 485 (Bio-Rad Laboratories, Hercules, CA). The quantity of protein loaded was 6.1 ± 0.2 μg per lane.

The following proteins were employed as molecular mass markers: myosin, 200 000; β-galactosidase, 116 250; phosphorylase b, 97 400; serum albumin, 66 200; ovalbumin, 45 000; carbonic anhydrase, 31 000; trypsin inhibitor, 21 500; lysozyme, 14 400; and aprotinin, 6500 (SDS−PAGE MW standards, Broad range, Bio-Rad Laboratories).

The electrophoresis was run for 60 min at constant voltage of 150 V. A Mini Protean II Slab Cell (Bio-Rad Laboratories) was used.

The proteins were stained by silver stain and quantified by densitometry in an Image Master VDS (Pharmacia Biotech Inc., U.S.A.) provided with the Image master VDS software (Pharmacia Biotech Inc.). A blank lane was used to obtain the background signal. The volume of protein band (integrated optical density, IOD) was represented by the following expression:

$$\text{IOD} = [\text{mean intensity (Im)} - \text{background (Ib)}] \times \text{band area}$$

The proportions of polypeptides relative to total protein in the corresponding lane were quantified as follows: IOD from each band/total IOD of the lane. The standard curve for silver staining IOD vs quantity of protein was performed using carbonic anhydrase (C 7025 Sigma Chemical Co.) as standard. The silver-stained IOD showed a linear response between 0.1 and 10 μg of protein with a regression coefficient of $r^2 = 0.915$ and a lineal equation of $y = 639.5x + 205.49$.

Three replicates of the granule protein extractions were done, and each one was run. The IOD value was the mean of three replicates.

Statistical Analysis. All reported results are the means of at least two replicates. The means were compared by the LSD Fisher test at a significance level of 0.05, while the relationship between measured parameters was assessed by Pearson's test, in both cases using the INFOSTAT statistical software (Facultad de Ciencias Agropecuarias, UNC, Argentina).

RESULTS AND DISCUSSION

To determine the presence of friabilins in the starch granule surface, the granule proteins obtained from eight triticale lines and two wheat cultivars were separated by SDS−PAGE gradient electrophoresis. The electropherogram of all samples showed a similar pattern for starch granule proteins (between M_r 66 000 and M_r 30 000). A band of approximately M_r 15 000 was present in LA6 soft wheat, which was used as positive control, meanwhile the friabilin band was absent in durum wheat, which was used as negative control. In all triticale samples, a double band of M_r 15 000 was observed (**Figure 1**). Originally, friabilin was defined as an approximately M_r 15 000 starch granule surface protein on the basis of its extractability from isolated starch and its mobility during SDS−PAGE electrophoresis (*6*). Later, Oda and Schofield (*30*) found, by means of two-dimensional electrophoresis, that friabilin comprises a mixture of proteins in which puroindoline polypeptides are important components.

Texture in triticale and in wheat samples was classified on the basis of PSI test as summarized in **Table 2**. Bushuk (*25*) reported that triticale presents soft texture grain, but in this study, the triticale lines analyzed presented a wide range of hardness, and they showed lower values of %PSI than soft wheat (LA6) and higher values than durum wheat (Topacio). These results agree with Williams findings (*26*) that reported a hardness range from 7.8 to 36.6 in PSI of 280 triticale cultivars. Another hardness parameter, percentage of damaged starch, was determined. The hard wheat showed the highest value, and the soft wheat had the lowest value; the triticale lines presented intermediate values of damaged starch, and they corresponded to PSI values (**Table 2**). The correlation between damaged starch and PSI for the triticale lines was $r = -0.60$ because a soft grain offers a lower resistance to milling and the flour obtained had a lower percentage of damaged starch. The friabilin band was quantified by densitometric analysis. All triticale lines analyzed presented values of IOD from 2.66 to 12.27% (**Table 2**). There is little information about friabilin content in triticale. In rye, friabilins are present on the starch granules and the corresponding gene is on chromosome 5R (*31*); triticale also has the R genome that is responsible for the presence of friabilin in triticale.

