Baking Quality of Bread Wheat Cultivars Damaged by *Nysius simulans*

Malena Moiraghi, Lorena S. Sciarini, Franco Gil, Gabriel Galván, and Gabriela T. Pérez†

Cereal Chem. 0(0):1–7

ABSTRACT

Wheat grain may be attacked by different insect species. Among them, some Heteroptera species (e.g., *Aelia* spp. and *Eurygaster* spp.) reduce wheat breadmaking quality; others, such as *Nysius simulans*, commonly extract water and nutrients from soy plants. The aim of this study was to assess the effect of *N. simulans* infestation on breadmaking quality of different bread wheat cultivars. Twelve wheat cultivars (damaged and undamaged by *N. simulans*) were studied. Infested grain percentage varied between 51 and 78%, depending on cultivar. Protein and gluten quantity and quality were significantly reduced in damaged flours, as shown by gluten index, solvent retention capacity, and SDS sedimentation index. SDS-PAGE from water-extractable proteins evidenced an important proteolytic activity in damaged samples. Dough rheological properties showed a reduced dough viscoelasticity in damaged samples. Microbread specific volume changed from 3.26 cm^3/g for samples made with undamaged flour to 2.77 cm^3/g for bread made with damaged flour. No evidence for modification in starch properties was found. The infestation by *N. simulans* reduced wheat breadmaking quality in all cultivars studied, as a result of proteolytic activity occurring after dough hydration. Results suggest that the presence of *N. simulans* should be considered as a factor affecting wheat crops, mainly those located next to soy crop areas, which is the usual host for this insect.

It is widely known that some heteropterous insects (*Aelia* spp., *Eurygaster* spp., *Stenotus binotatus*, and *Nysius huttoni*) attack growing wheat grains, causing important economic loss to millers and bakers (Lorenz and Meredith 1988; Critchley 1998; Pérez et al. 2005; Blandino et al. 2015). These herbivorous insects use multiple types of proteinases as digestive enzymes. The diversity and plasticity of proteases expressed in the bugs' alimentary canal enables them to hydrolyze proteins into short peptides or individual amino acids (Amiri et al. 2016). *Euygaster* spp. even have a specific gluten hydrolase, with important roles in digestion of wheat gluten (Konarev et al. 2011), which is responsible for severe quantitative and qualitative (destruction of gluten protein) damage to crops (sometimes up to 100%) by feeding on leaves, stems, and grains (Amiri and Bandani 2013; Yandamuri et al. 2014).

The quality of damaged wheat grains, as measured by test weight and 1,000-grain weight, decreases as infestation level increases (Karababa and Ozan 1998). However, when infestation takes place at the end of grain filling, grain keeps its normal shape and size, which makes it difficult to identify the attack before milling. Hence, a reduction in flour quality is observed after milling, obtaining flour with poor breadmaking performance owing to high concentration of proteolytic enzymes from bug saliva (Köksel et al. 2002).

Gluten quality is widely influenced by genotype and also by crop agronomy and the presence of biotic and abiotic factors (Triboï et al. 2000; Torbica et al. 2011). Gluten is a specific protein fraction of wheat responsible for viscoelastic properties of bread dough; it influences breadmaking performance and determines the quality of final products. It is thus because of gluten hydrolysis that wheat flour obtained from infested seeds shows a reduced dough strength and extensibility, and this effect is more evident after fermentation (Meredith and Best 1985; Cressey and McStay 1987; Pérez et al. 2005). Bread quality is consequently affected, leading to low specific volumes and inappropriate texture (Lorenz and Meredith 1988; Hariri et al. 2000). It has been usually accepted that the gliadin fraction from gluten is more resistant to the bugs' digestive enzymes, and that these are more specific for degrading high-molecular-weight (HMW) glutenins (Yakovenko et al. 1973; Cressey and McStay 1987). However, Sivri et al. (1998) reported that both fractions (gliadins and HMW glutenins) were affected by *Eurygaster maura*'s enzymes, and they found an intercultivar variation in susceptibility to hydrolysis by bug proteolytic enzymes. It has also been reported that *N. huttoni* and *Stenotus binotatus* affect starchy endosperm (Lorenz and Meredith 1988; Every et al. 1990).

Most Argentinian wheat is characterized by a high test weight, which allows a high flour extraction rate, with low ash content and low sprout probability. Although protein and gluten contents are usually low, environmental conditions during grain filling and good genetic varieties planted allow rheological parameters to be sufficiently high, leading to proper alveographic *W* and farinograph stability, with a tendency toward high dough tenacity (Molfese et al. 2015).

In Argentina, during the 2000–2010 decade, a shift in soil usage from wheat to soy crops took place. In 2000–2001, 6,496,600 ha was used for wheat, whereas only 4,582,250 was used for this culture in 2010–2011. On the other hand, 10,664,330 ha was used for soy in 2000–2001 and 18,886,634 ha in 2010–2011 (Sistema Integrado de Información Agropecuaria 2016). Some bug species, such as *Nysius simulans*, have been reported to extract water and nutrients from soy plants (Dalazen et al. 2014). Females lay eggs in the soil after being fertilized on the plant. In this regard, direct seeding, which allows keeping brush, provides a favorable environment for insect proliferation (Dughetti 2015). In 2010, in Marcos Juarez (Córdoba, Argentina), a high population of *N. simulans* was detected in soybean crops, leading to heavy infestation of wheat crops growing in experimental fields near this area. In this way, an increase in the area planted with soy may lead to higher infestation by *N. simulans*, which could also cause higher infestation in wheat crops. It is worth highlighting that there are no worldwide reports about *N. simulans* attack in wheat crops. It is thus the aim of this study to assess the effect of *N. simulans* infestation on the breadmaking quality of different Argentinian bread wheat cultivars.

