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3 1 **Effect of tef [*Eragrostis tef* (Zucc.) Trotter] grain flour addition on**
4 2 **viscoelastic properties and stickiness of wheat dough matrices and**
5 3 **bread loaf volume**
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3 **Abstract**
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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.

40 | Key-words: Bread, creep-recovery test, dough, oscillatory test, stickiness, tef
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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 **f**oods 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 **f**oods 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

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3 68 alternative ingredient in addressing the aforementioned demand. So far, some studies
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5 69 have been made to produce tef supplemented- and gluten-free western type breads from
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7 70 grain tef flours (Mohammed et al., 2009; Renzetti and Arendt, 2009 and Alaunyte et al.,
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9 71 2011) with encouraging results. However, these studies do not include information of
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11 72 either the tef varieties used or their effects on dough viscoelastic fundamental
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13 73 properties.

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16 74 The replacement of wheat in bakery products is a major technological challenge, as the
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18 75 wheat protein gluten is essential for structure-formation. The gluten matrix is a major
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20 76 determinant of the important rheological characteristics of dough, such as elasticity,
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22 77 extensibility, resistance to stretch, mixing tolerance, and gas holding ability. Tef is
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24 78 always consumed in the whole grain form (germ, bran and endosperm) and the
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26 79 composition and types of starch and proteins available are distinct from wheat.
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28 80 Consequently, dilution or removal of wheat gluten during supplementation and/or
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30 81 substitution in the dough system impairs proper dough development capacity during
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32 82 kneading, leavening and baking. Stickiness is a combination of adhesion, the interaction
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34 83 between a material and a surface, and cohesion, the interactions within the material.
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38 84 Therefore, in a dough system there is a combination of surface and rheological
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40 85 properties. Dough stickiness is a major problem in the industry, particularly in large
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42 86 mechanized bakeries, as sticky and poor machinable doughs lead to process disruption
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44 87 and product loss (Armero & Collar, 1997).

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47 88 Studying the rheological properties of wheat doughs supplemented with tef flours are of
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49 89 paramount importance because it may influence the machinability, elasticity,
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51 90 extensibility, resistance to stretch, mixing tolerance and the gas holding capacity of the
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53 91 dough and eventually the quality of the baked bread. Viscoelastic and stickiness
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55 92 properties of wheat flour dough matrices enriched with known Ethiopian grain tef flours

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3 93 varieties have not been explored so far. Hence, the effect of tef variety type and addition
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5 94 level in the flour blend on dough rheological properties and bread loaf volume were
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7 95 studied.
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11 97 **2. Materials and methods**

12 98 **2.1. Materials**

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15 99 Three tef varieties DZ-01-99 (brown grain tef), DZ-Cr-37 (white grain tef) and DZ-Cr-
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17 100 387 (Quncho, white grain tef) were obtained from the Debre Zeit Agricultural Research
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19 101 Center of the Ethiopian Institute of Agricultural Research (EIAR). Refined wheat flour
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21 102 was supplied by Emilio Esteban SA (Valladolid, Spain). Wheat flour alveographic
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23 103 characteristics were (supplier data): Tenacity (P) 129 mm; Extensibility (L) 107 mm,
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25 104 Energy of Deformation (W) 466×10^{-4} J; P/L ratio: 1.21. A general purpose bread
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27 105 improver *Toupan Puratos®* (Puratos, Barcelona, Spain) containing mono- and di-
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29 106 glyceride of fatty acids, ascorbic acid, α -amylase and xylanase was used. The chemical
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31 107 compositions of ~~the wheat and~~ the three tef variety grain flours ~~are summarized in Table~~
32
33 108 ~~and were reported in Abebe et al. (2015).~~ The wheat flour used in this study contained
34
35 109 12.2% moisture, 14.5% protein, 0.66% ash, 1.47% fat, 85.1% carbohydrate, 78.8%
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37 110 starch and its starch had 23.2% of amylase.
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42 111 **2.2. Milling**

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44 112 ~~Grain tef varieties were manually cleaned by siftings and winnowing before milling.~~
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46 113 ~~Disc attrition mill, being used traditionally in cottage tef grain-milling house (Bishoftu,~~
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48 114 ~~Ethiopia) to mill tef grain for injera-making in Ethiopia, was used to whole flour the tef~~
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50 115 ~~grain and immediately packed in airtight plastic bags and stored at 4°C until~~
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52 116 ~~analysis.~~ Grain tef was milled to whole flour by disc attrition mill, with two discs,
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54 117 traditionally used in the cottage tef grain-milling house (Bishoftu, Ethiopia) for injera
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3 118 making, immediately packed in airtight plastic bags and then stored at 4°C until
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5 119 analysis.
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7 120 The mean particle size (D_{50}) and size dispersion of the flours were reported in Abebe et
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9 121 al. (2015) as 90.7 μm and 2.17 for (DZ-01-99), 94.7 μm and 2.14 for (DZ-Cr-37) and
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11 122 94.2 μm and 2.10 for (DZ.Cr-387), respectively.
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14 123 **2.3. Dough preparation and breadmaking**

