Suitability of tef varieties in mixed wheat flour bread matrices: a physico-chemical and nutritional approach.

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Abstract

Wheat flour replacement from 0 to 40% by single tef flours from three Ethiopian varieties DZ-01-99 (brown grain tef), DZ-Cr-37 (white grain tef) and DZ-Cr-387 (Quncho, white grain tef) yielded a technologically viable *ciabatta* type composite bread with acceptable sensory properties and enhanced nutritional value, as compared to 100% refined wheat flour. Incorporation of tef flour from 30% to 40% imparted discreet negative effects in terms of decreased loaf volume and crumb resilience, and increase of crumb hardness in brown tef blended breads. Increment of crumb hardness on aging was in general much lower in tef blended breads compared to wheat bread counterparts, revealing slower firming kinetics, especially for brown tef blended breads. Blended breads with 40% white tef exhibited similar extent and variable rate of retrogradation kinetics along storage, while brown tef-blended breads retrograded slower but in higher extent than control wheat flour breads. Breads that contains 40% tef grain flour were found to contain five folds (DZ-01-99, DZ-Cr-387) to 10 folds (DZ-Cr-37) Fe, three folds Mn, twice Cu, Zn and Mg, and 1.5 times Ca, K, and P contents as compared to the contents found in 100% refined wheat grain flour breads. In addition, suitable dietary trends for lower rapidly digestible starch and starch digestion rate index were met from tef grain flour fortified breads.

Key words: Composite wheat breads, grain tef, nutritional profile, physical properties

List of abbreviations

AI: adequate intake

AR: antiradical activity

CE: catechin equivalents

dm: dry matter

DPPH• : 2,2-diphenyl-1-picrylhydrazyl

DSC: Differential Scanning Calorimeter

FGS: free sugar glucose

G_{120} : hydrolyzed glucose at 120 min

G_{20}: hydrolyzed glucose at 20 min

GA: gallic acid

H_{∞} : levelling-off value of melting enthalpy

H_o : melting enthalpy at initial time

$H_{t:}$: melting enthalpy at time t

k rate constant

LSD: Fisher’s least significant difference test

n: Avrami exponent

RAG: rapidly available glucose

RDA: Recommended Dietary Allowances

RDS: rapidly digestible starch

RS: resistant starch

SDRI: Starch digestibility rate index

SDS: slowly digestible starch

t: time

$t_{1/2}$: half-life

TG: total glucose

TPA: Texture Profile Analysis

TS: total starch

1. Introduction

With the constant search for diversity and innovation in foods, an alternative niche market for nutrient-dense fermented baked goods has emerged to satisfy the interest of health conscious people diet, which became the dietary needs of a significant part of the world human population. Tef (*Eragrostis tef*) is a nutritious cereal wheat type gluten-free grain indigenous to Ethiopia, rich in carbohydrates and fibre, microelements and phytochemicals (Baye, 2014) that contains superior amounts of iron, calcium and zinc than wheat, barley and sorghum (Abebe et al., 2007). The high nutritional profile makes tef a good candidate for designing innovative functional foods for health promotion and disease prevention.

In general, replacement of wheat by non-gluten forming cereals is a major technological challenge in breadmaking, as the wheat protein gluten is essential for structure-formation. Dilution of wheat gluten during supplementation and/or substitution at higher amounts in the dough system impairs proper dough development capacity during kneading, leavening and baking. Tef has been incorporated into breadmaking systems encompassing detrimental effects on bread physical and sensory quality when tef flour levels reached 20% (Mohammed et al., 2009) and 30% (Ben-Fayed et al., 2008; Alaunyte et al., 2012). Tef breads ~~deserved~~ showed significantly lower sensory scores, as only 10% and 5% tef breads had comparable acceptability scores to wheat bread in Ben-Fayed et al. (2008) and Mohammed et al. (2009) studies, respectively. More recently, a combination of enzymes has been successfully used to improve the quality of tef-enriched breads in terms of loaf volume and crumb firmness during storage in both straight dough and sourdough breadmaking processes (Alaunyte et al., 2012).

Since major challenge to include high levels of tef grain flours into breadmaking matrices relates the production of bread with good volume, textural and sensory attributes, changing the bread formulation and process conditions might be necessary. In addition, exploring the suitability of different tef varieties for bread formulation could be useful since most physicochemical, functional and nutritional properties of cereal-based goods are variety dependent. Therefore, in this study the impact of three Ethiopian grain tef varieties at different incorporation levels is evaluated for the physical, sensory and nutritional performance in making *ciabatta* type bread.

2. Experimental

2.1. Materials

Three tef grain varieties with brown and white seed colour named DZ-01-99 (brown tef), DZ-Cr-37 (white tef) and DZ-Cr-387 (Quncho, white tef), respectively, were obtained from the Debre Zeit Agricultural Research Center of the Ethiopian Institute of Agricultural Research (EIAR). Tef grain was manually cleaned by siftings and winnowing before milling. Disc attrition mill, being used traditionally in cottage tef grain-milling house (Bishoftu, Ethiopia) to mill tef grain for *injera* making in Ethiopia, was used to mill the tef grain. Tef grain flours (per 100 g, dry basis) from the different varieties (DZ-01-99, DZ-Cr-37, DZ-Cr-387) accounted for 8.9, 10.5, 8.9% protein, 2.8, 2.6, 3.2% fat, and 86, 83, 85% total carbohydrate, respectively as reported earlier (Abebe et al., 2015) were used. Wheat flour of extra-strength (14.5 % protein, 1.47% fat, and 85% total carbohydrate, Energy of Deformation (W) 466 x10^{-4}J, P/L ratio 1.21) was supplied by Emilio Esteban SA (Valladolid, Spain). A general purpose bread improver Toupan Puratos® (Puratos, Barcelona, Spain) containing mono- and di-glyceride of fatty acids, ascorbic acid, α-amylase and xylanase was used.

2.2. Dough preparation and breadmaking

A straight dough process for a *ciabatta* bread type was performed using the following formula on a 100g flour (tef + wheat) basis: 1.8% salt, 0.5% bread improver, 2% dry yeast and 85% water. Tef flours were incorporated at 0%, 10%, 20%, 30% and 40% of wheat flour replacement and mixed for 15 min. using a Chopin MR2L/MR10L mixer (Chopin Technologies, France). Dough (300 g) was placed into aluminium pans and proofed at 28ºC and (75 ± 5) % relative humidity for 40 min. Subsequently, baking was carried out in a Salva oven (Lezo, Spain) at 190ºC for 40 min, and resulting breads were left for one hour at room temperature before analysis. Control wheat breads for sensory evaluation were made from refined wheat flour 70% extraction rate (Control 1) and from a tailored mixture of 85% refined flour 70% extraction rate and 15% of added bran, provided by the supplier Emilio Estaban (Valladolid, Spain) (Control 2).

