Highlights

- Tef variety plays an important effect on dough and bread quality
- Tef flour addition had a structuring effect on maize starch gluten-free doughs
- The gelatinization temperature increased with the addition of tef flour
- Breads made with tef flour up to 75% level had high volume and good appearance
- DZ-Cr-37 variety led to the highest bread volume but to the less regular crumb

Tef [*Eragrostis tef* (Zucc.) Trotter] variety determines viscoelastic and thermal properties of gluten-free dough and bread quality

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Abstract

Tef flour has been considered a good alternative raw material to improve the quality of gluten-free bread due to its attractive nutritional profile. The influence of tef variety (DZ-Cr-37, DZ-Cr-387 and DZ-01-99) and incorporation level (100, 75 and 50%) on rheological and thermal properties of maize starch-based gluten-free doughs, and their impact on bread characteristics was investigated. The addition of tef brought about a structuring effect of doughs leading to higher viscoelastic moduli and steady-state viscosities and to lower $\tan \delta$ values and instantaneous and retarded compliances. These effects were magnified with the increase of tef level in the dough. Tef addition led to an increase in dough gelatinization temperature and a decrease of gelatinization enthalpy, especially in the case of DZ-01-99 variety. Tef incorporation at 50 and 75% level led to well-developed bread which volume and crumb grain properties were significantly affected by the tef variety. DZ-Cr-37 variety led to the highest bread volume but to a less regular and poorer crumb grain. However, the bread made with 100% DZ-Cr-37 reached the highest overall acceptance score. This work demonstrates tef flour incorporation up to 100% level is a feasible procedure to reach technologically viable and sensory acceptable gluten-free bread.

Keywords: tef varieties; dough rheology; thermal properties; gluten-free bread

1. Introduction

Over the last decade, the market for gluten-free (GF) products has grown considerably. This demand is related to better diagnostic methods identifying an increasing number of people suffering from coeliac disease and other gluten-related disorders, and people who intake GF products as a “healthier” lifestyle (Foschia, Horstmann, Arendt, & Zannini, 2016). However, the elimination of gluten often encompasses deleterious

37 effects on both quality attributes and consumer acceptance (Naqash, Gani, Gani, &
38 Masoodi, 2017). The nutritional value of GF products is also poorer due to the raw
39 materials used compared to wheat flour (Naqash et al., 2017). In addition deficient gas
40 retention and consequent low loaf volume are the major challenges to face in GF bread
41 production (Naqash et al., 2017). To solve this problem, several strategies in terms of
42 technological improvement have been developed so far (Ronda, Villanueva, & Collar,
43 2014).

44 From nutritional and health points of view, tef is an ancient grain that is increasingly
45 growing in popularity. Tef is always consumed as whole grain and is rich in
46 carbohydrate and fiber, presents equilibrated balance among the essential amino acids
47 and contains more iron, calcium and zinc than other cereal grains, including wheat,
48 barley and sorghum (Ronda, Abebe, Pérez-Quirce, & Collar, 2015). It has no gluten and
49 is suited for diabetic patients due to slow-release of their carbohydrates (Abebe &
50 Ronda, 2014) probably because of its lower starch content as consequence of its higher
51 dietary fiber and ash contents (Abebe et al., 2015a).

52 Tef cultivars are most commonly classified in terms of their brown or white seed
53 ~~colour~~color, and rarely according to their specific variety (Sliwinski, Hopfer, & Ziegler,
54 2019). However, the physico-chemical and nutritional quality of tef flour and their
55 derived products are highly dependent on the variety (Abebe, Collar, & Ronda, 2015a;
56 Abebe, Ronda, Villanueva, & Collar, 2015b; Bultosa & Taylor, 2004). Although tef
57 grain/flour characterization and their food applications are extensively studied, little
58 information is available about the impact of tef incorporation in GF bread formulations
59 depending on the variety. Abebe et al. (2015b) assessed wheat bread loaves made with
60 up to 30% of three varieties of tef flour and obtained higher volume than control,
61 ascribed to lower consistency and higher deformability of the doughs. GF bread with

100% white tef (of unspecified variety) flour have also been made (Hager et al., 2012). These authors reported the quality of these bread loaves were much lower than that of wheat bread, having much denser structure and low specific volume. Rheological and thermal properties of doughs are known to determine the physical quality of the final bread because they affect their mechanical behaviour and expansion capacity during dough processing and bread making (Moreira, Chenlo, & Torres, 2011; Ronda, Pérez-Quirce, & Villanueva, 2017). Hence, in order to develop new tef enriched GF bread, this work aimed at studying the effect of tef variety and its addition level on the viscoelastic properties and thermal transitions of maize starch-based gluten-free doughs and their correlation and impact on bread making performance.