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16 124 A straight dough process for a *ciabatta* bread type was performed using the following
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18 125 formula on a 100g flour basis: 1.8% salt, 0.5% bread improver, 2% dry yeast (~~added for~~
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20 126 ~~making bread~~) and 85% water. For dough rheological measurements, yeast-free samples
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22 127 were used in order to keep sample stability during test running. Each of the three tef
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24 128 varieties (DZ-01-99, DZ-Cr-37 and DZ-Cr-387) was incorporated at 0%, 10%, 20%,
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26 129 30% and 40% dose level and mixed with the wheat flour for 15 minutes using Chopin
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28 130 MR2L/MR10L mixer (Chopin technologies, France). The dough was prepared by
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30 131 blending the solid ingredients first in a kitchen-aid professional mixer (KPM5) for 2
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32 132 min. at speed 2. Then the kneading process was made in three phases: at speed 4 for 5
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34 133 min. by adding water during the first minute, at speed 6 for 1 min- and finally at speed 4
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36 134 for 8 min. ~~After mixing, the temperature of the dough was $25 \pm 1^\circ\text{C}$.~~ The dough, 300 g,
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38 135 was placed into aluminium pans and was proofed for 40 min at 28°C and $(75 \pm 5) \%$
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40 136 relative ~~moisture-humidity for 40 min~~. Subsequently, baking was carried out in a Salva
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42 137 oven (Lezo, Spain) at 190°C for 40 min. After baking, breads were left for one hour at
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44 138 room temperature before analysis. Bread volume was determined in duplicate using a
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46 139 volume analyser BVM-L370 TexVol Instruments (Viken, Sweden).
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142 2.4. Oscillatory and creep recovery tests

143 Oscillatory and creep-recovery tests were carried out with a RheoStress 1 rheometer
 144 (Thermo Haake, Karlsruhe, Germany) with parallel plate geometry (60 mm diameter) of
 145 serrated surface and with 3 mm gap. The excess of dough was removed and vaseline oil
 146 was applied to cover the exposed sample surfaces. All measurements were done at 25
 147 °C. Before each assay the dough was allowed to rest for 10 min for relaxation.
 148 Frequency sweeps were carried out from 10 to 0.1 Hz in the linear viscoelastic region
 149 (LVR). A constant stress value of 1 Pa was chosen for the frequency sweeps of all
 150 doughs after establishing this value fell in the LVR of all doughs by means of stress
 151 sweeps from 0.1 to 100 Pa at 1 Hz. From the curves, the maximum stress beyond which
 152 the dough structure was broken, τ_{max} , was established. Frequency sweep data were fitted
 153 to the power law model as in previous works (Ronda et al., 2011):

$$154 \quad G'(\omega) = G'_1 \cdot \omega^a \quad (1)$$

$$155 \quad G''(\omega) = G''_1 \cdot \omega^b \quad (2)$$

$$156 \quad \tan \delta(\omega) = \frac{G''(\omega)}{G'(\omega)} = \left(\frac{G''}{G'} \right)_1 \cdot \omega^c = (\tan \delta)_1 \cdot \omega^c \quad (3)$$

157 The coefficients G'_1 , G''_1 , and $(\tan \delta)_1$, stand for the elastic modulus, viscous modulus
 158 and the loss tangent at a frequency of 1 Hz. The a , b and c exponents quantify the
 159 degree of dependence of these moduli and the loss tangent with the oscillation
 160 frequency, ω expressed in Hz.

161 Creep tests were performed by imposing a sudden step shear stress in the LVR and
 162 outside the linear viscoelastic region (OLVR). For the creep study in the LVR a
 163 constant shear stress of 1Pa was applied for 120 s while in the recovery phase the stress
 164 was suddenly removed and the sample was allowed for 240 s to recover the elastic
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3 166 (instantaneous and retarded) part of the deformation. For the study OLVR a constant
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5 167 shear stress of 50Pa was applied for 60 s and the sample was allowed to recover for 200
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7 168 s after removing the load. Each test was performed in triplicate. The data from creep
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9 169 tests were modelled to the 4-parameter Burgers model (Lazaridou et al., 2007) given by:

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$$J_c(t) = J_{0c} + J_{1c} \left(1 - \exp\left(\frac{-t}{\lambda_{1c}}\right) \right) + \frac{t}{\mu_0} \quad (4)$$

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16 171 In the equation, $J_c(t)$ is the creep compliance (strain divided by stress), J_{0c} is the
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18 172 instantaneous compliance, J_{1c} is the retarded elastic compliance or viscoelastic
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20 173 compliances, λ_{1c} is the retardation time and μ_0 gives information about the steady state
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22 174 viscosity. Similar equations were used for the recovery compliance $J_r(t)$. As there is no
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24 175 viscous flow in the recovery phase, equations consist only of parameters describing the
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26 176 elastic response after removal of the shear stress. The data from creep tests were
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28 177 modelled to the 3- parameter Burgers model given by:

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

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36 179 J_{max} is the maximum creep compliance obtained at the end of the creep step. The steady-
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38 180 state compliance in recovery step, J_{steady} , was also calculated by subtracting the
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40 181 compliance value at the terminal region of curve (where dough recovery reached
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42 182 equilibrium) from the J_{max} . The ratio J_{steady}/J_{max} (elastic recovery) was also calculated
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44 183 and expressed as Recovery (%).

