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A novel α-amylase-lipase formulation as anti-staling agent in durum wheat bread

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Abstract

The aim of this work has been to evaluate the anti-staling effect exerted by a novel α-amylase-lipase enzyme formulation on durum wheat bread, in comparison with four different commercial amylase preparations and with control without added enzymes. Bread-making trials were carried out at industrial level. Sliced bread, packed under modified atmosphere, was analyzed for texture profile, moisture content, and water activity during 90 days. Crumb sections were submitted to environmental scanning electron microscopy at the end of the storage period. The α-amylase-lipase enzyme preparation showed synergistic interactions in preventing staling. In particular, bread added of these two enzymes in mixture was always softer and more chewable than either control or samples added of other enzymes. Moreover, α-amylase-lipase exhibited the most marked effect in slowing down both hardening and chewiness changes during time. Starting from 7 days of storage, both water activity and moisture content of bread added of α-amylase-lipase were higher than in control. Starting from 68 days the moisture content of α-amylase-lipase-added bread became lower than that of other enzyme-added breads, and at the end of storage also water activity was significantly lower. Pore morphology of α-amylase-lipase-added bread appeared markedly different from that of control bread.

Keywords: enzymes, durum wheat semolina, TPA, a_w, bread staling

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Introduction

Durum wheat (*Triticum turgidum* L. subsp. *durum* Desf.) is usually the raw material for pasta. In addition, it is also used in the production of various types of bread all over the Mediterranean area (Quaglia, 1988). This ancient habit is still particularly diffused in the Southern regions of Italy and in its main islands (Sicily and Sardinia), where the pedoclimatic conditions are more favorable to durum than soft wheat cultivation (Fadda, Santos, Piga, & Collar, 2010; Pasqualone, 2012).

Bread is a worldwide essential food but quickly loses its desirable qualities such as texture, flavor, and freshness. In fact, when stored at room temperature, most types of breads or bakery products with a spongy crumb undergo a progressive and often rapid deterioration of quality, commonly known as 'staling'. Some authors (Boyacioglu & D'Appolonia, 1994a) have investigated the staling behavior of bread made of durum wheat semolina or blends of durum and soft wheat flour. Durum wheat bread has been reported to have longer shelf-life than soft wheat bread, due to high water-binding capacity of semolina (Boyacioglu & D'Appolonia, 1994b).

The mechanism of bread staling involves the re-crystallization (retrogradation) of gelatinized starch, responsible for the texture changes that take place during bread storage (Gray & Bemiller, 2003). During baking, amylose and amylopectin tend to separate and to accumulate within the starch granules and in the intergranular space in the form of double helices (Conde-Petit, Nuessli, Handschin, & Escher, 1998; Hug-Iten, Escher, & Conde-Petit, 1999). After baking, amylose retrogrades very quickly, stabilizing the initial structure and forming a more rigid, insoluble network. The subsequent increase in bread firmness is due to further physic-chemical changes affecting the starch components, especially the amylopectin fraction.

To prevent these phenomena, anti-staling agents such as enzymes and emulsifiers are used in bread-making, as they interfere with the re-association of amylose, amylopectin, or both (Purhagen, Sjöö,

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& Eliasson, 2011). In particular, the enzymes most frequently used are α-amylases of fungal, microbial, or cereal origin (Rosell, Haros, Escrivà, & Benedito de Barber, 2001). These enzymes positively act on bread texture by producing low-molecular-weight dextrins that, in turn, interfere with the amylopectin retrogradation and with the protein-starch interactions occurring during bread storage (Martinez-Anaya, Devesa, Andreu, Escrivà, & Collar, 1999). Their use optimizes the amylase activity of flour and slows down bread staling (Goesaert et al., 2005). Moreover, the α-amylases reduce the firming rate (Rosell et al., 2001), increase bread volume, improve crumb grain, crust and crumb color, and contribute to flavor development (Martinez-Anaya et al., 1999).

A few studies, carried out mainly by micro baking tests, have been conducted on the lipases (Castello, Jollet, Potus, Baret, & Nicolas, 1998; Castello, Potus, Baret, & Nicolas, 2000; Schaffarczyk, Østdal, & Koehler, 2014). These enzymes have been reported to positively influence bread quality characteristics. In fact, they act on the lipid fraction by increasing the amount of molecules with emulsifying properties (diacylglicerols and monoacyldiglicerols) (Castello et al., 1998) which, in turn, positively influence bread volume (Castello et al., 2000). Increases of bread volume of 56-58% have been reported, depending on the type and concentration of the added lipase (Schaffarczyk et al., 2014). Moreover, exogenous lipases improve the rheological properties of the dough, and delay bread staling (Castello et al., 2000; Olesen, Si, & Donelyan, 2000).