2. Materials and methods

2.1 Raw materials

Three-grain tef varieties DZ-01-99 (brown tef), DZ-Cr-37 (white tef) and DZ-Cr-387 (white tef), were obtained from the Debre Zeit Agricultural Research Center of the Ethiopian Institute of Agricultural Research (EIAR). Selection was based on the seed color, the year of release and the popularity among the Ethiopian tef grain consumers and farming community. Grain tef was milled to whole flour by disk attrition mill, with two disks, traditionally used in the cottage tef grain milling house (Bishoftu, Ethiopia) for injera making, immediately packed in airtight plastic bags and then stored at 4 °C until analysis. The moisture content determined using method 44-10 of AACC (AACC, 1999) of the DZ-Cr-37, DZ-Cr-387, and DZ-01-99 tef flours were 12.02%, 11.80% and 10.99% respectively. Starch contents, measured with the optional rapid method for total starch described by Englyst, Hudson & Englyst (2006) were 77 ± 1 , 79 ± 1 and 80 ± 2 g/100 g dry matter basis. Maize starch was supplied by Ferrer Alimentación S.A. (Barcelona, Spain) (11.21% m.c.) and its total starch content was 99 ± 2 g/100g dry

matter. Proximal analysis of the flours of these three tef varieties have been reported previously (Abebe & Ronda, 2014; Ronda et al., 2015). Sunflower oil, sugar, and salt were purchased from the local market and hydroxy-propyl-methyl-cellulose (HPMC, Methocel K4M Food Grade) was provided freely by Dow Chemical (Midland, USA).

2.2 Dough preparation and bread making

Preliminary tests were carried out to determine the most suitable matrix to add tef flour to before establishing the formulation of bread doughs. The ingredients that usually lead to the best results in gluten-free baking were tested: rice flour and a mixture of rice flour and ~~corn~~maize starch (60:40). However, the matrix that mixed with tef produced the best bread loaves (higher volume and finer and regular crumb grain) was ~~corn~~maize starch, although when used alone it led to bread with unacceptable grain defects. In fact, for the sensory evaluation performed in this study ([see 2.6 section below](#)), the rice flour + ~~corn~~maize starch blend (60:40) was chosen to make the ~~control~~/reference bread, as [an example of ~~a commercial type~~ GF bread of good/suitable quality, similar to the quality of a commercial GF bread.](#)

A straight dough process was performed using the following formula on a 100 g maize starch or maize starch+tef flour (14% moisture) basis: 5 g sugar, 1.5 g salt, 2 g HPMC, 6 g of oil, 3% dried yeast and 90 g water. The water added to the dough was adapted depending on the starch or starch+flour moisture content in order to get the same final dough hydration. Tef flour was incorporated at 50%, 75% and 100% level. Mixtures of maize starch and tef flour were prepared by mixing both with a Chopin MR2L/MR19L mixer (Chopin technologies, France) for 20 minutes. For the rheological measurements no yeast was added to the dough to avoid changes in dough properties over time and to obtain stable readings. The GF dough and bread-making procedures are described in detail elsewhere (Villanueva, Harasym, Muñoz, & Ronda, 2019).

2.3 Oscillatory and creep–recovery tests

These tests were performed with a rheometer AR 2000EX (TA Instruments, New Castle, USA) using parallel plate geometry (20 mm diameter), with 2 mm gap. In all tests, the temperature was set at 25 °C. Excess dough after the application was removed and the exposed sample surfaces were covered with vaseline oil and the sample was allowed to relax for 6 min before each assay. The stress sweeps were performed in duplicate in the range of 0.3 to 1000Pa at 1 Hz. From these curves, the maximum stress (τ_{max}) beyond which the dough structure was broken and the linear viscoelastic zone (LVR) and the stress at the cross point ($G'=G''$) were established (Ronda et al., 2017). Frequency sweeps were carried out from 10 to 0.1Hz at a constant stress of 2Pa. Frequency sweep data were fitted to the power law model as in previous works (Ronda et al., 2014).

Creep tests were performed by imposing a sudden step shear stress outside the linear viscoelastic region (OLVR) of 100Pa for 60s. Then, the recovery phase was recorded for 200s. These tests were performed at least in triplicate. The data from creep-recovery tests were modelled to Burgers model as in previous works (Ronda et al., 2014). The Recovery (%) was calculated as $100 \cdot J_{steady}/J_{max}$, where J_{steady} is the steady-state compliance in recovery step. The stress value to carry out this test was selected in order to differentiate among samples in function of the stress at the cross point ($G'=G''$) established from stress sweeps. 100Pa was above this value in some samples and below it in others; only the samples of the second group would retain at 100 Pa a certain elastic component. For all the doughs the test allowed the evaluation of the steady-state viscosity from the inverse of the slope of the linear evolution of compliance versus time in the creep phase, that is a straight line since the beginning of the test in absence of elastic behavior.

2.4 Thermal Properties

Gelatinization and retrogradation transitions of doughs were determined using a differential scanning calorimeter (DSC) (DSC-822e, Mettler Toledo, SAE). Freeze-dried hydrated doughs (≈ 10 mg dry matter) were weighed into 40 μ l aluminum pans and distilled water was added to make 50% moisture content, simulating the moisture level in the dough. Subsequently they were scanned from 0 to 110°C at 5°C/min using an empty pan as reference. After the first gelatinization test, the aluminum pans were stored at 4°C for 7 days and measured again to evaluate the retrogradation transition. The enthalpy, (ΔH), onset (T_o), endset (T_e), and peak (T_p) temperatures and the difference of temperatures ($\Delta T = T_e - T_o$), as a measure of the width of the endothermic transition, were analyzed from both scans. ΔH was expressed in J/g starch in the dough. Tests were performed at least in duplicate.