45 46 47 184 **2.5. Stickiness**

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49 185 This assay was conducted by following the procedure proposed by Grausgruber et al.
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51 186 (2003) and used by Ronda et al. (2011). A texturometer TA-XT2 from Stable
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53 187 Microsystem (Surrey, UK) provided with a SMS/Chen-Hoseney device where the
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55 188 sample was placed, and a methacrylate 25 mm cylinder (P/25P) as compression cell,

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3 189 were used. The stickiness of the dough was determined at pre-test and test speed of 0.5
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5 190 mm/s, a post-test speed of 10.0 mm/ s and 40 g force. Three parameters were used to
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7 191 define stickiness: the positive maximum force or adhesive force, which is the measure
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9 192 of stickiness, the positive area under the curve or the adhesive energy, which is the work
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11 193 of adhesion, and the distance the sample is extended on probe return, which is an
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13 194 indication of sample cohesion/dough strength. Six replicates were carried out for all
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15 195 doughs.

16 17 18 196 **2.6. Statistical analysis**

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20 197 Experimental data were analyzed using two-way analysis of variance (MANOVA) and
21 198 then means were compared at $p < 0.05$ using Fisher's least significant difference (LSD)
22 199 test. Correlations among the viscoelastic parameters and bread volume were evaluated
23 200 at $p < 0.01$ and $p < 0.05$ using Pearson's correlation method. Statistical analysis was done
24 201 by Statgraphics Centurion XVI program (StatPoint Technologies, Inc. 1982-2010).
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35 203 **3. Results and discussions**

36 204 Tables 1 to 3 show the effects of tef grain flour dose and tef variety on bread dough
37 205 viscoelastic properties and stickiness. Second order interactions (tef dose x tef variety)
38 206 were not significant ($p > 0.05$) on these parameters; therefore, only single effects are
39 207 presented.

40 208 **3.1. Dynamic oscillatory rheology**

41 209 The results of the stress and frequency sweeps are presented in ~~Table 2~~Table 1. The
42 210 values of τ_{max} , G' and G'' exhibited by the control dough in this study were lower than
43 211 those reported for wheat flour doughs (Dobraszczyk & Morgenstern, 2003) due to the
44 212 higher amount of water used in the *ciabatta* formulation. Water plays an important role
45 213 in determining the viscoelastic properties of dough. Both G' and G'' values decreased

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3 214 with increasing water content, because either water can act as an inert filler causing the
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5 215 dynamic properties to reduce proportionally to moisture content or water can behave as
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7 216 a ~~lubricant-plasticizer~~ enhancing the relaxation phenomena (Masi et al., 1998). τ_{max}
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9 217 values of tef enriched doughs showed a significant decrease (>41% on average)
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11 218 regardless wheat substitution level compared to control counterpart (~~Table 2~~Table 1).
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13 219 Such lower breakpoint for the tef incorporated doughs might be due to the dilution and
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15 220 breaking of the former strong network formed during wheat flour dough development.
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17 221 The dose of tef addition and the variety type did not appreciably change the τ_{max} score
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19 222 of the doughs.
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21 223 Frequency sweeps showed that in the whole range of frequencies, the elastic (or storage)
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23 224 modulus, G' , was greater than the viscous (or loss) modulus, G'' for all dough
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25 225 formulations. This led all values of loss tangent, included those at a frequency of 1Hz,
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27 226 $(\tan\delta)_1$, to be lower than 1 suggesting a solid elastic-like behavior of dough
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29 227 formulations. Both moduli slightly increased with frequency. This variation, which is
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31 228 quantified by a and b exponents from G' and G'' fittings to power law (~~Table 2~~Table 1),
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33 229 decreased significantly with tef addition. The incorporation of tef flours also markedly
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35 230 reduced both viscoelastic moduli, G_1' and G_1'' , leading to values 20% and 30% lower,
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37 231 respectively, than the control dough regardless the wheat substitution level. It can be
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39 232 noted that 10% tef addition was enough to exert gluten dilution and further weakening
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41 233 effect of the gluten network. The additional increase of tef dose did not lead to the
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43 234 concomitant decrease of viscoelastic moduli. Even ~~though, a~~ slight, ~~although, a~~
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45 235 significant increase in G_1' was observed for samples with 40% tef addition with respect
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47 236 to lower doses. This could be explained by the higher water absorption capacity
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49 237 (+27%), water holding capacity (+38%) and swelling volume (+37%) of tef flour in
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51 238 comparison with wheat flour as was reported in previous studies (Abebe et al., 2015a).
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3 239 The explanation is consistent with the increase in dough consistency that may
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5 240 counteract the gluten dilution effect. Other authors have found higher viscoelastic
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7 241 moduli in rice-wheat composite doughs than in wheat doughs associated to stronger
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9 242 starch–gluten interactions in composite flour (Sivaramakrishnan et al., 2004). Authors
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11 243 also reported that, rice starch granules in the dough can act as filler that reinforces the
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14 244 gluten and produce strong bonds to given higher modulus. The viscous modulus
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16 245 decreased with tef addition in a greater extent than the elastic one (~~Table 2~~Table 1).
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18 246 Consequently, the loss tangent decreased significantly ($p < 0.05$) with tef addition, from
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20 247 0.34 (0% tef) to 0.27 (40% tef) implying an increase in the solid like behavior of tef-
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22 248 added doughs that increased with the tef level. This could be attributed to the
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24 249 differences in protein contents and profiles (Ronda et al., 2011; Hager et al., 2012;
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26 250 Abebe and Ronda, 2014). The marked variation in the lipid profiles, fiber, and shape
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28 251 and size of starch granules of wheat and tef flours observed by Hager et al. (2012) and
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30 252 Abebe & Ronda (2014) could also be a key factor. The c exponent, as was reported for
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32 253 a and b , also decreased with tef addition level, encompassing G_1''/G_1' ratio to have
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34 254 lower dependence on frequency (Ronda et al., 2011) associated to a lower frequency
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36 255 dependence structure (Sivaramakrishnan et al., 2004) in tef-supplemented doughs.
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38 256 Significant effect of tef variety type on G_1' and G_1'' was observed. The DZ-Cr-37 tef
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40 257 variety flour exhibited the highest G_1' and G_1'' average moduli, 14% higher than the
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42 258 remaining two tef varieties.

