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## Effect of tef [*Eragrostis tef* (Zucc.) Trotter] grain flour addition on viscoelastic properties and stickiness of wheat dough matrices <u>and</u> <u>bread loaf volume</u>

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#### 20 Abstract

Currently, consumers' preference towards baked goods with additional (functional and nutritional) value is increasing, leading food industries to look at natural nutrient-dense alternatives like tef grain. Impact of tef grain flour incorporation (three Ethiopian varieties: DZ-01-99, DZ-Cr-37 and DZ-Cr-387 at 10, 20, 30 and 40% levels) on dough viscoelastic profiles and stickiness of wheat-based dough matrices were investigated. Oscillatory and creep-recovery tests together with dough stickiness were performed. Incorporation of tef flours affected the structure of the dough matrices visibly by reducing viscoelastic moduli and the maximum stress doughs can tolerate before its structure is broken, and increased dough instantaneous and retarded elastic compliances. Effect of dose was not always significant in the parameters measured. Tef grain flour incorporation up to 30% level led to breads with higher loaf volume than the control associated to optimal consistency and higher deformability of doughs. Higher tef doses increased dough stickiness. This will affect dough handling and shaping/flattening to get continuous strands or thin sheets. On average, the DZ-Cr-37 supplemented doughs exhibited higher elastic and viscous moduli, lower compliances and higher steady state viscosity and led to significantly lower loaf bread volumes. Hence, based on dough viscoelastic and stickiness properties, incorporation of DZ-01-99 and DZ-Cr-387 into wheat flour based formulations could be more preferable. 

Key-words: Bread, creep-recovery test, dough, oscillatory test, stickiness, tef

#### 1. Introduction

- Understanding the rheological characteristics of food materials is necessary in designing new products. It is important to determine the rheological properties of doughs due to their effect on its processing and on bread final characteristics (Ronda et al., 2011). Obesity, type-2-diabetes, coronary heart disease and colo-rectal cancer are among the rising challenges of western population, due to changes in both life style and eating behavior (WHO, 2005). Currently consumers' awareness for wholesome fgoods to get a healthy life has changed their preferences considerably regarding cereal products. Accordingly, the interest for breads for special dietary requirements and with increased nutritional value is rising. Hence, nutrient-rich whole grain incorporated baked fgoods with low glycemic index and/or enriched with dietary fiber constitute are promising ways for producing healthy alternatives.

Tef [Eragrostis tef (Zucc.) Trotter] is a tropical cereal which has gained a rapidly growing global interest due to its nutritional composition and health benefits. Literature indicates it is gluten free, with equivalent protein content to other more common cereals like wheat and relatively richer than other cereals in the essential amino acid lysine (National Research Council, 1996; Dekking et al., 2005). It is composed of complex carbohydrates with slowly digestible starch (Wolter et al., 2013). It is also known to be a good source of essential fatty acids, fiber, minerals (especially calcium and iron), and some phytochemicals such as polyphenols and phytates (Baye, 2013). In addition, tef grain and derived starch have suitable techno-functional properties like high water absorption capacity, foaming stability and a slow amylose retrogradation, dependent on tef variety type, that could have a positive impact on the quality of cereal based products (Bultosa, 2007; Abebe et al., 2015). These merits of tef make the grain a good 

alternative ingredient in addressing the aforementioned demand. So far, some studies
have been made to produce tef supplemented- and gluten-free western type breads from
grain tef flours (Mohammed et al., 2009; Renzetti and Arendt, 2009 and Alaunyte et al.,
2011) with encouraging results. However, these studies do not include information of
either the tef varieties used or their effects on dough viscoelastic fundamental
properties.

The replacement of wheat in bakery products is a major technological challenge, as the wheat protein gluten is essential for structure-formation. The gluten matrix is a major determinant of the important rheological characteristics of dough, such as elasticity, extensibility, resistance to stretch, mixing tolerance, and gas holding ability. Tef is always consumed in the whole grain form (germ, bran and endosperm) and the composition and types of starch and proteins available are distinct from wheat. Consequently, dilution or removal of wheat gluten during supplementation and/or substitution in the dough system impairs proper dough development capacity during kneading, leavening and baking. Stickiness is a combination of adhesion, the interaction between a material and a surface, and cohesion, the interactions within the material. Therefore, in a dough system there is a combination of surface and rheological properties. Dough stickiness is a major problem in the industry, particularly in large mechanized bakeries, as sticky and poor machinable doughs lead to process disruption and product loss (Armero & Collar, 1997).

Studying the rheological properties of wheat doughs supplemented with tef flours are of paramount importance because it may influence the machinability, elasticity, extensibility, resistance to stretch, mixing tolerance and the gas holding capacity of the dough and eventually the quality of the baked bread. Viscoelastic and stickiness properties of wheat flour dough matrices enriched with known Ethiopian grain tef flours

 varieties have not been explored so far. Hence, the effect of tef variety type and addition
level in the flour blend on dough rheological properties and bread <u>loaf</u> volume were
studied.

#### 2. Materials and methods

#### 98 2.1. Materials

Three tef varieties DZ-01-99 (brown grain tef), DZ-Cr-37 (white grain tef) and DZ-Cr-387 (Quncho, white grain tef) were obtained from the Debre Zeit Agricultural Research Center of the Ethiopian Institute of Agricultural Research (EIAR). Refined wheat flour was supplied by Emilio Esteban SA (Valladolid, Spain). Wheat flour alveographic characteristics were (supplier data): Tenacity (P) 129 mm; Extensibility (L) 107 mm, Energy of Deformation (W)  $466 \times 10^{-4}$  J; P/L ratio: 1.21. A general purpose bread improver Toupan Puratos @ (Puratos, Barcelona, Spain) containing mono- and di-glyceride of fatty acids, ascorbic acid,  $\alpha$ -amylase and xylanase was used. The chemical compositions of the wheat and the three tef variety grain flours are summarized in Table +were reported in Abebe et al. (2015). -The wheat flour used in this study contained 12.2% moisture, 14.5% protein, 0.66% ash, 1.47% fat, 85.1% carbohydrate, 78.8% starch and its starch had 23.2% of amylase. 2.2. Milling Grain tef varieties were manually cleaned by siftings and winnowing before milling. 

