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Effectiveness of vital gluten and transglutaminase in the improvement of physico-chemical properties of fresh bread

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1 **Effectiveness of vital gluten and transglutaminase in the improvement of physico-chemical**
2 **properties of fresh bread**

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27 **Abstract**

28 Transglutaminase (TG) and vital gluten (VG) were tested as improver agents in bread. The
29 experimental design evaluated TG (0, 0.05, 0.1, 0.2 g/100g flour), VG (0 and 1 g/100g flour), and
30 wheat flour strength (FS: weak -WF, medium -MF, and strong -SF). Bread specific volume,
31 texture, color, moisture content and water activity were assessed and elaborated with multivariate
32 statistical analysis (MANOVA). TG had a significant ($p \leq 0.001$) impact on bread specific volume,
33 color, texture and water status, while VG significantly ($p \leq 0.001$) impacted crust color and texture.
34 A significant ($p \leq 0.05$) synergic effect (TGxVG) was observed on texture, crust color and water
35 activity, MANOVA showed different effects, depending on flour strength. Optimal addition levels
36 for best bread quality (low hardness and high specific volume) were selected for each flour: WF 0.2
37 g/100g flour TG and 1 g/100g flour VG; MF, 0.1 and 0.2 g/100g flour of TG with 1 g/100g flour
38 VG; SF 0.1 g/100g flour TG with 1 g/100g flour VG.

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41 **Keywords:** Bread; Supplementation; Quality; Multivariate analysis; Texture; Volume.

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53 **1. Introduction**

54 Bread is a common staple food around the world. Over the years, food manufactures kept seeking
55 for innovative technologies and/or additives that diversify their choices and increase their profits,
56 maintaining quality and competitiveness of the product (Seravalli, Iguti, Santana, & Filho, 2011).
57 Currently, there is a mounting trend towards the use of natural additives to improve technological
58 features and to preserve quality during shelf-life. Considering that the functional properties of bread
59 dough greatly depend on the gluten proteins (Bonet, Blaszcak, & Rosell, 2006), much interest has
60 been directed to gluten network improver agents.

61 In bakery products, proteins and enzymes are commonly used to ameliorate processability and
62 quality properties of bread. Vital wheat gluten (VG) is used to fortify flour with lower protein
63 content, that does not match the bread-making requirements or is considered inadequate for bread-
64 making (Ortolan & Steel, 2017). The addition of VG can increase dough and bread yields, and
65 enhance mixing tolerance, as well as bread texture, color, crumb grain, sensory attributes and
66 storage stability (Borla, Leonor Motta, Saiza, & Fritza, 2004; Giannou & Tzia, 2016). Besides VG,
67 transglutaminase (TG) is an effective ingredient to improve gluten quality (Moore, Heinbockel,
68 Dockery, Ulmer, & Arendt, 2006), throughout the catalysis of the reaction between a ϵ -amino group
69 on protein-bound lysine residues and a β -carboxyamide group on protein-bound glutamine residues
70 leading to covalent cross-linking of proteins (Huang et al., 2010). Consequently, the
71 supplementation of wheat flour with this enzyme can have an important impact on several physico-
72 chemical features and sensory attributes of food products (Kieliszek & Misiewicz, 2014). Bread
73 enriched with TG showed improvements in volume, crumb structure, firmness, elasticity, and
74 water-holding capacity (Veraverbeke & Delcour, 2002; Dłuzewska, Marciniak-Lukasiak, & Kurek,
75 2005). Hence, adding a mix of VG and TG might be an interesting approach to standardize the
76 quality of wheat flours, and optimize the baking process, as well as bread quality.

77 During the last decades, extensive studies were addressed to the investigation of a spectrum of
78 wheat flour based-bread improvers (enzymes, emulsifiers and hydrocolloids) and their

79 combinations (Moore, Heinbockel, Dockery, Ulmer, & Arendt, 2006; Guarda, Rosell, Benedito, &
80 Galotto, 2004; Benezam, Steffolani, & León, 2009; Aleid, Al-Hulaibi, Ghoush, & Al-Shathri, 2015;
81 Eduardo, Svanberg, & Ahrné, 2015). To the authors' best knowledge, the effect of the combination
82 of wheat gluten and crosslinking enzymes on bread properties has not yet been considered in the
83 scientific literature. In this regard, the present work aimed to develop the optimal combination of
84 TG and VG for bread-making based on the quality evaluation of fresh bread made with flour of
85 different strength (weak, medium and strong).