Hence, the aim of the present work has been to evaluate the anti-staling effect exerted by a novel α-amylase-lipase enzyme formulation in comparison with four different commercial α-amylase preparations, and with control without added enzymes. The enzymes were tested in durum wheat bread-making trials carried out at industrial level. Sliced bread, packed under modified atmosphere, was analyzed for texture profile, moisture content, and water activity during 90 days. The environmental scanning electron microscopic analysis of crumb sections was also carried out at the end of storage to point out morphological differences of crumb pores.

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2. Materials and methods

2.1 Enzymes

Four commercial enzyme preparations and a novel formulation were used: (i) the maltogenic amylase NM 15 new (Ri.fra, Marsala, Italy) (NM 15), containing 1500 α-amylase units/g; (ii) the maltogenic amylase MAX LIFE P15 (Danisco, Cernusco sul Naviglio, Italy), (ML P15) containing 1500 glucan 1,4-α-maltohydrolase units/g; (iii) the maltogenic amylase VERON x Tender (AB enzymes, Darmstad, Germany) (VxT) containing 1000 bacterial α-amylase units/g; (iv) the maltogenic amylase VERON MAC (AB enzymes, Darmstad, Germany) (VMAC) containing 1000 fungal α-amylase units/g; (v) the novel α-amylase-lipase enzyme formulation Prozyme's 3010 fresh (Bontà Infinite, Terme Vigliatore, Messina, Italy) (3010) containing 150 MANU/g (α-amylase 0.67 FAU-F/g, lipase 0.19 KLU/g). All the enzyme preparations were used according to the recommended manufacturer's dosage.

2.2 Sample preparation

Commercial durum wheat (*Triticum turgidum* L. subsp. *durum* Desf.) re-milled semolina was provided by Molino S. Paolo of Mario Paolo Gallo & C. S.p.A. (Noto, Italy). Compressed yeast (AB Mauri, Casteggio, Italy) and NaCl employed in bread-making process were purchased at a local retailer. Bread was prepared at a local bread-making factory (Valle del Dittaino Società Cooperativa Agricola, Assoro, Italy) according to a consolidated industrial process based on the following formulation, with no added fat: durum wheat semolina, water (66% semolina basis), compressed yeast (0.47% semolina basis), NaCl (2.2% semolina basis). Six types of bread were produced: the control (without the addition of enzymes), and five α-amylase-added types (NM 15,

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ML P15, VxT, VMAC, and 3010), each containing one of the five tested enzyme preparations added – at the manufacturer's recommended dosage – to the above formulation without modifications. The ingredients were mixed for 17 min by means of a diving arms mixer (Pietro Berto, Marano Vicentino, Italy). The final dough temperature was 26±1 °C. The dough was rested in bulk for 15 min, then was scaled by the automatic volumetric divider Omega (Pietro Berto, Marano Vicentino, Italy) into 145 portions weighting 1160±20 g, that were automatically shaped into round loaves by the conical rounder CO 3000 (Turri, Costa di Rovigo, Italy), and were placed for 150 min into a proofer (Pavailler Engineering, Galliate, Italy) set at 32 °C and 66% RH. The subsequent baking was carried out at 240 °C for 60 min, in an industrial tunnel oven (Pavailler Engineering, Galliate, Italy). The baked loaves, weighting approximately 1 kg each, were automatically transported to a cooling chamber (S.L.C, Copit, Trecate, Italy) set at 20±2 °C, where were kept for 120 min. After cooling, the loaves were sliced by means of an automatic slicing machine (Brevetti Gasparin, Marano Vicentino, Italy) to 11±1 mm thickness.

2.3 Packaging and storage conditions

About 500 g of sliced bread per loaf were packaged under modified atmosphere (70:30 N_2:CO_2). The packages were constituted by two plastic films (kindly provided by Cryovac Sealed Air, Elmwood Park, NJ, USA), one for the lid and another for the bottom. The latter was thermoformed (MIX 9000, Tecnosistem, Coccaglio, Italy) into a bowl before inserting the bread slices. The characteristics of thickness, water vapor transmission rate (WVTR, g/m^2/24 h), and oxygen transmission rate (OTR, cm^3/m^2/24 h) of the bottom film (type T6011B, Cryovac Sealed Air, Elmwood Park, NJ, USA) were: 275 µm thickness, WVTR ≤ 10, OTR = 1; those of the lid film (type T9250B, Cryovac Sealed Air, Elmwood Park, NJ, USA) were: 125 µm thickness, WVTR < 10, OTR < 3. As conventionally done by the producer, food-grade ethanol was sprayed on the slices

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into the packages, whose internal atmosphere was then replaced by a 70:30 N_2:CO_2 gas mixture (MAP MIX 9001 ME, Dansensor Italia, Segrate, Italy). Nitrogen and carbon dioxide for bread packaging and ethanol were respectively supplied by SOL (Monza, Italy) and Distillerie F.lli Russo (Santa Venerina, Italy). The packaged samples were stored up to 90 days at 20±2°C and 60±2% RH. Sample withdrawals were carried out, for the subsequent analyses, at 0, 7, 14, 21, 28, 48, 68, and 90 days.