259 3.2. Creep-recovery tests

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

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3 264 provide additional information to those of the creep phase. The dough had typical
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5 265 viscoelastic creep-recovery curves combining both viscous and elastic components.
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7 266 Both tef incorporation and variety affected creep-recovery parameters. However, as it
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9 267 was observed in oscillatory tests, the effect on creep results was not proportional to tef
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11 268 addition. The incorporation of 10% tef flour to replace wheat made creep phase
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13 269 instantaneous (J_{0c}) and retarded (J_{1c}) elastic compliances to increase significantly (28%
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15 270 and 33% in LVR and 53% and 46% OLVR) with respect to control dough values. The
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17 271 increase of compliances in the recovery phase was 66% and 38% for J_{0r} and J_{1r} with
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19 272 respect to the control dough values. This indicates that tef enriched doughs had higher
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21 273 instant and retarded deformations when subjected to a constant stress and higher
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23 274 recoveries when the stress was removed. Higher levels of tef in the flour blend, in
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25 275 general, did not lead to significant increases in the elastic or viscoelastic answers
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27 276 obtained in the LVR and OLVR with respect to that of the 10% tef-supplemented
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29 277 dough. The J_{0c} and J_{1c} compliances in the LVR tended to decrease again with tef dose
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31 278 attaining at 40% level very similar values to the control dough (+12% and +6%
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33 279 respectively). The steady-state viscosity, μ_{st} , which gave the flowability of the material
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35 280 at the end of the applied load decreased with 10% tef addition being significantly higher
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37 281 lower than the control dough for the OLVR measurement (-35%). For durum wheat
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39 282 doughs, it was found that the entire elastic compliance curve was shifted to higher
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41 283 values as the strength of the dough (measured by extensigraph) decreased (Edwards et
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43 284 al., 2001), while the steady-state viscosity increased with strength (Edwards et al.,
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45 285 2001). Authors interpreted the differences in creep behavior in terms of differences in
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47 286 strength of the associative network established by non-covalent intermolecular
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49 287 associations within gluten chains. 10% Whole grain tef flour at 10% addition
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51 288 represents a supply of insoluble fiber that could explain the wheat gluten network
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3 289 disruption. The non-proportional effects of tef substitution levels could be due to
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5 290 differences in tef functional properties with respect to wheat (Abebe et al., 2015)
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7 291 dependent on their different composition and particularly their different protein and
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9 292 starch nature (Sivaramakrishnan et al., 2004). The λ values (~~Table 3~~ Table 2) calculated
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11 293 did not show any significant variation with tef addition. The maximum creep
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13 294 compliances, both in and outside the LVR assays, increased with 10% tef addition
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15 295 although it was much more pronounced (13% versus 30%) in OLVR assays. An
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17 296 additional increase of 30% in the maximum creep compliance in OLVR assays was
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19 297 observed in 40% tef added dough. This can be partly attributed to its higher flowability
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21 298 (lower η_0) and partly to its higher viscoelastic deformation (higher J_{Ic}). The total elastic
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23 299 compliance ($J_{0c}+J_{Ic}$) represented 56% of the maximum creep compliance in wheat
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25 300 dough. This ratio did not vary with tef addition in the LVR measurements meanwhile
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27 301 increased significantly in OLVR test, increasing until 64% independently of the dose of
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29 302 tef. In the recovery phase approximately 55% and 65% elastic recovery could be seen
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31 303 for pure and 10% tef-added wheat doughs respectively. This means a lower viscous
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33 304 characteristics of tef added doughs which is coherent with the lower $\tan \delta$ values already
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35 305 reported in the oscillatory tests.