MATERIALS AND METHODS

Materials. Wheat grain samples were provided by Estación Experimental Marcos Juárez (Marcos Juárez, Córdoba, Argentina; 32°42′ S, 62°07′ W). Twelve wheat cultivars were naturally infested by *N. simulans*. This natural infestation occurred because next to this plot there were soybean plots surrounded by

† Corresponding author. Phone: +54 351 4334116, ext. 255.
E-mail: gaperez@agro.unc.edu.ar

Instituto de Ciencia y Tecnología de los Alimentos Córdoba (ICYTAC-UNC-CONICET), Cátedra de Química Biológica, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Av. Valparaíso y Rogelio Martínez, 5000 Córdoba, Argentina.

http://dx.doi.org/10.1094/CCHEM-11-16-0272-R

weeds, attacked by *N. simulans* first, when soybean seedlings were growing. Bugs then infested wheat plants when they were at the end of the grain filling period. The field was monitored weekly, and bugs were seen on the spikes and on the field soil, and they were captured in order to be identified. When wheat grains were harvested, grains showed puncture marks (black spots). The same 12 wheat cultivars were sowed in another plot in the same field, although not contiguous to soybean plots. This part of the field was clean of weeds, and no bugs were observed during the weekly monitoring. Harvested grains did not show any puncture marks. All samples (damage and undamaged) correspond to the 2010–2011 harvest period. Cultivars studied were ACA 304, ACA 315, Baguette Premium 11, Baguette 30, Baguette 31, Biointa 2004, Biointa 3004, Biointa 3005, Buck Charrua, Buck Taita, Klein Pantera, and SRM Nogal.

Refined flour was obtained from the 24 samples (12 infested, 12 uninfested) after conditioning grain moisture up to 14% by adding tap water and milling with a laboratory mill (AG AQC 109, Agromatic, Laupen, Switzerland) provided with a 250 µM sieve used to separate pericarp from endosperm.

Infested Grain Percentage (IG%). The insect damage levels of these grains were visually determined. The damaged kernels that showed characteristic puncture marks (black spots) were counted from three sets of 100 grains to determine IG%.

Flour Analysis. *Protein Content.* Nitrogen content was determined following the micro-Kjeldahl method modified with boric acid (AACC International Approved Method 46-13.01). The sample was digested (digester, Raypa, Barcelona, Spain) for 20 min and then distilled with a UDK126A distillation unit (VELP Scientifica, Milan, Italy). Nitrogen was collected in a boric acid solution, and the crude protein was calculated as N × 5.7. Protein content was determined in duplicate.

Wet Gluten, Dry Gluten, and Gluten Index (GI). These parameters were evaluated with a GI meter (China) (AACCI Approved Method 38-12.02), keeping the volume of wash solution at 4.8 mL for all samples. These tests were carried out in triplicate.

Proteolytic Activity. Soluble protein extraction was performed according to the method of Aja et al. (2004) with slight modifications. Wet gluten from damaged and undamaged wheat was obtained by hand washing. Wet gluten (100 mg) was incubated at 37°C in 2 mL centrifuge tubes for different time intervals (0, 2, 6, and 18 h) and then suspended in 0.5 mL of distilled water, mixed for 5 min on a vortex mixer, and then centrifuged at 15,700 × *g* for 2 min. Soluble proteins were then precipitated with acetone; briefly, supernatant was mixed with three volumes of acetone and kept overnight at –18°C and then centrifuged at 10,000 × *g* for 5 min. The supernatant was discarded, and the precipitate was suspended in sample buffer with 2-mercaptoethanol and then vortexed for 1 h. Samples were then taken to a boiling water bath for 5 min and then centrifuged.

Electrophoresis. According to the method of Aja et al. (2004), water extracts were dissolved in 0.125M Tris/HCl (pH 6.8) containing 2% SDS, 10% glycerol, 0.05% bromophenol blue, and 2% β-mercaptoethanol (reducing conditions); protein composition was analyzed by SDS-PAGE (stacking gel of 4% w/v acrylamide and separating gel of 12% w/v acrylamide) according to the method of Laemmli (1970). A Mini Protean II dual slab cell (Bio-Rad Laboratories, Hercules, CA, U.S.A.) was used to perform electrophoretic runs, conducted at constant voltage (150 V) until the front reached the end of the gel. Proteins were stained with Coomassie brilliant blue.

SDS Sedimentation Index (SDS-SI). SDS-SI values were determined by using 1 g of flour moistened in a 25 mL cylinder with 8 mL of 10 mg/L Coomassie blue solution. The sample was left to stand for 3 min 40 s and then vortexed for 5 s, left to stand for 1 min 55 s, and then vortexed again. An SDS-lactic acid reagent (12 mL) was added immediately afterward and agitated for 1 min in a horizontal agitator. The SDS-lactic acid reagent was prepared by mixing 20 mL of lactic acid solution (10% v/v) with 970 mL of SDS solution (2% w/v). The resulting suspension was left to stand for 14 min, and the volume of moistened flour was measured. The results were expressed in cubic centimeters (Dick and Quick 1983). SDS-SI was performed in triplicate.

Solvent Retention Capacity (SRC). Lactic acid (SRC-lac) and carbonate (SRC-car) retention capacities were measured according to AACCI Approved Method 56-11.01. Flour samples (5 g) were suspended with 25 g of 5% sodium carbonate and 5% lactic acid. Samples were hydrated for 20 min and centrifuged at 1,000 × *g* for 15 min. Each precipitate obtained was weighed, and the SRC of each sample was calculated. SRC was performed in triplicate.

Dough Analysis. *Small Deformation Rheology.* Rheological assays were performed with an oscillatory rheometer (Anton Paar, Ostfildern, Germany). Frequency sweeps were carried out at 0.1–10 Hz, 0.05% strain, and 30°C (linear viscoelastic range was determined with a previous strain sweep from 0.1 to 100%, at a constant frequency of 1 Hz). Plate-plate geometry (25 mm diameter) was used, with a 1 mm gap. Samples were prepared for breadmaking, but without yeast addition. Dough was allowed to rest for 15 min and then placed between plates, and excess sample was carefully trimmed. To avoid water loss during determination, the exposed edges of dough were covered with Vaseline petroleum jelly. Before starting the assay, samples were rested for 5 min to allow residual stress relaxation. Dough preparation was performed in triplicate.