2.4 Chemical and rheological analyses of durum wheat semolina

Protein content was determined by means of Infratec 1241 Grain Analyzer (Foss Tecator, Höganäs, Sweden), based on Near Infrared Transmittance. A calibration curve (range 8.3-15.3) was previously set up on the results of Kjeldahl nitrogen method and validated according to ISO 12099:2010 method (ISO, 2010) on a large set of samples of durum wheat grain and semolina. Ash and moisture content were determined according to the AACC 44–19 and AACC 08–01 methods (AACC, 2000), respectively. Dry gluten and gluten index were determined by using a Glutomatic System consisting of Glutomatic 2200, Centrifuge 2015, Glutork 2020 (Perten Instruments AB, Huddinge, Sweden), according to the UNI 10690 method (UNI, 1979). The α-amylase activity was determined by using the Falling Number 1500 apparatus (Perten Instruments AB, Huddinge, Sweden), following the ISO 3093:2009 method (ISO, 2009). The color parameters in the color space *L**, *a**, *b** were determined by Chromameter CR-300 (Minolta, Osaka, Japan), under the illuminant D65. The farinograph indices were determined according to the AACC 54-21 method (AACC, 2000) by a farinograph (Brabender instrument, Duisburg, Germany), equipped with the software Farinograph®. Alveograph trials were performed according to the 10453 method (UNI, 1995) using an alveoconsistograph, equipped with the software Alveolink NG (Tripette et Renaud, Villeneuve-la-Garenne, France).

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164

165 *2.5 Determination of moisture content and water activity of bread crumb*

166 Moisture content of bread crumb was determined by oven drying at 105 °C until constant weight
167 according to AOAC method no. 945.15 (AOAC, 2000). Three bread slices (11±1 mm thickness)
168 were used, and moisture was determined on a crumb cylinder (40 mm diameter) taken from the
169 center of each slice. Water activity (a_w) was determined by Hygropalm 40 AW (Rotronic
170 Instruments Ltd, Crawley, UK) according to manufacturers' instructions. Three bread slices (11±1
171 mm thickness) were used, after removal of the crust. For each set of determinations, separate loaves
172 were considered.

173

174 *2.6 Texture Profile Analysis of bread*

175 The Texture Profile Analysis (TPA) of bread was carried out by means of an Universal Testing
176 machine (model 3344, Instron, Norwood, MA, USA), equipped with a 5.0 cm diameter cylindrical
177 probe and a 2000 N load cell. Data were acquired through Bluehill® 2 software (Instron, Norwood,
178 MA, USA). Cyclic compression tests (30s gap between first and second compression) were set up:
179 the crosshead speed was 3.3 mm/s, the force required to compress the samples by 40% was
180 recorded on 5-cm side square portions of 24-mm thick slices, and the average value of five
181 replicates was taken.
182 Four primary TPA parameters (hardness, cohesiveness, springiness, and resilience), and two
183 derived parameters (gumminess and chewiness) were calculated: hardness (N), defined as the peak
184 force during the first compression cycle; cohesiveness, i.e. the adimensional ratio of the positive
185 force area during the second compression cycle to the positive force area recorded during the first
186 compression cycle, or Area 2/Area 1; springiness (mm), i.e. the elastic recovery that occurs when
187 the compressive force is removed, defined as the height to which the food recovers during the time

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that elapses between the end of the first and the start of the second compression; resilience, defined as the adimensional ratio between the negative force input and the positive force input during the first compression, or Area 5/Area 4; chewiness (N), defined as the product of hardness, resilience and springiness.

2.7 Headspace gas composition analysis

The internal O_2 and CO_2 composition of packages was determined by means of Dansensor Checkpoint portable gas analyzer (Dansensor, Ringsted, Denmark). Ten mL of headspace were analyzed, with three replications.

2.8 Environmental scanning electron microscopy

At 90 days of storage, bread crumb was submitted to environmental scanning electron microscopy (ESEM), carried out by means of the Inspect S50 electron microscope (FEI, Hillsboro, Oregon, USA) operating at an accelerating voltage of 15 kV. Before testing, the first layer of each sample was carefully removed. Then, the sample (small portions of bread cut with razor blade) were prepared for ESEM analysis. Bread samples were mounted on specific stubs using a thin spatula and transferred to the microscopy support. Digital images at 100× were acquired by using the Imix software (Princeton Gamma Tech, Princeton, USA).

2.9 Statistical analysis

The statistical analyses were performed by using the CoStat Anova Statistic Software version 6.311 (Cohort, Monterey, CA, USA) for Windows. Textural properties, moisture content, a_w and pCO_2 data were submitted to analysis of variance (ANOVA) using Duncan's multiple range test.