2.3. Bread physical characteristics

Bread volume was determined in duplicate using a volume analyser BVM-L370 TexVol Instruments (Viken, Sweden). Bread mechanical properties -firmness (N), cohesiveness, springiness, resilience and chewiness- were determined in fresh and 7 days stored breads using a TA-XT2 texture analyzer (Stable Microsystems, Surrey, UK) fitted with the “Texture Expert” software. A 25-mm diameter cylindrical aluminium probe was used in a Texture Profile Analysis (TPA) with double compression test to penetrate to 50% of the sample depth at a test speed of 2 mm/s and with a 30 s delay between first and second compressions. Analysis were carried out at (20 ± 2) °C on two slices of 20 mm thickness taken from the centre of the loaf of two breads (2x2) per sample, taking the average of the 4 measurements. Crumb and crust moisture contents were determined by drying the samples in an oven for 24 hours at 105 ºC. Color was measured using a Minolta spectrophotometer CN-508i (Minolta, Co.LTD, Tokyo, Japan). Results were expressed in the CIE $L^*a^*b^*$ colour space and were obtained using the D65 standard illuminant, and the 2º standard observer. Color determinations were made 4x5 times on each bread loaf (two breads per formula): crumb and crust color was checked at four different points per loaf, and five measurements per point were made.

2.4. Mineral determination

Mineral content (Ca, Cr, Cu, Fe, K, Mg, Mn, Na, P, Zn) of flours and breads were determined using a Radial Simultaneous inductively coupled plasma optical emission spectrometry (ICP-OES) Varian 725-ES spectrophotometer (Agilent Technologies, Santa Clara, CA, US). Aliquots of flours and freeze-dried breads (0.5 g) were placed in Teflon cups, diluted with 6 mL of 65% HNO_3 and 2 mL of 30% H_2O_2, heated for 6 min up to 200ºC and hold for 15 min at 200ºC for mineralisation in a microwave digester (MLS 1200 mega, Milestone, Shelton, CN, US) and finally diluted to 25 mL.

2.5. Starch digestibility

In vitro starch digestibility of breads was measured according to the modified method by Englyst et al. (2000), as previously applied by Ronda et al. (2012). The hydrolyzed glucose at 20 min (G_{20}) and 120 min (G_{120}) and the total glucose (TG) were determined by the glucose oxidase/peroxidase colorimetric method. The free sugar glucose (FGS) content was also determined through a separate test following the procedure proposed by Englyst et al. (2000). From the above results, rapidly digested starch (RDS) = 0.9 * (G_{20}− FGS), slowly digestible starch (SDS) = 0.9 * (G_{120}− G_{20}), resistant starch (RS) = 0.9 * (TG − G_{120}), total starch (TS) = 0.9 * (TG − FGS) and rapidly available glucose (RAG) = G_{20} were calculated. Starch digestibility rate index (SDRI) was computed from the percentage of RDS in TS in the flours.

2.6. Amylopectin retrogradation

A Metller Toledo Differential Scanning Calorimeter DSC 822e (Schwerzenbch, Switzerland) equipped with a ceramic sensor (FSR5) of high sensitivity, liquid nitrogen cooling system and nitrogen purge gas was used. Bread crumb samples (20-25 mg) taken from the center of the bread loaf were hermetically sealed in aluminum pans (40 µL) and stored in the refrigerator at 4ºC from 0 to 9 days. Starch retrogradation was analyzed from DSC endotherms obtained for crumb samples during temperature scanning from 0ºC to 105ºC at a heating rate of 5ºC/min. Each measurement was performed at least in duplicate. The melting enthalpy was expressed in J/g of solids. Crystallization data using melting enthalpies after storage were fitted to the Avrami equation:

$$\frac{H_\infty - H_t}{H_\infty - H_o} = e^{-kt^n}$$

where *t* is time, *k* is a rate constant, and *n* is the Avrami exponent describing the type of crystal growth, H_∞ is the levelling-off value of melting enthalpy, H_t is the melting enthalpy at time t, and H_o is the melting enthalpy at initial time. The values of the constants *k* and *n* were used to calculate the half-life, $t_{1/2}$ (Ronda and Roos, 2011) according to:

$$t_{1/2} = \left(-\frac{\ln 0.5}{k} \right)^{\frac{1}{n}} .$$

2.7. Extraction and determination of polyphenols

Extractable (soluble) phenols from bread samples were extracted by concentrated hydrochloric acid:methanol:water (1:80:10,v/v) mixture at room temperature for 5 h, as reported by Milella et al. (2011). *Hydrolyzable (insoluble) phenolics* extraction was conducted with methanol and concentrated sulfuric acid (10:1, v/v) at 85ºC for 20 h according to the procedure of Hartzfeld et al. (2002). Total phenolic content was calculated as the sum of extractable and hydrolyzable polyphenolic fractions as suggested by Perez-Jiménez and Saura-Calixto (2005).

Bioaccessible phenol determination was carried out by conducting an “in vitro” digestive enzymatic mild extraction that mimics the conditions in the gastrointestinal tract according to Angioloni and Collar (2011a). *Polyphenols content* were determined according to the Folin-Ciocalteau procedure as described by Singleton et al. (1999). Results were expressed as gallic acid (GA) equivalents.

For the detection of *flavonoids*, 1 g of ground freeze-dried bread was extracted in 10 ml of 40% (v/v) ethanol for 30 min at room temperature according to Collar et al (2014a). The results expressed as mg of catechin equivalents (CE) per g of dry matter (dm).

2.8. Anti-radical activity

The stable 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical was used to measure the radical scavenging capacity of bread samples according to the DPPH• method adapted by Collar et al. (2014b). Plots of µmol DPPH *vs* time (min) were drawn, and calculations were made to know the antiradical activity (AR). AR= $[([DPPH]_{INITIAL} - [DPPH]_{PLATEAU}) \times 100] / [DPPH]_{INITIAL}$.