Disc attrition mill, being used traditionally in cottage tef grain milling house (Bishoftu,
Ethiopia) to mill tef grain for *injera* making in Ethiopia, was used to whole flour the tef
grain and immediately packed in airtight plastic bags and stored at 4°C until
analysisGrain tef was milled to whole flour by disc attrition mill, with two discs,
traditionally used in the cottage tef grain-milling house (Bishoftu, Ethiopia) for injera

118 <u>making, immediately packed in airtight plastic bags and then stored at 4°C until</u>
119 analysis.

120 The mean particle size  $(D_{50})$  and size dispersion of the flours were reported in Abebe et

- 121 al. (2015) as 90.7 μm and 2.17 for (DZ-01-99), 94.7 μm and 2.14 for (DZ-Cr-37) and
- 122 94.2 μm and 2.10 <u>for (</u>DZ.Cr-387<u>), respectively</u>.

#### **2.3. Dough preparation and breadmaking**

A straight dough process for a *ciabatta* bread type was performed using the following formula on a 100g flour basis: 1.8% salt, 0.5% bread improver, 2% dry yeast (added for making bread) and 85% water. For dough rheological measurements, yeast-free samples were used in order to keep sample stability during test running. Each of the three tef varieties (DZ-01-99, DZ-Cr-37 and DZ-Cr-387) was incorporated at 0%, 10%, 20%, 30% and 40% dose level and mixed with the wheat flour for 15 minutes using Chopin MR2L/MR10L mixer (Chopin technologies, France). The dough was prepared by blending the solid ingredients first in a kitchen-aid professional mixer (KPM5) for 2 min. at speed 2. Then the kneading process was made in three phases: at speed 4 for 5 min. by adding water during the first minute, at speed 6 for 1 min- and finally at speed 4 for 8 min. After mixing, the temperature of the dough was 25±1°C. The dough, 300 g, was placed into aluminium pans and was proofed for 40 min at 28°C and  $(75 \pm 5)$  % relative moisture humidity for 40 min. Subsequently, baking was carried out in a Salva oven (Lezo, Spain) at 190°C for 40 min. After baking, breads were left for one hour at room temperature before analysis. Bread volume was determined in duplicate using a volume analyser BVM-L370 TexVol Instruments (Viken, Sweden). 

#### **2.4.** Oscillatory and creep recovery tests

Oscillatory and creep-recovery tests were carried out with a RheoStress 1 rheometer (Thermo Haake, Karlsruhe, Germany) with parallel plate geometry (60 mm diameter) of serrated surface and with 3 mm gap. The excess of dough was removed and vaseline oil was applied to cover the exposed sample surfaces. All measurements were done at 25 °C. Before each assay the dough was allowed to rest for 10 min for relaxation. Frequency sweeps were carried out from 10 to 0.1 Hz in the linear viscoelastic region (LVR). A constant stress value of 1 Pa was chosen for the frequency sweeps of all doughs after establishing this value fell in the LVR of all doughs by means of stress sweeps from 0.1 to 100 Pa at 1 Hz. From the curves, the maximum stress beyond which the dough structure was broken,  $\tau_{max}$ , was established. Frequency sweep data were fitted to the power law model as in previous works (Ronda et al., 2011): 

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$$G'(\omega) = G'_1 \cdot \omega^a \qquad ($$

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$$G'(\omega) = G'_1 \cdot \omega^a$$
 (1)  
 $G''(\omega) = G''_1 \cdot \omega^b$  (2)  
 $G''(\omega) = G''_1 \cdot \omega^b$  (2)

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$$\tan \delta(\omega) = \frac{G''(\omega)}{G'(\omega)} = \left(\frac{G''}{G'}\right)_1 \cdot \omega^c = (\tan \delta)_1 \cdot \omega^c \qquad (3)$$

The coefficients  $G'_{l}$ ,  $G''_{l}$ , and  $(\tan \delta)_{1}$ , stand for the elastic modulus, viscous modulus and the loss tangent at a frequency of 1 Hz. The a, b and c exponents quantify the degree of dependence of these moduli and the loss tangent with the oscillation frequency,  $\omega$  expressed in Hz.

Creep tests were performed by imposing a sudden step shear stress in the LVR and outside the linear viscoelastic region (OLVR). For the creep study in the LVR a constant shear stress of 1Pa was applied for 120 s while in the recovery phase the stress was suddenly removed and the sample was allowed for 240 s to recover the elastic 

(instantaneous and retarded) part of the deformation. For the study OLVR a constant
shear stress of 50Pa was applied for 60 s and the sample was allowed to recover for 200
s after removing the load. Each test was performed in triplicate. The data from creep
tests were modelled to the 4-parameter Burgers model (Lazaridou et al., 2007) given by:

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$$J_{c}(t) = J_{0c} + J_{1c} \left( 1 - \exp\left(\frac{-t}{\lambda_{1c}}\right) \right) + \frac{t}{\mu_{0}}$$
 (4)

In the equation,  $J_c(t)$  is the creep compliance (strain divided by stress),  $J_{0c}$  is the instantaneous compliance,  $J_{Ic}$  is the retarded elastic compliance or viscoelastic compliances,  $\lambda_{Ic}$  is the retardation time and  $\mu_0$  gives information about the steady state viscosity. Similar equations were used for the recovery compliance  $J_r(t)$ . As there is no viscous flow in the recovery phase, equations consist only of parameters describing the elastic response after removal of the shear stress. The data from creep tests were modelled to the 3- parameter Burgers model given by:

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$$J_r(t) = J_{\text{max}} - J_{0r} - J_{1r} \left( 1 - \exp\left(\frac{-t}{\lambda_{1r}}\right) \right)$$
 (5)

 $J_{max}$  is the maximum creep compliance obtained at the end of the creep step. The steady-180 state compliance in recovery step,  $J_{steady}$ , was also calculated by subtracting the 181 compliance value at the terminal region of curve (where dough recovery reached 182 equilibrium) from the  $J_{max}$ . The ratio  $J_{steady}/J_{max}$  (elastic recovery) was also calculated 183 and expressed as Recovery (%).