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3. Results and discussion

3.1 Durum wheat re-milled semolina composition

The compositional data of durum wheat re-milled semolina are shown in Table 1. Protein content and farinograph parameters were in the ranges previously observed in quality surveys about Southern Italian re-milled semolina used for bread-making (Pasqualone, Caponio, & Simeone, 2004; Raffo et al., 2003). The observed values of gluten content and gluten index, however, were borderline (Brescia et al., 2007; Pasqualone et al., 2004); in particular, gluten content was low and coupled to very high gluten index, so as to induce expecting a very compact bread. Also the alveograph parameters, especially the P/L ratio – high, although in line with durum milling products (Brescia et al., 2007; Pasqualone et al., 2004; Raffo et al., 2003) – further sustained these expectations. The high value of Falling Number indicated a limited fermentative activity, suitable to be supplemented by exogenous amylases. The bright yellow color, due to carotenoid pigments, is a typical feature of re-milled semolina that is partly transferred to final bread crumb (Pasqualone et al., 2004; Pasqualone, Summo, Bilancia, & Caponio, 2007) and is very appreciated by consumers. Similar yellow levels were previously observed (Brescia et al., 2007; Fadda et al., 2010; Pasqualone et al., 2004).

3.2 Crumb moisture content and water activity of bread

The variations of moisture content of food are related to its stability and quality (Pomeranz & Meloan, 1994). Moistness is a favorable sensory attribute for baked goods because it is synonymous of soft and tender products. Moreover, moisture has a plasticizing effect on the crumb network, and its loss contributes to crumb hardening. This event, together with starch recrystallization, is one of

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the major factors involved in bread staling (Piazza & Masi, 1995). Usually, lower moisture variations correspond to slower firming rate.

As shown in Table 2, all the samples exhibited a decrease of moisture content during storage, but at different extents. At the beginning, the highest moisture content was observed in NL P15-added bread. The control bread contained 36.37% moisture (not significantly different than 3010 and VxT-added breads), and showed a marked decrease during 90 days. The value reached after 7 days was in the range reported in literature for 1 kg-sized durum wheat sourdough-based bread after 9 days of unpackaged storage, without slicing (Raffo et al., 2003).

The variations of moisture content were less pronounced in presence of the enzyme preparations. With the only exception of VxT-added bread, starting from 7 days of storage all the enzyme-added bread types appeared moister than control. However, bread supplemented by the 3010 α-amylase-lipase agent showed, starting from 68 days, a lower moisture content than the other enzyme-added samples, probably due to minor amylase activity of this formulation, leading to lower formation of moisturizing dextrines. At the end of the storage period, NM 15-added bread showed the moister crumb, followed by VMAC, NM 15 and ML P15 samples – without significant differences among them – and by 3010-added bread.

The changes of a_w, summarized in Table 3, basically paralleled the variations in moisture content. At the beginning of storage, control bread showed no significant differences respect to NM 15, ML P15, and VMAC-added breads while, starting from 7 days, it exhibited a_w values significantly lower than those of the other samples. Among the enzyme-added breads, after 90 days the lowest values of a_w were observed in bread supplemented by 3010 α-amylase-lipase formulation.

3.3 Textural characteristics of bread

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Texture is an important characteristic in consumer's perception of food and influences the purchasing decisions. Meilgaard, Civille, & Carr (1991) define food texture as the sensory manifestation for the structure of products in terms of their: (i) reaction to stress by the kinesthetic sense in the muscles of the hand, fingers, tongue, jaw, or lips (e.g. adhesiveness, cohesiveness, hardness, etc.), and (ii) tactile feel properties measured by the tactile nerves in the surface of the skin of the hand, lips, or tongue (e.g. oiliness, tenderness, moistness, etc.). The TPA results, obtained by means of double cycle compressions at 40% depth, evidenced the structural changes that affected the samples during storage and clearly differentiated control from the various enzyme-added samples (Figure 1). Crumb hardness showed significant differences (p<0.01) among the five treatments and the control. These differences, less evident at the beginning, were particularly marked starting from 7 days of storage. In fact, in spite of MAP, the control bread showed 80 N hardness – not easily acceptable by consumers – after only 7 days. This value was higher than those reported in literature for 1 kg-sized durum wheat sourdough-based breads after 9 days of unpackaged storage, without slicing (Raffo et al., 2003). After 28 days, control bread crumb even reached 180 N hardness, that was maintained for the rest of the storage period as it corresponded to complete hardening. The particularly high hardness values observed in control bread during storage were probably imputable to the characteristics of starting re-milled semolina that, in absence of α-amylase corrections, coupled a low fermentative activity with low gluten content and excessive gluten tenacity.

During the whole storage period considered, the enzyme-containing breads were markedly softer than control, and only a slight increase of hardness was observed, demonstrating the anti-staling effectiveness of all the enzymes tested. The VxT-added bread even reported a decrease in hardness after 14 days. As regards to the 3010-added bread, its consistency was almost constant throughout the whole storage period, so that after 90 days it still showed a hardness value lower than 30 N.