2.9. Sensory analysis

Laboratory acceptance panels were used to give an indication of consumer acceptance of the tef breads under study that were baked the day before sensory testing and were served at room temperature. Bread samples were Control 1 (100% refined wheat flour), Control 2 (tailored mixture of 85% refined wheat flour 70% extraction rate and 15% wheat bran, 10%, 20%, 30% and 40% DZ-01-99 (brown grain tef flour) addition to refined wheat flour, respectively, 10%, 20%, 30% and 40% DZ-Cr-37 (white grain tef flour) addition to refined wheat flour, respectively, and 10%, 20%, 30% and 40% DZ-Cr-387 (white grain tef flour) addition to refined wheat flour, respectively. Tef-added breads were analyzed in three sessions. A serving of four randomized bread samples and controls were simultaneously served per session. Servings were approximately 1-cm-thick slices from loafs. Panelists (60 volunteers from laboratory staff) were presented the test samples in individual panel booths under normal (daylight) illumination. Evaluation was for quality attributes: visual appearance, odour intensity, texture, taste intensity, persistency of taste and overall acceptability. Score of each quality attribute was rated on a nine-point hedonic scale, and ratings were converted to numerical scores where 1 = *very much disliked* and 9 = *very much liked*. Tef breads were considered acceptable if their mean scores were above 5 (*neither like nor dislike*). When necessary, brief explanations of terms were given.

2.10. Statistical analysis

Experimental data were analysed using single and multivariate analysis of variance, and then means were then compared at $p<0.05$ using Fisher's least significant difference (LSD) test. Statistical analysis was performed by using the Statgraphics Centurion XVI program (StatPoint Technologies, Inc. 1982-2010).

3. Results and Discussion

3.1. Physicochemical pattern and sensory performance of tef-wheat blended breads

The effect of wheat flour replacement from 0% up to 40% by tef flours with brown grain (DZ-01-99) and white grain (DZ-Cr-37, DZ-Cr-387) varieties on physico-chemical analysis (Table 1), sensory acceptability (Table 2), staling kinetics (Table 3) and images of the tef-wheat blended breads (Fig. 1) are discussed below.

Physical characteristics data (Table 1), showed that replacing wheat flour with tef up to the level of 30% in straight dough breads did not affect either loaf volume (DZ-Cr-37, DZ-Cr-387) or crumb hardness and cohesiveness, and even provided 10% higher volume blended breads when a brown tef variety was used (DZ-01-99). Further incorporation of tef grain flour from 30% to 40% had negative effects in terms of decreasing 3.4 % loaf volume regardless the tef variety used, 17% increase of crumb hardness in brown grain tef flours (DZ-01-99) blended breads, and 15 % decrease in the crumb resilience, irrespective of grain tef variety. Increments of crumb hardness at 7 days of storage were in general much lower in tef blended breads compared to wheat bread counterparts, revealing slower firming kinetics, especially for brown grain tef

(DZ-01-99) blended breads. A dramatic deleterious effect of tef flour incorporation up to 20-30% has been previously reported for mixed breads quality (Alaunyte et al., 2012; Ben-Fayed et al., 2008; Mohammed et al., 2009), particularly regarding reduced bread volume, harder texture and compact crumb structure. The wheat flour type used in our study (extra-strength), capable of standing the gluten dilution, instead of a common bread making flour may explain these differences. Close examination of bread crumb grain (Fig. 1) by visual inspection revealed changes in cell features depending on both the tef variety and the tef addition. Slice area decreased significantly in 40% tef breads, particularly for the brown grain tef variety DZ-01-99, in good accordance with data for specific volume (Table 1). Breads with 40% tef exhibited a more open and coarse crumb structure with less and larger cells, and thicker cell walls, particularly pronounced for the brown grain tef variety DZ-01-99 compared to their respective wheat counterparts and to breads with 10% tef.

Slice brightness (L*) significantly decreased gradually in both crumb and crust with increased levels of brown tef flour from 0% up to 40% (Table 1). L* values ranged from 63.8 to 42.9 (crumb) and from 58.5 to 49.1 (crust) in brown grain tef variety DZ-01-99. In white grain tef breads, a slight decrease in crumb brightness was only observed from 30% to 40% of tef addition ranging from -19% (DZ-Cr-37) to -14% (DZ-Cr-387). Earlier reports (Alaunyte et al.,, 2012; Ben-Fayed et al., 2008) found similar decreases of slice brightness with grain tef flour addition, attributed to bran particles in wholegrain flours causing a darker crumb colour (Fig. 1). The crumb hue (h), associated to the original colour of ingredients, decreased from -14% to -29% with brown tef (DZ-01-99) addition, denoting a significantly loss of the pure yellow hue of the control bread (h=88 degrees) to evolve toward reddish (Figure 1). White grain tef varieties slightly decreased the crumb hue, although the variety DZ-Cr-387 led to the closer colour of wheat bread. The crust hue, more affected by Maillard reactions, only varied slightly with tef addition. The crumb Chroma (C^*) increased with tef addition, reaching the maximum at 30% addition, and denoting more vivid colors than control breads. In the crust, only brown tef incorporated breads had visibly decreased C^*.

Sensory evaluation of blended breads (Table 2) revealed that increased tef grain flour levels from 0% to 30%, provided in general no dramatic decrease in sensory ratings, particularly for breads blended with white grain tef flours of DZ-Cr-37 and DZ-Cr-387 DZ-Cr-37 and DZ-Cr-387 which their scores were being similar or discreetly lower than the control bread processed from refined wheat grain flours. Blended breads with brown grain tef flour (DZ-01-99) at 30% produced poor ratings on visual appearance and overall acceptability as compared the whole wheat control bread. Increased tef addition from 30 to 40% encompassed significant lower scores on odour and overall acceptability of DZ-Cr-37 white breads, and similar ratings for brown DZ-01-99 and white DZ-Cr-387 blended breads. Previous results by Ben-Fayed et al. (2008), Mohammed et al. (2009), and Alaunyte et al. (2012) reported bread processed by substitution of grain tef flour from 10, 5% (basic formulation), and 30% (with enzyme addition) respectively were acceptable as that of control bread. In this work even the 40% grain tef flours blended breads were rated >5 and judged acceptable for all sensory attributes. Scores for overall acceptability depended on the tef grain variety used, and followed the order: DZ-01-99 ≈DZ-Cr-387> DZ-Cr-37 (Table 2). Breads made with 40% grain tef flour DZ-01-99 deserved average ratings on overall acceptability very closed to the respective control wheat flour breads (6.0 *vs* 6.2), and were statistically non significant ($p>0.05$).