86

87 **2. Materials and methods**

88 **2.1. Materials**

89 Three wheat flours (type 00) with different strength (weak – WF, medium – MF, and strong – SF
90 (Molino Agugiaro & Figna, Collecchio, PR, Italy) were used. Chemical and rheological parameters
91 of flours, as provided by the producer, are reported in Table 1. A bacterial Transglutaminase HI-
92 NET 001 (40 IU/g) and vital wheat gluten (Vitalor, Champtor, Moisture 6 g / 100 g, Protein: 77
93 g/100 g dry matter, Ash: 1g/100 g dry matter, Fat: 5g / 100 g dry matter, Starch: 11 g /100 g dry
94 matter) were obtained from Hi-Food (Parma, Italy) and Molino Agugiaro & Figna, (Collecchio, PR,
95 Italy), respectively. Sunflower oil (Eulip, Italy), sugar (Tereos, Italy), and dry yeast (Aliseo, Italia)
96 were purchased from a local market and a baking improver [Soft'r Panbauletto 1%; ingredients:
97 wheat flour, ascorbic acid (E300), enzymes (Amylase, Xylanase, Lipase, phospholipase)] was
98 provided by Puratos (Italy).

99 **2.2. Bread-making: formulation and production**

100 A multi-factorial experimental design was set up to study and optimize the level of addition of TG
101 and VG for bread-baking quality improvement. The two factors were tested using three flours with
102 different strength. Different levels of TG (0, 0.05, 0.1, and 0.2 g/100g flour) and VG (0 and 1
103 g/100g flour) were tested in a standard bread recipe used in industrial applications. The obtained
104 bread formulations were named, F0 (control), F1 (supplemented with 1 g/100g flour VG), F2

105 (supplemented with 0.05 g/100g flour TG), F3 (supplemented with 0.05 g/100g flour TG and 1
106 g/100g flour VG), F4 (supplemented with 0.1 g/100g flour TG), F5 (supplemented with 0.1 g/100g
107 flour TG and 1 g/100g flour VG), F6 (supplemented with 0.2 g/100g flour of TG), and F7
108 (supplemented with 0.2 g/100g flour TG and 1 g/100g flour of VG) as summarized in Table 2.
109 Dry ingredients (flour, yeast, sugar, salt and the improver, TG and VG) were placed in a spiral
110 mixer (MFITALY, Vicenza, Italy) and mixed at speed of 105 rpm for 2 min. Liquid ingredients
111 (water and oil) were then added and kneaded at speed of 210 rpm for 8 min. The dough was then
112 portioned into 550 g pieces, laminated (Econom 4000, Rondo, Burgdorf, Switzerland) into
113 rectangular shape (490*160*6 mm) and placed into plastic molds. For rising, molds were left under
114 controlled conditions (35 °C, 85% Relative Humidity) for 70 min. Afterward, doughs were baked
115 for 35 min at the temperature of 220 °C (Modus, Logiudice, Verona, Italy). The bread loaves were
116 cooled at room temperature for 3 h before analysis.

117 Two loaves for each sample were produced and two bread productions were carried out for a total
118 of 4 bread loaves for each sample.

119

120 **2.3. Bread characterization**

121 **2.3.1. Specific volume**

122 Specific volume was obtained by dividing loaf volume (determined with a standard rapeseed
123 displacement method) (AACC, 2001) for the corresponding loaf weight. Four measurements were
124 carried out for each sample. Specific volume was expressed in cm³/g.

