Impact of variety type and particle size distribution on starch enzymatic hydrolysis and functional properties of tef flours

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Abstract

Tef grain is becoming very attractive in the Western countries since it is a gluten-free grain with appreciated nutritional advantages. However there is little information of its functional properties and starch digestibility and how they are affected by variety type and particle size distribution. This work evaluates the effect of the grain variety and the mill used on tef flour physico-chemical and functional properties, mainly derived from starch behaviour. *In vitro* starch digestibility of the flours by Englyst method was assessed. Two types of mills were used to obtain whole flours of different granulation. Rice and wheat flours were analyzed as references. Protein molecular weight distribution and flour structure by SEM were also analyzed to justify some of the differences found among the cereals studied. Tef cultivar and mill type exhibited important effect on granulation, bulking density and starch damage, affecting the processing performance of the flours and determining the hydration and pasting properties. The colour was darker although one of the white varieties had a lightness near the reference flours. Different granulation of tef flour induced different *in vitro* starch digestibility. The disc attrition mill led to higher starch digestibility rate index and rapidly available glucose, probably as consequence of a higher damaged starch content. The results confirm the adequacy of tef flour as ingredient in the formulation of new cereal based foods and the importance of the variety and the mill on its functional properties.

Keywords: tef; *in vitro* starch digestibility; milling; functional properties

1. Introduction

Tef [*Eragrostis tef* (Zucc.)Trotter] grain, originated from Ethiopia, is becoming a very attractive cereal in the Western world since it is a gluten-free grain encompassing highly appreciated nutritional advantages. Tef grain size is known to be extremely small with mean length ranging 0.61-1.17 mm and mean width ranging 0.13-0.59 mm, that gives an average thousand kernel weight of 0.264 g (Bultosa, 2007). Tef grain anatomy studies by Parker et al. (1989) and Umeta and Parker (1996) indicate that the embryo, rich in protein and lipid, occupies a relatively large part of the grain. The aleurone layer is one cell thick and rich in protein lipid bodies. The testa is located within the pericarp and its thickness varies with the color of the grain. The testa of red tef is thicker than that of white tef and it is filled with pigmented material as tannins or polyphenolic compounds (Umeta and Parker, 1996). Tef grain is consumed as a whole meal and has more iron, calcium and zinc than other cereal grains, including wheat, barley and sorghum (Abebe, Bogale, Hambidge, Stoecker, Bailey & Gibson, 2007). The grain proteins offer an excellent balance among the essential amino acids (Yu, Sun, Rota, Edwards, Hailu, & Sorrells, 2006). Tef has recently been receiving global attention as a "healthy food", suitable for its employment in novel foods such as baby foods and gluten-free based goods (Dekking, Winkelaar, & Koning, 2005).

Different milling or grinding processes have been shown to produce different flours with different particle size and degree of damage of starch granules in flour, depending on the mechanical forces and temperature during the grinding process (Kadan, 2008). The kinetics of starch digestion by alpha amylase of barley and sorghum flours were found to be dependent on the particle size of flours (Al-Rabadi Gilbert, & Gidley, 2009). Starch damage encompasses disruption of the granular structure (Level 5) of the starch (Tran, Shelat, Tang, Li, Gilbert, & Hasjim, 2011), the extent being dependent on the starch size, botanical source and milling condition (Li, Dhital, & Hasjim, 2014). The extent of starch damage is known to affect the quality and functionality of the flours.

In Ethiopia tef is mainly processed to injera after milling with disc attrition mills available in cottage grain mill houses. Injera with much and evenly spread eyes, soft texture, easily rollable and bland after taste is rated as excellent. Intrinsic tef flour quality factors which favor these quality aspects include starch granule characteristics and the higher water solubility index of tef flour which positively influence injera quality (Yetneberk, Rooney, & Taylor, 2005).

The effect of milling method on starch damage, flour physical and functional properties and end product quality for common cereals like wheat and rice is well known (Kadan, Bryant, & Miller, 2008; Al-Rabadi et al., 2009; Tran et al., 2011). However, despite the nutritional interest and peculiarities of tef grain, information available on the functional properties and starch digestibility and its dependence on grain variety and granulation are still lacking. Therefore, the objective of this research was to identify the influence of two types of mills on the physical and functional properties and the starch digestibility of flours from three Ethiopian tef varieties, to properly assess the end use of tef flours thereof. Protein molecular weight distribution and flour structure by SEM were also evaluated to establish their significance on functional properties.