The DSC thermal analysis data for 40% grain tef flour-blended breads generated from storage after 9 days were defined according to the tef variety used, and the kinetics of amylopectin recrystallization on aging were modelled using the Avrami equation. Results on the model factors H_0, H_∞, n, and k for the enthalpy of amylopectin retrogradation are compiled in Table 3. Compared to the control wheat breads, white grain tef flour-blended breads (DZ-Cr-37, DZ-Cr-387) exhibited similar extent (H_∞: 5.63, 5.97 J/g vs 5.79 J/g) and variable rate (n: 0.69, 0.83 vs 0.85) of retrogradation kinetics with storage. Whereas the brown grain tef flour-blended breads (DZ-01-99) retrograded slower (k: 0.31 vs 0.49; n: 0.66 vs 0.85; $t_{1/2}$: 3.52 vs 1.51) but in higher extent (H_∞: 8.19 J/g vs 5.79 J/g) than control wheat breads.

3.2. Nutritional features/profile of tef-wheat blended breads

3.2.1. Mineral elements

Potassium, P, Mg, and Ca are the most abundant minerals in wheat flour (Piironen et al. 2009; De Brier et al., 2015), while tef grain flours have a higher Fe, Ca, Zn and Cu content than other common cereals including wheat (Hager et al., 2013). The mineral contents are dependent on the genetic and environmental factors (Baye, 2014). Incorporation of 40% (Table 4) in the bread resulted into significantly higher amounts of micro-elements compared to the refined wheat, in agreement with previous studies (Alaunyte et al., 2012). This is especially true for Ca, Cu, Fe, K, Mg, Mn and P, regardless the tef variety used for blending (Table 4). Breads that contain 40% tef grain flour were found to contain five folds (DZ-01-99, DZ-Cr-387) to 10 folds (DZ-Cr-37) Fe, three folds Mn, twice Cu, Zn and Mg, and 1.5 times Ca, K, and P contents as compared to the contents found in 100% refined wheat grain flour breads. The most noticeable difference in contribution between wheat and tef breads was the dietary iron, which would be notably higher if tef breads were incorporated as a part of the diet, particularly using DZ-Cr-37 variety. If bioavailability of iron is assumed 100%, based on Recommended Dietary Allowances (RDA) for adequate intake (AI) of Fe (female = 18 mg/day and male = 8 mg/day), daily consumption of 170-180 g of tef-wheat (40-60%) breads depending on tef grain variety will satisfy 60% and 135% (DZ-01-99), 141% and 318% (DZ-Cr-37) and 54% and 123% (DZ-Cr-387), for female and male adults, respectively. Similarly, if copper bioavailability is assumed 100%, copper requirements (0.9 mg/day) can be met by 43% (DZ-01-99, DZ-Cr-37) and 48% (DZ-Cr-387) by consumption of 170-180 g blended breads. However, daily consumption of 170-180 g of wheat bread can only delivers 13% (female) and 29% (male) of the required amount of iron and less than 28% of copper daily requirements.

3.2.2. Starch digestibility

Rate of starch hydrolysis and the subsequent nutritionally relevant starch fractions obtained from tef-wheat (40:60) blended breads are presented in Table 3. Significant differences ($p < 0.05$) in free sugar glucose (FSG) contents (%, d. b.) of tef enriched breads were observed (1.30-1.58%), in accordance with similar observed in the grain tef flours (1.48-1.86%) of the different varieties (Abebe et al., 2015). Starch fractions (RDS, SDS and RS), rapidly available glucose (RAG) and starch digestion rate index (SDRI) did not show dependence on tef variety. Amounts of digestible starch (RDS +SDS) of mixed breads were significantly lower than values found for the reference wheat bread (71.1-72.5% *vs* 77.1%). This is probably because of the relatively lower starch contents (74.0-75.5% vs 78.8%) and higher dietary fiber and ash contents in the respective tef flours (Abebe et al., 2015) as compared to wheat flours (Collar and Angioloni, 2014). Results are in accordance with the superior total starch content (TS) found in wheat breads (75.6%) compared to tef-enriched breads (71.4-72.3%) (Table 3). Suitable dietary trends for lower RDS and SDRI, and higher SDS contents (statistically non significant) in tef-enriched breads (67.5-68.2%, 94.2-94.7%, 3.5-4.3%) compared to wheat breads (74.3%, 98.3%, 2.8%) were found. In addition to the interference by dietary fibre, a stronger and denser mixed protein network may be formed hindering the starch availability to enzyme attack (Hager et al., 2013), which may contribute to the reduced rate of enzymatic starch hydrolysis.

3.2.3. Polyphenol fractions and anti-radical activity of tef-wheat blended breads

The profile of phenolic fractions and subfractions and anti-radical activity of 100% wheat bread used as a control, and tef enriched breads from three tef grain flour varieties are given in Table 5. Contents of extractable, hydrolyzable and total polyphenols of tef-enriched breads were higher than those of wheat flour bread counterparts (0% tef), regardless the tef variety and the percent of wheat flour replacement used. When the tef blended wheat breads are compared to that of control breads values (mg GA/100 g sample, d.b.) the extractable polyphenols ranged from 391 (DZ-01-99, 20%) to 585 (DZ-Cr-387, 40%) *vs* 308 (0% tef), and the hydrolyzable polyphenols varied from 1942 (DZ-Cr-387, 40%) to 2505 (DZ-Cr-37, 10%) *vs* 1958 (0% tef). The estimated total polyphenol content ranged from 2481 (DZ-Cr-387, 10%) to 2912 (DZ-Cr-37, 10%) *vs* 2265 (0% tef). The results show that the content of extractable polyphenols increased with an increase in the dosages of grain tef flours from 10 to 40% leading to a concomitant decrease of hydrolyzable polyphenol contents except for DZ-01-99 tef enriched samples. Compared to wheat flour breads counterparts, the larger increase in extractable polyphenols corresponded to 40%-DZ-Cr-387 breads (+90%), followed by 40%-DZ-Cr-37 (+65%) and 40%-DZ-01-99

(+61%) breads, while the larger decrease in hydrolyzable polyphenols with dose (from 10 to 40%) was observed for DZ-Cr-37 breads (-11%). As a result of the translocation of insoluble polyphenols to accumulation of soluble components in tef-enriched breads, total polyphenol content changed little with dose for each tef variety: 2913-2743 mg/100g (DZ-Cr-37), 2481-2527 mg/100g (DZ-Cr-387), and 2700-2893 mg/100g (DZ-01-99) (Table 5). Values for extractable polyphenols of tef-enriched samples changed little according to the tef variety, covering similar ranges: 408-508 mg/100g (DZ-Cr-37), 445-585 mg/100g (DZ-Cr-387), and 401-496 mg/100g (DZ-01-99), while content of hydrolyzable polyphenols followed the decreasing order: DZ-Cr-37 (2505-2235 mg/100g) > DZ-01-99 (2299-2397 mg/100g) >DZ-Cr-387 (2036-1942 mg/100g).