2.5. Bread volume and crumb characteristics

The bread volume was determined from two replicates using a Volscan-profiler-300 (Stable Microsystems, Surrey, UK) analyzer. Crumb grain characteristics were established from two central slices of two loaves taken from each elaboration. The images were acquired with Hp Scanjet G3110 scanner and were processed with ImageJ image analysis program from 15mm x 50mm sections taken from the center of slices. Cells above 10 mm were considered as defects and discarded from the calculation of cell density (number of cells per cm^2 area) and mean cell area (mm^2).

2.6. Sensory evaluation

Hedonic sensory evaluation was performed on bread samples using a multisample difference test following the guidelines suggested by Meilgaard et al. (2006). One hundred twenty-seven volunteers, between the ages of 15–64 and with different

socioeconomic backgrounds, rated the overall acceptance on a non-structured scale ranging from 0 (I like it much less than R) to 10 (I like it much more than R), where R was ~~the~~ reference ~~bread that~~ was positioned in the middle of the scale. This means that reference bread was arbitrarily assigned a score of 5. R was a gluten-free bread made with a ~~conventional~~ mixture of rice flour and ~~corn~~maize-starch (60:40 w:w) following the same procedure described for the remaining bread samples (see the section- 2.2). Panelists were offered pieces of 40x40 mm (that included both crumb and crust) of the bread loaves made with 50, 75 and 100% tef flour of the three varieties. The control bread (100% maize starch) was also included in the sensory evaluation. Bread samples were evaluated in three sessions. The R bread (60:40 rice flour: maize starch) was included in the three sessions and used by panelist as reference to locate the center of the overall acceptability scale. Panelists had a free writing area on the bread evaluation form to make any comments on the aspects of the bread that had contributed most to their score.

2.7 Statistical analysis

Statistical analyses were performed using software Statgraphics Centurion XVIII (Bitstream, Cambridge, MN, USA) for non-linear regression and Pearson correlation matrix. The range of Pearson correlation coefficient is from -1 to +1, and measures the strength of the linear relationship between the variables. Only correlation coefficients with a p-value <0.05 have been considered. The significance of the differences was determined based on the multifactor analysis of variance (ANOVA) and LSD (Least Significant Difference) test was used to evaluate significant differences (p<0.05) between samples.

3. Results and discussion

3.1 Oscillatory tests

Rheological parameters from dynamic tests performed on doughs are summarized in Table 1. The ANOVA study showed a significant effect of tef level ($p < 0.001$) and tef variety ($p < 0.01$) on G'_1 , G''_1 and $(\tan \delta)_1$. Second-order interactions (variety x level) were not significant ($p > 0.05$).

The mean value of G'_1 and G''_1 of all tef-containing doughs were significantly higher than those obtained with 100% maize starch. The incorporation of 50% tef flour increased viscoelastic moduli, G'_1 and G''_1 , 241% and 178% respectively, with regard to the control. The additional increase of tef in the dough led to the concomitant increase in both moduli. This could be due to the higher water absorption capacity of tef flour (with protein and fiber) than maize starch (Bultosa, 2007) that would explain the lower amount of free water in the dough and its higher consistency. Maize starch alone fails to impart dough viscoelasticity and it needs to be paired with other ingredients (Federici, Jones, Selling, Tagliasco, & Campanella, 2020). However, it resulted to be a good diluter of tef flour. The highest dough consistency and the lowest loss tangent was reached with DZ-Cr-387 variety (see Table 1 and Figure 1a). The $(\tan \delta)_1$ mean value decreased following the opposite order than both viscoelastic moduli. Differences among tef varieties must be related to their particular protein, fiber and fat contents (Bultosa & Tylor 2004; Abebe & Ronda, 2014, Abebe et al., 2015b), and their effect on the water absorption capacity of the flours (Bultosa, 2007). The increased contribution of G' to the overall viscoelastic response of doughs supplemented with tef can be explained by its insoluble ~~fibrefiber~~ contribution (Kiewlicz & Rybicka, 2020), with high hydration ability, as reported Djordjević et al. (2018) for maize-based GF bread enriched with sugar beet ~~fibrefiber~~.

G' and G'' values slightly increased with frequency as denote the a and b exponents, always positive (Table 1). The dependence of the elastic modulus on frequency decreased with the addition of tef, in particular in the DZ-Cr-387 variety, denoting more structured doughs. It was concluded that the higher the elastic modulus the lower the frequency dependence, as demonstrates the significant ($p<0.001$) negative Pearson coefficient between G_1' and a ($r=-0.87$). However, the viscous modulus was hardly dependent on frequency.

The maximum stress that the dough could withstand before breaking its structure, τ_{max} , depended significantly on both the variety of tef and the level of addition and on their double interaction (variety x level). The τ_{max} values ranged between 1.2Pa (100% maize starch) and 3.8Pa (100% Dz-Cr-387) (Table 1) and revealed that doughs with a higher percentage of tef had a more stable and robust structure. This is depicted in Fig.1b where the stress sweeps obtained for the control dough and doughs made with the variety Dz-Cr-37 (at 100% and 50% addition level) are shown.