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Springiness did not provide information of great value as it did not present significant variations during storage (data not shown). On the contrary, the resilience, that shows how well a product 'fights to regain its original position after a stress' (Abdelghafor, Mustafa, Ibrahim, & Krishnan, 2011), was very informative. The resilience of control decreased significantly with storage time, indicating a marked tendency to become crumblier, with a less cohesive structure. At 28 days this kind of bread reached the lowest possible resilience value, accounting for about 0.35, and became extremely fragile and crumbly. The resilience of all the enzyme-supplemented bread loaves remained quite high during the whole storage period, with a slightly worst ageing pattern for VMAC-added bread, as was observed for hardness.

The results evidenced that all the enzymes tested had positive anti-staling effects on durum wheat bread. The addition of α-amylase significantly slowed down the water loss and firmness increase, confirming the literature data (Błaszczak et al., 2004; Bollaín, Angioloni & Collar et al., 2005; Jiménez & Martínez-Anaya, 2001). The 3010 α-amylase-lipase enzyme preparation showed synergistic interactions in preventing staling: it exhibited the most marked effect in slowing down both hardening and chewiness during storage. The VxT-supplemented bread also showed an interesting anti-staling effect and could be considered in future researches in combination with 3010 agent to achieve further improvement of durum wheat bread shelf-life.

3.4 Headspace gas composition analysis

The packaging material showed good barrier performances. In fact, O_2 was not detectable inside the packages through the whole duration of the shelf-life test. Also, the internal content of CO_2 accounted for 31.46% at the beginning, and decreased to 24.26% after 90 days, probably mainly due to gas dissolution into the food matrix during storage. Furthermore, no significant differences were observed among CO_2 values in the different bread types (data not shown).

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307 *3.5 Morphological features of crumb pores*

308 The microstructure of bread samples was studied after 90 days of storage in order to visualize the
309 effect of different enzymes used in bread formulation on the morphological features of crumb pores.
310 The ESEM micrographs of crumb section (Figure 2), able to give qualitative information on pore
311 structure (Datta, Sahin, Sumnu, & Keskin, 2007), showed that all breads were characterized by
312 pores of different size, heterogeneously distributed. However, in presence of 3010, the pores
313 appeared to be slightly smaller and more spherical as compared to other samples. This is consistent
314 with the lower level of moisture and a_w value observed in 3010 bread at the end of the storage
315 period: water loss probably induced slightly greater pore shrinkage than in other samples. The pores
316 of bread supplemented by NM 15 appeared to be the largest, while those of breads treated with ML
317 P15 and VxT appeared to be elongated. The most irregular pore distribution was observed in
318 VMAC-added breads, where a population of larger cells coexisted with smaller ones. Control bread
319 appeared markedly different from the enzyme-supplemented breads: it showed a more opened
320 structure, and a dry and opaque crumb, with rigid and fragile features typical of retrograded starch.
321 Other authors (Błaszczak, Sadowska, Rosell, & Fornal, 2004) observed similar structural
322 differences, although less evident, between 5-days stored white breads added of α-amylases of
323 fungal and bacterial origin and samples obtained without the addition of enzymes.
324 Finally, it has to be reported that, although sensory analysis was not carried out, at the end of
325 storage period all the samples appeared devoid of anomalies, apart the unacceptable hardness of
326 control, with no anomalous colors and/or odors (data not shown).

327

328 **4. Conclusions**

329 The evolution of textural properties, crumb moisture, and a_w during bread storage confirmed that

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amylases are effective in slowing down bread staling also in durum wheat bread, and pointed out the significantly greater effect provided by the 3010 α-amylase-lipase combination, that positively modified textural and crumb grain properties of bread. The experimental data also indicated the close connection between moisture content and textural properties, with special regard to crumb hardness, resilience, and chewiness.

The obtained results have an immediate practical application, since all the trials have been directly carried out at industrial level. Hence, the producers may take advantage of the increases in shelf-life to enhance the diffusion and marketing of durum wheat bread far from the areas of production.

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Figure captions

Figure 1. Trends of variations of hardness, resilience, and chewiness, in five enzyme-supplemented durum wheat breads and control (without enzymes).

Figure 2. Environmental scanning electron microscopy images of crumb of five enzyme-supplemented durum wheat breads and control (without enzymes).