The result shows the contents of non-extractable (hydrolyzable) phenolics were significantly higher than the soluble phenolic fraction (from 3.7-fold in 30%-DZ-Cr-387 sample to 6.1-fold in sample containing 10% DZ-Cr-37 tef flour) vs 6.4-fold in the control wheat flour sample (Table 5). The average ratio between hydrolyzable and extractable phenolic content in the present samples was very similar to the one obtained by Saura-Calixto et al. (2007) for cereal grain products. Amounts of phenolic fraction and subfractions were substantially higher than expected from sum of the respective values of the flours. This fact can be ascribed to the breadmaking process, mainly through the mixing and baking stages that encompass mechanical and thermal input, respectively. Both breadmaking steps may favour either depolymerization/unfolding and linkage breaking of insoluble, bound forms and further release, or may increase the accessibility of soluble free compounds and soluble conjugates. The content of flavonoids (mg CE/100 g sample, d.b.) was significantly higher in bread samples enriched with brown tef DZ-01-99 (115-155 mg/100g) compared to control wheat flour sample (97 mg), values being higher with tef flour dose. The tef grain flours dose with white tef varieties (DZ-Cr-37, DZ-Cr-387) within the range 10 to 40% had insignificant effect ($p > 0.05$) on the total flavonoids contents of the bread (80-100 mg/100g) opposite to dosing effect of brown grain tef variety. This is in agreement with the high flavonoid contents in brown grain tef flour variety (DZ-01-99) of 266 mg/100g as compared to white grain tef varieties (DZ-Cr-37 = 117 mg/100g and DZ-Cr-387 = 108 mg/100g).

The bioaccessible polyphenol content (mg GA/100 g sample, d.b.) of the blended breads decreased with an increase of tef grain flours doses from 10 to 40%, ranging from 1810 to 1608 mg/100g (DZ-Cr-37), from 1862 to 1612 mg/100g (DZ-Cr-387) and from 1628 to 1591 mg/100g (DZ-01-99). The bioaccessible polyphenols contents of the control wheat flour breads (1747 mg/100g) were found to be higher than the breads processed by enriching with different grain tef varieties (1249 mg/100g for DZ-Cr-37-, 1496 mg/100g for DZ-Cr-387, 1406 mg/100g for DZ-01-99) (Table 5). Accumulation of bioaccessible polyphenols from flour to bread is in line with previous results observed on multigrain blended breads (Angioloni and Collar, 2011b; Collar et al., 2014b). Mechanical input during mixing and thermal treatment during baking may induce depolymerization of the constituents, mainly fibre, and hence may favour bread accessibility to enzyme attack and the subsequent release of fibre-associated polyphenols. In addition, Maillard reactions during bread baking can result in the synthesis of substances with antioxidant properties (Vogrincic et al., 2010). Nevertheless, replacement of wheat flour by increasing amounts of tef flour resulted in either a decline in the absolute level of bioaccessible polyphenols (DZ-Cr-37, DZ-Cr-387) or a reduction in the percentage of bioaccessible compounds with respect to total polyphenol content (DZ-Cr-37, DZ-01-99). This fact possibly attributed to a physical/sterical interference by tef grain flour constituents, particularly dietary fibre, that may hinder the accessibility of pepsin and pancreatin to achieve gastric and intestinal digestion. It has been stated that other compounds of proven resistance to the action of digestive enzymes, such as resistant starch, resistant protein, Maillard reaction compounds and other associated compounds, may reduce the bread phenol bioaccessibility (Saura-Calixto et al., 2000).

Anti-radical activity was determined by the extent of the reduction of the stable DPPH• radical, and results expressed as the remaining unreacted DPPH• amount when 0.247 μmols of the free radical are initially available to react with enzyme extracts from 2.5 mg flour or freeze-dried bread. Anti-radical activity for flours and for breads ranged from 24 to 36.3% (Table 5). It should be noticed the superior anti-radical activity of brown DZ-01-99 flour (32%) compared to white tef flours DZ-Cr-37 and DZ-Cr-387 that showed 27% in good accordance with the higher flavonoid content that are known to be good radical scavengers due to the presence of polyhydroxyl groups in the molecule. This resulted in a concomitant higher anti-radical activity in DZ-01-99 tef-blended breads (32-36%) regardless the dose of wheat flour replacement, compared to control wheat flour breads (29%) and white tef-blended breads (24-32%) (Table 5). For white tef-blended samples, irrespective of the dose of addition, incorporation of tef flour into formulations did not induced/contributed to enhanced anti-radical activity of breads. The observation, can be ascribed, to the changes occurring over breadmaking steps in terms of oxidation of phenolic compounds by coupled reaction due to substantial incorporation of oxygen in the dough during mixing (Eyoum et al., 2003), and to losses or degradation of phenolic compounds during baking (Angioloni and Collar, 2011b) as a result of the susceptibility of phenolic acids and flavonoids to heat.

4. Conclusions

Wheat flour replacement from 0% up to 40% by single tef flours from three Ethiopian varieties DZ-01-99 (brown grain tef), DZ-Cr-37 (white grain tef) and DZ-Cr-387 (Quncho, white grain tef) yielded technologically viable and sensory acceptable *ciabatta* type blended breads with enhanced nutritional value, as compared to the 100% refined wheat flour breads. Addition of tef grain flours up to 30% had insignificant effects on either loaf volume (DZ-Cr-37, DZ-Cr-387) or crumb hardness and cohesiveness, and provided even 10% higher volume when brown grain tef flour (DZ-01-99) was used as compared to the control bread. Further incorporation of tef flour from 30% to 40% imparted discreet negative effects in terms of decreasing loaf volume and crumb resilience regardless the tef variety used, and increase of crumb hardness in brown tef blended breads. Increment of crumb hardness on aging was in general much lower in tef blended breads as compared to wheat bread counterparts, revealing slower firming kinetics, especially for brown grain tef flour blended breads. Blended breads with 40% white grain tef flour exhibited similar extent and variable rate of retrogradation kinetics along storage, while brown tef-blended breads retrograded slower although in higher extent than control wheat flour breads. If the bioavailability can be assumed 100%, a daily intake of 170-180 g of tef-wheat (40-60%) blended breads can provide from 60 to 135% (DZ-01-99), from 141 to 318% (DZ-Cr3-7) and from 54 to 123% (DZ-Cr-387) of the amount of iron recommended for adults, depending on the tef flour variety and the gender, while copper requirements can be met from 43% (DZ-01-99, DZ-Cr-37) to 48% (DZ-Cr-387). In addition, suitable dietary trends for lower rapid digestible starch and starch digestion rate index can be fulfilled. The content of flavonoids and the anti-radical activity were significantly higher in bread samples enriched with brown grain tef flour compared to control wheat flour sample, values for flavonoids being higher with tef flour dose.