The shear stress at the cross point ($G'=G''$, $\tan\delta=1$), considered as a yield stress where dough passes from a solid-like to a liquid-like behavior, is also useful to evaluate dough structure stability. In general, the increase in tef addition led to a concomitant increase in this yield stress value of the dough (Figure 1). Major and minor flour components and their interaction with water can strongly impact dough structure by creating a homogenous phase, resulting in a creation of stable GF structure (Renzetti & Rosell, 2016). The control dough had the lowest yield stress (12Pa), while the highest was obtained for 100% Dz-Cr-387 (238Pa). This parameter was significantly ($p<0.001$) and positively correlated with τ_{max} ($r=0.85$), G_1' ($r=0.95$), G_1'' ($r=0.92$) and negatively correlated with $(\tan\delta)_1$ ($r=-0.83$).

3.2 Creep-recovery tests

234 The creep-recovery tests were carried out by application of 100 Pa, because stresses
235 applied outside the LVR are closer to those experienced by the dough during bread
236 making (Federici et al., 2020; Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007).
237 Only the curves obtained from doughs made with 75% and 100% tef showed an elastic
238 component different than zero (Figure 1c) in good accordance with other authors
239 (Lazaridou et al., 2007; Villanueva et al., 2019). Values of Burgers model parameters
240 obtained from creep-recovery tests are presented in Table 2. The instantaneous, or
241 elastic (J_0), retarded, or viscoelastic (J_1), compliances and the retardation time (λ) for
242 dough samples made with 100% maize starch and 50% tef flour + 50% maize starch
243 (regardless of the tef variety) could not be estimated with the nonlinear regression
244 performed to fit compliance data to Burgers model. Only their steady-state viscosities
245 (μ_0) were determined as stated in the materials and methods section. These samples, at
246 100Pa, only exhibited a viscous behavior. Both studied factors, tef incorporation level
247 and variety, affected significantly to creep-recovery parameters. Among the tef-
248 containing samples, those with DZ-Cr-387 variety showed the lowest J_0 and J_1 values,
249 both in creep and recovery phases. This indicates that these tef-enriched doughs had
250 lower instant and retarded deformations when subjected to a constant stress and lower
251 recoveries when the stress was removed when compared to doughs made with the other
252 tef varieties. This means these doughs will expand less under the pressure of the
253 fermentation gas during proofing and baking. At the same time, the steady-state
254 viscosity, μ_0 , increased significantly for this tef variety. This variety also showed the
255 highest dependence of the tef dose on the creep test parameters. Villanueva, Pérez-
256 Quirce, Collar, & Ronda (2018) also reported a marked decrease in creep/recovery
257 | compliances and an increase of the steady-state viscosity measured OLVR of ~~corn~~maize
258 starch doughs when exogenous proteins were added which suggests that the

presence/increase in protein content leads to the creation of a robust cross-linked dough structure. The retardation times (λ) found in both phases were mainly dependent on tef variety. Doughs made with DZ-Cr-37 tef variety showed the highest retardation time values in both phases, denoting the time required to get the retarded elastic deformation under the application of the stress was longer (around 30% in the creep phase) than that needed when the other two varieties were used.

Pearson correlation analysis showed, as expected, a significant correlation between J_{0c} and J_{1c} ($r=0.94$, $p<0.01$). The viscosity at steady state (μ_0) strongly decrease with increasing J_{0c} and J_{1c} ($r=-0.92$ and $r=-0.91$, respectively). According to Abebe et al. (2015b), the G_1' and G_1'' showed a negative correlation with the values of the instantaneous compliance in the creep phase (J_{0c}) ($r=-0.97$ at $p<0.01$ for both G_1' and G_1'') and retarded elastic compliance (J_{1c}) ($r=-0.93$ $p<0.01$, for G_1' and $r=-0.87$ $p<0.05$, for G_1''). The steady-state viscosity (μ_0) was also significantly ($p<0.01$) and positively correlated with τ_{max} ($r=0.85$), G_1' ($r=0.94$), G_1'' ($r=0.92$) and negatively correlated with $(\tan\delta)_1$ ($r=-0.69$). In agreement with Ronda et al. (2014), the higher maximum stress τ_{max} explaining structure integrity of the doughs increased in parallel with dynamic moduli and decreased with instantaneous and retarded compliance. The creep compliance parameters showed strongly significant correlation with recovery phase counterparts ($r > 0.94$, $p<0.01$). The recovery capacity of doughs after releasing the applied stress, ranged between 1 and 29% (Table 2). The very low value observed for doughs made with 100% and 50% maize starch confirm they behaved at 100Pa like a viscous material and their elastic components were near zero under that conditions. These results are in agreement with Federici et al. (2020) who reported that dough made with maize starch did not have good recovery after the applied stress of 100Pa was removed compared to other starch sources. They explained that it is possible that the

large size of maize starch granule (up to 20 μm) did not contribute to the formation of a continuous matrix, leading to lower elasticity. The elastic recovery was strongly dependent on tef variety type ($p < 0.001$). The recovery capacity of the doughs made with 75% and 100% tef flour of the DZ-Cr-387 variety doubled that of the other varieties at the same substitution level.