#### **2.5. Stickiness**

This assay was conducted by following the procedure proposed by Grausgruber et al. (2003) and used by Ronda et al. (2011). A texturometer TA-XT2 from Stable Microsystem (Surrey, UK) provided with a SMS/Chen-Hoseney device where the sample was placed, and a methacrylate 25 mm cylinder (P/25P) as compression cell,

were used. The stickiness of the dough was determined at pre-test and test speed of 0.5 mm/s, a post-test speed of 10.0 mm/s and 40 g force. Three parameters were used to define stickiness: the positive maximum force or adhesive force, which is the measure of stickiness, the positive area under the curve or the adhesive energy, which is the work of adhesion, and the distance the sample is extended on probe return, which is an indication of sample cohesion/dough strength. Six replicates were carried out for all doughs.

**2.6. Statistical analysis** 

Experimental data were analyzed using two-way analysis of variance (MANOVA) and then means were compared at p<0.05 using Fisher's least significant difference (LSD) test. Correlations among the viscoelastic parameters and bread volume were evaluated at p<0.01 and p<0.05 using Pearson's correlation method. Statistical analysis was done by Statgraphics Centurion XVI program (StatPoint Technologies, Inc. 1982-2010).

#### **3. Results and discussions**

Tables 1 to 3 show the effects of tef grain flour dose and tef variety on bread dough
 viscoelastic properties and stickiness. Second order interactions (tef dose x tef variety)
 were not significant (p>0.05) on these parameters; therefore, only single effects are
 presented-.

#### **3.1. Dynamic oscillatory rheology**

The results of the stress and frequency sweeps are presented in Table 2<u>Table 1</u>. The values of  $\tau_{max}$ , G' and G'' exhibited by the control dough in this study were lower than those reported for wheat flour doughs (Dobraszczyk & Morgenstern, 2003) due to the higher amount of water used in the *ciabatta* formulation. Water plays an important role in determining the viscoelastic properties of dough. Both G' and G'' values decreased

with increasing water content, because either water can act as an inert filler causing the dynamic properties to reduce proportionally to moisture content or water can behave as a lubricant-plasticizer enhancing the relaxation phenomena (Masi et al., 1998).  $\tau_{max}$ values of tef enriched doughs showed a significant decrease (>41% on average) regardless wheat substitution level compared to control counterpart (Table 2 Table 1). Such lower breakpoint for the tef incorporated doughs might be due to the dilution and breaking of the former strong network formed during wheat flour dough development. The dose of tef addition and the variety type did not appreciably change the  $\tau_{max}$  score of the doughs. 

Frequency sweeps showed that in the whole range of frequencies, the elastic (or storage) modulus, G', was greater than the viscous (or loss) modulus, G'' for all dough formulations. This led all values of loss tangent, included those at a frequency of 1Hz,  $(tan\delta)_1$ , to be lower than 1 suggesting a solid elastic-like behavior of dough formulations. Both moduli slightly increased with frequency. This variation, which is quantified by a and b exponents from G' and G'' fittings to power law (Table 2 Table 1), decreased significantly with tef addition. The incorporation of tef flours also markedly reduced both viscoelastic moduli, G<sub>1</sub>' and G<sub>1</sub>'', leading to values 20% and 30% lower, respectively, than the control dough regardless the wheat substitution level. It can be noted that 10% tef addition was enough to exert gluten dilution and further weakening effect of the gluten network. The additional increase of tef dose did not lead to the concomitant decrease of viscoelastic moduli. Even though, a slight, although, a significant increase in G<sub>1</sub>' was observed for samples with 40% tef addition with respect to lower doses. This could be explained by the higher water absorption capacity (+27%), water holding capacity (+38%) and swelling volume (+37%) of tef flour in comparison with wheat flour as was reported in previous studies (Abebe et al., 2015a). 

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The explanation is consistent with the increase in dough consistency that may counteract the gluten dilution effect. Other authors have found higher viscoelastic moduli in rice-wheat composite doughs than in wheat doughs associated to stronger starch-gluten interactions in composite flour (Sivaramakrishnan et al., 2004). Authors also reported that, rice starch granules in the dough can act as filler that reinforces the gluten and produce strong bonds to given higher modulus. The viscous modulus decreased with tef addition in a greater extent than the elastic one (Table 2 Table 1). Consequently, the loss tangent decreased significantly (p < 0.05) with tef addition, from 0.34 (0% tef) to 0.27 (40% tef) implying an increase in the solid like behavior of tef-added doughs that increased with the tef level. This could be attributed to the differences in protein contents and profiles (Ronda et al., 2011; Hager et al., 2012; Abebe and Ronda, 2014). The marked variation in the lipid profiles, fiber, and shape and size of starch granules of wheat and tef flours observed by Hager et al. (2012) and Abebe & Ronda (2014) could also be a key factor. The c exponent, as was reported for a and b, also decreased with tef addition level, encompassing  $G_1$ "/ $G_1$ " ratio to have lower dependence on frequency (Ronda et al., 2011) associated to a lower frequency dependence structure (Sivaramakrishanan et al., 2004) in tef-supplemented doughs. Significant effect of tef variety type on  $G_1$ ' and  $G_1$ '' was observed. The DZ-Cr-37 tef variety flour exhibited the highest  $G_1$ ' and  $G_1$ '' average moduli, 14% higher than the remaining two tef varieties.