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41 306 The study effect of tef variety type on creep-recovery properties demonstrated that DZ-
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43 307 Cr-37 behaved differently, as was already commented with respect to oscillatory test
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45 308 results and the differences were more marked in OLVR assays. Accordingly, flour of
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47 309 this variety led to significantly lower average elastic compliances (-23% for J_{0c} and J_{0r} ,
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49 310 -30% for J_{Ic} and J_{Ir} , -33% for J_{max} in the creep phase, and -23% for J_{steady} from recovery
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51 311 phase) and higher average steady-state viscosity (+49%) than DZ-Cr-387 tef flour
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53 312 doughs. Though DZ-Cr-387 is a white tef variety like as DZ-Cr-37, incorporation of
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55 313 DZ-Cr-387 and DZ-01-99 (brown tef variety) grain flours changed the resulting dough
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3 314 | ~~creep-recovery characteristics in a closer manner while DZ-Cr-37 incorporated dough~~
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5 315 | ~~behaved differently, showed the maximum difference with it in all the creep-recovery~~
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7 316 | ~~parameters meanwhile gave similar values to the brown grain tef variety, DZ-01-99.~~ In
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10 317 | the LVR the creep compliances of different tef varieties doughs gave maximum average
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12 318 | differences of 16 – 19% and non-significant differences among steady-state viscosities.
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14 319 | The relatively higher consistency (higher G_1' and η_0 values) of DZ-Cr-37 cultivar and
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16 320 | its lower deformability versus a stress may explain the lower dough development during
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18 321 | proofing and baking, resulting in lower bread volumes. Tef variety type did not
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20 322 | significantly affect the retardation time (λ_c) in the creep phase of the test carried out in
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22 323 | the LVR. However, in OLVR tests impact of tef variety was significant on λ_c and DZ-
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24 324 | Cr-37 showed the lowest value indicating that the retardation time of the elastic retarded
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26 325 | response was smaller than the doughs with the remaining varieties. No significant
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28 326 | difference was observed in the retardation time of the recovery phase. Previous works
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30 327 | have correlated the retardation times after creep with the bread volume reporting lower
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32 328 | bread volumes for doughs with faster recoveries (Van Bockstaele et al., 2011). In this
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34 329 | work, differences in retardation times, both in the creep or recovery phases were too
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36 330 | small to explain the differences found in bread volume.
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40 331 | Although significant effects ($p < 0.05$) were observed among the doughs different in tef
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42 332 | flour dose level –or tef variety type on some creep parameters obtained from LVR
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44 333 | assays, it can be concluded their effect in OLVR were much more pronounced allowing
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46 334 | better dough discrimination. Probably the general thought that higher correlation
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48 335 | between dough creep parameters and bread volume are obtained outside the LVR can be
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50 336 | partly due to the more marked differences found among samples in the latter test. In any
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52 337 | case, very high correlations between all the parameters obtained in and outside LVR
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54 338 | were found.
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339 3.3. Dough stickiness

340 Results of the stickiness test on the formulated doughs are disclosed in ~~Table 4~~Table 3.

341 The stickiness (adhesive force) of the tef enriched doughs was lower than the control at

342 lower doses, mainly at 10% and 20%, ~~and conversely the cohesiveness of these doughs~~

343 ~~were higher (Hoseney and Smewing, 1999).~~ However the adhesive forces recorded

344 tended to rise with tef dose level so that, 40% tef-added doughs showed considerably

345 higher average stickiness (+36%) than the control. The adhesive energy and the distance

346 on return also showed a marked decrease since the smallest tef addition but, in this case,

347 they continued decreasing until the 30% dose, and started to rise at the highest tef

348 content. Tef grain flour supplemented doughs did not overpass the control adhesive

349 energy and the distance on return values. Then, the three dough stickiness parameters

350 showed similar tendency with a minimum value versus tef concentration, shifted toward

351 higher concentrations in the case of adhesiveness energy and distance on return.

352 The study shows that incorporation of tef at higher percentage significantly increases

353 the adhesive force and this may affect the handling and shaping/flattening purposes to

354 get continuous strands or thin sheets of the doughs. In any case, stickiness did not

355 overpass the 100 g value, discarding important dough handling problems (Chen &

356 Hoseney 1995; Armero y Collar, 1997). Slight variations due to tef variety were also

357 observed, in accordance with earlier observations reported on wheat revealing that

358 varieties, growing season, protein concentration, water absorption, milling process and

359 extraction rate may influence dough stickiness (Van Velzen et al., 2003; Yildiz et al.,

360 2012).