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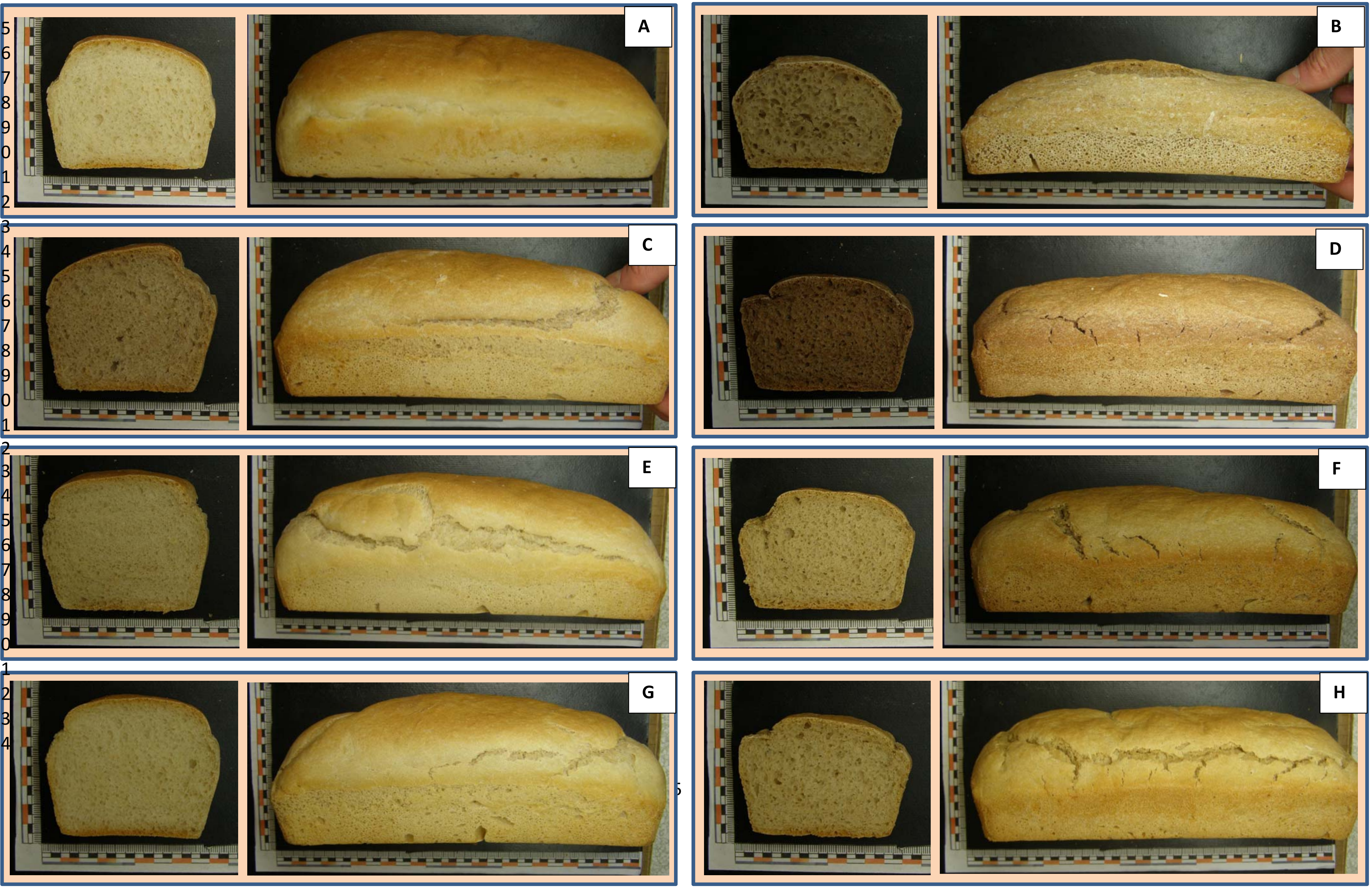
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Figure 1.- Effect of tef addition on the external appearance and internal structures of refined wheat bread depending on the addition level and tef variety . A) Control 1: 100% refined wheat bread; B) Control 2: mixture of 85% refined wheat flour 70% extraction rate and 15% wheat bran; C) 10% DZ-01-99 (brown grain tef flour) addition to refined wheat bread flour D) 40% DZ-01-99 (brown grain tef flour) addition to refined wheat bread E) 10% DZ-Cr-37 (white grain tef flour) addition to refined wheat bread flour F) 40% DZ-Cr-37 (white grain tef flour) addition to refined wheat bread flour; G) 10% DZ-Cr-387 (white grain tef flour) addition to refined wheat bread flour F) 40% DZ-Cr-387 (white grain tef flour) addition to refined wheat bread flour.

Table 1: Physical properties of composite breads processed by substitution with 10, 20, 30 and 40% three grain tef flour varieties to refined wheat grain flours[a]