3.3 Thermal properties of doughs

Thermal properties of doughs are presented in Table 3. Gelatinization (fresh dough) and retrogradation (gelatinized sample, stored 7 days) scans were carried out on doughs made with individual varieties at 100%, 50%, and 0% tef flour (control) level. According to Biliaderis (2009), in pure systems, when the water/starch ratio is < 1.5 , two endothermic transitions appear. The magnitude of the first endothermic peak, related to the less organized starch molecules within the granule, decreases progressively with a concomitant development of a second high temperature endothermic transition, related to melting of ordered chain domains of amylopectin. In this work, only one endothermic peak was obtained. Wang & Copeland (2013) suggested that the second high temperature endothermic peak does not always appear, especially with rapid heating rates. The presence of sugar, oil, salt and HPMC in the dough also could have had influence in the gelatinization and retrogradation of starch because they can restrict granules from swelling (Villanueva et al., 2018). A strong interaction between hydrocolloids and starch that induces the formation of a stable structure that makes starch require higher temperatures to start its gelatinization has been reported (Sansano et al., 2018). Neither the gelatinization nor the retrogradation thermograms included the amylose-lipid complex dissociation peak, unlike was observed by Abebe & Ronda (2015) when measured tef flour. This could be due to the limiting water content in the

sample which increases the complex dissociation temperature above the value applied in our assay (Eliasson, 1994).

The interaction (variety x level) had no significant effect on any of thermal properties of doughs (Table 3). The incorporation of DZ-01-99 (50% and 100%) increased significantly ($p < 0.05$) the gelatinization onset temperature from 68°C (control) to 70°C. The other two tef varieties did not cause appreciable variation. The peak and endset temperatures, T_p , T_e , and the temperature range, ΔT , of gelatinization, increased significantly with tef addition level. ΔT increased from 11°C (control) to 14-17°C, in all tef-enriched doughs. A wider melting range might implies amylopectin crystals with a larger variation in stability, denoting less homogeneous quality of crystals in the tef granules compared to maize starch (Ratnayake, Hoover, Shahidi, Perera, & Jane, 2001). The higher gelatinization temperature of doughs with 100% tef flour, would allow a greater development of the dough during baking before the fixation of the crumb structure upon baking. However, a significant correlation between T_o or T_p and bread volume was not found. Probably other factors, mainly related to the rheology of the dough and its capacity of retaining gas and expanding as result of its pressure, had a more marked effect on dough development during baking. A slight but significant decrease in gelatinization enthalpy was observed as a consequence of the addition of tef in the doughs, with a 37% reduction in the case of 100% Dz-01-99 dough compared to the control. Slight/Some differences were observed compared to the results obtained by Abebe & Ronda (2015). They obtained higher gelatinization enthalpy values (ΔH_{gel}) for the three varieties studied, and the temperatures T_o , T_e , T_p were lower than those obtained in this study. Maleki et al. (2012) found that the presence of hydrocolloids limited the water mobility and reduced water availability to starch during gelatinization process, by decreasing the ΔH . This phenomenon was explained in terms of an

incomplete starch gelatinization as a result of limited water availability (Eliasson, 1980) or lower degree of disorder (Biliaderis, 2009) that could justify the differences observed.

The second scan applied to gelatinized samples led also to a visible peak associated to the melting of the recrystallized amylopectin during the sample staling. It appeared at a notably lower transition temperature ($\sim 54^{\circ}\text{C}$) than in the initial scan of gelatinization due to the smaller and less perfect crystallites of amylopectin recrystallized during the storage (Biliaderis, 2009). Concomitantly ΔT increased, being 1.5-2 times higher than those obtained in the gelatinization phase, indicating less uniformity of recrystallized amylopectin. The enthalpy values recorded in the retrogradation scans were also lower than in the first scan. Such lower enthalpies and melting temperatures could be due to the formation of smaller and/or less perfect crystalline regions during storage (Biliaderis et al., 1986). As can be seen in Table 1, ΔH_{ret} followed the same trend observed in the gelatinization phase. The lowest retrogradation extent was obtained for the DZ-01-99 tef variety regardless the addition level to the dough.

3.4 Bread volume and crumb grain characteristics

Bread made with 100% maize starch showed deficiencies in their crumb structure as depicted in Figure 2. Figure 3a shows the volume of bread made at different doses of the three tef varieties studied. Bread made from 100% tef flour had always lower specific volume than those formulated from their mixture with maize starch (see Fig. 2 and Fig. 3) regardless its variety. The high consistency of the doughs that hinders its expansion during fermentation may explain this result. It also could be possible that the bran particles present in whole-meal flour puncture and break a high number of the gas bubbles, which results in a lower specific volume as reported Hager et al. (2012) for wheat whole-meal bread. A negative correlation of G_1' , τ_{max} , yield stress and μ_0 with the