361 3.4. Bread volume

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

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

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3 364 volume of bread ($p < 0.001$). The substitution of wheat flour by tef flour until the 30%
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5 365 level, led to *ciabatta* type breads with significantly higher ($p < 0.05$) specific volume
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7 366 than the control wheat flour bread. The highest effect on volume was obtained with 10%
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10 367 or 20% additions (+12% on average) depending on the tef variety, but still 30% tef-
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12 368 enriched breads showed a significant ($p < 0.05$) 5% volume increase with respect to the
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14 369 control breads. Previous studies reported that the lower polymerization, hydrophobicity
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16 370 and denaturation temperature of tef prolamins probably make them somewhat functional
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18 371 in bread making (Adebowale et al., 2011). The loaves with 40% tef flour showed a small
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20 372 (-2%) although significant, lower volume than the wheat counterpart. Aluyante et al.
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22 373 (2012) showed that replacing wheat flour by tef grain flour up to 10% in straight dough
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24 374 bread making did not affect loaf volume, while larger incorporations had a detrimental
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26 375 effect. Mohammed et al., (2009) obtained declining breads volumes on additions to
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28 376 wheat flour higher than 10-15% tef. The higher amount of admitted tef in the present
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30 377 samples could be due to bread formulation and the tef varieties used. However,
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32 378 probably the most important factor was the type of wheat flour used in the blend. In our
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34 379 case, high grade wheat flour was used while previous authors reported to use all-
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36 380 purpose wheat flour (Mohammed et al., 2009). The very high gluten content of the
37
38 381 wheat flour (14.5% protein), too high for general breadmaking proposes, withstood the
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40 382 dilution with tef leading, until a certain substitution level, to suitable dough rheological
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42 383 characteristics, less tough and with higher development capacity under the gas
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44 384 expansion effect during proofing and baking. The higher gelatinization temperature of
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46 385 tef starch than wheat starch (Whistler and Be Miller 1997, Bultosa et al., 2002) could
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48 386 also explain the higher volume of tef enriched breads as a higher dough development is
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50 387 allowed in the oven due to the gas expansion retained in the dough before reaching a
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3 388 rigid structure. Tef variety type exerted remarkable effects on bread loaf volume in the
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5 389 order of: DZ-01-99 > Quncho DZ-Cr-387 > DZ-Cr-37.
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9 391 **3.5. Correlations among rheological properties and bread volume**

10 392 Pearson correlation analysis showed a significant interdependence among the oscillatory
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12 393 and creep-recovery parameters (Table 45). As reported Ronda et al. (2014) both, the
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14 394 storage and loss moduli, showed strong interdependence. The loss tangent ($\tan \delta$)_l was
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16 395 more dependent on loss moduli than the storage modulus. The creep compliances
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18 396 parameters showed strongly significant correlations with recovery phase counterparts (r
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20 397 > 0.92; $p < 0.01$). In the LVR the viscosity at steady state (μ_0) was only dependent on the
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22 398 maximum creep compliance J_{max} ($r = -0.40$, $p < 0.01$). However, for measurements
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24 399 outside the LVR, μ_0 strongly decreased ($r > -0.81$, $p < 0.01$) with increasing J_{max} , J_0 and J_l .
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26 400 In agreement with Ronda et al (2014), the higher maximum stress (τ_{max}) explaining
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28 401 structural integrity of the doughs, increased in parallel with dynamic moduli, and
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30 402 decreased with instantaneous compliance. Bread volume was negatively correlated with
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32 403 the elastic modulus G_1' ($r = -0.5$, $p < 0.01$) and positively with elastic compliances, J_{oc}
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34 404 and J_{lc} in the LVR assays ($r = 0.3$, $p < 0.5$). This can be explained by the dilution of the
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36 405 strong gluten network of wheat dough due to tef addition which lowered the dough
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38 406 consistency and increased its deformation capacity versus a constant stress. The
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40 407 condition allowed a higher dough development as a consequence of the gas production
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42 408 during proofing and its further expansion during baking (Villanueva et al., 2015). Bread
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44 409 volume had a highly significant positive correlation with dough elastic recovery after
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46 410 creep (Recovery) ($p < 0.01$; $r = 0.62$) and with the ratio $(J_{oc} + J_{lc})/J_{maxc}$ in the creep phase
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48 411 ($p < 0.01$; $r = 0.45$) both in OLVR assays. This means that doughs with smaller relative
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50 412 viscous parts led to higher bread volumes. Bread volume showed also a highly negative
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3 413 correlation with dough stickiness (adhesive force) ($r=-0.87$; $p<0.01$) showing that
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5 414 doughs with the highest level of tef that became stickier gave lower bread volumes.
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7 415 Armero & Collar (1997) recommended ~~for~~to maximized dough cohesiveness and
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9 416 minimized dough stickiness for providing good bread-making performance. Therefore,
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11 417 dough stickiness could be one of the drawbacks of incorporating tef flours at higher
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13 418 percentages.
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19 420 **4. Conclusions**