2. Materials and methods

2.1. Material

Three tef varieties DZ-01-99 (brown tef), DZ-Cr-37 (white tef) and DZ-Cr-387 (Qouncho, white tef) were obtained from the Debre Zeit Agricultural Research Center of the Ethiopian Institute of Agricultural Research (EIAR). Rice flour, whole wheat and refined wheat flours used as references were supplied by Emilio Esteban SA

(Valladolid, Spain). The proximal composition of the flours from the tef grains and the reference flours are shown in Table 1. Moisture, ash, fat and protein contents of the flours were determined using methods 44-19, 08-01, 30-25 and 46-11A of AACC (AACC, 2000) respectively. Total carbohydrates were determined by difference to 100% (FAO, 2003). Starch content was determined by Fraser, Brendon-Bravo & Holmes (1956) method and amylose and amylopectin with the Megazyme assay kit (Megazyme Bray, Ireland). All the assays were conducted in duplicate.

2.2. Milling process

The tef grains were manually cleaned by sifting and winnowing before milling. Two types of mills were used to obtain the whole flour of the three tef varieties. The first one was Cyclotech Sample mill (Foss Tecator, Häganäs Sweden) fitted with a 0.5 mm opening screen size (Mill 1). The second mill was a disc attrition mill (Mill 2) which is the type traditionally used in cottage tef grain-milling house (Bishoftu, Ethiopia) to mill tef grain for *injera* making in Ethiopia. The moisture content levels of the three tef cultivar grains were equivalent (10.3-10.5%, $p>0.05$) and in a normal range for field dried tef grains (Bultosa, 2007).

2.3. Protein characterization

All gels were run in minislabs (Bio-Rad Mini Protean II Model). Sodium dodecyl sulphate (SDS)-PAGE was performed according to Laemmli´s method (1970) using continuous gels (12%). Flour samples (1%, w/v) were dissolved in 0.125 M Tris-HCl, pH 6.8 buffer containing 0.02% (v/v) glycerol, 0.1% (w/v) SDS and 0.05% (w/v) bromophenol blue, and centrifuged at 15800 x g for 5 min at 4ºC. Supernatants were loaded onto the gel (30-40 μg of protein per lane). Samples to be run under reducing conditions were boiled for 1 min in 0.005% (v/v) 2-mercaptoethanol (2-ME) buffer before centrifugation. Electrophoresis was conducted for 1 h at a constant voltage of

200 V. The following molecular weight standards were used to estimate the molecular masses of polypeptides: phosphorylase b (94 kDa); bovine serum albumin (67 kDa); ovalbumin (45 kDa); carbonic anhydrase (30 kDa); trypsin inhibitor (20.1 kDa); α-lactalbumin (14.4 kDa), (Pharmacia Hepar Inc, Franklin, OH, U.S.A).

2.4. Granulation and density of flours

Flour particle size distribution was measured using a Sympatec Particle size and shape analyser (Sympatec GmbH, Germany) using diffraction of laser light and controlled by HELOS particle size analysis Window 5 software. The particle size distribution was characterized by the mean diameter (D_{50}) and the dispersion ((D_{90} –D_{10})/D_{50}) as described in Landillon, Cassan, Morel & Cuq (2008). Bulk density (BD) of the flours was determined according to Kaushal et al. (2012). Flour samples were gently poured into previously tared 10 ml graduated cylinders. The final volume reading was taken after vibrating the sample until constant value. Flour true density (TD) was determined by liquid displacement method with toluene as described in Deshpande & Poshadri (2011) by using 50ml pycnometers for the determination.

2.5. Flour Color

A Minolta spectrophotometer CN-508i (Minolta, Co.LTD, Japan) was used for flour color measurements. Results were obtained in the CIE L*a*b coordinates using the D65 standard illuminant, and the 2º standard observer. The hue (h) and the chroma (C*) were calculated from the equations 1 and 2 respectively. The spectrophotometer was programmed to report an average of 5 measurements.

$$h = \tan^{-1}\left(\frac{b*}{a*}\right) \quad (1)$$

$$C* = \left((a*)^2 + (b)^2\right)^{1/2} \quad (2)$$

2.6. Damaged starch

The damaged starch content in flour samples was determined in accordance with the American Association of Cereal Chemists (AACC) method (AACC, 2012), by using Megazyme starch damage kit (Megazyme International Ireland Ltd, Co. Wicklow, Ireland). Absorbance was read at 510 nm in a microplate reader BIOTEK EPOCH (Izasa, Barcelona, Spain). The damaged starch was determined as percentage of flour weight on a dry basis. Three replicates were made for each sample.