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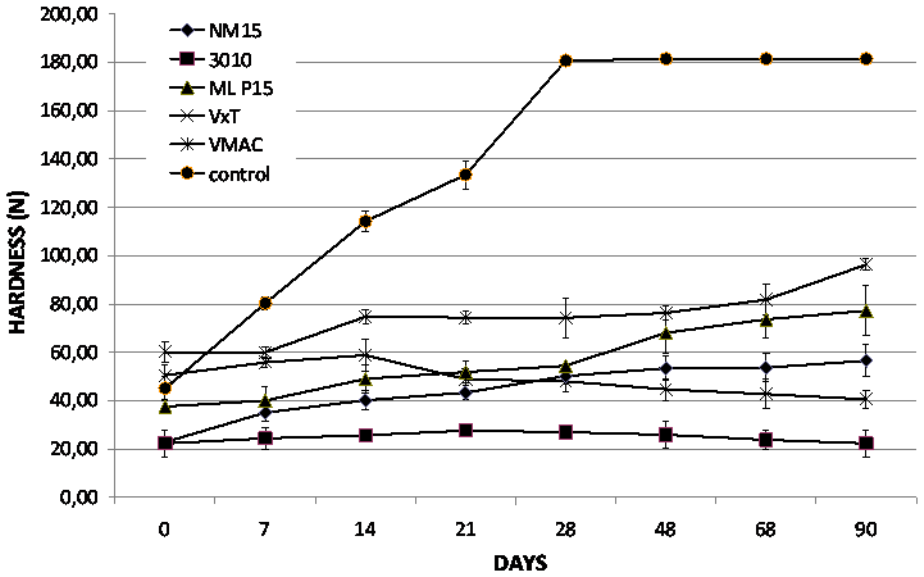

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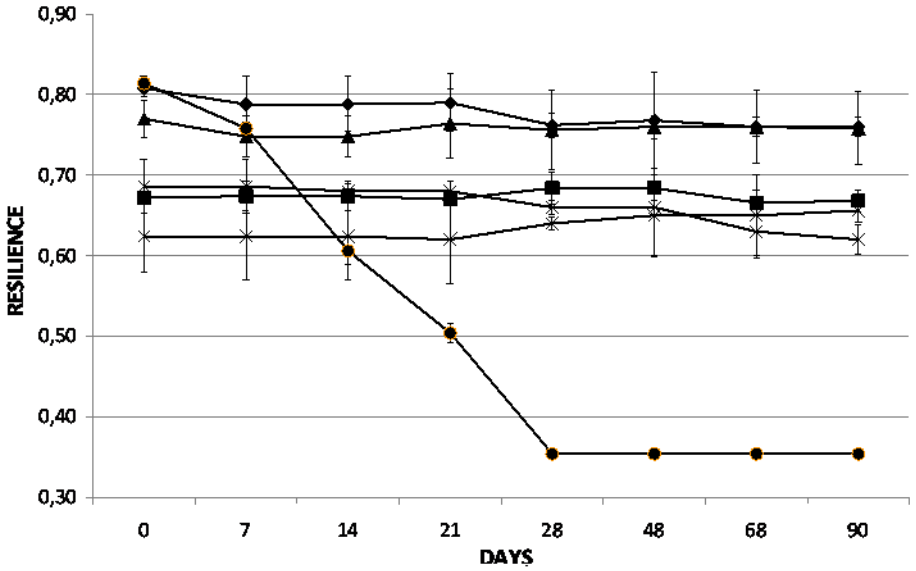

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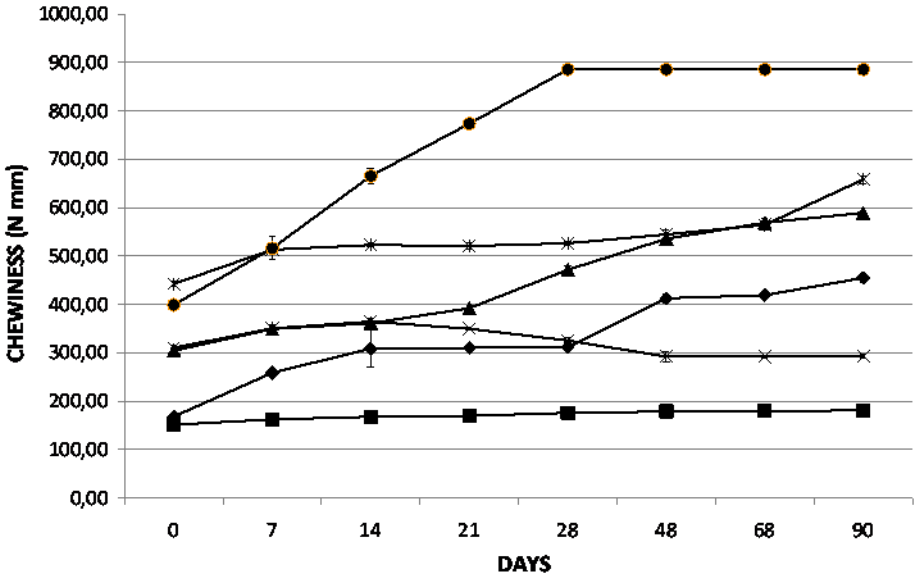