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21 421 In general, incorporation of tef flours affected the structure of the dough matrices
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23 422 visibly in terms of lower viscoelastic moduli and τ_{max} values and larger instantaneous
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25 423 and retarded elastic compliances. Effect of dose level on these parameters was also
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27 424 significant. Tef flour supplemented breads up to 30% level had higher volume than the
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29 425 control ascribed to lower consistency and higher deformability of the doughs. However,
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31 426 at 40% tef dose the bread volume decreased to lower values than wheat bread.
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33 427 Viscoelastic properties do not explain easily this observation, as in general fundamental
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35 428 properties did not change markedly in samples over 10% tef addition. The elastic
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37 429 recovery capacity after creep and stickiness strongly correlated with bread volume. The
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39 430 present study also show that incorporation of tef at higher percentage (40%) increases
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41 431 dough stickiness and this may affect the handling and shaping/flattening purposes to
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43 432 obtain continuous strands or thin sheets of the doughs. On average, the DZ-Cr-37
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45 433 supplemented doughs showed higher elastic and viscous moduli, lower compliances,
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47 434 and higher steady state viscosity ~~and~~ in both LVR and OLVR than those supplemented
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49 435 with other tef varieties. In addition, DZ-Cr-37 supplemented doughs also led to breads
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51 436 with lower volume. However, tef variety type did not appreciably affect dough
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53 437 stickiness. Hence, based on the dough viscoelastic and surface-related handling
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3 438 properties studied, the incorporation of DZ-01-99 and DZ-Cr-387 could be more
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5 439 preferable 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 (%)	Carbohydrates (%)	Starch (%)	Amylose (% of starch)
Tef brown (DZ-01-99)	10.5±0.1a	8.9±0.3a	2.71±0.19b	2.84±0.08e	85.6±0.6b	75.5±0.1b	21.6±0.3a
Tef white (DZ-Cr-37)	10.3±0.1a	10.5±0.2b	3.52±0.01e	2.63±0.06b	83.4±0.2a	74.0±0.3a	21.8±0.3a
Tef white (DZ-Cr-387)	10.4±0.1a	8.9±0.2a	2.63±0.09b	3.24±0.06d	85.3±0.3b	75.5±0.4b	21.1±0.4a
Wheat	12.2±0.1b	14.5±0.2d	0.66±0.01a	1.47±0.06a	85.1±0.2b	78.8±0.4c	23.2±0.5b

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'_i (Pa)	a	G''_i (Pa)	b	$(\tan \delta)_i$	c	τ_{\max} (Pa)
<i>Tef dose (%)</i>							
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
<i>Tef variety</i>							
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. $G'(\omega) = G'_i \cdot \omega^a$; $G''(\omega) = G''_i \cdot \omega^b$; $\tan \delta(\omega) = (\tan \delta)_i \cdot \omega^c$. τ_{\max} was obtained from stress sweeps. Data are the mean ± 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.

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

Parameter	LVR						OLVR										
	Creep phase						Creep phase					Recovery phase					Recovery (%)
J_{0c} (10^{-5} Pa $^{-1}$)	J_{1c} (10^{-5} Pa $^{-1}$)	λ_c (s)	μ_{0c} (10^5 Pa·s)	J_{maxc} (10^{-5} Pa $^{-1}$)	J_{e-c}/J_{maxc} (%)	J_{0c} (10^{-5} Pa $^{-1}$)	J_{1c} (10^{-5} Pa $^{-1}$)	λ_c (s)	μ_{0c} (10^5 Pa·s)	J_{maxc} (10^{-5} Pa $^{-1}$)	J_{e-c}/J_{maxc} (%)	J_{0r} (10^{-5} Pa $^{-1}$)	J_{1r} (10^{-5} Pa $^{-1}$)	λ_r (s)	J_{steady} (10^{-5} Pa $^{-1}$)		
<i>Tef Dose (%)</i>																	
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
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
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
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
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
<i>Tef variety</i>																	
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±7B	58±3A
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
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

LVR: Results obtained in the Linear Viscoelastic Region (at 1Pa); OLVR: Results obtained from creep test carried out Outside the Linear Viscoelastic Region (at 50Pa); J_{maxc} , J_{0c} , and J_{1c} = maximum, instantaneous and retarded compliances (respectively), λ_c = retardation time and μ_{0c} = steady state viscosity in the creep phase; J_{steady} , J_{0r} , and J_{1r} = steady-state, instantaneous and retarded compliances (respectively), λ_r = retardation time and μ_{0r} = steady state viscosity in the recovery phase; J_{e-c} : Elastic compliance in creep phase= $J_{0c}+J_{1c}$; Recovery: $100*J_{steady}/J_{maxc}$
Data are the mean± 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 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)
<i>Tef dose (%)</i>			
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
<i>Tef variety</i>			
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 ± 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.

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Table 54. Correlations between viscoelastic properties and bread volume.