Breadmaking. *Microbaking Test.* A microscale baking test with 20 g of flour was carried out according to the method of Selmair and Koehler (2008) with modifications as follows. The ingredients used were (on a flour basis): NaCl, 1.5%; sucrose, 1%; dry baker's yeast, 2.5%; sodium stearoyl lactylate, 0.5%; and water, 54–60% (optimum level). Ingredients were mixed for 2 min in a manual mixer (Supermix 130, Moulinex, Buenos Aires, Argentina). The resulting dough was taken to a first proof for 20 min at 30°C in a water-saturated atmosphere. The dough was then manually degassed and sheeted with a Pastalinda machine (Buenos Aires, Argentina) to form an oval dough piece. This was folded twice into halves. The dough was then divided into 10 g pieces, rolled up, and placed in a baking pan (40 × 25 × 20 mm). After a fermentation period of 35 min at 30°C in a water-saturated atmosphere, dough was baked for 12 min at 200°C. The oven (Pauna, Buenos Aires, Argentina) was steamed prior to baking. Breadmaking was carried out in duplicate.

Specific Bread Volume (SBV). The volume of each bread loaf was determined by rapeseed displacement 2 h after baking. Specific volume was obtained as bread volume/bread weight ratio. Three measurements of each breadmaking batch were performed.

Statistical Analysis. Data were subjected to one-way analysis of variance considering the wheat cultivar and the infestation status (damaged versus undamaged). Results of the analysis were compared by the DGC means-comparison test of Di Rienzo et al. (2002) with a P value < 0.05 to compare samples. Pearson correlation coefficients between variables were also calculated. Principal component analysis was run using the difference in selected variables (flour quality parameters wet gluten, GI, SDS-SI, and SRC-lac, rheological parameter tan δ, and breadmaking performance parameter SBV) between damaged and undamaged samples. All analyses were performed with INFOSTAT statistical software (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina).

RESULTS AND DISCUSSION

IG%, protein content, and gluten quality of damaged and undamaged cultivars are presented in Table I. IG% was evaluated as the number of grains showing black spots (associated with bug damage, Figure 1) from a batch of 100 grains. This parameter varied from 51.3 to 78.3%; with Biointa 3005 being the least affected and

Biointa 2004 and Baguette 31 the most affected cultivars (Table I). In general, damaged samples showed reduced protein content, the reduction degree depending on wheat cultivar. ACA 304, Biointa 3004, and SRM Nogal protein content was not affected, whereas those most affected were Baguette 30 and Baguette 31; on average, protein reduction was 16.7% (range 0–40%). For wet gluten, reduction was not significant for ACA 304, Biointa 3005, and Buck Taita cultivars, whereas Baguette 31 and Biointa 2004 were the most affected (53 and 62% reduction, respectively); average reduction was 26.1%. GI is a measure of gluten strength; gluten separated from wheat flour with a Glutomatic device is centrifuged to force wet gluten through a specific sieve under standardized conditions. The percentage of wet gluten remaining on the sieve after centrifugation is defined as the GI. The higher the GI, the higher the gluten quality. On average, GI was reduced 55.7% after bug damage, Biointa 3004 being the most affected flour, with almost no gluten remaining after centrifugation, whereas Buck Charrua showed only 13% diminution (Table I). Torbica et al. (2014) also found a decrease in GI for bug-damaged wheats, this reduction being around 20%. It must be pointed out that damaged and undamaged samples were grown in different plots, so differences between sound and damaged grains could also be owing to soil differences. It is well known that total protein and gluten contents are strongly affected by environmental conditions (Blumenthal et al. 1993; Graybosch et al. 1995). However, it is to be noted that although environmental effects are expectable, all 12 cultivars presented marked differences after bug attack. Souza et al. (2004) reported an important effect of environment on wheat protein quality, determining the final end-use of wheat, but when genotype and location were considered together cultivar selection (genotype) was critical for achieving a desired end use, with location effects (environment) being of secondary importance.

No significant correlations were found between IG% and protein content or gluten quantity/quality. It should be considered that the determination of grain infestation was not necessarily related to the degree of damage, because some cultivars may have a lower number of infested grains but with a higher enzymatic concentration.

The SRC test is a solvation assay for flour based on the enhanced swelling behavior of individual polymer networks in selected single diagnostic solvents—water, 5% w/w lactic acid in water (for glutenin), 5% w/w sodium carbonate (Na_2CO_3) in water (for damaged starch), and 50% w/w sucrose in water (for pentosans)—which are used to predict the functional contribution of each individual flour component (Kweon et al. 2011). SDS-SI is widely used to predict gluten strength (Clarke et al. 2010). Table II presents SRC-lac, SRC-car, and SDS-SI values for damaged and undamaged samples. Undamaged cultivars showed different SRC-lac values, between

Fig. 1. Representative image of damaged (left) and undamaged (right) wheat grains corresponding to ACA 315 cultivar. Q:13

BW in print

TABLE I
Infested Grain Percentage, Protein and Gluten Contents, and Gluten Quality of the 12 Wheat Cultivars Under Study[z]

Cultivar	State	IG%	Proteins (%)	Wet Gluten (%)	Dry Gluten (%)	Gluten Index (%)
ACA 304	U	…	12.48Ac	31.36Ab	11.45Aa	94.77Ba
	D	56.5b	13.14Af	32.28Ag	11.11Ac	60.89Ac
ACA 315	U	…	13.06Bd	36.62Bc	12.85Bb	93.16Ba
	D	65.3c	9.58Ac	25.03Ae	8.67Ab	62.51Ac
Baguette 11	U	…	13.17Bd	32.87Bb	11.75Ba	98.16Ba
	D	61.3b	10.63Ad	25.35Ae	8.88Ab	80.11Ad
Baguette 30	U	…	12.01Ba	31.54Bb	12.10Ba	92.82Ba
	D	60.0b	7.93Ab	19.42Ac	7.14Ab	30.23Ab
Baguette 31	U	…	11.82Bb	31.22Bb	10.92Ba	92.32Ba
	D	75.0d	7.15Aa	14.68Ab	5.52Aa	25.35Ab
Biointa 2004	U	…	11.06Ba	26.95Ba	11.16Ba	97.67Ba
	D	78.3d	8.24Ab	10.12Aa	3.85Aa	5.14Aa
Biointa 3004	U	…	11.61Ab	29.96Bb	10.19Ba	88.13Ba
	D	59.3b	11.20Ae	23.21Ad	8.46Ab	0.48Aa
Biointa 3005	U	…	11.95Bb	29.44Ab	10.28Ba	96.04Ba
	D	51.3a	9.98Ac	23.80Ad	7.73Ab	25.71Ab
Buck Charrua	U	…	14.63Be	41.75Ad	13.89Ab	92.92Aa
	D	59.0b	10.89Ad	25.94Be	11.95Ac	79.83Ad
Buck Taita	U	…	12.67Bc	31.56Ab	13.24Ab	96.15Ba
	D	65.5c	11.48Ae	30.65Af	12.15Ac	38.71Ab
Klein Pantera	U	…	12.43Bc	30.60Bb	13.13Bb	94.48Ba
	D	58.0b	11.01Ad	26.74Ae	9.88Ab	30.55Ab
SRM Nogal	U	…	11.09Aa	30.37Bb	10.53Aa	93.82Ba
	D	67.0c	11.09Ad	25.87Ae	10.00Ab	61.55Ac
Average	U	…	12.33B	31.94B	11.79B	94.17B
	D	63.1	10.19A	23.59A	8.78A	41.74A