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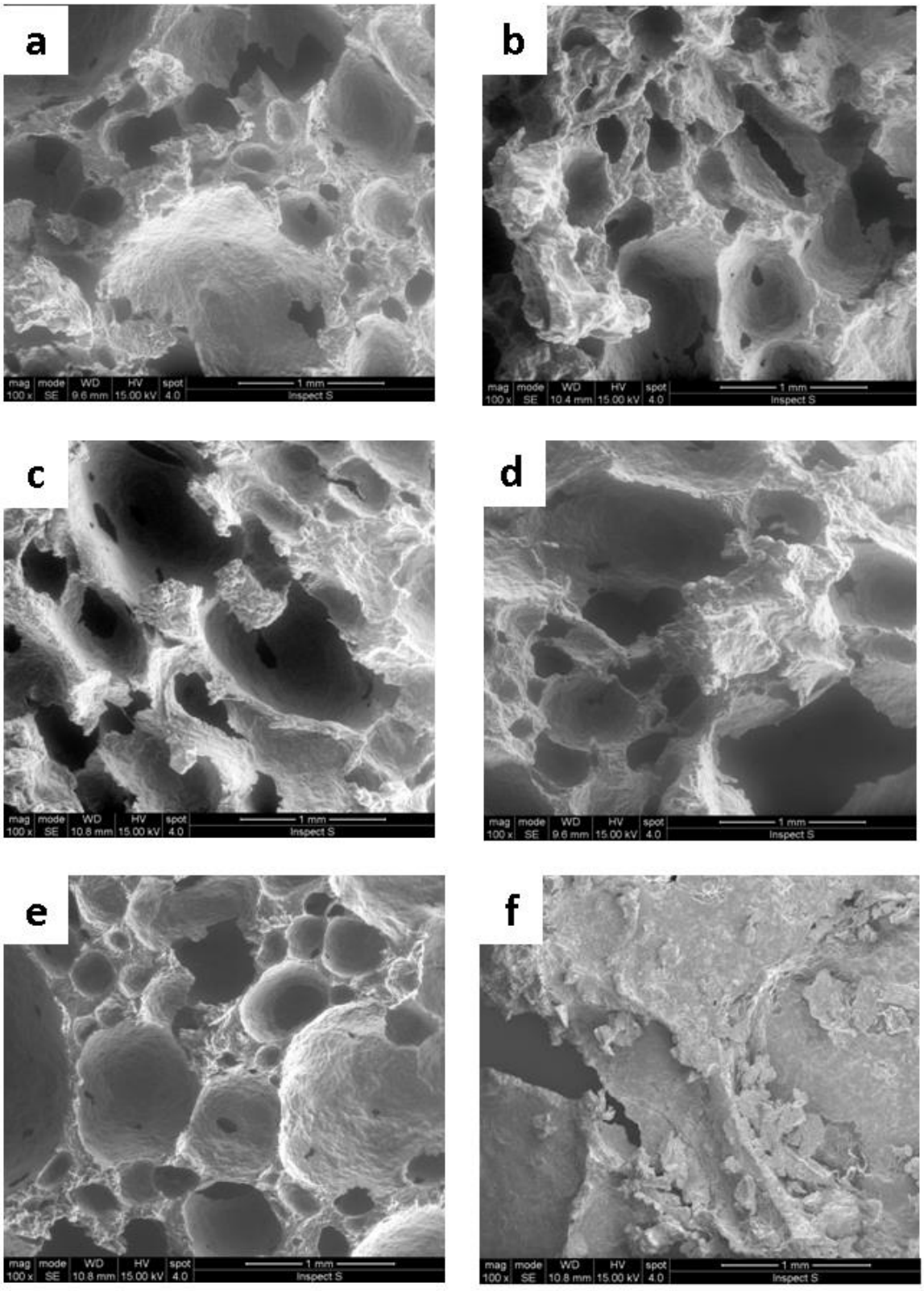

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457 **Table 1.** Quality attributes of durum wheat re-milled semolina used in the bread-making trials (n = 3). B.U. = Brabender Units
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Parameter	Value
Chemical composition	
Protein (% dry basis)	11.05±0.07
Ash (% dry basis)	0.82±0.03
Falling Number (s)	487±1.41
Gluten (% dry basis)	7.77±0.26
Gluten Index	96±1.41
Colorimeter indexes	
Luminosity (*L**)	89.31 ±0.01
Red index (*a**)	-2.38 ±0.05
Yellow index (*b**)	21.24 ±0.04
Farinograph parameters	
Water absorption at 500 B.U. (%)	54.70±0.28
Development time (s)	90±4.24
Dough stability (s)	342±4.24
Softening index (B.U.)	46±2.83
Alveograph trial	
Deformation energy (10^{-4} J) [W]	159.00±1.41
Configuration ratio curve (P/L)	2.17±0.05

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Table 2. Crumb moisture content (%) of five enzyme-supplemented durum wheat breads and control (without enzymes) during 90 days of storage (n = 3). Values followed by different lower case letters in column, and upper case letters in row, indicate significant differences (p<0.05)