Variety/ Dose (%)	Specific volume (mL/g)	Hardness (N)	Cohesiveness	Resilience	ΔFirmness 7 days (N)	Crumb			Crust		
						L*	h	C*	L*	h	C*
Control	3.21±0.09b	2.42±0.19de	0.69±0.01f	0.54±0.02f	5.95±0.26f	63.8±2.8g	87.50±0.94i	11.27±0.74a	58.5±3.8de	67.2±2.0bc	30.1±1.4ef
DZ-01-99											
10	3.57±0.03ef	2.08±0.23abc	0.69±0.01ef	0.53±0.01ef	2.99±0.30ab	59.5±1.3f	75.43±0.47d	12.83±0.62bc	57.0±3.4cd	67.5±2.8bc	26.7±5.1b
20	3.58±0.06ef	1.74±0.39^{a}	0.72±0.04g	0.55±0.04f	2.82±0.53^{a}	52.4±2.4cd	69.18±0.52c	13.90±0.61de	55.4±1.5bc	66.7±1.0ab	28.7±0.8bcde
30	3.54±0.07de	2.31±0.28cde	0.66±0.01abcd	0.48±0.01bcd	3.73±0.15abc	47.9±1.0b	65.61±0.66b	14.53±0.99de	52.8±2.2b	65.6±1.2ab	26.8±2.6bc
40	3.09±0.01a	2.83±0.20f	0.65±0.02abc	0.47±0.03abc	3.78±0.53abc	42.9±3.4^{a}	61.94±0.82a	14.33±1.49de	49.1±3.2^{a}	65.0±1.0a	23.0±2.0a
DZ-Cr-37											
10	3.49±0.02cd	2.37±0.17cde	0.68±0.01def	0.50±0.01de	5.29±0.28def	60.2±3.1f	85.82±0.57h	12.61±0.74b	59.6±1.2def	69.6±1.4de	31.8±1.3g
20	3.42±0.01c	2.46±0.08cdef	0.67±0.02cde	0.50±0.02cd	4.11±0.89bc	60.8±2.7f	83.61±0.99g	14.68±1.18ef	56.8±5.8cd	70.8±1.3def	30.3±2.4efg
30	3.20±0.01b	2.21±0.32bcd	0.67±0.02bcde	0.49±0.03cd	5.49±0.55ef	60.7±2.2f	82.57±1.28f	15.98±0.89g	62.3±2.3f	73.9±1.5g	29.2±1.2def
40	3.11±0.02a	2.60±0.37ef	0.65±0.02ab	0.45±0.02^{a}	6.04±0.53f	49.3±8.2bc	79.71±1.33e	13.63±1.44cd	57.7±2.9cde	71.5±1.0ef	27.2±1.6bcd
DZ-Cr-387											
10	3.53±0.05de	2.16±0.22bcd	0.69±0.01ef	0.54±0.02f	4.27±0.72cd	58.7±5.3ef	87.49±1.04i	12.15±1.19b	55.1±4.3bc	68.9±4.8cd	31.0±2.7fg
20	3.64±0.06f	1.87±0.16ab	0.67±0.01bcde	0.50±0.01d	3.30±0.90abc	55.3±4.3de	86.90±0.66i	12.55±0.76b	59.4±3.2def	71.5±2.5ef	30.9±2.5fg
30	3.26±0.01b	2.20±0.20bcd	0.68±0.01def	0.506±0.02de	4.35±0.24cde	62.2±2.8fg	85.94±0.72h	15.56±0.66fg	59.6±2.7def	72.4±2.3fg	29.1±1.4def
40	3.09±0.01a	2.35±0.12cde	0.64±0.01a	0.456±0.01ab	3.90±0.53abcd	53.6±3.5d	83.10±1.57fg	13.69±0.99cd	61.0±3.3ef	72.3±1.4fg	28.9±1.4cdef

Control: 100% wheat bread, ΔFirmness 7 day: Firmness increase over 7 storage days.

(a) Mean values ± standard deviation. Values with the same letters in a column are not significantly different ($p > 0.05$).

Table 2: Sensory properties of composite breads processed by substitution with 10, 20, 30 and 40% three grain tef flour varieties to refined wheat grain flours and two type control breads

Variety/ Dose (%)	Appearance	Odour	Texture	Taste	Persistency	Overall acceptability
Control -1	7.1 e	6.5 cd	6 de	6.4 cd	6.2 ab	6.9 e
Control- 2	6.4 cd	6.3 abcd	5 a	6.2 bcd	6.3 b	6.2 bcd
DZ-01-99						
10	6.0 abcd	6.6 cd	6 de	6.2 bcd	6.0 ab	6.5 cde
20	5.6 ab	5.8 a	6 cde	6.5 cd	6.0 ab	6.3 cde
30	5.7 ab	6.2 abcd	5 ab	5.8 abc	5.7 ab	5.7 ab
40	5.5 a	6.6 cd	6 abc	5.4 a	5.7 ab	6.0 abc
DZ-Cr-37						
10	6.6 de	6.5 bcd	7 de	6.2 bcd	6.0 ab	6.7 de
20	6.4 bcd	6.2 abcd	6 bcd	6.2 bcd	6.1 ab	6.4 cde
30	6.2 abcd	6.8 d	6 bcd	6.1 abcd	6.1 ab	6.5 cde
40	5.7 ab	5.9 ab	5 ab	5.7 ab	5.6 a	5.6 a
DZ-Cr-387						
10	6.8 de	6.0 abc	7 e	6.6 d	6.2 ab	6.9 e
20	6.8 de	6.1 abc	6 cde	6.4 bcd	6.1 ab	6.4 cde
30	5.9 abc	6.1 abc	6 cde	5.9 abcd	6.1 ab	6.1 abc
40	5.7 ab	6.1 abc	5 a	5.9 abcd	5.7 ab	5.9 abc
SD	0.26	0.25	0.27	0.27	0.28	0.24

Control 1 = 100% refined wheat bread and control 2 = wheat bread processed from 85% wheat and 15% wheat bran. Values with the same letters in a column are not significantly different (p > 0.05).

Table 3. - Starch fractions, FSG, RAG and SDRI expressed in % referred to dry matter, and values of Avrami model factors for crumb amylopectin recrystallization in terms of melting enthalpy (*H*) of tef-wheat (40:60) blended breads

Tef variety	FSG* (%)	RAG* (%)	RDS* (%)	SDS* (%)	RS* (%)	TS* (%)	SDRI* (%)	H_0 (J/g solids)	H_∞ (J/g solids)	k (d^{-n})	n	$t_{1/2}$ (d)	R^2
Control (0%)	0.11 ± 0.01a	82.7 ± 3b	74.3 ± 2b	2.8 ± 3a	-1.4 ± 1.9a	75.6 ± 0.6b	98.3 ± 0.8b	1.22±0.40b	5.79±0.83a	0.49±0.17a	0.85±0.40b	1.51±0.4a	0.975
DZ-01-99	1.58 ± 0.05c	76.7 ± 3a	67.6 ± 3a	3.5 ± 2a	0.3 ± 0.7a	71.4 ± 0.7a	94.7 ± 0.9a	1.10±0.34b	8.19±2.9b	0.31±0.21a	0.66±0.33a	3.52±0.5b	0.981
DZ-Cr-37	1.30 ± 0.03b	76.2 ± 4a	67.5 ± 2a	3.6 ± 2a	0.8 ± 1.8a	71.4 ± 1.7a	94.6 ± 2.3a	0.63±0.24a	5.63±0.10a	0.78±0.11b	0.69±0.10a	0.84±0.4a	0.999
DZ-Cr-387	1.47 ± 0.04c	77.2 ± 5a	68.2 ± 4a	4.3 ± 1a	-0.1 ± 1.0a	72.3 ± 1.0a	94.2 ± 1.3a	0.26±0.22a	5.97±0.48a	0.60±0.19b	0.83±0.22b	1.19±0.4a	0.999

*All results are expressed as the mean of six replicates ± standard deviation

FSG: Free glucose and sucrose; RDS = rapidly digestible starch, SDS = slowly digestible starch, RS = resistant starch, TS = total starch, RAG = rapidly available glucose, and SDRI = starch digestion rate index.

