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Original
Effectiveness of vital gluten and transglutaminase in the improvement of physico-chemical properties of fresh bread / Boukid, F.; Carini, E.; Curti, E.; Bardini, Gloria; Pizzigalli, E.; Vittadini, E.. - In: LEBENSMITTEL-WISSENSCHAFT + TECHNOLOGIE. - ISSN 0023-6438. - (2018). [10.1016/j.lwt.2018.02.059]

Availability:
This version is available at: 11381/2840430 since: 2021-12-02T11:30:46Z

Publisher:
Academic Press

Published
DOI:10.1016/j.lwt.2018.02.059

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09 July 2025

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

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Abstract

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

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

1. Introduction

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

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

During the last decades, extensive studies were addressed to the investigation of a spectrum of wheat flour based-bread improvers (enzymes, emulsifiers and hydrocolloids) and their combinations (Moore, Heinbockel, Dockery, Ulmer, & Arendt, 2006; Guarda, Rosell, Benedito, & Galotto, 2004; Benejam, Steffolani, & León, 2009; Aleid, Al-Hulaibi, Ghoush, & Al-Shathri, 2015; Eduardo, Svanberg, & Ahrné, 2015). To the authors' best knowledge, the effect of the combination of wheat gluten and crosslinking enzymes on bread properties has not yet been considered in the scientific literature. In this regard, the present work aimed to develop the optimal combination of TG and VG for bread-making based on the quality evaluation of fresh bread made with flour of different strength (weak, medium and strong).

2. Materials and methods

2.1. Materials

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

2.2. Bread-making: formulation and production

A multi-factorial experimental design was set up to study and optimize the level of addition of TG and VG for bread-baking quality improvement. The two factors were tested using three flours with different strength. Different levels of TG (0, 0.05, 0.1, and 0.2 g/100g flour) and VG (0 and 1 g/100g flour) were tested in a standard bread recipe used in industrial applications. The obtained bread formulations were named, F0 (control), F1 (supplemented with 1 g/100g flour VG), F2 (supplemented with 0.05 g/100g flour TG), F3 (supplemented with 0.05 g/100g flour TG and 1 g/100g flour VG), F4 (supplemented with 0.1 g/100g flour TG), F5 (supplemented with 0.1 g/100g flour TG and 1 g/100g flour VG), F6 (supplemented with 0.2 g/100g flour of TG), and F7 (supplemented with 0.2 g/100g flour TG and 1 g/100g flour of VG) as summarized in Table 2. Dry ingredients (flour, yeast, sugar, salt and the improver, TG and VG) were placed in a spiral mixer (MFITALY, Vicenza, Italy) and mixed at speed of 105 rpm for 2 min. Liquid ingredients (water and oil) were then added and kneaded at speed of 210 rpm for 8 min. The dough was then portioned into 550 g pieces, laminated (Econom 4000, Rondo, Burgdorf, Switzerland) into rectangular shape (490*160*6 mm) and placed into plastic molds. For rising, molds were left under controlled conditions (35 °C, 85% Relative Humidity) for 70 min. Afterward, doughs were baked for 35 min at the temperature of 220 °C (Modus, Logiudice, Verona, Italy). The bread loaves were cooled at room temperature for 3 h before analysis.

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

2.3. Bread characterization

2.3.1. Specific volume

Specific volume was obtained by dividing loaf volume (determined with a standard rapeseed displacement method) (AACC, 2001) for the corresponding loaf weight. Four measurements were carried out for each sample. Specific volume was expressed in cm^3/g.

2.3.2. Texture profile analysis

Texture properties were determined using TA.XT2 Texture Analyzer (Stable Micro Systems, Godalming, UK) and analyzed using Texture Expert software (Stable Micro Systems, Godalming, UK). Bread loaves were portioned mechanically into 1.2 cm thick slices (bread slicing machine, MAC.PAN, Vicenza, Italy). Two overlapped slices were subjected to a Texture Profile Analysis test, at 40 % strain with a compression plate (P/75) at 5 mm/s speed. At least eight measurements were taken for each bread production. The obtained textural parameters were hardness (peak force of the first compression cycle, N), cohesiveness (ratio of positive area during the second to that of the first compression cycle), springiness (ratio of the distance from the start of the second area up to the second probe reversal over the distance between the start of the first area and the first probe reversal), chewiness (hardness x cohesiveness x springiness, N mm), and resilience (area during the withdrawal of the penetration, divided by the area of the first penetration) (Alvarez, Canet, & López, 2002).