125 **2.3.2. Texture profile analysis**

126 Texture properties were determined using TA.XT2 Texture Analyzer (Stable Micro Systems,
127 Godalming, UK) and analyzed using Texture Expert software (Stable Micro Systems, Godalming,
128 UK). Bread loaves were portioned mechanically into 1.2 cm thick slices (bread slicing machine,
129 MAC.PAN, Vicenza, Italy). Two overlapped slices were subjected to a Texture Profile Analysis
130 test, at 40 % strain with a compression plate (P/75) at 5 mm/s speed. At least eight measurements

131 were taken for each bread production. The obtained textural parameters were hardness (peak force
132 of the first compression cycle, N), cohesiveness (ratio of positive area during the second to that of
133 the first compression cycle), springiness (ratio of the distance from the start of the second area up to
134 the second probe reversal over the distance between the start of the first area and the first probe
135 reversal), chewiness (hardness x cohesiveness x springiness, N mm), and resilience (area during the
136 withdrawal of the penetration, divided by the area of the first penetration) (Alvarez, Canet, &
137 López, 2002).

138 **2.3.3. Color**

139 Color analysis was determined for the crust and crumb of each bread loaf. The measurements were
140 carried out using a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka, Japan) equipped with a
141 standard illuminant D65 and a 10° position of the standard observer. The results were expressed in
142 accordance with the CIE Lab system. The parameters determined were: L^* [0 (black) - 100 (white),
143 brightness], a^* ($-a^*$ = greenness and $+a^*$ = redness) and b^* ($-b^*$ = blueness and $+b^*$ = yellowness).
144 At least twelve determinations were performed for each loaf, evenly distributed on crust and crumb.

145 **2.3.4. Water activity and moisture content**

146 Water activity was measured at 25 °C with an Aqualab 4 TE (Decagon Devices, Inc. WA, USA).
147 The moisture content of crust and crumb (from loaf center) (g/100 g) was evaluated following the
148 standard method (AACC, 2001). At least three measurements were taken for two bread loaves for a
149 total of twelve measurements for each sample.

150 **2.4. Statistical analysis**

151 For each parameter, a Multivariate Analysis of Variance (MANOVA) model was used considering
152 FS, TG, and VG as fixed effects. The first MANOVA (3-way ANOVA) included the entire
153 experimental setup (FS, TG and VG), to investigate the variance and the significance of the studied
154 parameters as a function of all the factors at a significance level of $\alpha=0.05$. Then, three 2-way
155 ANOVAs (TG and VG) were conducted to assess the effects of the factors on the physico-chemical
156 properties of bread made with each flour. The percentages of total variations were computed to

157 explain the variance of each parameter, as a function of the main and interaction effects to evaluate
158 the contribution of each factor on its variability. Significant differences among the mean values
159 were calculated using Duncan's test. All experimental data were statistically analyzed using SPSS
160 version 19.0 (SPSS Inc., Chicago, IL, USA).

161

162 **3. Results and discussion**

163 **3.1. Multivariate assessment of FS, TG and VG effects on bread properties**

164 To assess the effectiveness of VG and TG as improving agents of fresh bread properties, a
165 multivariate analysis of variance was conducted. This analysis was based on the quantitative data of
166 the breads physico-chemical properties obtained from three flours of different strength and
167 supplemented with VG and TG. The obtained results (Table 3) revealed that TG and FS had
168 significant effects on bread specific volume, while VG and TGxVG did not show a significant
169 effect. Regarding the textural properties, TG, VG and FS and their interactions had a significant
170 effect. Color was significantly influenced by flour strength, TG and VG and TGxVG. Moisture
171 content depended exclusively on FS and TG, while water activity was significantly affected by all
172 the parameters except from VG. In the following sections, every bread property was studied
173 focusing on the significant effects identified (Table 3) to optimize TG and VG supplementation as a
174 function of flour strength.

175

176 **3.2. Effect of VG and TG supplementation on bread specific volume**

177 MANOVA results (Table 3) showed the significant effect of FS and its interaction with TG and VG
178 on specific volume. The contribution of TG and VG on each flour type was studied and the results
179 are reported in Figure 1A. Bread made with SF was found to have the highest specific volume.
180 VG alone (1 g/100g flour) did not have a significant effect on bread's specific volume (Table 3),
181 while its interaction with FS (VGxFS) was significant (Table 3). Therefore, a three 2-ways
182 ANOVA, considering the effect of TG and VG on specific volume for each flour, was carried out.