358 volume of bread ($r=-0.75$ at $p<0.05$ for these parameters) was observed and confirms
359 the lower the consistency of the dough, the greater the volume of the bread. It has been
360 reported that a greater consistency of the dough helps to retain the gas formed during
361 fermentation and prevents its coalescence and loss during both fermentation and baking,
362 allowing a higher volume of bread. Excessive dough consistency may have detrimental
363 effects and lead to smaller bread because the dough cannot sufficiently expand as a
364 result of the pressure produced by the gas (Ronda et al., 2017). In agreement with this
365 hypothesis, the lowest bread volume was obtained for 100% Dz-Cr-387 tef which
366 corresponds to the dough with the highest G'_1 and G''_1 values. In addition, a positive
367 correlation ($p<0.01$) of $(\tan\delta)_1$ and J_{1r} ($r=0.86$ and $r=0.73$, respectively) and a negative
368 correlation ($p<0.05$) of T_e and ΔT ($r=-0.81$ and $r=-0.76$) with the volume of bread were
369 observed. An increase in the gelatinization T_e means a higher temperature/longer time
370 needed in the oven to get a fixed structure in the bread crumb. This delay can allow the
371 leak of gas and the fall of the loaf during baking. The blend of tef flour with 25% maize
372 starch increased significantly the volume of bread (19%, in average). An additional
373 increase in maize percentage in the blend to 50% hardly affected the final bread volume.
374 Previous works also confirmed the viability of wheat bread enriched with tef flour up to
375 a level of 30% without a significant detriment of its quality (Ronda et al., 2015).
376 Bread crumb structure also provides useful information on bread quality. Figure 4
377 represents the cell density and the mean cell area of bread crumbs. Tef incorporation
378 level, tef variety, and their interaction (variety x level) affected significantly on crumb
379 grain parameters. Significant ($p<0.01$) and negative correlation was found between cell
380 density and cell area ($r=-0.81$). The cell density in bread samples made with 50% tef
381 was significantly higher than in those made with greater additions. They showed also
382 the smaller mean cell area, indicating a finer and more compact and uniform structure

(see Figure 2). The increase of tef level up to 75% reduced the cell density of crumb and increased markedly the mean cell area, showing a more open structure. The additional increase of tef up to 100% hardly had any additional effect on crumb grain. A positive correlation between the onset temperature of gelatinization (T_o) ($r=0.73$, $p<0.05$) and the mean cell area of the crumb was obtained. This indicates that the delay in the formation of crumb structure makes easier a higher expansion of gas into the cells and therefore the increase in their size.

3.5 Overall Acceptance

Figure 5 shows the overall acceptance scores obtained from the sensory evaluation of bread. The tef variety and its level of addition did not have an effect on the overall acceptance of the bread, while the second order interaction (level x variety) did affect significantly ($p<0.01$). The highest score was given to 100% of DZ-Cr-37 (6.4), that was significantly higher than those of the remaining tested bread samples, including the reference/commercial type bread, which was arbitrarily placed in the middle of the scale and assigned a score of 5. Bread loaves made with 75% of DZ-Cr-387 and 50% of ~~DZ~~DZ-01-99 obtained the following higher scores, 5.7 and 5.4 respectively, although they were not significantly higher than the ~~corn~~maize starch bread, that reached a score of 4.8. The relatively high score of ~~corn~~maize starch bread can be explained by the fact that panelists were offered only a piece of bread (that included both crumb and crust) and were not able to include in their perception of acceptability the marked crumb grain defect of ~~corn~~maize starch bread. Consumers, in the free writing area of the sensory evaluation form, reported that taste, color and texture were the properties that most influenced their scores. The overall acceptance of the bread made from the brown tef variety (DZ-01-99) decreased with the increase in its addition level due to the dark color of the crumb. So, 50% was the preferred addition for this variety. However, in the

case of DZ-Cr-387 variety, the highest score was obtained for the 75% addition level. A positive correlation between general acceptance and bread volume ($r=0.67$, $p<0.05$) was obtained. However, no significant correlation was obtained between general acceptance and any of the physical properties measured in dough. Flavor, taste, color and texture were the attributes that most influenced the score that consumers gave to bread samples in their evaluation, as commented in their evaluation reports. However, in our study, a positive correlation between the overall acceptance and the bread volume ($r=0.67$, $p<0.05$) was obtained. In general, tef flour addition led to higher scores than that of the maize starch bread, denoting that gluten-free tef bread satisfied the perception of the potential consumers. Ronda et al (2015) showed that tef flour reduced the overall acceptability of wheat bread when added at a level $\geq 30\%$. In that case, the addition level of tef flour significantly affected the overall acceptability of the bread, while the tef variety had no significant effect. Probably the high quality standard of wheat bread versus GF bread may explain these different results.

Comment [MVB1]: No se si aquí se podría decir también que fueron iguales o más altos que los obtenidos para el 100% maize starch bread y el pan comercial.