In agreement with other authors (*6*, *7*), the results showed the absence of friabilin on the starch granule of durum wheat (Topacio) and the presence of this protein in the soft wheat (LA6) and triticale lines, which presented a wide range of hardness measured as PSI. However, the correlation between friabilin content on starch granules and grain hardness of triticale was not found (**Table 3**).

In previous works, Jolly et al. (*8*) have reported that friabilins are accumulated in hard and soft wheat, but they found a weak correlation between friabilin quantity on kernel and grain texture.

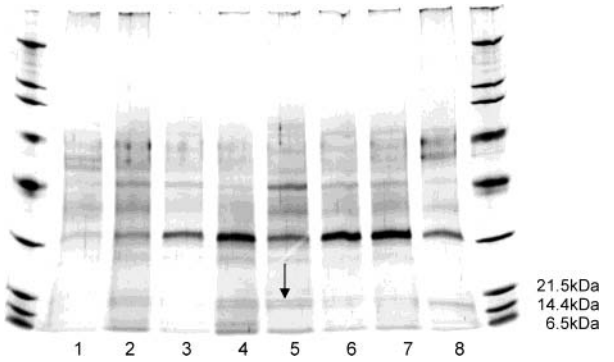

Figure 1. Electropherogram of proteins from starch granules from six triticale lines, 397j95 (2), 392j95 (3), 390j95 (4), Tatú (5), Remedios (6), Boaglio (7), and from control wheats, Topacio (1) and LA6 (8). Lane 9 has molecular weight markers of 200 000, 116 000, 97 400, 66 200, 45 000, 31 000, 21 500, 14 400, and 6500. Friabilin is indicated by the arrow.

Table 2. Grain Hardness Determined as PSI, Percentage of Damaged Starch, and Amount of M_r 15 000 Protein Analyzed by Densitometry in Eight Triticales Lines and Two Wheat Cultivars[a]

sample	PSI (%)	relative hardness	damaged starch (%)	IOD of M_r 15 000 (%)
La 6	23.9 ± 0.8	medium soft	5.75 ± 0.39	7.76 ± 1.70
Boaglio	15.4 ± 1.2	hard	9.04 ± 0.76	7.27 ± 1.67
Remedios	16.1 ± 0.8	hard	9.44 ± 0.28	6.70 ± 2.25
Tatú	20.2 ± 1.0	medium hard	5.88 ± 0.48	8.13 ± 2.22
383	19.4 ± 1.9	medium hard	9.03 ± 0.82	6.38 ± 2.81
393	19.1 ± 1.3	medium hard	7.35 ± 0.52	2.66 ± 0.14
390j95	21.8 ± 1.5	medium soft	6.92 ± 0.08	11.04 ± 2.65
392j95	16.6 ± 1.1	hard	7.76 ± 0.46	5.67 ± 1.77
397j95	16.3 ± 0.6	hard	7.35 ± 0.46	12.27 ± 2.88
Topacio	7.5 ± 0.8	very hard	11.66 ± 1.19	0.00 ± 0.00

[a] The values are the mean of two measurements with the standard error.

Table 3. Correlation Values Calculated between Studied Parameters of the Eight Triticale Lines

	protein	starch damage	SDS-SI	AWRC	cookie factor	PSI	SRC sodium carbonate	SRC lactic acid	SRC water	SRC sucrose	IOD
protein	1.00										
starch damage	–0.12	1.00									
SDS-SI	–0.22	–0.82	1.00								
AWRC	0.18	0.16	0.03	1.00							
cookie factor	–0.21	–0.44	0.30	–0.70	1.00						
PSI	–0.02	–0.60	0.52	–0.55	0.89	1.00					
SRC sodium carbonate	–0.08	0.49	–0.44	0.82	–0.53	–0.58	1.00				
SRC lactic acid	0.51	–0.29	0.70	0.56	–0.30	–0.02	0.06	1.00			
SRC water	0.00	0.22	–0.13	0.96	–0.76	–0.64	0.85	0.37	1.00		
SRC sucrose	0.30	–0.16	0.24	0.69	–0.13	–0.17	0.62	0.43	0.55	1.00	
IOD	–0.02	–0.28	0.66	0.00	–0.10	0.09	–0.45	0.65	–0.07	–0.27	1.00