183 Specific volume of breads made from WF and MF were significantly influenced by TG, but not by
184 VG. However, in the case of SF, both TG and VG had significant effects: the addition of VG
185 showed a significant increase in the average specific volume from $4.74 \pm 0.03 \text{ cm}^3/\text{g}$ (0% VG) to
186 $5.20 \pm 0.32 \text{ cm}^3/\text{g}$ (1% VG).

187 The effect of TG supplementation was assessed for each type of flour and a significant interaction
188 was observed between FS and TG (Table 3). In general, it was observed that the effect of TG on
189 specific volume was correlated to flour type and levels of addition, as previously reported (Basman,
190 Köksel, & Ng, 2002; Steffolani, Ribotta, Pérez, & León, 2010; Gujral & Rosell, 2004). Specific
191 volume increased significantly as a function of TG addition, and was maximum at the highest TG
192 supplementation (0.2 g/100g flour) for WF (Figure 1B), possibly due to the ability of TG to
193 strengthen a weak gluten (Basman, Köksel, & Ng, 2002; Pongjaruvat, Methacanon, Seetapan,
194 Fuongfuchat, & Gamonpilas, 2014). Specific volume significantly increased at TG 0.1 g/100g flour,
195 and remained constant at 0.2 g/100g flour addition for MF with no significant differences between
196 the two levels (Figure 1C). In the case of SF based-bread (Figure 1D), specific volume increased
197 until 0.1, and then slightly decreased at 0.2 g/100g flour of TG addition, as previously reported (0.5
198 g/100g flour TG addition; Steffolani et al., 2010). This result might be possibly attributed to a too
199 strong dough, due to excessive crosslinking (Mohammadi, Azizi, Neyestani, Hosseinid, &
200 Mortazavian, 2015), which hindered its expansion during fermentation (Basman, Köksel, Ng, 2002;
201 Schoenlechner, Szatmari, Bagdi, & Tomoskozi, 2013). In general, it is possible to conclude that
202 enhancement of fresh bread specific volume was reached at low levels of TG substitution
203 (Schoenlechner, Szatmari, Bagdi, & Tomoskozi, 2013).

204

205 **3.3. Effect of TG and VG supplementation on fresh bread texture**

206 Textural properties of TG and VG breads are shown in Supplementary Material 1, and they were
207 affected by the addition of both VG and TG by themselves or in combination.

208 The sum square percent of the studied factors FS, TG and VG, as well as their interactions, were
209 calculated based on the results of tests of between-subjects' effects (Table 4). Results revealed that
210 all the textural attributes were significantly influenced by TG and FS. TG reduced hardness,
211 independently of flour strength, possibly because of TG crosslinking action that favoured gas
212 expansion and volume extension (Pongjaruvat, Methacanon, Seetapan, Fuongfuchat, &
213 Gamonpilas, 2014). VG significantly decreased only bread springiness. Likewise, the interaction
214 TGxVG significantly influenced all the studied parameters, especially springiness, indicating a
215 synergic effect of the two additives.

216 Three MANOVAs were conducted to investigate the contribution of TG, VG and their interaction
217 on the variability of the textural attributes for each flour type (Table 4). In the case of WF, TG
218 mainly controlled hardness (88.54%), cohesiveness (90.86%), chewiness (87.15%) and resilience
219 (57.09%), while chewiness was controlled by VG (89.10%). The interaction TGxVG had a
220 significant contribution in the variability of hardness (11%) and chewiness (12%). The contribution
221 of TG in MF was quite similar, in terms of hardness, chewiness and resilience, to WF. However,
222 springiness was controlled at 54% by TG and 39% by VG. Remarkably, cohesiveness was almost
223 equally controlled by TG and TGxVG interaction. Regarding SF, TG was the most important factor,
224 while springiness was controlled at 55% by TG, 16% VG and 28% by TGxVG.