2.7. Technological functional properties

Foaming capacity (FC) and foam stability (FS) were determined as described by Collar & Angioloni (2014a, b) based on the methods used by Alu'datt, Rababah, Ereifej, Alli, Alrababah, Almajwal, Masadeh, & Alhamad (2012). Briefly, 2 g of flour sample was mixed with 40 ml distilled water at 30°C in a 100 ml measuring cylinder. The suspension was stirred and shaken manually for 5 min to produce foam. The volume of foam was measured after 0 min (VT) and 60 min (V1). FC was calculated directly from VT while FS was calculated from 100*(V1/VT).

The water holding capacity (WHC), the amount of water retained by the sample without being subjected to any stress, was determined with slight modification of the method used by Nelson (2001). Samples (2.000g ± 0.005g) were mixed with distilled water (20 ml) and kept at room temperature for 24 h. The supernatant was removed and WHC was measured as grams of water retained per gram of solid. The swelling volume (SV) was obtained by dividing the total volume of the swollen sample by the original dry weight of the sample (Nelson, 2001).

Water absorption capacity (WAC) and oil absorption capacity (OAC) of the flours were determined by the centrifugation method described by Beuchat (1977). Two grams of flour were mixed with 20 mL of distilled water or corn oil in 50 mL centrifuge tubes. The dispersions were occasionally vortexed while they were held at room temperature for 30 min, followed by centrifugation for 30 min at 3000 x *g* (Orto Alresa, Spain). The supernatant was removed and weighed and results were expressed as grams of water or oil retained per gram of flour.

Water absorption index (WAI) and water solubility index (WSI) of the flours were measured as described in Kaushal, Kumar & Sharma (2012). 2.5 g of flour sample (w_0) was dispersed in 30 ml of distilled water, using a glass rod, in tared centrifuge tubes; then cooked at 90^{o}C for 10min, cooled to room temperature and centrifuged at 3000 x *g* for 10 min. The supernatant was poured into a pre-weighed evaporating dish to determine its solid content and the sediment was weighed (w_{ss}). The weight of dry solids was recovered by evaporating the supernatant overnight at 110^{o}C (w_{ds}). WAI, WSI and swelling power (SP) were calculated from the equations:

$$\mathrm{WAI(g/g)} = \frac{w_{ss}}{w_0} \qquad (3)$$

$$\mathrm{WSI\ (g/100g)} = \frac{w_{ds}}{w_0} \mathrm{x}100 \qquad (4)$$

$$\mathrm{SP\ (g/g)} = \frac{w_{ss}}{w_0 - w_{ds}} \qquad (5)$$

2.8. Pasting properties of flours

Pasting properties were studied by using Rapid Visco Analyzer (RVA-4, Newport Scientific Pvt. Ltd, Australia) using ICC standard method 162. Parameters recorded were pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), final viscosity (FV), breakdown viscosity (BV), setback viscosity (SV), and peak time (Pt).

RVA parameters were calculated from the pasting curve using Thermocline v. 2.2 software. Analysis was done in triplicate.

2.8. Scanning electron microscopy (SEM)

A Scanning Electron Microscope (SEM) model Quanta 200-F (FEI, Oregon, USA) was used to examine the flours. This microscope was equipped with an X-ray detector and allowed the analysis of samples of low conductivity without prior metallization. The samples were directly mounted on stubs. Observations were made with an accelerating voltage of 1.5 keV.

2.9. Starch fractions analysis

In vitro starch digestibility was measured according to Englyst, Kingman, & Cummings (1992), including the latest modifications (Englyst, K., Englyst, H., Hudson, Cole, & Cummings,1999; Englyst, K., Hudson, & Englyst, H., 2000) as previously applied Ronda, Rivero, Caballero, & Quilez (2012). The hydrolysed glucose at 20 min (G_{20}) and 120 min (G_{120}) and the total glucose (TG) were determined by glucose oxidase colorimetric method and with six repetitions for each. 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 starch (RAG) = G_{20} were calculated. Starch digestibility rate index (SDRI) was computed from the percentage of RDS in TS in the flours.