[z] Different uppercase letters within a cultivar represent significant differences between damaged (D) and undamaged (U) samples ($P < 0.05$). Different lowercase letters represent significant differences among cultivars (damaged and undamaged samples were analyzed separately) ($P < 0.05$). IG% = infested grain percentage.

93.87 (Biointa 3004) and 130.66% (Baguette 11), with an average value of 118.00%. The same was true for SDS-SI, with values between 12.25 (Buck Taita) and 19.00 mL (ACA 304) (average 17.19 mL). Both parameters showed significant ($P < 0.05$) differences between undamaged and damaged wheat. For damaged samples, a decrease in these values was observed, with reduction ranging from 3.98 (Klein Pantera) to 26.34% (Biointa 3005) for SRC-lac, and from 2.63% (ACA 304) to 61.46% (Baguette 31) for SDS-SI. Taken together, both tests (SRC-lac and SDS-SI) highlight the fact that gluten quality is dramatically affected after bug infestation.

An effect of bug infestation on starch fraction structure and properties has already been reported for *N. huttoni* in Australia and New Zealand (Lorenz and Meredith 1988; Every et al. 1990), although no differences in amylase activity were assessed. Starch granules from flour infested by *Aelia* spp. or *Eurygaster* spp., as examined by scanning electron microscopy, were intact in the surrounding areas of the insect puncture (Rosell et al. 2002). To check whether *N. simulans* had an effect on starch properties, SRC-car was assessed (as already explained, SRC-car correlates with damaged starch content in flour). Thus, differences among cultivars are expected, and these differences are usually related to grain hardness, with harder grains showing higher damaged starch fraction. No differences were found between damaged and sound samples, on average (Table II); however, undamaged ACA 315, Baguette 31, Biointa 2004, Biointa 3004, Biointa 3005, and Buck Taita showed higher Na_2CO_3 absorption than their damaged counterparts. Nonetheless, no differences in DSC and Rapid Visco Analyzer profiles were observed in these samples (data not shown). It is thus unlikely that differences in SRC-car are a result from different damaged starch content. More likely, differences in carbonate absorption are owing to a "dilution" effect: damaged flour has lower protein content, which in turn leads to higher starch/protein ratio, which may explain the higher Na_2CO_3 absorption.

SDS-PAGE from aqueous extracts obtained from isolated gluten of damaged and sound wheat under increasing incubation times is shown in Figure 2 (a representative image was selected, corresponding to Biointa 3005 because these findings were similar for all cultivars, data not shown). It can be observed that undamaged

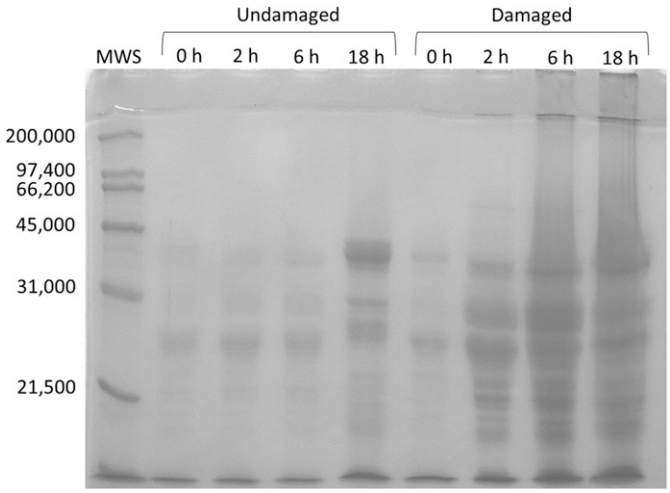


Fig. 2. Representative SDS-PAGE of water-soluble proteins extracted from undamaged and damaged wheat gluten (Biointa 3005) after 0 (control), 2, 6, and 18 h of incubation at 37°C. Molecular weight standard (MWS) was as follows: myosin (200,000), β-galactosidase (116,250), phosphorylase b (97,400), serum albumin (66,200), ovalbumin (45,000), soybean trypsin inhibitor (21,500), and lysozyme (14,400). Q:14

TABLE II
Solvent Retention Capacity (Lactic Acid and Carbonate) and SDS Sedimentation Index of the 12 Wheat Cultivars Under Study[z]

Cultivar	State	SRC-Lac (%)	SRC-Car (%)	SDS-SI (mL)
ACA 304	U	124.23Ac	76.25Ab	19.00Bd
	D	116.91Ag	76.78Av	18.50Ae
ACA 315	U	124.47Bc	78.49Bc	18.25Bc
	D	95.17Ad	72.55Aa	11.50Ac
Baguette 11	U	130.66Bc	78.73Ac	18.00Bc
	D	96.63Ad	75.54Ab	11.75Ac
Baguette 30	U	121.62Bc	79.88Ac	17.50Bc
	D	89.69Ac	77.26Ab	9.75Ab
Baguette 31	U	111.58Bb	77.25Bb	18.50Bc
	D	82.40Ab	72.35Aa	7.13Aa
Biointa 2004	U	101.25Bc	93.67Af	18.25Bc
	D	94.49Ad	83.43Ac	9.00Ab
Biointa 3004	U	93.87Ba	75.17Bb	14.25Bb
	D	75.85Aa	71.51Aa	9.00Ab
Biointa 3005	U	112.51Bb	80.86Bc	17.50Bc
	D	82.87Ab	73.49Aa	9.25Ab
Buck Charrua	U	119.88Bc	89.81Ae	17.00Bc
	D	95.50Ad	86.57Ad	12.25Ac
Buck Taita	U	112.44Bb	86.29Bd	12.25Ba
	D	105.10Af	83.14Ac	9.25Ab
Klein Pantera	U	111.90Ab	73.31Aa	18.00Bc
	D	107.45Ae	76.11Ab	14.25Ad
SRM Nogal	U	124.80Bc	80.42Ac	17.75Bc
	D	99.99Ae	83.15Ac	13.50Ad
Average	U	118.00B	80.84A	17.19B
	D	94.44A	77.66A	11.27A