	G_1'	a	G_1''	b	$(\tan \delta)_1$	c	τ_{max}	J_{maxcl}	J_{0cl}	J_{1cl}	μ_{0cl}	J_{cl}/J_{maxcl}	J_{maxeO}	J_{0eO}	J_{1eO}	λ_{eO}	μ_{0eO}	J_{eO}/J_{maxeO}	$J_{steadyO}$	J_{0rO}	J_{1rO}	λ_{rO}	Rec	F	A	D
Volume	-0.40*	-	-0.33*	0.33*	-	0.55**	-	-	0.33*	0.28*	-	-	-	-	-	-	-	0.45**	-	-	-	-	0.62**	-0.87**	-0.56**	-0.42*
G_1'		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*	-
G_1''				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*	-
$(\tan \delta)_1$						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_{max}								-	-	-	-	-	-0.52**	-0.55**	-0.44**	-	0.44**	-0.34*	-0.6**	0.48**	-0.33*	-	-	-	0.66**	0.52**
J_{maxcl}								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*	-0.42**	-	-	-	-
J_{0cl}									0.74*	-	-	0.76**	0.87**	0.77**	-	-0.64**	-	0.79**	0.82**	0.78**	-	-	-	-	-0.40**	-0.37**
J_{1cl}										-	-	0.52**	0.59**	0.56**	-	-0.47**	-	0.55**	0.57**	0.54**	-	-	-	-	-	-
μ_{0cl}													-0.37**	-0.29*	-0.39**	-0.44**	0.35**	-	-0.33*	-0.29*	-0.33*	0.31*	-	-	-	0.28*
J_{cl}/J_{maxcl}													-	-	-0.50**	0.34**	0.50**	-	-	-	0.31*	0.46**	-	-	-	-
J_{maxeO}													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**	
J_{0eO}														0.94**	0.30*	-0.81**	-	0.95**	0.97**	0.92**	-0.40**	-0.30*	-	-0.46**	-0.44**	
J_{1eO}															0.52**	-0.88**	-	0.94**	0.95**	0.95**	-0.50**	-0.44**	-	-0.36**	-0.38**	
λ_{eO}																-0.55**	-0.27*	0.36**	0.35**	0.42**	-0.33*	-0.60**	0.36**	-	-	
μ_{0eO}																	0.26*	-0.83**	-0.83**	-0.81**	0.51**	0.42**	-	0.43**	0.40**	
J_{eO}/J_{maxeO}																			-	-	0.33*	0.70**	-	-	-	-
$J_{steadyO}$																				0.95**	0.93**	-0.50**	-0.30*	-	-0.45**	-0.43**
J_{0rO}																				0.96**	-0.52**	-0.30*	-	-0.45**	-0.44**	
J_{1rO}																					-0.60*	-	-	-0.31*	-0.34*	
λ_{rO}																										
Rec																								-0.32*	-	-
F1																										
A1																										
D1																										0.83**

J_{maxcl} , J_{0cl} , and J_{1cl} = maximum, instantaneous and retarded compliances (respectively), λ_{cl} = retardation time and μ_{0cl} = steady state viscosity in the creep phase obtained from creep test in linear viscoelastic region (at 1Pa); J_{maxeO} , J_{0eO} , and J_{1eO} = maximum, instantaneous and retarded compliances (respectively) outside the linear VR, λ_{eO} = Retardation time and μ_{0eO} = 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, λ_{rO} = Retardation time in the recovery phase obtained from test outside the viscoelastic region (at 50Pa). J_{e-c} : Elastic compliance in creep phase = $J_{0e} + J_{1e}$; Rec: Recovery: J_{steady}/J_{max} ; G_1' , G_1'' and $(\tan \delta)_1$ 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; τ_{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 ($p > 0.05$).

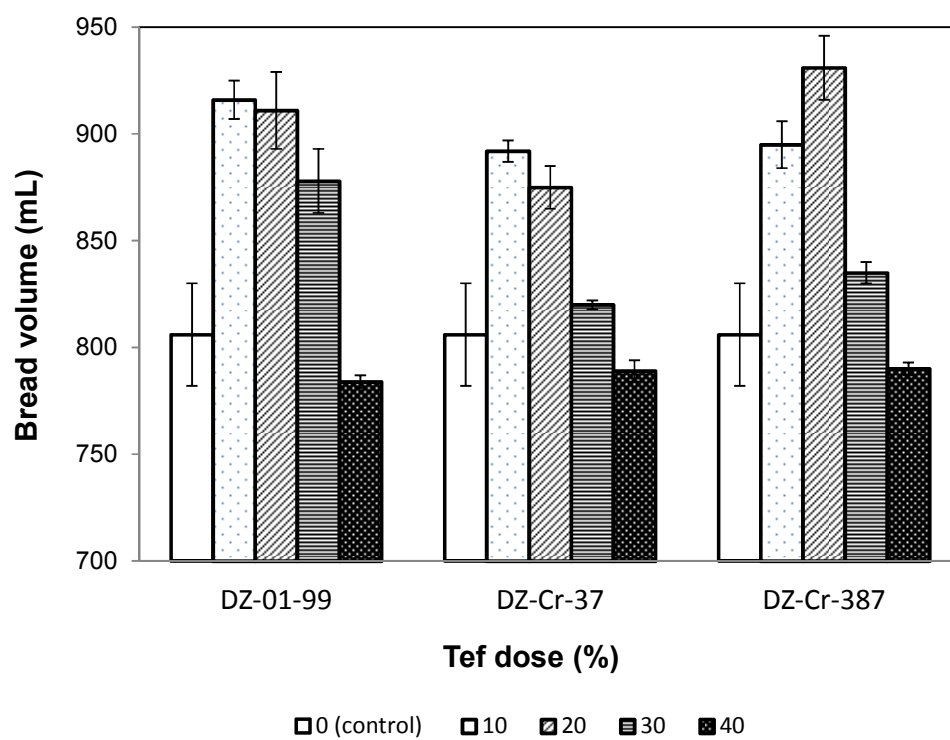


Figure 1. Evolution of bread volume with tef dose of three different varieties