2.10. Statistical analysis

Experimental data were analyzed using two-way analysis of variance (MANOVA) and then means were then compared at $p<0.05$ using Fisher's least significant difference

(LSD) test. Statistical analysis was done by Statgraphics Centurion XVI program (StatPoint Technologies, Inc. 1982-2010).

3. RESULTS AND DISCUSSION

3.1. Protein Characterization

The three tef cultivars showed similar protein profiles which were different from the reference flours (Figure 1). Under non-reducing conditions, polypeptides of 67-65, 56, 52, 35, 28, 25 and <20 kDa were observed in the three tef flours. The polypeptide of 52 kDa (Figure 1a, arrow 1) was dissociated by 2-ME reducing agent in tef flours while an increase in the intensity of bands between 20 and 30 kDa was observed under reducing conditions (Figure 1b), denoting the presence of disulfide bridges. Rice showed similar protein profile to tef under non-reducing conditions except two new polypeptides at 32 kDa (arrow 2) and 20 kDa (arrow 3). Under reducing conditions the 32 kDa band increased in intensity and a new polypeptide of 25 kDa appeared. As for most other cereals, prolamins are major storage proteins in tef (Adebowale, Emmambux, Beukes, & Taylor, 2011). However, protein fractions in tef are less complex than those of wheat, in terms of their apparent molecular size differences, and resemble more the pattern found in maize (Shewry & Tatham, 1990; Hager, Wolter, Jacob, Zannini, & Arendt, 2012).

3.2. Granulation and density of flours

Granulation and uniformity of particle size have long been assumed to be important factors affecting the processing performance of flours. The mean diameters of flour particles (D_{50}) of tef flours varied significantly (Table 2) in the order DZ-01-99 (92.4 µm) < DZ-Cr-387 (94.9 µm) < DZ-Cr-37 (96.6 µm), noting also significantly higher values for mill 1 (96.2 µm) than mill 2 (93.3 µm). The D_{50} of the tef flours was higher than in wheat flour (56.8 µm) and lower than in rice flour (142.4 µm). However, earlier work on three common wheat flours showed D_{50} values ranging from 64 to 99 µm (Landillon et al., 2008) indicating the high dependence of wheat flour particle size on variety type. The size dispersion of tef cultivar flours (2.32 – 2.36) was notably higher than those of wheat and rice flours. This difference could be attributed to continuous sieving processes during industrial milling of the reference flours. Mill 2 led to significantly lower size dispersion (2.13) than mill 1 (2.55) which shows that the discs mill gave flour of more uniform size. For the three tef cultivars mill 1 generated flours with bimodal particle size distribution (4.5- 150 µm and 150-850 µm). In both, D_{50} and size dispersion, significant ($p<0,01$) variety x mill interaction was observed. The less pronounced effect of mill type on D_{50} was observed in the DZ-Cr-387 variety while the most impact on the size dispersion was detected for DZ-01-99.

The bulk densities (BD) and true densities (TD) of the tef cultivar flours showed significant ($p<0.01$) variations depending on the variety and the mill. DZ-01-99 flour obtained from the mill 2 had the lowest values (Table 2). BD can be used to predict packaging requirements of the flours (Akubor, 2007). Tef flours from mill 1 had significantly ($p<0.01$) higher mean BD (0.86 g/cm^3) than those from mill 2 (0.80 g/cm^3) and the mill type influence being more visible on DZ-Cr-387 than on the other tef cultivars. This could be due to the fact that mill 1 led to flours with higher average particle size than mill 2 and agrees with the statement of Brown & Richards (1970) describing powders with a fine structure pack loosely than aggregated granules and samples of larger particle size will give higher densities. As it could be expected, the type of mill did not affect TD as it is mainly dependent on flour composition but not on particle size.

3.3 Flour color

The average lightness (L*) of grain flours from the three tef varieties varied markedly ($p<0.01$) in the order DZ-01-99 (67.4) < DZ-Cr-37 (78.0) < DZ-Cr-387(82.4) (Table 2). The hue angle (h) of the tef flours also varied from reddish to the yellowish in the order: DZ-01-99< DZ-Cr-37<DZ-Cr-387. However, compared with wheat and rice flours they all showed lower L* and h. A similar trend of L* and h was recorded on the gels from the three tef cultivars (Abebe & Ronda, 2014). DZ-01-99 grain flour exhibited the darkest and most red flour that could be due to tannin or polyphenol compounds (Umeta & Parker, 1996). The average chroma (C*) of DZ-Cr-387 (15.2) and DZ-Cr-37 (15.2) grain flours obtained from the two mills were significantly higher than that of DZ-01-99 (13.7) indicating more vivid colors. Rice and wheat flours were paler, with significant ($p<0.05$) higher L* values than tef flours, which could be because they are refined flours or with very little amount of bran components. Among the tef cultivars effect of mill type was not significant only on DZ-Cr-37 flour color. Such effect of mill type could probably be related to degree of breaking and pulverisation of the bran of the tef grains. However, although significant, the flour color differences attributed to the mill could hardly be detected by eye.