Conclusion

The results of this study revealed that tef variety and its addition level modified the rheological and thermal properties of maize-based gluten-free bread doughs and determined the quality of bread obtained from them. Higher doses of tef flour affected the structure of the doughs in terms of higher viscoelastic moduli and $\tau_{\max}$ values and lower instantaneous and retarded elastic compliances obtained outside the Linear Viscoelastic Region. Higher gelatinization temperatures and lower enthalpies were obtained in tef-added doughs. The addition of tef flour to maize starch made the gluten-free bread viable. The excessive consistency of doughs made with 100% tef flour could explain its lower expansion during fermentation and the lower volume of the bread

loaves. However, the addition of 75% and, particularly, 50%, led to well-developed bread of good appearance. DZ-Cr-37 (white) and DZ-01-99 (brown) varieties led to the greater bread volume. However, bread made with DZ-Cr-387 had the finest and most regular grain structure. ~~It should be emphasized that maize starch GF bread can be enriched with tef flour, in particular from white varieties, up to a high level, with a great consumer acceptance. Brown tef flour, probably due to its dark color, should be limited to 50% addition.~~ There was no clear trend in terms of overall acceptability, because each variety was preferred at a different level of addition. ~~However, it can be concluded that tef flour, at a certain level of addition (from 50% to 100%) depending on the tef variety, gave rise to bread that surpassed the standard quality of a commercial gluten-free bread. This denotes that GF tef bread satisfied the perception of the potential consumers.~~ The formulated bread represents an important improvement for celiac patients with respect to the common products usually found in the market. Additional studies are still needed to quantify also the nutritional improvement.

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Table 1. Effect of tef incorporation level and variety type on dynamic parameters of maize starch-based doughs

Incorporation level	Tef variety	G' ₁ (Pa)		a		G'' ₁ (Pa)		b		(tanδ) ₁		c		τ _{max} (Pa)		Cross point (Pa)	
100%	DZ-Cr-37	5968	fg	0.25	ab	2317	f	0.34	abc	0.39	b	0.09	bc	2.4	c	150	d
	DZ-Cr-387	8285	h	0.22	a	2836	g	0.31	a	0.34	a	0.08	bc	3.8	d	238	e
	DZ-01-99	4746	e	0.25	ab	1858	ef	0.35	bc	0.39	bc	0.10	c	2.4	c	135	cd
75%	DZ-Cr-37	4901	ef	0.28	bc	2186	fg	0.33	ab	0.45	de	0.05	bc	2.1	c	154	d
	DZ-Cr-387	6294	g	0.27	bc	2687	fg	0.33	abc	0.42	cd	0.06	bc	2.3	c	83	bc
	DZ-01-99	3217	d	0.28	c	1455	de	0.36	bcd	0.45	de	0.08	bc	2.4	c	85	bc
50%	DZ-Cr-37	1992	bc	0.32	d	1055	bc	0.37	cd	0.53	f	0.04	b	2.1	c	31	ab
	DZ-Cr-387	2783	cd	0.28	bc	1315	cd	0.34	abc	0.47	e	0.06	bc	1.3	ab	61	ab
	DZ-01-99	1844	b	0.32	d	945	b	0.38	d	0.51	f	0.07	bc	1.7	b	24	a
100%	Maize starch	914	a	0.38	e	618	a	0.34	abc	0.68	g	-0.03	a	1.2	a	12	a
SE		372		0.01		196		0.01		0.01		0.02		0.1		18	
Analysis of variance and significance (p-values)																	
Tef variety		***		*		**		*		**		ns		*		*	
Tef incorporation level		***		***		***		*		***		ns		***		***	
Tef variety * Incorporation level		ns		ns		ns		ns		ns		ns		***		*	

SE: Pooled standard error obtained from ANOVA analysis. τ_{max} was obtained from strain sweeps. The power law model was fitted to experimental results from frequency sweeps:
 $G'(\omega) = G'_1 \cdot \omega^a$; $G''(\omega) = G''_1 \cdot \omega^b$; $\tan \delta(\omega) = (\tan \delta)_1 \cdot \omega^c$.

Values with the same letters in a column are not significantly different (p > 0.05). Significance level: *** p<0.001. ** p<0.01. * p<0.05. ns: not significant.

Table 2. Effects of tef incorporation level and variety type on the creep-recovery parameters obtained outside the Linear Viscoelastic Region of maize starch-based doughs

Incorporation level	Tef variety	Creep phase				Recovery phase			
		J_0 ($10^{-5} Pa^{-1}$)	J_1 ($10^{-5} Pa^{-1}$)	λ (s)	μ_0 ($10^2 Pa \cdot s$)	J_0 ($10^{-5} Pa^{-1}$)	J_1 ($10^{-5} Pa^{-1}$)	λ (s)	Recovery (%)
100%	DZ-Cr-37	22 b	93 b	2.2 b	75 e	30 b	66 b	6.8 f	12 bc
	DZ-Cr-387	13 a	37 a	1.4 a	206 f	23 a	41 a	5.9 e	21 de
	DZ-01-99	32 cd	136 cd	1.7 ab	46 d	39 c	79 c	6.6 ef	9 abc
75%	DZ-Cr-37	27 bc	160 de	2.9 c	66 de	32 b	78 c	7.7 g	14 cd
	DZ-Cr-387	23 b	106 bc	2.2 b	126 f	32 b	78 c	6.7 ef	29 e
	DZ-01-99	37 d	186 e	1.3 a	17 c	51 d	99 d	4.4 d	6 abc
50%	DZ-Cr-37	-	-	-	5 b	-	-	-	2 a
	DZ-Cr-387	-	-	-	12 c	-	-	-	4 ab
	DZ-01-99	-	-	-	4 ab	-	-	-	1 a
100%	Maize starch	-	-	-	2 a	-	-	-	1 a
SE		2	12	0.2	21	2	3	0.3	4
Analysis of variance and significance (p-values)									
Tef variety		***	***	***	***	***	***	***	***
Tef incorporation level		**	***	*	***	***	***	*	***
Tef variety * Incorporation level		ns	ns	*	**	**	***	*	ns

J_0 : Instantaneous compliances and J_1 : retarded compliances, λ : retardation time and μ_0 : steady-state viscosity in the creep phase.