**3.2.** Creep-recovery tests

The results of the analysis of creep curves obtained both in LVR and OLVR are summarized in Table 3 Table 2. The strong correlation (p < 0.001) found for all creep compliance parameters and the equivalents for the recovery phase in the LVR (Ronda et al., 2014) suggested the omission of those data in Table 3 Table 2 since they do not

264	provide additional information to those of the creep phase. The dough had typical
265	viscoelastic creep-recovery curves combining both viscous and elastic components.
266	Both tef incorporation and variety affected creep-recovery parameters. However, as it
267	was observed in oscillatory tests, the effect on creep results was not proportional to tef
268	addition. The incorporation of 10% tef flour to replace wheat made creep phase
269	instantaneous $(J_{0c})$ and retarded $(J_{1c})$ elastic compliances to increase significantly (28%)
270	and 33% in LVR and 53% and 46% OLVR) with respect to control dough values. The
271	increase of compliances in the recovery phase was 66% and 38% for $J_{0r}$ and $J_{1r}$ with
272	respect to the control dough values. This indicates that tef enriched doughs had higher
273	instant and retarded deformations when subjected to a constant stress and higher
274	recoveries when the stress was removed. Higher levels of tef in the flour blend, in
275	general, did not lead to significant increases in the elastic or viscoelastic answers
276	obtained in the LVR and OLVR with respect to that of the 10% tef-supplemented
277	dough. <u>The</u> $J_{0c}$ and $J_{1c}$ compliances in the LVR tended to decrease again with tef dose
278	attaining at 40% level very similar values to the control dough (+12% and +6%
279	respectively). The steady-state viscosity, $\underline{\mu}_{\eta_0}$ , which gave the flowability of the material
280	at the end of the applied load decreased with 10% tef addition being significantly higher
281	lower than the control dough for the OLVR measurement (-35%). For durum wheat
282	doughs, it was found that the entire elastic compliance curve was shifted to higher
283	values as the strength of the dough (measured by extensigraph) decreased (Edwards et
284	al., 2001), while the steady-state viscosity increased with strength (Edwards et al.,
285	2001). Authors interpreted the differences in creep behavior in terms of differences in
286	strength of the associative network established by non-covalent intermolecular
287	associations within gluten chains. $\frac{10\%}{W}$ whole grain tef flour <u>at 10%</u> addition
288	represents a supply of insoluble fiber that could explain the wheat gluten network

disruption. The non-proportional effects of tef substitution levels could be due to differences in tef functional properties with respect to wheat (Abebe et al., 2015) dependent on their different composition and particularly their different protein and starch nature (Sivaramakrishnan et al., 2004). The  $\lambda$  values (Table 3 Table 2) calculated did not show any significant variation with tef addition. The maximum creep compliances, both in and outside the LVR assays, increased with 10% tef addition although it was much more pronounced (13% versus 30%) in OLVR assays. An additional increase of 30% in the maximum creep compliance in OLVR assays was observed in 40% tef added dough. This can be partly attributed to its higher flowability (lower  $\eta_0$ ) and partly to its higher viscoelastic deformation (higher  $J_{Ic}$ ). The total elastic compliance  $(J_{0c+}J_{1c})$  represented 56% of the maximum creep compliance in wheat dough. This ratio did not vary with tef addition in the LVR measurements meanwhile increased significantly in OLVR test, increasing until 64% independently of the dose of tef. In the recovery phase approximately 55% and 65% elastic recovery could be seen for pure and 10% tef-added wheat doughs respectively. This means a lower viscous characteristics of tef added doughs which is coherent with the lower tan  $\delta$  values already reported in the oscillatory tests. 

The study effect of tef variety type on creep-recovery properties demonstrated that DZ-Cr-37 behaved differently, as was already commented with respect to oscillatory test results and the differences were more marked in OLVR assays. Accordingly, flour of this variety led to significantly lower average elastic compliances (-23% for  $J_{oc}$  and  $J_{or}$ , -30% for  $J_{1c}$  and  $J_{1r}$ , -33% for  $J_{max}$  in the creep phase, and -23% for  $J_{steady}$  from recovery phase) and higher average steady-state viscosity (+49%) than DZ-Cr-387 tef flour doughs. Though DZ-Cr-387 is, a white tef variety like as-DZ-Cr-37, incorporation of DZ-Cr-387 and DZ-01-99 (brown tef variety) grain flours changed the resulting dough 

314	creep-recovery characteristics in a closer manner while DZ-Cr-37 incorporated dough
315	behaved differently.showed the maximum difference with it in all the creep recovery
316	parameters meanwhile gave similar values to the brown grain tef variety, DZ-01-99. In
317	the LVR the creep compliances of different tef varieties doughs gave maximum average
318	differences of 16 – 19% and non-significant differences among steady-state viscosities.
319	The relatively higher consistency (higher $G_1$ ' and $\eta_o$ values) of DZ-Cr-37 cultivar and
320	its lower deformability versus a stress may explain the lower dough development during
321	proofing and baking, resulting in lower bread volumes. Tef variety type did not
322	significantly affect the retardation time ( $\lambda_c$ ) in the creep phase of the test carried out in
323	the LVR. However, in OLVR tests impact of tef variety was significant on $\lambda_c$ and DZ-
324	Cr-37 showed the lowest value indicating that the retardation time of the elastic retarded
325	response was smaller than the doughs with the remaining varieties. No significant
326	difference was observed in the retardation time of the recovery phase. Previous works
327	have correlated the retardation times after creep with the bread volume reporting lower
328	bread volumes for doughs with faster recoveries (Van Bockstaele et al., 2011). In this
329	work, differences in retardation times, both in the creep or recovery phases were too
330	small to explain the differences found in bread volume.
331	Although significant effects ( $p < 0.05$ ) were observed among the doughs different in tef
332	flour_dose_level_or tef variety type on some creep parameters obtained from LVR
333	assays, it can be concluded their effect in OLVR were much more pronounced allowing

assays, it can be concluded their effect in OLVR were much more pronounced allowing
better dough discrimination. Probably the general thought that higher correlation
between dough creep parameters and bread volume are obtained outside the LVR can be
partly due to the more marked differences found among samples in the latter test. In any
case, very high correlations between all the parameters obtained in and outside LVR
were found.