3.4. Damaged starch

The damaged starch (DS) determined in tef cultivars varied significantly ($p<0.001$) with the tef variety and the mill used (Table 2). The mean DS varied with variety in the order DZ-Cr-387 (5.33%) >DZ-01-99 (4.14%) = DZ-Cr-37 (4.02%). Notably higher ($p<0.01$) DS was exhibited by mill 2 (5.72%) than mill 1 (3.27%). DS in the tef flours increased with decreasing D_{50} ($r= 0.6$, $p<0.05$). This agrees with report by Lijuan, Guiying, Guoan, & Zaigui (2007) stating under the same milling conditions milling to smaller flour particle sizes caused higher DS. Tef variety and mill type interaction effect was also significant ($p<0.01$). The level of DS in DZ-Cr-387 flour from mill 1 was much higher than the remaining tef cultivars. DS in tef flours from mill 2 were apparently higher than the DS in wheat flour and lower than DS in rice flour evaluated together.

3.5. Technological functional properties

Technological functional properties are summarized in Table 3. Cultivar and mill type did not show significant ($p>0.05$) effect on foaming capacity (FC) and foaming stability (FS) of the tef flours. However, FC values exhibited by the flours from tef cultivars were 1.7 times lower than wheat flours and 1.8 times higher than rice flours. Flour foaming occurs mainly due to a continuous cohesive film formed around the air bubbles in the foam. Similarity in the protein type available in the three tef cultivars and their difference with the reference flours discussed earlier may justify the observed FC's of the flours (Kaushal et al., 2012). The FC score of tef flours could indicate their better suitability than rice in gluten-free food systems that require aeration for textural and leavening properties. The FS of tef flours was much higher than wheat and rice indicating their ability to maintain the foam. Therefore, tef flour could be a better ingredient in gluten-free food system, such as ice-cream, cakes or toppings and confectionary products, which require aeration for textural and leavening properties.

Flour hydration properties were significantly affected by both type of tef cultivar and mill type (Table 3). Among the tef cultivars, DZ-Cr-387 had relatively higher mean water holding capacity (WHC), swelling volume (SV), water absorption index (WAI), water solubility index (WSI) and swelling power (SP) while it scored lower mean water absorption capacity (WAC). The wheat and rice flours had notably lower WHC and SV than tef flours. The higher fiber content in tef flours, as whole meal (Collar and Angioloni, 2014b), could also explain its higher water binding capacity with respect to refined wheat and rice flours (Santos, Rosell, & Collar, 2008). Tef flours from mill 2 also had significantly higher WHC, SV, OAC, WAI, WSI and SP. The probable reason for these results could be the smaller flour particle size of flours from mill 2 giving greater surface area for binding water molecules inducing higher water or oil uptake. The significant negative correlation ($p<0.01$, $r= -0.7$) observed between the D_{50} of tef flours and their WHC confirms the relationship.

The WAC values of tef flours were apparently higher than wheat flour and lower than the rice flour. WAC has fundamental importance in viscous foods such as soups, sauces, doughs and baked products in which good protein-water interaction is required (Granito Guerra, Torres, & Guinand., 2004) making tef to be a more suitable ingredient than rice in gluten free formulation. Effect of mill type on OAC of the tef flours was significant ($p<0.05$). Flours from mill 1 had lower OAC (0.83g/g) than those from mill 2 (0.86g/g). The tef flours had apparently similar OAC to the reference flours. Higher OAC in DZ-Cr-387 and DZ-01-99 than DZ-01-37 and in mill 2 than mill 1 can partly be attributed to the lower particle size because oil absorption also depends on the physical entrapment of oil. Flours with high OAC are potentially useful in food products for flavour retention, improvement of palatability and extension of shelf life, mainly in bakery and meat products. High OAC makes the flour suitable in facilitating enhancement in mouthfeel when used in food preparations. Therefore, products from DZ-Cr-387 and DZ-01-99 may better have these quality attributes than DZ-01-37.