Table 4. Baking Quality Parameters of Eight Triticale Lines and Two Wheat Cultivars[a]

	protein (%)	SRC sodium carbonate (%)	SRC lactic acid (%)	SRC water (%)	SRC sucrose (%)	SDS SI (cm^3)	AWRC (%)	cookie factor
La 6	14.42 ± 0.20^{d}	68.70 ± 1.30^{a}	96.01 ± 2.12^{g}	56.39 ± 0.67^{b}	99.80 ± 1.01^{e}	11.0 ± 0.0^{e}	61.20 ± 0.25^{b}	7.50 ± 0.21^{a}
Boaglio	9.82 ± 0.47^{b}	78.11 ± 1.31^{f}	76.41 ± 2.28^{c}	61.70 ± 0.73^{c}	97.86 ± 0.30^{d}	5.1 ± 0.0^{a}	67.02 ± 0.72^{de}	4.74 ± 0.17^{b}
Remedios	9.82 ± 0.63^{b}	78.61 ± 0.24^{fg}	77.40 ± 0.65^{cd}	62.07 ± 0.34^{c}	98.23 ± 0.79^{de}	5.5 ± 0.0^{b}	68.13 ± 0.43^{e}	4.65 ± 0.41^{b}
Tatú	9.98 ± 0.37^{b}	72.33 ± 0.18^{c}	81.08 ± 0.51^{e}	56.83 ± 0.08^{b}	103.17 ± 0.93^{f}	9.3 ± 0.3^{d}	64.08 ± 0.51^{c}	5.63 ± 0.11^{c}
383	8.02 ± 0.10^{a}	73.52 ± 0.06^{d}	59.66 ± 0.23^{a}	54.29 ± 0.18^{a}	92.03 ± 0.02^{b}	5.0 ± 0.0^{a}	59.52 ± 1.12^{a}	5.80 ± 0.04^{c}
393	8.73 ± 0.11^{a}	79.54 ± 0.05^{g}	64.84 ± 0.18^{b}	62.35 ± 0.21^{cd}	99.62 ± 0.38^{e}	5.5 ± 0.0^{b}	66.78 ± 0.41^{d}	5.32 ± 0.14^{d}
390j95	9.57 ± 0.17^{b}	68.80 ± 0.05^{a}	77.08 ± 0.86^{c}	55.13 ± 0.57^{a}	88.44 ± 1.06^{a}	8.0 ± 0.0^{c}	60.84 ± 0.69^{b}	5.53 ± 0.05^{cd}
392j95	9.29 ± 0.17^{b}	70.99 ± 0.30^{b}	60.23 ± 0.10^{a}	56.23 ± 0.06^{b}	89.99 ± 0.92^{a}	5.0 ± 0.0^{a}	60.37 ± 0.97^{ab}	5.00 ± 0.19^{bd}
397j95	8.17 ± 0.06^{a}	75.63 ± 0.20^{e}	79.34 ± 0.36^{de}	62.97 ± 0.03^{d}	95.49 ± 0.37^{c}	7.8 ± 0.3^{c}	67.54 ± 0.22^{de}	4.77 ± 0.12^{b}
Topacio	11.72 ± 0.00^{c}	88.12 ± 0.71^{h}	88.88 ± 0.06^{f}	73.78 ± 0.45^{e}	104.39 ± 0.57^{f}	5.5 ± 0.0^{b}	71.75 ± 0.51^{f}	4.50 ± 0.01^{b}

[a] Values followed by the same letter in the same column are not significantly different ($p < 0.05$).