225 Concluding, regardless of flour type, TG, VG had, in most cases, a synergic effect on texture. For
226 WF, TG reduced hardness and increased cohesiveness and chewiness. No significant difference was
227 observed between 0.1 and 0.2 g/100g flour TG supplementation (F4 and F6) on springiness. VG
228 decreased springiness. For MF, TG reduced hardness. An increase in cohesiveness and resilience
229 was also observed, consistently with the findings of Wang, Huang, Kim, Liu, & Tilley (2011).
230 However, at high level of TG, VG addition increased hardness, suggesting a negative effect on
231 bread texture, possibly due to excessive crosslinking (Basman, Köksel, & Ng, 2003; Gerrard et al.,
232 1998). Regarding bread made from SF, VG induced a slight increase in springiness and
233 cohesiveness, whereas TG influenced all parameters. The addition of 0.1 g/100g flour of TG and 1

234 g / 100 g flour of VG showed the lowest hardness and chewiness and the highest springiness.
235 Springiness was increased due to enhanced elasticity of the gluten network conferred by TG and
236 VG (Wu & Corke, 2005). As for hardness, 0.2 g/100g flour TG addition (F6) resulted in a
237 significant increase compared to 0.1 g/100g flour of TG and 1 g / 100 g flour of VG (F5), as well as
238 to 0.2 g/100g flour of TG and 1 g / 100 g of VG (F7). However, with added VG, hardness
239 significantly decreased, confirming the synergic effect between exogenous enzymes and proteins.
240 According to Basman, Köksel, & Ng (2002), the optimum TG level for the best bread quality can
241 be obtained from maximum volume and from the minimum hardness versus addition level. This
242 assumption was based on the fact that hardness is inversely related to volume, as previously
243 evidenced in several studies (Miñarro, Albanell, Aguilar, Guamis, & Capellas, 2012; Sabanis &
244 Tzia, 2016), and was consistent with the findings of this work. Indeed, Pearson's correlation
245 coefficient indicated that hardness was inversely correlated to specific volume ($r=-0.776$).
246 Concluding, for WF, optimum formulation was F7. In the case of MF based-bread, F5 and F7 flour
247 gave similar results, and F5 enabled the best enhancement for SF based-bread.

248

249 **3.4. Effect of TG and the VG supplementation on color of fresh bread**

250 Color is an important quality trait of bread. Crust is associated with a brown surface of bread, while
251 crumb is the inner white spongy structure beneath the crust (Mohd Jusoh, China, Yusof, & Abdul
252 Rahmana, 2009). Color properties of fresh bread supplemented with TG and VG are summarized in
253 Supplementary Material 2.

254 Regarding crumb color, VG addition showed slight but significant effect on SF based-bread crumb
255 yellowness (b^*) and brightness (L^*). However, in the case of WF and MF based-breads, VG did not
256 have significant influence. Such result might be attributed to the fact that 1 g/100g flour VG
257 enrichment was not enough to contribute in color changes. Indeed, it was revealed VG had
258 significant effect on crumb yellowness after 4 g/100g flour for white flour and 6 g/100g flour
259 supplementation for whole wheat flour (Giannou & Tzia, 2016). Crumb color of bread made from

260 WF showed no significant changes with TG, while for MF and SF, no clear trend was observed. TG
261 supplementation led to a decrease in crumb redness (a^*), yellowness (b^*) and brightness (L^*), as
262 compared to the control.

263 As for crust color, VG slightly increased SF and MF crust based-bread yellowness and brightness,
264 while redness was significantly increased, in concordance with the findings of Giannou & Tzia
265 (2016). Also, TG enhanced crust yellowness and brightness, particularly at 0.1 g/100g flour TG
266 with the most relevant influence regardless the type of flour. Such result might be attributed to the
267 fact TG and VG contributed to the formation of a network, and consequently less amino acids were
268 available for Maillard reaction (Moore, Heinbockel, Dockery, Ulmer, & Arendt, 2006). These
269 findings underlined the fact that the addition of TG at lower levels had positive effects on color, in
270 concordance with Basman, Köksel, & Ng (2002) while VG impact was less relevant.

271

272 **3.5. Effects of TG and VG on moisture content and water activity**

273 TG and VG bread macroscopic water status parameters are reported in Table 5. Moisture content
274 and water activity were significantly influenced by TG and VG, as well as FS. However, the overall
275 variability was low, possibly due to the low doses of TG and VG used. In fact no significant
276 differences were observed in the supplemented samples, compared to the control breads.