#### **3.3. Dough stickiness**

Results of the stickiness test on the formulated doughs are disclosed in Table 4Table 3. The stickiness (adhesive force) of the tef enriched doughs was lower than the control at lower doses, mainly at 10% and 20%. - and conversely the cohesiveness of these doughs were higher (Hoseney and Smewing, 1999). However the adhesive forces recorded tended to rise with tef dose level so that, 40% tef-added doughs showed considerably higher average stickiness (+36%) than the control. The adhesive energy and the distance on return also showed a marked decrease since the smallest tef addition but, in this case, they continued decreasing until the 30% dose, and started to rise at the highest tef content. Tef grain flour supplemented doughs did not overpass the control adhesive energy and the distance on return values. Then, the three dough stickiness parameters showed similar tendency with a minimum value versus tef concentration, shifted toward higher concentrations in the case of adhesiveness energy and distance on return. 

The study shows that incorporation of tef at higher percentage significantly increases the adhesive force and this may affect the handling and shaping/flattening purposes to get continuous strands or thin sheets of the doughs. In any case, stickiness did not overpass the 100 g value, discarding important dough handling problems (Chen & Hoseney 1995; Armero y Collar, 1997). Slight variations due to tef variety were also observed, in accordance with earlier observations reported on wheat revealing that varieties, growing season, protein concentration, water absorption, milling process and extraction rate may influence dough stickiness (Van Velzen et al., 2003; Yildiz et al., 2012).

**3.4. Bread volume** 

362 Figure 1 represents the bread volume evolution for different doses and tef variety. Both,

tef variety type and its content in the formulation, had a significant effect on the specific

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364	volume of bread (p <0.001). The substitution of wheat flour by tef flour until the $30\%$
365	level, led to <i>ciabatta</i> type breads with significantly higher ( $p < 0.05$ ) specific volume
366	than the control wheat flour bread. The highest effect on volume was obtained with 10%
367	or 20% additions (+12% on average) depending on the tef variety, but still 30% tef-
368	enriched breads showed a significant (p<0.05) 5% volume increase with respect to the
369	control breads. Previous studies reported that the lower polymerization, hydrophobicity
370	and denaturation temperature of tef prolamins probably make them somewhat functional
371	in bread making (Adebowale et al., 2011). The loaves with 40% tef flour showed a small
372	(-2%) although significant, lower volume than the wheat counterpart. Aluyante et al.
373	(2012) showed that replacing wheat flour by tef grain flour up to 10% in straight dough
374	bread making did not affect loaf volume, while larger incorporations had a detrimental
375	effect. Mohammed et al., (2009) obtained declining breads volumes on additions to
376	wheat flour higher than 10-15% tef. The higher amount of admitted tef in the present
377	samples could be due to bread formulation and the tef varieties used. However,
378	probably the most important factor was the type of wheat flour used in the blend. In our
379	case, high grade wheat flour was used while previous authors reported to use all-
380	purpose wheat flour (Mohammed et al., 2009). The very high gluten content of the
381	wheat flour (14.5% protein), too high for general breadmaking proposes, withstood the
382	dilution with tef leading, until a certain substitution level, to suitable dough rheological
383	characteristics, less tough and with higher development capacity under the gas
384	expansion effect during proofing and baking. The higher gelatinization temperature of
385	tef starch than wheat starch (Whistler and Be Miller 1997, Bultosa et al., 2002) could
386	also explain the higher volume of tef enriched breads as a higher dough development is
387	allowed in the oven due to the gas expansion retained in the dough before reaching a

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rigid structure. Tef variety type exerted remarkable effects on bread loaf volume in the
order of: DZ-01-99 > Quncho DZ-Cr-387 > DZ-Cr-37.

#### **3.5.** Correlations among rheological properties and bread volume

Pearson correlation analysis showed a significant interdependence among the oscillatory and creep-recovery parameters (Table 45). As reported Ronda et al. (2014) both, the storage and loss moduli, showed strong interdependence. The loss tangent  $(tan \delta)_l$  was more dependent on loss moduli than the storage modulus. The creep compliances parameters showed strongly significant correlations with recovery phase counterparts (r > 0.92; p<0.01). In the LVR the viscosity at steady state ( $\mu_0$ ) was only dependent on the maximum creep compliance  $J_{max}$  (r =-0.40, p<0.01). However, for measurements outside the LVR,  $\mu_0$  strongly decreased (r>-0.81, p<0.01) with increasing  $J_{max}$ ,  $J_0$  and  $J_1$ . In agreement with Ronda et al (2014), the higher maximum stress ( $\tau_{max}$ ) explaining structural integrity of the doughs, increased in parallel with dynamic moduli, and decreased with instantaneous compliance. Bread volume was negatively correlated with the elastic modulus  $G_1$ ' (r = -0.5, p<0.01) and positively with elastic compliances,  $J_{oc}$ and  $J_{lc}$  in the LVR assays (r =0.3, p<0.5). This can be explained by the dilution of the strong gluten network of wheat dough due to tef addition which lowered the dough consistency and increased its deformation capacity versus a constant stress. The condition allowed a higher dough development as a consequence of the gas production during proofing and its further expansion during baking (Villanueva et al., 2015). Bread volume had a highly significant positive correlation with dough elastic recovery after creep (Recovery) (p<0.01; r=0.62) and with the ratio  $(J_{oc}+J_{lc})/J_{maxc}$  in the creep phase (p<0.01; r= 0.45) both in OLVR assays. This means that doughs with smaller relative viscous parts led to higher bread volumes. Bread volume showed also a highly negative 413 correlation with dough stickiness (adhesive force) (r=-0.87; p<0.01) showing that 414 doughs with the highest level of tef that became stickier gave lower bread volumes. 415 Armero & Collar (1997) recommended for-to maximized dough cohesiveness and 416 minimized dough stickiness for providing good bread-making performance. Therefore, 417 dough stickiness could be one of the drawbacks of incorporating tef flours at higher 418 percentages.