2.3.3. Color

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

2.3.4. Water activity and moisture content

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

2.4. Statistical analysis

For each parameter, a Multivariate Analysis of Variance (MANOVA) model was used considering FS, TG, and VG as fixed effects. The first MANOVA (3-way ANOVA) included the entire experimental setup (FS, TG and VG), to investigate the variance and the significance of the studied parameters as a function of all the factors at a significance level of α=0.05. Then, three 2-way ANOVAs (TG and VG) were conducted to assess the effects of the factors on the physico-chemical properties of bread made with each flour. The percentages of total variations were computed to explain the variance of each parameter, as a function of the main and interaction effects to evaluate the contribution of each factor on its variability. Significant differences among the mean values were calculated using Duncan's test. All experimental data were statistically analyzed using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

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

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

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

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

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

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

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

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

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

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

Concluding, regardless of flour type, TG, VG had, in most cases, a synergic effect on texture. For WF, TG reduced hardness and increased cohesiveness and chewiness. No significant difference was observed between 0.1 and 0.2 g/100g flour TG supplementation (F4 and F6) on springiness. VG decreased springiness. For MF, TG reduced hardness. An increase in cohesiveness and resilience was also observed, consistently with the findings of Wang, Huang, Kim, Liu, & Tilley (2011). However, at high level of TG, VG addition increased hardness, suggesting a negative effect on bread texture, possibly due to excessive crosslinking (Basman, Köksel, & Ng, 2003; Gerrard et al., 1998). Regarding bread made from SF, VG induced a slight increase in springiness and cohesiveness, whereas TG influenced all parameters. The addition of 0.1 g/100g flour of TG and 1 g / 100 g flour of VG showed the lowest hardness and chewiness and the highest springiness. Springiness was increased due to enhanced elasticity of the gluten network conferred by TG and VG (Wu & Corke, 2005). As for hardness, 0.2 g/100g flour TG addition (F6) resulted in a significant increase compared to 0.1 g/100g flour of TG and 1 g / 100 g flour of VG (F5), as well as to 0.2 g/100g flour of TG and 1 g / 100 g of VG (F7). However, with added VG, hardness significantly decreased, confirming the synergic effect between exogenous enzymes and proteins. According to Basman, Köksel, & Ng (2002), the optimum TG level for the best bread quality can be obtained from maximum volume and from the minimum hardness versus addition level. This assumption was based on the fact that hardness is inversely related to volume, as previously evidenced in several studies (Miñarro, Albanell, Aguilar, Guamis, & Capellas, 2012; Sabanis & Tzia, 2016), and was consistent with the findings of this work. Indeed, Pearson's correlation coefficient indicated that hardness was inversely correlated to specific volume (r=-0.776). Concluding, for WF, optimum formulation was F7. In the case of MF based-bread, F5 and F7 flour gave similar results, and F5 enabled the best enhancement for SF based-bread.

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

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

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

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

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

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

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

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

4. Conclusions

VG and TG (as well as their combinations) were assessed as quality-improving agents of fresh bread, in terms of physico-chemical properties. MANOVA results showed that TG significantly influenced all the parameters and VG influenced color and texture. It revealed also in a significant synergic effect (TGxVG) on crust color and texture.

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

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

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

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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)	$10.5^{a}\pm0.6$	$11.7^{b}\pm0.6$	$13.7^{c}\pm0.7$
Rheological properties	Falling number (s)	$338^{a}\pm17$	$363^{b}\pm22$	$368^{c}\pm19$
	W (10^{4} J)	$118^{a}\pm6$	$227^{b}\pm11$	$358^{c}\pm18$
	P/L (dimensionless)	$0.46^{a}\pm0.02$	$0.64^{b}\pm0.03$	$0.63^{b}\pm0.04$
	Water absorbance (%)	$53^{a}\pm3$	$55.2^{ab}\pm5$	$58.7^{b}\pm7$
	Stability (min)	$2.5^{a}\pm0.1$	$6.4^{b}\pm0.3$	$16.3^{c}\pm0.8$

*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 ($p\leq0.05$)

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

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							

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 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

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 ≤ 0.05; **: p ≤ 0.01; ***: p≤ 0.001; SS: sum of squares

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

*Different superscript letters at each row indicate significant differences ($p \leq 0.05$).

Figure captions

Figure 1: Specific volume (SV) as a function of flour strength (FS) (A) and transglutaminase (TG) for weak (B), medium (C) and strong (D) flours.