The water absorption index (WAI) measures the volume occupied by the gelatinized starch and denatured protein and other components after swelling in excess water maintaining the integrity of starch in aqueous dispersion (Marson & Hoseney, 1986). Compared to wheat and rice flours, the mean values of the WAI of the flours from three tef varieties were apparently lower. WSI of the three tef cultivars was apparently higher than that of wheat and especially that of rice flours indicating the presence of higher soluble matter content in the tef flours. Tef flours from mill 1 had significantly ($p<0.01$) lower WAI, WSI and SP (5.71 g/g, 5.21 g/100 g and 6.02 g/g respectively) than from mill 2 (6.20 g/g, 5.83 g/100 g and 6.58 g/g respectively). The value of WSI positively correlated with DS ($r=0.63$, $p<0.05$) because damaged granules hydrate readily and are susceptible to amylolytic hydrolysis. Similarly the effect of flour mean particle size was important ($p<0.05$ and $r=-0.5$ to -0.6) on gel hydration properties of the tef flours and this could be due to higher surface area being exposed for water binding. Earlier work by Yetneberk et al. (2005) shows that in sorghum and tef composite flours the WSI increased progressively with increasing proportion of tef, giving injera better quality. The increase in WSI agreed with the observation that, during mixing, compared with sorghum, tef dough tended to be stickier and water-soluble components in the tef flour could have modified the dough rheology and the texture of injera positively (Yetneberk, et al., 2005). In evaluating injera making potentials of sorghum varieties higher WSI gave more fluffy, soft and rollable injera (Yetneberk, 2004). In addition, in flat breads superior quality is associated with wheat flours with high damaged starch content and water absorption (Qarooni, Posner, & Ponte, 1993). Therefore, based on WSI, starch damage level and water absorption injera from DZ-Cr-387 could be more fluffy, soft and rollable followed by DZ-01-99 and then DZ-Cr-37. At the same time mill 2 seems more suitable for preparation of tef flours for injera.

3.5. Pasting properties

Among the tef flours the pasting viscosity (PV) of DZ-Cr-387 (1647mPa.s) was 20% higher than the equivalent PV of DZ-01-99 and DZ-Cr-37 (Table 4). Trough viscosity (TV) was similar for the three tef varieties, with an average value of 830 mPa.s. The mill type influenced the TV of the tef flours in which mill 1 led to the higher value, 862 mPa.s versus 799 mPa.s of mill 2. The breakdown viscosity (BV) of DZ-Cr-387 (794 mPa.s) was about 60% higher than that of the other two varieties. This means that this white tef variety showed the highest disintegration degree of the swollen systems and alignment of amylose and other linear components in the direction of shear. Mill 2 led to flours with a mean BV value 16% higher than mill 1. Consequently, flour from mill 1 had higher thermostability and lower shear thinning and disintegration of swollen systems than from mill 2. The BV of wheat flour was similar to that of tef; however, the rice flour BV was 3.5–5 times higher. Hence, the result obtained supports the suggestion by Bultosa (2007) indicating the potential of tef to be used under high shear conditions. Final viscosity (FV) shows the ability of the material to form a viscous paste and it is mainly determined by the retrogradation of soluble amylose in the process of cooling and tef cultivar type did not influence it. However, the effect of mill type was significant where flours from Mill 1 had FV 10% higher than mill 2. Setback viscosity (SV) shows how the viscosity of the paste of the flour suspensions recovered during the cooling period. The average SV of DZ-Cr-37 flour was 18% and 10 % higher than that of flours from DZ-01-99 and DZ-Cr-387 respectively. The mill used also affected significantly the SV of the flours and mill 1 led to flours with SV values 10% higher than mill 2. The remarkably lower SV of the tef flours with respect to wheat and rice flours is related to amylose retrogradation and confirm that tef flours retrograde to less extent than other cereals. Such lower reterogradation tendency in the tef flours could make them to be advantageous in formulation of different food products.

The peak time (Pt) and pasting temperature (PT) were also dependent on tef variety. Tef flour from DZ-Cr-37 showed the highest Pt (8.62 min) and PT (83.1 ^{o}C) and the results lie in the range reported by Bultosa (2007). The Pt of the tef flours were lower than both wheat and rice flours. Mean Pt and PT of tef flours from mill 1 (8.58 min and 77 ^{o}C) were also significantly higher than that of mill 2 (8.44 min and 75 ^{o}C).