SE: Pooled standard error obtained from ANOVA analysis. Values with the same letters in a column are not significantly different ($p > 0.05$).

Significance level: *** $p < 0.001$. ** $p < 0.01$. * $p < 0.05$. ns: not significant

Table 3. Effects of tef incorporation level and variety type on the thermal properties of maize starch-based doughs

Incorporation level (%)	Tef variety	Gelatinization						Retrogradation													
		T _o (°C)		T _e (°C)		T _p (°C)		ΔT (°C)		ΔH _{gel} (J/g starch)		T _o (°C)		T _e (°C)		T _p (°C)		ΔT (°C)		ΔH _{ret} (J/g starch)	
100	DZ-Cr-37	68.2	a	84.7	d	74.7	c	16	cd	11.9	c	41	a	64.8	a	52	a	24	ab	7.8	d
	DZ-Cr-387	68.0	a	85.1	d	74.8	c	17	d	9.5	b	44	ab	65.3	ab	53	a	21	ab	7.0	abc
	DZ-01-99	70.2	c	84.4	cd	75.9	d	14	bcd	7.2	a	44	ab	65.5	ab	55	a	22	ab	6.3	a
50	DZ-Cr-37	68.5	ab	82.3	bc	73.7	b	14	abcd	12.2	c	41	ab	65.9	bc	52	a	25	b	7.5	cd
	DZ-Cr-387	68.4	ab	82.0	b	73.8	b	14	abc	10.3	bc	44	ab	65.6	ab	54	a	21	a	6.5	ab
	DZ-01-99	69.9	bc	80.7	ab	73.8	b	11	a	11.3	bc	45	ab	65.3	ab	55	a	21	a	6.3	a
100	Maize starch	67.6	a	79.0	a	72.5	a	11	ab	11.5	bc	44	ab	66.6	c	54	a	23	ab	7.3	bcd
SE		0.5		0.8		0.3		1		0.7		1		0.3		1		1		0.2	
Analysis of variance and significance (p-values)																					
Tef variety		**		ns		ns		ns		**		ns		ns		*		ns		**	
Tef incorporation level		ns		**		***		**		*		ns		ns		ns		ns		ns	
Tef variety * Incorp. level		ns		ns		ns		ns		ns		ns		ns		ns		ns		ns	

T_o: onset temperature, T_e: endset temperature, T_p: peak temperature, ΔT: T_e - T_o, and ΔH: enthalpy.

SE: Pooled standard error obtained from ANOVA analysis. Values with the same letters in a column are not significantly different (p > 0.05). Significance level:

*** p<0.001. ** p<0.01. * p<0.05. ns: not significant.

Figure 1. A) Frequency sweeps of doughs with 100g/100g of Dz-Cr-387 tef flour (■ □), 100g/100g of Dz-Cr-37 tef flour (▲ Δ), 100g/100g of Dz-01-99 tef flour (◆ ◇) and 100g/100g maize starch (control) (●). B) Stress sweeps of doughs with 100g/100 g of Dz-Cr-37 tef flour (▲ Δ, continuous line), 50g/100g of Dz-Cr-37 tef flour (▲ Δ, discontinuous line), and maize starch (control) (●). In A) and B) figures, elastic modulus G' is represented by solid symbols and the viscous modulus, G'' by void symbols. C) Creep-recovery tests of maize starch (control) doughs (●), Dz-Cr-387 tef flour (■ □), Dz-Cr-37 tef flour (▲ Δ), Dz-01-99 tef flour (◆ ◇). Symbols connected by segments represent the level of 50g/100g; unconnected symbols represent the level of 100g/100g (these are also shown in the amplified graph).

Figure 2. Effect of tef flour incorporation level and variety on the volume of maize starch gluten-free breads. A1: 100% DZ-Cr-37 tef flour, B1: 75% DZ-Cr-37 tef flour, C1: 50% DZ-Cr-37 tef flour, A2: 100% DZ-Cr-387 tef flour, B2: 75% DZ-Cr-387 tef flour, C2: 50% DZ-Cr-387 tef flour, A3: 100% DZ-01-99 tef flour, B3: 75% DZ-01-99 tef flour, C3: 50% DZ-01-99 tef flour, D: 100% maize starch bread.

Figure 3. Evolution of bread volume with tef dose of the three different varieties studied

Figure 4. Effect of the level of addition and the tef variety on crumb grain properties of gluten-free breads A) Cell density and B) Mean cell area

Figure 5. Effect of the level of addition and the tef variety on overall acceptance of gluten-free bread samples

Author contributions

Marina Villanueva: Methodology, Investigation, Formal analysis, Data curation, Writing - original draft, Writing - review & editing.

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Conflict of Interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.