Values with a letter in common in the same column are not significantly different (p>0.05)

Table 4.- Moisture (%) and micro-element contents (mg/100g) of 40% tef- 60% wheat blended breads.

	Moisture (%)	Ca	Cu	Fe	Cr	K	Mg	Mn	P	Zn
Breads										
Control (0%)	43.4±0.7 b	151±1 a	<0.25 a	2.3±0.2 a	<0.25	201±5 a	50±1 a	0.86±0.02 a	208±1 a	1.56±0.03 a
DZ-01-99	42.5±0.6 ab	191±14 b	0.40±0.03 b	10.8±0.2 b	<0.25	347±35 b	105±7 b	3.02±0.11 c	319±25 b	2.35±0.09 b
DZ-Cr-37	41.5±0.7 a	209±2 b	0.39±0.01 b	25.4±1.5 c	<0.25	319±2 b	102±4 b	2.98±0.26 c	295±2 b	1.99±0.18 ab
DZ-Cr-387	42.8±0.7 ab	208±6 b	0.43±0.02 b	9.8±0.3 b	<0.25	358±31 b	101±2 b	2.47±0.04 b	313±13 b	2.35±0.24 b
Flours										
DZ-01-99	10.5±0.1 A	129±2 A	0.63±0.01 A	17.4±2.1 A	<0.25	475±1 B	172±1 B	6.07±0.05 B	455±2 C	2.80±0.01 C
DZ-Cr-37	10.3±0.1 B	138±2 B	0.68±0.01 B	77.8±1.5 C	<0.25	375±5 A	156±2 A	6.66±0.07 C	357±2 A	2.40±0.02 B
DZ-Cr-387	10.4±0.1 B	137±1 B	0.65±0.01 A	22.9±0.5 B	<0.25	467±2 B	171±1 B	4.51±0.02 A	409±2 B	2.20±0.02 A

Mean values (two replicates) ± standard deviation. Within columns, values with the same following letter do not differ significantly from each other ($p > 0.05$). Lower case letters are used to compare bread contents and capital letters to compare flour amounts.

Table 5.- Polyphenols fractions and subfractions and anti-radical activity of tef-wheat blended breads[a]

Variety/ Dose (%)	Extractable Polyphenols		Hydrolyzable Polyphenols		Total Polyphenols	Hydrolyzable/ Extractable	Flavonoids		Bioaccessible Polyphenols		Bioaccessible polyphenols,	Anti-radical activity DPPH•*		
	mg gallic acid/100 g sample, d.b.		mg gallic acid/100 g sample, d.b.		mg gallic acid/100 g sample, d.b.		mg catechin equivalents/100 g sample, d. b.		mg gallic acid/100 g sample, d.b.		% of total	remaining μmolDPPH at steady state		%
Breads														
Control 0%	308 ± 40	a	1958 ± 40	a	2265	6.4	97 ± 3	a	1747 ± 25	b	77	0.1802 ± 0.0061	a	28.9
DZ-01-99														
10	401 ± 34	bc	2299 ± 7	b	2700	5.7	115 ± 4	a	1628 ± 42	a	60	0.1710 ±0.0054	a	32.6
20	391 ± 2	b	2352 ± 48	b	2744	6.0	121 ± 0	b	1603 ± 47	a	58	0.1616 ± 0.0037	a	36.3
30	459 ± 51	cd	2320 ± 32	b	2780	5.1	132 ± 3	c	1611 ± 53	a	58	0.1646 ± 0.0034	a	35.1
40	496 ± 6	d	2397 ± 55	b	2893	4.8	155 ± 6	d	1591 ± 49	a	55	0.1729 ± 0.0090	a	31.8
DZ-Cr-37														
10	408 ± 21	b	2505 ± 72	d	2913	6.1	95 ± 1	a	1810 ± 59	b	62	0.1907 ± 0.0084	a	24.8
20	417 ± 6	b	2479 ± 11	d	2896	5.9	102 ± 4	a	1658 ± 51	a	57	0.1926 ± 0.0027	a	24.1
30	456 ± 25	c	2399 ± 67	c	2855	5.3	92 ± 6	a	1647 ± 51	a	58	0.1847 ± 0.0112	a	27.2
40	508 ± 9,70	d	2235 ± 16	b	2743	4.4	103 ± 16	a	1608 ± 60	a	59	0.1824 ± 0.0104	a	28.1
DZ-Cr-387														
10	445 ± 25	b	2036 ± 61	b	2481	4.6	83 ± 33	a	1862 ± 29	d	75	0.1816 ± 0.0048	a	28.4
20	413 ± 49	b	2037 ± 27	b	2450	4.9	91 ± 9	a	1790 ± 12	c	73	0.1715 ± 0.0044	a	32.4
30	539 ± 33	c	1984 ± 32	ab	2523	3.7	102 ± 9	a	1703 ± 18	ab	67	0.1705 ± 0.0112	a	32.8
40	585 ± 6	d	1942 ± 35	a	2527	4.3	87 ±2	a	1612 ± 97	a	64	0.1741 ± 0.0062	a	31.4
Flours														
DZ-01-99	907 ± 177	b	1971 ± 155	a	2879	2.2	266 ± 5	b	1406 ± 13	b	49	0.1724 ± 0.0057	a	32.0

DZ-Cr-37	685 ± 56	a	1972 ± 78	a	2657	2.9	117 ± 22	a	1249 ± 44	a	47	0.1852 ± 0.0033	b	27.0
DZ-Cr-387	670 ± 23	a	1840 ± 147	a	2510	2.7	108 ± 4	a	1496 ± 108	b	60	0.1849 ± 0.0044	b	27.1

(*) Corresponding to 2,5 mg flour or freeze-dried bread that consumed DPPH when 0.247 μmol of the free radical are initially available to react. The plateau was decided at 90 min of reaction.

(a) Mean values ± standard deviation. Within columns, values (mean of two replicates) with the same following letter do not differ significantly from each other ($p > 0.05$).