#### 420 4. Conclusions

In general, incorporation of tef flours affected the structure of the dough matrices visibly in terms of lower viscoelastic moduli and  $\tau_{max}$  values and larger instantaneous and retarded elastic compliances. Effect of dose level on these parameters was also significant. Tef flour supplemented breads up to 30% level had higher volume than the control ascribed to lower consistency and higher deformability of the doughs. However, at 40% tef dose the bread volume decreased to lower values than wheat bread. Viscoelastic properties do not explain easily this observation, as in general fundamental properties did not change markedly in samples over 10% tef addition. The elastic recovery capacity after creep and stickiness strongly correlated with bread volume. The present study also show that incorporation of tef at higher percentage (40%) increases dough stickiness and this may affect the handling and shaping/flattening purposes to obtain continuous strands or thin sheets of the doughs. On average, the DZ-Cr-37 supplemented doughs showed higher elastic and viscous moduli, lower compliances, and higher steady state viscosity and in both LVR and OLVR than those supplemented with other tef varieties. In addition, DZ-Cr-37 supplemented doughs also led to breads with lower volume. However, tef variety type did not appreciably affect dough stickiness. Hence, based on the dough viscoelastic and surface-related handling

properties studied, the incorporation of DZ-01-99 and DZ-Cr-387 could be morepreferable than DZ-Cr-37.

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Table 1: Chemical composition of tef and wheat flours (% on dry basis, except moisture).													
Flour	Moisture	Proteins	Ash	Fat	<b>Carbohydrates</b>	Starch	Amylose						
	<del>(%)</del>	<del>(%)</del>	<del>(%)</del>	<del>(%)</del>	<del>(%)</del>	<del>(%)</del>	<del>(% of starc</del> l						
Tef-brown (DZ-01-99)	<del>10.5±0.1a</del>	<del>8.9±0.3a</del>	<del>2.71±0.19b</del>	<del>2.84±0.08c</del>	<del>85.6±0.6b</del>	<del>75.5±0.1b</del>	<del>21.6±0.3a</del>						
Tef-white (DZ-Cr-37)	<del>10.3±0.1a</del>	<del>10.5±0.2b</del>	<del>3.52±0.01c</del>	<del>2.63±0.06b</del>	<del>83.4±0.2a</del>	<del>74.0±0.3a</del>	<del>21.8±0.3a</del>						
Tef-white (DZ-Cr-387)	<del>10.4±0.1a</del>	<del>8.9±0.2a</del>	<del>2.63±0.09b</del>	<del>3.24±0.06d</del>	<del>85.3±0.3b</del>	<del>75.5±0.4b</del>	<del>21.1±0.4a</del>						
Wheat	<del>12.2±0.1b</del>	<del>14.5±0.2d</del>	<del>0.66±0.01a</del>	<del>1.47±0.06a</del>	<del>85.1±0.2b</del>	<del>78.8±0.4c</del>	<del>23.2±0.5b</del>						

Data are the mean ± standard deviation. Values with a letter in common in the same column are not significantly different (p<0.05)

 Table 21. Single-Main effects of tef grain flour incorporation level and tef variety on dynamic parameters of bread doughs.

	$G_1^{'}$ (Pa)	a) a $G_1^{"}$		b	$(\tan \delta)_1$	с	$\tau_{\rm max}$ (Pa)
Tef dose (%)							
0	4314±83c	0.20±0.02c	1472±41b	0.260±0.0203c	0.341±0.006e	0.058±0.009c	3.31±0.38c
10	3293±342a	0.18±0.02b	1001±114a	0.249±0.030c	0.304±0.007d	0.067±0.014d	1.94±0.65b
20	3405±235a	0.17±0.01b	1039±105a	0.222±0.022b	0.300±0.007c	0.048±0.012b	1.93±0.62b
30	3560±350a	0.17±0.02ab	1021±98a	0.211±0.017ab	0.287±0.006b	0.037±0.006a	1.36±0.26a
40	3678±525b	0.16±0.01a	1007±146a	0.192±0.019a	0.273±0.008a	0.031±0.007a	1.29±0.23a
Tef variety							
DZ-01-99	3540±446A	0.18±0.02A	1077±210A	0.226±0.032A	0.302±0.023A	0.047±0.015A	1.92±0.76A
DZ-Cr-37	4019±379B	0.18±0.02A	1224±168B	0.224±0.034A	0.300±0.025A	0.044±0.017A	1.45±0.24A
DZ-Cr-387	3549±524 A	0.18±0.02A	1088±256A	0.236±0.035A	0.303±0.028A	0.055±0.016B	1.52±0.46A

The power law model was fitted to experimental results from frequency sweeps.  $G'(\omega) = G'_1 \cdot \omega^*$ ;  $G''(\omega) = G''_1 \cdot \omega^*$ ;  $\tan \delta(\omega) = (\tan \delta)_1 \cdot \omega^*$ .  $\tau_{max}$  was obtained from stress sweeps. Data are the mean  $\pm$  standard deviation. Values with the same letter in a column are not significantly different (p>0.05). Lower case letters are used to compare the effect of tef level and capital letters to compare the effect of variety.

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### Table 32. Single-Main effects of tef dose and grain tef flour variety on the creep-recovery parameters of bread doughs.