[z] Different upper-case letters within a cultivar represent significant differences between damaged (D) and undamaged (U) samples ($P < 0.05$). Different lower-case letters represent significant differences among cultivars (damaged and undamaged samples were analyzed separately) ($P < 0.05$). SRC-Lac = lactic acid solvent retention capacity; SRC-Car = carbonate solvent retention capacity; and SDS-SI = SDS sedimentation index.

TABLE III
Rheological Parameters of Dough Prepared with Damaged and Undamaged Wheat Flour and Specific Bread Volume (SBV)[z]

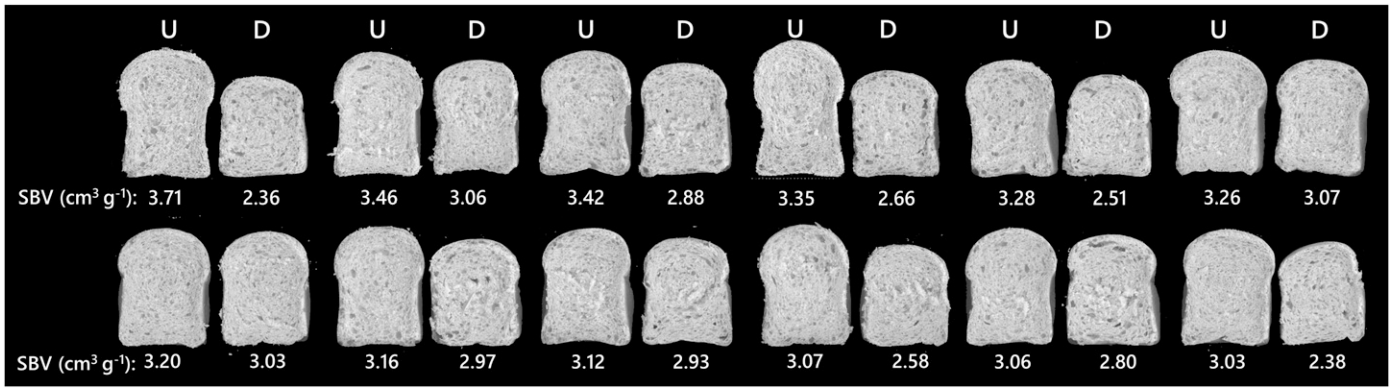
Cultivar	State	G' (Pa)	G'' (Pa)	Tan δ	SBV (cm^3/g)
ACA 304	U	24,700Bb	8,485Ab	0.343Ab	3.26Aa
	D	15,000Ab	19,850Bd	0.418Ab	3.07Ab
ACA 315	U	13,200Aa	13,200Bc	0.340Ab	3.71Ba
	D	19,850Ac	4,470Aa	0.425Bb	2.36Aa
Baguette 11	U	18,300Aa	5,420Aa	0.295Aa	3.06Ba
	D	15,100Ab	5,720Ab	0.379Bb	2.80Ab
Baguette 30	U	18,450Aa	6,415Aa	0.348Ab	3.28Ba
	D	24,050Bc	9,040Bc	0.376Ab	2.51Aa
Baguette 31	U	22,950Ab	9,360Ab	0.407Ab	3.16Aa
	D	19,850Ac	8,210Ac	0.414Ab	2.97Ab
Biointa 2004	U	21,800Ab	7,000Aa	0.321Ab	3.03Ba
	D	20,700Ac	8,285Ac	0.402Bb	2.38Aa
Biointa 3004	U	23,900Ab	6,470Aa	0.271Aa	3.12Aa
	D	17,750Ab	6,810Ab	0.385Bb	2.93Ab
Biointa 3005	U	16,350Aa	5,840Aa	0.361Ab	3.35Ba
	D	16,800Ab	7,505Ac	0.447Bc	2.66Aa
Buck Charrua	U	13,400Aa	4,505Aa	0.337Ab	3.07Ba
	D	13,900Ab	5,635Ab	0.390Bb	2.58Aa
Buck Taita	U	20,400Ab	5,810Aa	0.285Aa	3.20Ba
	D	19,700Ac	6,680Ab	0.339Ba	3.03Ab
Klein Pantera	U	20,800Bb	6,915Aa	0.333Ab	3.46Ba
	D	9,340Aa	4,170Aa	0.401Bb	3.06Ab
SRM Nogal	U	14,550Aa	4,745Aa	0.328Ab	3.42Ba
	D	14,800Ab	6,025Ab	0.407Bb	2.88Ab
Average	U	19,066A	7,013A	0.330A	3.26B
	D	16,920A	7,700A	0.400B	2.77A

[z] Different uppercase letters within a cultivar represent significant differences between damaged (D) and undamaged (U) samples ($P < 0.05$). Different lowercase letters represent significant differences among cultivars (damaged and undamaged samples were analyzed separately) ($P < 0.05$). G' and G'' = elastic and viscous moduli, respectively; and Tan δ = tangent of phase angle.

samples exhibited similar protein profile after 0, 2, and 6 h of incubation. However, after 18 h, numerous and intense protein bands were present, which correspond to the hydrolysis products resulting from the intrinsic proteolytic activity of the undamaged gluten; these results are in agreement with those of Aja et al. (2004).