Greenblat et al. (*9*) observed that friabilin occurs in the soft and hard wheat endosperm in approximately equal amounts, whereas when the friabilin was extracted from starch granules, a higher level of this protein was observed in soft cultivars than in hard ones. The association of friabilin with the starch granules surface seems to involve polar lipids. Giroux and Morris (*15*) reported that the quantitative level of friabilin present on the surface of starch granules is highly correlated ($r = -0.780$) with wheat grain softness when chromosome 5D recombinant substitution lines were studied. They suggested that the mutation of Pin B observed in hard wheat might alter the protein structure and reduce the strength of the lipid binding of this form of Pin B. Igrejas et al. (*32*) did not find correlation between puroindoline content in flour and grain hardness when they analyzed soft and hard wheat cultivars separately.

Grain hardness has been associated with the absence of the puroindoline a and glycine to serine mutation in Pin B (*15*, *33*); however, results obtained by Giroux et al. (*34*) indicated that most of the genetic variation in grain hardness among the hard red spring wheat populations was a result of factors other than Pin A and Pin B. Some hard wheat cultivars from Australia have the soft Pin B allele while Pin A is present (*13*).

In triticale, the amount of friabilin in the starch granule was not related to grain texture indicating that it would not be a "nonstick" protein. The puroindoline alleles, which are present in triticale, have not been studied. Triticale grain texture could be related with the presence of wild or mutated puroindoline alleles as it has been reported for wheat (*33*, *35*). These results suggest that the role of puroindolines in hardness is not so simple as could be expected, they are not directly involved in endosperm texture, but they could be markers of the hardness gene.

Table 4 shows the breadmaking quality parameters of flours obtained from eight triticale lines and wheat cultivars. SRC establishes a practical flour quality and functionality profile useful for predicting baking performance (*29*, *36*). A direct correlation between gluten content and percentage of total proteins has been observed in wheat (*37*). The lactic acid SRC is associated with glutenin characteristic, sodium carbonate SRC with levels of damage starch, and sucrose SRC with pentosan characteristics; water SRC is influenced by all of the flour constituents (*36*). A positive correlation between total proteins and lactic acid SRC was observed for triticale (**Table 3**), and the total proteins are poorly dependent on gluten protein because triticale has a high percentage of hydrosoluble protein (*38*). Guttieri et al. (*39*) reported that lactic acid SRC was independent of flour protein concentration in soft wheat. The grain hardness expressed as PSI percentage had a correlation with sodium carbonate SRC and water SRC (**Table 3**); these correlations account for the relationship between grain hardness, damaged starch, and the higher capacity of water absorption of starch. These results are supported by Guttieri et al. (*39*), who observed a significant correlation between single kernel characterization system hardness with water SRC and sodium carbonate SRC in 26 genotypes of soft wheats.

The baking quality of flour was characterized through AWRC, SDS-SI, and cookie factor. The AWRC values of triticales vary from 59.52 to 68.13%. Seven out of eight cultivars showed higher values than LA6 soft wheat. This is in agreement with values previously reported for triticale cultivars (*24*, *40*).

The flour fraction consisting of pentosans, proteins, glycoproteins, and damaged starch is thought to be responsible for the retention of alkaline water (*41*). In **Figure 2**, a positive correlation between AWRC with sodium carbonate SRC (associated with levels of damaged starch) can be observed. Also, a positive correlation was encountered among AWRC with sucrose SRC (associated with pentosans characteristic) and with water SRC.

The flours obtained from triticales under study showed a negative correlation between AWRC and cookie factor (**Table**

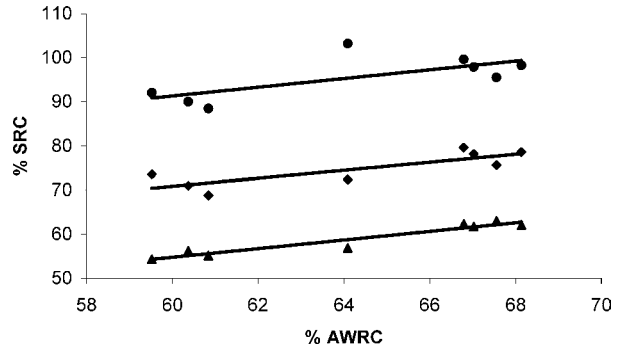

Figure 2. Correlation between AWRC with sodium carbonate SRC (♦, $r = 0.825$), sucrose SRC (●, $r = 0.694$), and water SRC (▲, $r = 0.961$).