9		LVR						OLVR										
10	Parameter	ameter Creep phase					Cre	ep phase			Recovery phase							
11 12		$J_{0c}$ (10 <sup>-5</sup>	$J_{1c}$ (10 <sup>-5</sup>	$\lambda_{c}$	$\mu_{0c}$ (10 <sup>5</sup>	$J_{\text{maxe}}$ (10 <sup>-5</sup>	J <sub>e-c</sub> /J <sub>maxc</sub>	$J_{0c}$ (10 <sup>-5</sup>	$J_{1c}$ (10 <sup>-5</sup>	$\lambda_{c}$	$\mu_{0c}$ (10 <sup>5</sup>	J <sub>maxc</sub> (10 <sup>-5</sup>	$J_{e\text{-}c\!/}J_{maxc}$	J <sub>0r</sub> (10 <sup>-5</sup>	$J_{1r}$ (10 <sup>-5</sup>	$\lambda_{r}$	J <sub>steady</sub> (10 <sup>-5</sup>	Recovery
13		Pa <sup>-1</sup> )	Pa <sup>-1</sup> )	(s)	Pa·s)	Pa <sup>-1</sup> )	(%)	Pa <sup>-1</sup> )	Pa <sup>-1</sup> )	(s)	Pa∙s)	Pa <sup>-1</sup> )	(%)	Pa <sup>-1</sup> )	Pa <sup>-1</sup> )	(s)	Pa <sup>-1</sup> )	(%)
14	Tef Dose (%	%)																
15 16	0	30±1a	51±7a	15±2a	2.1±0.5b	142±8 a	57±2ab	45±2a	93±7a	7.9±0.2a	0.75±0.03c	248±13a	56±2a	76±3a	61±2a	23.8±0.3b	137±5a	55±2a
17	10	38±3c	68±6b	15±1a	1.9±0.6ab	160±15b	62±2b	69±3b	136±9b	7.8±0.4a	0.49±0.05ab	322±19bc	64±7a	126±5b	84±4b	23.1±0.7ab	210±9b	65±3a
18	20	35±5bc	50±7a	14±2a	1.7±0.2a	158±20ab	52±3a	69±3b	133±9b	7.9±0.3a	0.58±0.05b	315±19b	65±3a	122±5b	81±4b	23.6±0.6ab	203±9b	64±3a
20	30	36±4bc	50±6a	15±1a	1.7±0.6ab	158±32b	56±3ab	75±3b	157±7bc	7.8±0.5a	0.47±0.05ab	367±19c	64±8a	138±6b	93±5b	22.0±0.7a	231±11b	63±3a
21	40	33±7ab	48±8a	15±2a	1.8±0.4ab	145±27ab	56±3ab	76±3b	178±7c	8.1±0.4a	0.42±0.05ab	420±19bc	61±3a	125±7b	89±6b	21.9±0.8a	214±12b	51±4a
22 23	Tef variety																	
24	DZ-01-99	35±3B	52±6AB	15±1A	1.8±0.5A	153±20B	56±2A	69±2B	140±7B	8.1±0.3B	0.50±0.03A	358±14B	61±6A	120±4B	86±3B	22.6±0.5A	$206\pm7B$	58±3A
25 26	DZ-Cr-37	32±5A	49±9A	17±2A	2.0±0.5A	138±18A	57±2A	57±2A	105±7A	7.7±3.5A	0.67±0.03B	261±14A	63±5A	101±4A	67±3A	23.7±0.5A	168±7A	64±3A
27	DZ-Cr-387	38±5C	59±7B	16±2A	1.8±0.6A	160±19B	57±2A	75±2B	150±7B	8.0±0.4B	0.45±0.04A	384±14B	61±6A	131±4B	92±3B	22.3±0.5A	223±7B	58±3A
28 20	LVR: Results	s obtained	l in the Line	ear Viscoe	lastic Region	n (at 1Pa); O = retardatic	LVR: Resu	ilts obtain	ed from cr dv state vi	eep test car	ried out Outsid	e the Linear	Viscoelast L = step	ic Region (	at 50Pa): stantane	J <sub>maxe</sub> , J <sub>0c</sub> , and	$J_{1c} = maxided complia$	imum, ances
30	(respectively	), $\lambda_r = reta$	rdation tim	e and $\mu_{0r}$ =	steady state	viscosity in	the recover	ry phase;	J <sub>e-c</sub> : Elastic	complianc	e in creep phas $(p>0.05)$ Low	$e = J_{0c} + J_{1c}; R$	ecovery: 1	00*J <sub>steady</sub> /J	maxc	ant of tof love	l and conit	l lattara tha
31	effect of vari	ety.		iation. vai	ues with the	same ieuer		ii are not	signinean	ly unicient	(p>0.05). Low	er ease iene	is are used	to compa	e uie en		r and capita	in letters the
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# Table-43. Single-Main effects of tef grain flour dose and tef variety on bread dough stickiness.

	Adhesive Force (N)	Adhesive energy (Positive area) (mN·s)	Distance on return (mm)
Tef dose (%)			
0	0.58±0.05b	_113±2d	4.39±0.03e
10	0.47±0.05a	63±2b	3.56±0.05c
20	0.50±0.07a	52±2a	3.16±0.05b
30	0.64±0.08c	51±2a	2.64±0.05a
40	0.79±0.13d	75±2c	3.87±0.05d
Tef variety			
DZ-01-99	0.59±0.01A	66±2A	3.40±0.90A
DZ-Cr-37	0.58±0.01AB	76±2A	2.63±0.10A
DZ-Cr-387	0.61±0.01B	70±2A	2.54±0.94A

Data are the mean  $\pm$  standard deviation. Values with the same letter in a column are not significantly different (p>0.05). Lower case letters are used to compare the effect of tef level and capital letters the effect of variety.

 Table 54. Correlations between viscoelastic properties and bread volume.