Damaged wheat showed a similar pattern to that of undamaged wheat when considering no incubation time (0 h). The water-soluble compounds extracted from incubated damaged gluten showed several bands between 42,000 and 27,000 that progressively increased in intensity during the incubation. In addition, a few bands with molecular mass lower than 21,000 appeared, but their intensity did not substantially change from 2 to 18 h of incubation. Also, a band retained between the stacking and the resolving gel was observed for damaged extracts when incubation time was long enough (from 2 h on) and most likely represented aggregated proteins that were released from gluten, most likely owing to the *Nysius* enzymatic system. Evidence of proteolysis has also been found in electropherograms for *N. huttoni* (Every et al. 1989), *Aelia* spp., and *Eurygaster* spp. (Sivri et al. 1998; Aja et al. 2004; Torbica et al. 2014). Q:8

Table III presents the rheological parameters for dough obtained from different samples. The tangent delta (tan δ), which equals the ratio of the viscous to the elastic modulus (G''/G'), reflects the balance between the viscous and elastic character of the viscoelastic material. A sample with a high degree of crosslinking would be expected to have a low tan δ (Tronsmo et al. 2003). The viscoelastic response (tan δ) of undamaged samples showed significant differences ($P < 0.05$), with values ranging from 0.271 for Biointa 3004 to 0.407 for Baguette 31. This parameter was drastically affected by bug infestation, increasing its value from 1.72% (Baguette 31) up to 42.07% (Biointa 3004). On average, tan δ increased 21.21% after damage. This increase is related to a more viscous response, compared with undamaged samples, possibly resulting from gluten hydrolysis.



Q:15 **Fig. 3.** Representative images of microbreads made with damaged (D) and undamaged (U) wheat samples. Top row, from left to right, ACA 315, Klein Pantera, SRM Nogal, Biointa 3005, Baguette 30, and ACA 304; bottom row, from left to right, Buck Taita, Baguette 31, Biointa 3004, Buck Charrua, Baguette 11, and Biointa 2004. SBV = specific bread volume.

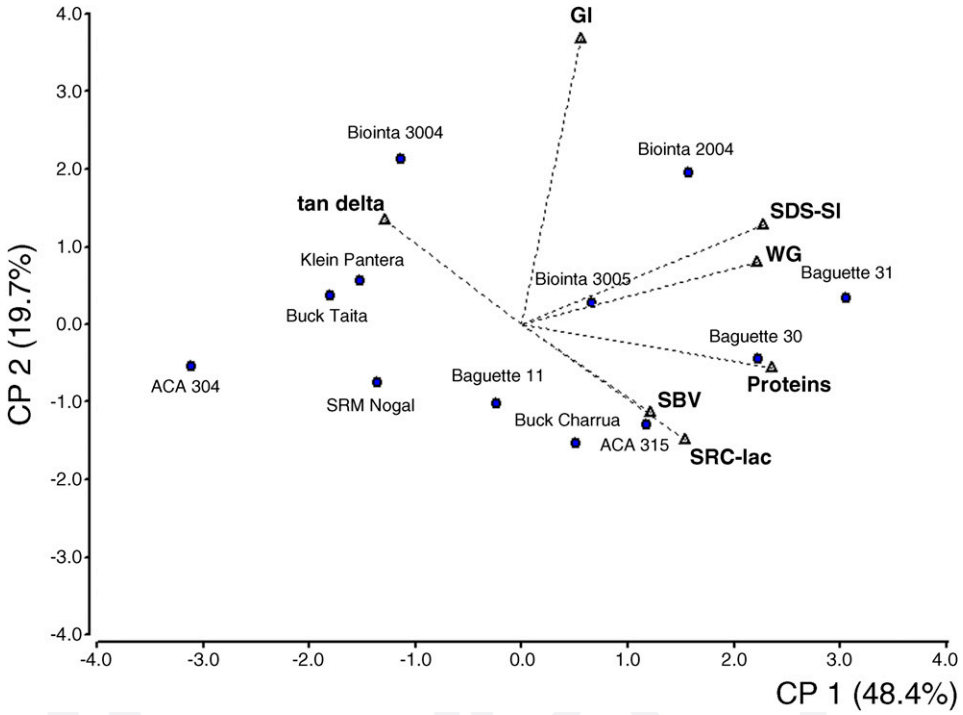


Fig. 4. Plot for principal component 1 (CP 1) versus 2 (CP 2). Cultivar discrimination was performed considering reduction on selected variables after *Nysius simulans* damage. SRC-lac = lactic acid solvent retention capacity; SDS-SI = SDS sedimentation index; tan delta = tangent of phase angle; WG = wet gluten; and SBV = specific bread volume.

BW in print

Figure 3 shows a representative image of microbreads formulated with different flours. A significant decrease in SBV was observed for damaged wheat flour, with a reduction of 15.03%. On average, ACA 315 was the most affected flour (SBV was reduced by 36.4%). On the other hand, ACA 304, Biointa 3004, and Baguette 31 showed no significant decrease in SBV when damaged flour was used (Table III). No significant differences in SBV were found between different cultivars ($P > 0.05$, Table III).

Torbica et al. (2014) found differences in dough viscoelastic properties (as measured with a farinograph, extensigraph, and alveograph) after attack by wheat bugs (*Eurygaster* spp. and *Aelia* spp.), along with a decrease in GI, and they related these differences to an increase in relative amount of gliadins with molecular mass below 30,000 and decrease in the relative amount of gliadins in the molecular mass range 30,000–75,000.

Significant correlations ($P < 0.05$) between SBV and protein content ($r = 0.65$), wet gluten ($r = 0.57$), GI ($r = 0.60$), SRC-lac ($r = 0.67$), SDS-SI ($r = 0.68$), and tan δ ($r = –0.56$) were found. Although a significant decrease in protein content was observed after *N. simulans* attack, more drastic effects were found on GI, SDS-SI, dough viscoelastic properties, and SBV.

Principal component analysis was carried out to assess the overall effect of insect infestation on flour quality, while the effect on each particular cultivar can be observed. To this end, the increase or decrease percentage in each variable after infestation was calculated. Figure 4 shows the score plot for principal component 1 versus principal component 2. As shown, 68.1% of whole data variability was explained by both axes. Cultivars located in positive values of the axes underwent a considerable modification in the variables measured when infested with *N. simulans*. Thus, Baguette 31 was the most affected cultivar, whereas ACA 304 showed the highest stability toward bug presence. SBV and SRC-lac were closely related variables, with a positive association, whereas tan δ showed a strong negative association with these variables.