	Storage time (days)							
	0	7	14	21	28	48	68	90
NM 15	$37.49^{bA} \pm 0.16$	$36.64^{aA} \pm 0.08$	$36.97^{aAB} \pm 0.74$	$36.70^{aAB} \pm 0.23$	$35.87^{aBC} \pm 0.36$	$35.05^{aC} \pm 0.70$	$35.07^{aC} \pm 0.64$	$34.11^{aD} \pm 0.45$
3010	$36.26^{cA} \pm 0.26$	$35.04^{cB} \pm 0.17$	$34.86^{bcB} \pm 0.54$	$33.99^{cC} \pm 0.57$	$33.84^{cC} \pm 0.29$	$32.10^{cD} \pm 0.43$	$29.98^{dE} \pm 0.08$	$29.94^{cE} \pm 0.91$
ML P15	$39.54^{aA} \pm 0.23$	$36.49^{aB} \pm 0.07$	$35.49^{bC} \pm 0.35$	$35.49^{bC} \pm 0.35$	$34.54^{bD} \pm 0.00$	$32.68^{cE} \pm 0.60$	$31.19^{cF} \pm 0.58$	$31.31^{bF} \pm 0.35$
VxT	$36.41^{cA} \pm 0.26$	$34.29^{dB} \pm 0.10$	$34.18^{cB} \pm 0.38$	$32.91^{dC} \pm 0.22$	$32.78^{dC} \pm 0.24$	$32.02^{cC} \pm 0.47$	$32.67^{bD} \pm 0.61$	$31.13^{bE} \pm 0.23$
VMAC	$37.18^{bA} \pm 0.28$	$36.18^{bB} \pm 0.24$	$34.31^{cC} \pm 0.21$	$34.31^{cC} \pm 0.21$	$34.25^{bcC} \pm 0.08$	$33.91^{bC} \pm 0.08$	$33.49^{bD} \pm 0.08$	$31.25^{bE} \pm 0.42$
Control	$36.37^{cA} \pm 0.21$	$34.54^{dB} \pm 0.08$	$33.24^{dC} \pm 0.14$	$31.36^{dD} \pm 0.17$	$28.15^{eE} \pm 0.30$	$23.42^{dF} \pm 0.10$	$23.42^{eF} \pm 0.10$	$23.42^{dF} \pm 0.10$

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Table 3. Water activity (a_w) of five enzyme-supplemented durum wheat breads and control (without enzymes) during 90 days of storage (n = 3). Values followed by different lower case letters in column, and upper case letters in row, indicate significant differences (p<0.05)

	Storage time (days)							
	0	7	14	21	28	48	68	90
NM 15	0.946^{aA} ±0.001	0.940^{abB} ±0.001	0.940^{aB} ±0.002	0.938^{aB} ±0.001	0.935^{aC} ±0.001	0.931^{aD} ±0.001	0.922^{aE} ±0.003	0.920^{aE} ±0.001
3010	0.940^{bA} ±0.001	0.935^{cB} ±0.001	0.921^{cC} ±0.001	0.920^{dC} ±0.001	0.919^{cC} ±0.002	0.920^{bC} ±0.000	0.913^{bD} ±0.002	0.906^{bE} ±0.001
ML P15	0.947^{aA} ±0.001	0.941^{aB} ±0.001	0.941^{aB} ±0.001	0.936^{bC} ±0.001	0.930^{abD} ±0.001	0.929^{aD} ±0.001	0.919^{aE} ±0.001	0.919^{aE} ±0.001
VxT	0.940^{bA} ±0.001	0.936^{bcAB} ±0.006	0.930^{bB} ±0.001	0.930^{cB} ±0.001	0.920^{cC} ±0.001	0.921^{bC} ±0.002	0.920^{aC} ±0.003	0.921^{aC} ±0.002
VMAC	0.946^{aA} ±0.001	0.940^{abB} ±0.001	0.930^{bC} ±0.001	0.930^{cC} ±0.001	0.924^{bcD} ±0.000	0.921^{bE} ±0.002	0.922^{aEF} ±0.002	0.919^{aF} ±0.001
Control	0.947^{aA} ±0.001	0.929^{dB} ±0.001	0.917^{dC} ±0.001	0.909^{eD} ±0.002	0.829^{dE} ±0.001	0.744^{cF} ±0.001	0.744^{cF} ±0.001	0.744^{cF} ±0.001

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Table 4. Correlation coefficients (*r*) among textural parameters, moisture content, and water activity measured in five enzyme-supplemented durum wheat breads and control (without enzymes) during 90 days of storage

	Resilience	Hardness (N)	Chewiness (N mm)
Hardness (N)			0.96**
Resilience		-0.81**	-0.65**
Moisture content (%)	0.82*	-0.74*	-0.63*
Water activity	0.85*	-0.83*	-0.68*

**Significant correlation at $p < 0.01$; *significant correlation at $p < 0.05$