Significant correlations ($p<0.05$) were obtained between the mean particle size of tef flours and its pasting properties, mainly FV, SV, Pt and PT (in all cases $r>0.6$). A similar trend was reported for PT and FV of rice flours by Hasjim, Li, & Dhital (2013). Tef flours with higher WAI, WSI and SP tend to have higher PV and BV ($p<0.05$, $r\geq0.6$) and lower FV, SV, PT and Pt ($p<0.05$, $r\geq -0.6$).

3.7. Scanning electron microscopy (SEM)

Like the other cereal species, tef starch is organized to form starch compound granules of the endosperm (Figure 2). The polygonal shaped starch is clearly seen packed together and protein seems to attach outside of the compound starch granule. In both mill types some of these compound granules were pulverized and individual starch granules are released. However, in mill 2 the starch granule pulverization was more pronounced. Hence, compared to the tef flours from mill 1, tef flours from mill 2 had smaller particle size and closer size distribution and this corroborates the results discussed earlier. In addition both large lenticular starch granules (A-granules) and smaller spherical granules (B-granules) can be observed in wheat. Rice flour particles were the larger having very small polyhedral starch granules.

3.8. Starch fractions and *in vitro* starch digestibility

The three tef varieties had similar contents of free sugar glucose (FGS), starch fractions (RDS, SDS and RS), rapidly available glucose (RAG) and starch digestion rate index (SDRI) (Table 5). However, the effect of mill type on starch vulnerability to the attack of digestive enzymes was significant: mill 2 led to higher RAG, RDS, and RS and lower SDS. As TS was not dependent on milling SDRI was also higher in flours from mill 2. Li, Dhital, & Hasjim (2014) indicate that damaged starch granules in flour (level 6 structure) have greater enzyme digestibility than intact native starch granules and starch digestibility of flours from milled cereal grains increases with the decreasing flour size.

Tef flours from mill 2 have the lower mean particle size and higher starch damage (Table 2). The damaged starch content had a significant positive correlation with SDRI and RAG ($p<0.01$, $r= 0.6$ in both cases). Apparently higher SDRI in the two white tef cultivars (DZ-Cr-37 and DZ-Cr-387) flours from mill 2 than in wheat flour could also be attributed to the higher damaged starch available in them. The lower RDS content in tef flour versus rice makes this cereal particularly interesting for celiac patients that frequently suffer diabetes of type I besides the celiac disease. However it is necessary to demonstrate the same behavior in final products to establish this conclusion as definitive. The FSG content of the three tef cultivar flours (1.5 % dry basis) was more than three and seven times higher than those of wheat and rice flours respectively. Higher FSG available in tef could probably be the reason why cooked tef grain tends to have sweet taste.

4. Conclusions

The protein profiles of the three tef cultivars were similar, but different from wheat and rice analyzed as reference. Tef cultivar and mill type used exhibited important effect on flour granulation and uniformity of particle size, starch damage and densities. These parameters were important factors affecting the processing performance of the flours by determining the absorbed water and dissolved flour components and the pasting properties of tef flours. A lighter product could be obtained from DZ-Cr-387 followed by DZ-Cr-37 and then DZ-01-99. This corroborates the report by Fufa, Behute, Simons & Berhe (2011) stating the higher preference and value of DZ-Cr-387 than DZ-Cr-37 giving brighter or whiter *injera* which is more preferable to Ethiopian consumers. Western consumers, more accustomed to white and refined cereals, could also prefer this variety. Based on WSI, starch damage level and water absorption results, injera from DZ-Cr-387 could be more fluffy, soft and rollable followed by DZ-01-99 and then

DZ-Cr-37. At the same time, compared to the Cyclotech Sample mill used in this experiment, the disc mill which is currently being used in Ethiopia for milling tef grain seems more suitable for preparation of tef flours for injera. The results confirm the adequacy of tef flours as ingredients in the formulation of new cereal based foods and the importance of the variety and the mill used on its functional properties. Starch fractions available in the three tef cultivars and indices indicating the *in vitro* starch digestibility of their flours were equivalent. The effect of damaged starch was more important and tef flours from the disc attrition mill had higher RAG and SDRI. Starch digestibility in the tef flours tended to be lower than the reference flours. Extensively higher FSG in tef may indicate its potential to develop products with different taste.