CONCLUSIONS

These results demonstrate that *N. simulans* infestation significantly reduced wheat breadmaking quality. This effect was a result of proteolysis of gluten proteins, with a sharp decrease in protein quantity and quality. In this regard, the most affected parameters were GI (indicative of gluten quality), SDS-SI, and tan δ. No evidence for modification of starch properties was found. It is interesting to note that, despite being usually related to soy crops, *N. simulans* infestation has a marked effect on wheat flour quality, as evidenced herein. Moreover, although to a different degree, all 12 cultivars analyzed showed negative changes after bug damage. The presence of this insect should be considered as a factor affecting wheat crops, mainly those located next to soy crop areas.

ACKNOWLEDGMENTS

This work was funded by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina) and Secretaría de Ciencia y Técnica (SeCyT-FCA). The authors thank Fernando Flores for providing wheat samples.

LITERATURE CITED

AACC International. Approved Methods of Analysis, 11th Ed. Method 38-12.02. Wet gluten, dry gluten, water-binding capacity, and gluten index. Approved November 8, 2000. Method 46-13.01. Crude protein—Micro-Kjeldahl method. Approved October 8, 1976. Method 56-11.01. Solvent retention capacity profile. Proposed November 3, 1999. [Archived method.] Available online only. AACCI: St. Paul, MN.

Aja, S., Pérez, G., and Rosell, C. M. 2004. Wheat damage by *Aelia* spp. and *Erygaster* spp.: Effects on gluten and water-soluble compounds released by gluten hydrolysis. J. Cereal Sci. 39:187-193.

Amiri, A., and Bandani, A. R. 2013. Comparison of energy reserves in prediapause and diapausing adult Sunn pest, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae). J. Agric. Sci. Technol. 15:435-444.

Amiri, A., Bandani, A. R., and Alizadeh, H. 2016. Molecular identification of cysteine and trypsin protease, effect of different hosts on protease expression, and RNAi mediated silencing of cysteine protease gene in the Sunn pest. Arch. Insect Biochem. Physiol. 91: 189-209.

Blandino, M., Marinaccio, F., Ingegno, B. L., Pansa, M. G., Vaccino, P., Tavella, L., and Reyneria, A. 2015. Evaluation of common and durum wheat rheological quality through Mixolab® analysis after field damage by cereal bugs. Field Crops Res. 179:95-102.

Blumenthal, C. S., Barlow, E. W. R., and Wrigley, C. W. 1993. Growth environment and wheat quality: The effect of heat stress on dough properties and gluten proteins. J. Cereal Sci. 18:3-21.

Clarke, F. R., Clarke, J. M., Ames, N. A., Knox, R. E., and Ross, J. R. 2010. Gluten index compared with SDS sedimentation volume for early generation selection for gluten strength in durum wheat. Can. J. Plant Sci. 90:1-11.

Cressey, P. J., and McStay, C. L. 1987. Wheat-bug damage in New Zealand wheats I. Development of a simple SDS-sedimentation test for bug damage. J. Sci. Food Agric. 38:357-366.

Critchley, B. R. 1998. Literature review of Sunn pest *Eurygaster integriceps* Put. (Hemiptera, Scutelleridae). Crop Prot. 17:271-287.

Dalazen, G., Guedes, J. V. C., Carpintero, D. L., Stacke, R. F., and Cagliari, D. 2014. Populational fluctuation of *Nysius simulans* associated with soybean and hairy fleabane in Brazil. Interciencia 39: 391-394.

Di Rienzo, J., Guzman, A., and Casanoves, F. 2002. A multiple-comparisons method based on the distribution of the root node distance of a binary tree. J. Agric. Biol. Environ. Stat. 7:129-142.

Dick, J., and Quick, J. 1983. A modified screening test for rapid estimation of gluten strength in early-generation durum wheat breeding lines. Cereal Chem. 60:315-318.

Dughetti, A. C. 2015. La chinche diminuta *Nysius simulans*: Plaga emergente en quinua y otros cultivos en el valle bonaerense del Río Colorado. AgroUNS 24:6-8.

Every, D., Farrell, J. A. K., and Stufkens, M. W. 1989. Effect of *Nysius huttoni* on the protein and baking properties of two New Zealand wheat cultivars. N. Z. J. Crop Hortic. 17:55-60.

Every, D., Farrell, J. A. K., and Stufkens, M. W. 1990. Wheat bug damage in New Zealand wheats: The feeding mechanism of *Nysius huttoni* and its effect on the morphological and physiological development of wheat. J. Sci. Food Agric. 50:297-309.

Graybosch, R. A., Peterson, C. J., Baenziger, P. S., and Shelton, D. R. 1995. Environmental modification of hard red winter wheat flour protein composition. J. Cereal Sci. 22:45-51.

Hariri, G., Williams, P. C., and El-Haramein, F. J. 2000. Influence of pentatomid insect on the physical dough properties and two-layered flat bread baking quality of Syrian wheat. J. Cereal Sci. 31:111-118.

Karababa, E., and Ozan, A. N. 1998. Effect of wheat bug (*Eurygaster integriceps*) damage on quality of a wheat variety grown in Turkey. J. Sci. Food Agric. 77:399-403.

Köksel, H., Atli, A., Dag, A., and Sivri, D. 2002. Commercial milling of suni-bug (*Eurygaster* spp.) damaged wheat. Nahrung 46:25-27.

Konarev, A. V., Beaudoin, F., Marsh, J., Vilkova, N. A., Nefedova, L. I., Sivri, D., Köksel, H., Shewry, P. R., and Lovegrove, A. 2011. Characterization of a glutenin-specific serine proteinase of Sunn bug *Eurygaster integriceps* Put. J. Agric. Food Chem. 59:2462-2470.

Kweon, M., Slade, L., and Levine, H. 2011. Solvent retention capacity (SRC) testing of wheat flour: Principles and value in predicting flour functionality in different wheat-based food processes and in wheat breeding—A review. Cereal Chem. 88:537-552.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.