	G1'	а	G1"	b	(tan ó)1	c	$\tau_{\text{max}}$	J <sub>maxeL</sub>	J <sub>0cL</sub>	$J_{1cL}$	$\mu_{0cL}$	$J_{ecL}\!/J_{maxeL}$	J <sub>maxeO</sub>	$J_{0cO}$	$J_{1cO}$	$\lambda_{cO}$	$\mu_{0cO}$	$J_{ecO}/J_{maxcO}$	J <sub>steadyO</sub>	$J_{0rO}$	$J_{\rm 1rO}$	$\lambda_{rO}$	Rec	F	А	D
Volume	-0.40*	-	-0.33*	0.33*		0.55**	-		0.33*	0.28*	-	-	-	-	-	-		0.45**	-	-	-	-	0.62**	-0.87**	-0.56**	-0.42*
G1'		0.33*	0.91**	-	$0.42^{**}$	-	0.42**	-0.50**	-0.82**	-0.45**	-	-	-0.74**	-0.82**	-0.72**	-	0.65**	-	-0.81**	-0.80**	-0.70**	$0.32^{*}$	-	-	0.66**	$0.56^{**}$
a			0.51**	$0.90^{**}$	0.63**	$0.52^{**}$	0.59**	-	-0.39*	-	-	-	-0.35*	-0.51**	-0.41**	-0.31*	0.53**	-	-0.35*	-0.38*	-	-	-	-	$0.45^{*}$	-
G1"				$0.39^{*}$	$0.74^{**}$		$0.66^{**}$	-0.32*	-0.71**	-0.31*	-	-	-0.79**	-0.85**	-0.74**	-	$0.70^{**}$	-	-0.86**	-0.81**	-0.66**	0.36**	-	-	0.74**	0.56**
b					0.65**	$0.84^{**}$	0.55**	-	-	-	-	-	-0.35*	-0.43**	-0.39*	-0.32**	0.46**	-	-	-0.31**	-	-	-	-0.41*	$0.37^{*}$	-
(tan ó)1						0.51**	0.83**	-	-			-	-0.52**	-0.54**	-0.45**	-	0.49**	-	-0.55**	-0.47**	-0.30*	-	-	-0.34*	0.59**	0.43*
c							$0.34^{*}$	-	-		-	-	-	-	-0.30*	-0.31*	$0.29^{*}$	-	-	-	-	-	-	-0.50**	-	-
τ <sub>max</sub>										-		-	-0.52**	-0.55**	-0.44**	-	0.44**	-0.34*	-0.6**	0.48**	-0.33*	-	-	-	0.66**	0.52**
Jmaxal									0.57**	0.64**	-0.40**	-0.48**	$0.67^{**}$	0.63**	$0.70^{**}$	0.50**	-0.57**	-0.26*	0.64**	$0.62^{**}$	0.66**	-0.32*	-042**	-	-	
Joa										0.74*			0.76**	0.87**	0.77**	-	-0.64**	-	0.79**	0.82**	0.78**	-	-	-	-0 40**	-0.37**
Ju												. ·	0.52**	0.59**	0.56**	-	-0.47**		0.55**	0.57**	0.54**	-	-	-	-	-
Ho-t													-0.37**	-0.29*	-0.39**	-044**	0.35**		-0.33*	-0.29*	-0.33*	0.31*	-	-	-	$0.28^{*}$
Lat / Lang													-		-	-0.50**	0.34**	0.50**	-	-	-	0.31*	0.46**	-	-	-
Ject/Jmaxel														0.93**	0.97**	0.47**	-0.82**	-0.30**	0.96**	0.94**	0.94**	-0.51**	-0.53**	0.29*	-0.38**	-0.40**
Jmaxe0														0.75	0.94**	0.30*	-0.81**	-0.50	0.95**	0.97**	0.97**	-0.40**	-0.30*	0.27	-0.56	-0.44**
Lo															0.74	0.52**	-0.88**	_	0.94**	0.95**	0.95**	-0.40	-0.44**	_	-0.40	-0.38**
31e0 2																0.52	-0.55**	-0.27*	0.36**	0.35**	0.42**	-0.33*	-0.44	0.36**	-0.50	-0.50
N <sub>cO</sub>																	-0.55	0.26*	0.83**	0.83**	0.91**	0.51**	0.42**	0.50	0.43**	0.40**
μ <sub>0c0</sub> L <sub>o</sub> /L <sub>o</sub>																		0.20	-0.85	-0.85	-0.81	0.22*	0.42	-	0.45	0.40
Ject/Jmaxe0																			-	-	-	0.55	0.70	-	- 45**	- 42**
J steadyO																				0.95	0.95	-0.50	-0.30	-	-0.45	-0.45
J <sub>0rO</sub>																					0.96	-0.52	-0.30	-	-0.45	-0.44
J <sub>1rO</sub>																						-0.60	-	-	-0.51	-0.34
Λ <sub>rO</sub> Doo																							-	0.22*	-	-
Rec																								-0.32	-	-
FI																									-	-
Al																										0.83
D1																										

 $J_{maxcl}$ ,  $J_{0cl}$ , and  $J_{1cL}$  = maximum, instantaneous and retarded compliances (respectively),  $\lambda_{cl}$  = retardation time and  $\mu_{0cL}$  = steady state viscosity in the creep phase obtained from creep test in linear viscoelsatic region (at 1Pa);  $J_{maxcO}$ ,  $J_{0cO}$ , and  $J_{1cO}$  = maximum, instantaneous and retarded compliances (respectively) outside the linear VR,  $\lambda_{cO}$  = Retardation time and  $\mu_{0cO}$  = steady state viscosity in the creep phase obtained from creep test outside the viscoelastic region (at 50Pa) and  $J_{steadyO}$ ,  $J_{0rO}$  and  $J_{1rO}$  = steady-state, instantaneous and retarded compliances (respectively) in the recovery phase outside,  $\lambda_{ro}$  = Retardation time in the recovery phase obtained from test outside the viscoelastic region (at 50Pa). Jeec: Elastic compliance in creep phase= J<sub>0c</sub>+J<sub>1c</sub>; Rec: Recovery: J<sub>steady</sub>/J<sub>maxc</sub>; G<sub>1</sub>', G<sub>1</sub>'' and (tan ó)<sub>1</sub> are the elastic and viscous moduli and the loss tangent at 1Hz and a, b, c are the exponents obtained after power law fitting of frequency sweeps data;  $\tau_{max} = the maximum$  stress the dough can tolerated in the LVR. F1 = Adhesive force; A = Adhesive energy, and D = Distance on return \* = p < 0.05 and \*\* = p < 0.01, - = not significant (p>0.05).



Figure 1. Evolution of bread volume with tef dose of three different varieties