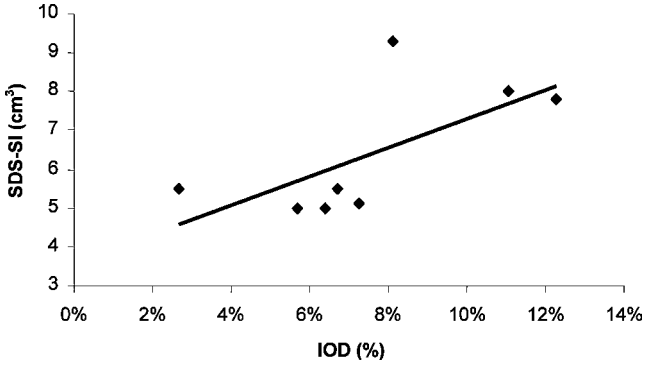

Figure 3. Correlation between IOD % of M_r 15 000 protein and SDS-SI for eight triticale lines; $r = 0.656$.

3) in agreement with other authors who found a negative correlation between AWRC and cookie quality in wheat (*3*, *40*, *42*, *43*).

The SDS-SI is a breadmaking quality parameter associated with quantity and quality of gluten protein and their capacity to form a network that produces sponged bread. SDS-SI values obtained for triticales were lower than those for the soft wheat because the soft wheat presented a high protein content (**Table 4**). Moreover, it has been reported that triticale has a lower concentration of gluten protein than wheat (*44*). This index was correlated to lactic acid SRC for the triticale lines (**Table 3**).

The quality parameters were analyzed in relationship to grain texture and friabilin content. Grain texture (PSI) correlated positively to cookie factor (**Table 3**) because soft grains produce a flour with a small particle size and a low level of damaged starch (*45*); however, the friabilin content extracted from starch granules of triticales did not correlate to the cookie factor (**Table 3**). It is consistent with the absence of correlation between texture and friabilin content in the triticale lines studied, whereas the amount of friabilin could be related to the breadmaking quality of flour because a correlation between IOD of M_r 15 000 protein and SDS-SI was observed (**Figure 3**). Puroindolines have intrinsically good foaming properties and their lipid binding capacity confers friabilin the ability to stabilize protein foam (*16*−*19*). Furthermore, puroindolines produce an increase of tenacity in the dough, which suggests an increase in protein−protein interactions (*20*). Because triticale flours have a higher proportion of hydrosoluble proteins, the SDS-SI is less dependent on gluten proteins and might be influenced by the content of friabilins.

The effect of puroindoline on breadmaking has been reported by Dubreil et al. (*20*) who found that the addition of low amounts of puroindoline to flours obtained from puroindoline free cultivars produced changes on the rheological properties associated to the flour quality. Flour with poor breadmaking quality supplemented with puroindolines showed a decrease in bread volume, while in medium quality flour, the presence of puroindoline induced an increase in bread volume. The improvement of crumb texture was less dependent on the flour quality, and the puroindoline addition produced breads with a fine crumb structure resulting from the fine gas bubbles that are formed by puroindoline foams in the aqueous phase of dough. The friabilin content in triticale and other soft grains could be considered as a breadmaking quality parameter since the gluten proteins have less influence than in hard grains.

The triticale lines have a wide range of hardness, which presented correlation with baking quality parameters such as damaged starch, sodium carbonate SRC, sucrose SRC, and water SRC. All studied triticale lines contained friabilins on the starch granules. However, the correlation between hardness and friabilin content was not observed suggesting that these proteins would not be directly involved in the grain texture determination of these triticale lines. Consequently, the amount of starch granule friabilins did not have any relationship with cookie quality in triticale flours, but they could be related to breadmaking quality, because they have a positive correlation with SDS-SI. Further studies are needed to determine which puroindoline alleles are present in triticale lines and their relationship to grain texture.

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