Lorenz, K., and Meredith, P. 1988. Insect-damaged wheat effects on starch characteristics. Starch/Stärke 40:136-139.

Meredith, P., and Best, S. M. 1985. Studies on bug damage in New Zealand wheats. Pages 115-123, in: Proceedings of the Second Biennial Cereal Science Conference. P. J. Cressey, W. H. Swallow, T. N. Lindley, N. G. Larsen, and R. W. Cawley, eds. Wheat Research Institute: Christchurch, New Zealand.

Molfese, E. R., Seghezzo, M. L., and Astiz, V. 2015. Monitoring farmers' wheat grain quality in the center-south of Buenos Aires Province, Argentina. http://inta.gob.ar/documentos/relevamiento-de-la-calidad-del-

trigo-pan-en-lotes-de-produccion-del-centro-sur-de-la-provincia-de-buenos-aires-argentina. INTA: Buenos Aires, Argentina.

Pérez, G., Bonet, A., and Rosell, C. M. 2005. Relationship between gluten degradation by *Aelia* spp. and *Eurygaster* spp. and protein structure. J. Sci. Food Agric. 85:1125-1130.

Rosell, C. M., Aja, S., and Sadowska, J. 2002. Amylase activities in insect (*Aelia* and *Eurygaster*)-damaged wheat. J. Sci. Food Agric. 82:977-982.

Selmair, P. L., and Koehler, P. 2008. Baking performance of synthetic glycolipids in comparison to commercial surfactants. J. Agric. Food Chem. 56:6691-6700.

Sistema Integrado de Información Agropecuaria. 2016. Datos abiertos agroindustria. Ministerio de Agroindustria de la Nación. https://datos.magyp.gob.ar

Sivri, D., Köksel, H., and Bushuk, W. 1998. Effects of wheat bug (*Eurygaster maura*) proteolytic enzymes on electrophoretic properties of gluten proteins. N. Z. J. Crop Hortic. 26:117-125.

Souza, E. J., Martin, J. M., Guttieri, M. J., O'Brien, K. M., Habernicht, D. K., Lanning, S. P., McLean, R., Carlson, G. R., and Talbert, L. E. 2004. Influence of genotype, environment, and nitrogen management on spring wheat quality. Crop Sci. 44:425-432.

Torbica, A., Mastilović, J. Pojić, M., and Kevrešan, Ž. 2014. Effects of wheat bug (*Eurygaster* spp. and *Aelia* spp.) infestation in preharvest period on wheat technological quality and gluten composition. Sci. World J. 2014:148025.

Torbica, A., Živančev, D., Mastilović, J., Knežević, D., and Bodroža-Solarov, M. 2011. Impact of changes in climate conditions on the technological quality of wheat. Pages 617-621 in: Proceedings of the 46th Croatian and 6th International Symposium on Agriculture, Opatija, Croatia.

Triboï, E., Abad, A., Michelena, A., Lloveras, J., Ollier, J. L., and Daniel, C. 2000. Environmental effects on the quality of two wheat genotypes: 1. Quantitative and qualitative variation of storage proteins. Eur. J. Agron. 13:47-64.

Tronsmo, K. M., Magnus, E. M., Baardseth, P., Schofield, J. D., Aamodt, A., and Færgestad, E. M. 2003. Comparison of small and large deformation rheological properties of wheat dough and gluten. Cereal Chem. 80:587-595.

Yandamuri, R. C., Gautam, R., Darkoh, C., Dareddy, V., El-Bouhssini, M., and Clack, B. A. 2014. Cloning, expression, sequence analysis and homology modeling of the prolyl endoprotease from *Eurygaster integriceps* Puton. Insects 5:762-782.

[Received November 17, 2016. Accepted February 28, 2017.]

AUTHOR QUERIES

PLEASE RESOLVE ALL QUERIES 1

Q: 1_Thank you for publishing with Cereal Chemistry. Please feel free to contact me, Carol Duling (cduling@scisoc.org), with questions and feedback. In addition to reviewing the galley proof, please be sure also to complete the copyright form and return it, because publication cannot proceed without it. You can retrieve the copyright form from the section below where you retrieved the galley proof. Here are suggestions for marking the galley proof. Please mark changes clearly. In general, please use the insertion, deletion, and replacement annotation tools rather than sticky notes to mark corrections. (Shortcuts: position the cursor and press the insert button on your keyboard to add text, select text and press insert to replace it, and select text and press delete to remove it.) Because making significant changes at the galley proof stage is expensive, please help us by limiting your changes whenever possible (for example, by only correcting errors). Please take the time to review the article carefully, including checking author names and affiliations, because any changes requested after the galley proof stage are at the discretion of the publisher and might incur a charge. If you happen to experience difficulty uploading your corrected proof to the system, please send me two emails: one stating the problem and a second with the files attached. Also, if you happen to send me a fax, please also email to confirm receipt. Thank you.

Q: 2_Names OK as listed? We prefer full given (first) names, but initials are OK if that is your preference.

Q: 3_The in-text citation "Yakovenko et al. 1973" is not in the reference list. Please correct the citation, add the reference to the list, or delete the citation.

Q: 4_Revision OK? β-mercaptoethanol

Q: 5_A trade name must always be followed by a common noun. Thus, Vaseline petroleum jelly.

Q: 6_Revision OK? steamed

Q: 7_Revision OK? Universidad Nacional de Córdoba

Q: 8_Revisions OK? electropherograms, *Eurygaster*

Q: 9_Revision OK? alveograph

Q: 10_Revision OK? SBV

Q: 11_Please confirm that the archived method 56-11.01 was used. 56-11.02 is the current method.

Q: 12_The URL http://www.siia.gov.ar/ has been redirected to https://datos.magyp.gob.ar/. Please verify the URL. Also, added “Datos abiertos agroindustria” – OK?

Q: 13_Figures will be in black and white in print and in color online.

Q: 14_Please check the MWS description, because β-galactosidase (116,250) and lysozyme (14,400) aren’t indicated on the figure, and there’s a 31,000 MWS shown that isn’t included in the list.

AUTHOR QUERIES

PLEASE RESOLVE ALL QUERIES **2**

Q: 15_Revision OK?