277 Water activity was slightly but significantly decreased after VG addition, while it was slightly but
278 significantly increased in the presence of TG. As regards moisture content, it did not vary with VG
279 and it showed only small changes with TG. The observed differences suggest that both TG and VG
280 did not markedly affect the water-solids interactions in the bread matrix.

281

282 **4. Conclusions**

283 VG and TG (as well as their combinations) were assessed as quality-improving agents of fresh
284 bread, in terms of physico-chemical properties. MANOVA results showed that TG significantly

285 influenced all the parameters and VG influenced color and texture. It revealed also in a significant
286 synergic effect (TGxVG) on crust color and texture.

287 TG supplementation increased bread specific volume regardless of the flour type.

288 The effect of supplementation strongly depended on flour strength. Hardness and springiness were
289 mainly controlled by TG and VG, respectively, while VGxTG greatly influenced cohesiveness and
290 resilience. Crust yellowness was increased by TG and VG addition, while water activity/content
291 were more influenced by TG and TGxVG.

292 This study allowed a better understanding of the interaction between TG and VG effect on fresh
293 bread quality, as well as for the determination of optimized formulation based on TG and VG
294 addition as a function of flour strength. Optimum addition levels were selected for each flour
295 strength: 0.2 % TG and 1% VG for WF; 0,1 and 0.2 % of TG flour with 1% VG for MF; 0.1% TG
296 with 1% VG for SF.

297

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379 **Table 1:** Compositional and rheological characteristics of the three flours types*

		Weak	Medium	Strong
Chemical composition	Moisture content (%)	14.8±0.8	14.6±0.8	14.7±0.5
	Ash content (% , dry basis)	0.51±0.02	0.53±0.03	0.52±0.03
	Protein content (% , dry basis)	10.5 ^a ±0.6	11.7 ^b ±0.6	13.7 ^c ±0.7
Rheological properties	Falling number (s)	338 ^a ±17	363 ^b ±22	368 ^c ±19
	W (10⁴ J)	118 ^a ±6	227 ^b ±11	358 ^c ±18
	P/L (dimensionless)	0.46 ^a ±0.02	0.64 ^b ±0.03	0.63 ^b ±0.04
	Water absorbance (%)	53 ^a ±3	55.2 ^{ab} ±5	58.7 ^b ±7
	Stability (min)	2.5 ^a ±0.1	6.4 ^b ±0.3	16.3 ^c ±0.8

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*Values are shown as means ± standard deviation. Three measurements were performed for each sample.

Different letter in the same section of the same column are significantly different (p≤0.05)

382 **Table 2:** Bread formulations (g/100g).

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Formula	F0	F1	F2	F3	F4	F5	F6	F7
Flour	100	100	100	100	100	100	100	100
TG	0	0	0.05	0.05	0.1	0.1	0.2	0.2
VG	0	1	0	1	0	1	0	1
Water				48				
Oil				3.4				
Yeast				3.2				
Sugar				1.5				
Salt				1.9				

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396 **Table 3:** Multivariate analysis based on Pillai's Trace test of the quality characteristics of fresh bread made from different flour strength (FS) supplemented with
 397 VG and TG*

		FS	TG	VG	FSxTG	FSxVG	TGxVG	FSxTGxVG
Specific volume	F value	16.49	23.11	2.38	4.50	8.83	0.32	0.311
	Significance	***	***	ns	**	***	ns	ns
Color	F value	47.68	5.90	14598	4.87	8.33	4.52	5.23
	Significance	***	***	***	***	***	***	***
TPA	F value	92.89	35.05	14.32	10.62	16.12	2.06	6.60
	Significance	***	***	***	***	***	**	***
MC	F value	12.81	27.91	21.98	37.64	26.03	24.28	14.58
	Significance	***	***	ns	ns	ns	ns	ns
a_w	F value	840.11	36.37	9.19	950.76	7.91	35.06	6.39
	Significance	***	***	ns	***	***	**	***

*ns: not significant; *:p ≤0.05; **: p ≤0.01; ***: p ≤0 .001

398 **Table 4:** F significance level and sum square percent of the studied parameters and their interactions effects on textural attributes^a

			Hardness	Springiness	Cohesiveness	Chewiness	Resilience
Overall	FS	SS%	21.76	17.41	30.82	22.39	16.14
		sig	***	***	***	***	***
	TG	SS%	51.21	27.21	33.84	49.79	37.17
		sig	***	***	***	***	***
	VG	SS%	0.03	8.62	0.21	0.04	0.8
		sig	ns	***	ns	ns	ns
	FSxTG	SS%	16.61	6.08	22.26	17.79	23
		sig	***	ns	***	***	***
	FSxVG	SS%	2.54	28.01	1.3	2.69	3.06
		sig	***	***	ns	***	*
	TGxVG	SS%	1.13	1.36	4.28	0.87	6.44
		sig	**	**	***	***	**
	FSxTGxVG	SS%	6.73	11.31	7.29	6.42	13.38
		sig	***	**	***	***	***
WF	TG	SS%	88.54	6.51	90.86	87.15	57.09
		sig	***	NS	***	***	***
	VG	SS%	0.01	89.10	1.43	0.76	29.97
		sig	NS	***	NS	NS	**
	TGxVG	SS%	11.45	4.38	7.71	12.10	12.94
		sig	***	NS	NS	***	NS
MF	TG	SS%	87.52	54.26	55.31	89.16	45.98
		sig	***	***	***	***	***
	VG	SS%	1.63	38.99	0.03	2.37	0.67
		sig	**	***	ns	***	ns
	TGxVG	SS%	10.85	6.75	44.66	8.47	53.35
		sig	***	ns	***	***	***
SF	TG	SS%	74.43	55.19	93.95	75.5	94.81
		sig	***	***	***	***	***
	VG	SS%	12.5	16.44	4.05	10.87	0.06
		sig	***	**	ns	***	ns
	TGxVG	SS%	13.07	28.37	2.01	13.64	5.13
		sig	***	**	ns	***	ns

^a ns= not significant; * : $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$; SS: sum of squares

399 **Table 5:** Effects of TG and VG supplementation on bread with weak (WF), medium (MF) and strong (SF) flours water activity and moisture
 400 content.*

	F0	F1	F2	F3	F4	F5	F6	F7
Water activity								
WF	0.965±0.001 ^b	0.961±0.001 ^a	0.964±0.002 ^b	0.960±0.001 ^a	0.966±0.007 ^b	0.961±0.000 ^a	0.965±0.001 ^b	0.962±0.005 ^a
MF	0.948±0.00 ^a	0.962±0.001 ^b	0.958±0.00 ^a	0.958±0.00 ^a	0.959±0.001 ^{ab}	0.962±0.00 ^b	0.959±0.00 ^{ab}	0.963±0.00 ^b
SF	0.961±0.000 ^a	0.963±0.001 ^a	0.964±0.001 ^{ab}	0.962±0.001 ^a	0.966±0.003 ^b	0.963±0.001 ^a	0.966±0.007 ^a	0.961±0.001 ^a
Moisture content (%)								
WF	39.3±0.4	39.2±0.2	39.1±0.8	38.7±0.3	39.1±0.4	38.9±0.2	39.2±0.2	39.1±0.1
MF	39.1±0.3 ^{ab}	39.0±0.1 ^b	39.6±0.3 ^b	39.0±0.4 ^b	39.7±0.3 ^b	39.0±0.6 ^b	39.6±0.2 ^b	37.1±0.3 ^a
SF	36.4±0.3 ^a	38.7±0.5 ^{ab}	39.0±0.5 ^b	38.5±0.3 ^{ab}	39.2±0.5 ^b	39.0±0.5 ^b	39.6±0.4 ^{bc}	38.6±0.3 ^{ab}

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*Different superscript letters at each row indicate significant differences (p≤ 0.05).

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405 **Figure captions**

406 **Figure 1:** Specific volume (SV) as a function of flour strength (FS) (A) and transglutaminase

407 (TG) for weak (B), medium (C) and strong (D) flours.

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