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Table 1: Chemical composition of tef flours (% on dry basis). Wheat and rice flours were included and considered as references

Flour	Moisture (%)	Proteins (% w/w)	Ash (% w/w)	Fat (% w/w)	Carbohydrates (% w/w)	Starch (% w/w)	Amylose (% of starch)
Tef-brown (DZ-01-99)	10.5±0.1a	8.9±0.3b	2.71±0.19c	2.84±0.08d	85.6±0.6c	75.5±0.1c	21.6±0.3a
Tef-white (DZ-Cr-37)	10.3±0.1a	10.5±0.2c	3.52±0.01d	2.63±0.06c	83.4±0.2b	74.0±0.3b	21.8±0.3a
Tef-white (DZ-Cr-387)	10.4±0.1a	8.9±0.2b	2.63±0.09c	3.24±0.06e	85.3±0.3c	75.5±0.4c	21.1±0.4a
Wheat	12.1±0.1b	12.7±0.2d	0.69±0.01a	1.47±0.06a	85.1±0.2c	78.8±0.4d	23.2±0.5b
Rice	12.2±0.1b	7.8±0.3a	0.67±0.01a	1.35±0.04a	90.5±0.3d	87.7±0.4e	21.7±0.1a

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

Table 2. Physical properties of the flours and damaged starch level

Variety	Mill	Average particle size		Bulk density (g/cm^3)	True density (g/cm^3)	Damaged starch (%)	L*	a*	b*	h	C*
		D_{50} (µm)	Dispersion $((D_{90}-D_{10})/D_{50})$								
DZ-01-99	1	94.1±0.8b	2.51±0.02d	0.85±0.01b	1.43±0.01ab	2.48±0.28a	67.1±0.3a	5.08±0.07e	12.1±0.1a	67.3±0.1a	13.1±0.1a
DZ-01-99	2	90.7±0.6a	2.17±0.01c	0.79±0.01a	1.42±0.01a	5.56±.14c	67.8±0.1b	4.83±0.04d	13.4±0.1b	70.1±0.1b	14.2±0.1b
DZ-Cr-37	1	98.4±0.9d	2.58±0.01f	0.87±0.01c	1.47±0.04b	2.43±0.16a	78.1±0.1c	1.97±0.02c	15.2±0.2de	82.6±0.1c	15.4±0.2de
DZ-Cr-37	2	94.7±0.1bc	2.14±0.01b	0.81±0.01a	1.46±0.01ab	5.85±0.04c	78.0±0.1c	1.96±0.02c	14.9±0.1cd	82.5±0.1c	15.0±0.1cd
DZ-Cr-387	1	95.5±0.6c	2.55±0.03e	0.88±0.01c	1.44±0.01ab	4.91±0.04b	83.2±0.1e	1.19±0.03a	14.6±0.1c	85.3±0.1d	14.6±0.1bc
DZ-Cr-387	2	94.2±0.5b	2.10±0.01a	0.79±0.01a	1.44±0.01ab	5.75±0.01c	81.7±0.1d	1.31±0.01b	15.7±0.4e	85.2±0.2d	15.4±0.4e
Wheat		56.8±0.1	1.88±0.01	0.76±0.01	1,42±0.01	5.27±0.28	94.4±0.1	0.60±0.01	9.7±0.1	86.5±0.1	9.7±0.1
Rice		142.7±0.3	1.70±0.01	0.84±0.01	1,43±0.01	6.51±0.57	93.9±0.1	-0.14±0.01	7.4±0.1	91.0±0.1	7.4±0.1
Variety		**	**	**	*	**	**	**	**	**	**
Mill		**	**	**	ns	**	**	ns	**	**	**
Variety x Mill		*	**	**	ns	**	**	*	*	**	**

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

*, ** and ns indicate the level of significance in the effects of tef variety, mill and their interaction. * $p<0.05$, ** $p<0.01$ and ns= not significant ($p>0.05$).

Where: L*, a*, and b* are CIE coordinates, h = hue and C* = chroma.

Table 3. Functional characteristics of flours.

Variety	Mill	FC (mL)	FS (%)	WAC (g/g)	OAC (g/g)	WHC (g/g)	SV (ml/g)	WAI (g/g)	WSI (g/100g)	SP (g/g)
DZ-01-99	1	6.5±2.1a	28.8±12.4	0.89±0.02a	0.83±0.02abc	2.07±0.12a	3.10±0.03cd	5.57±0.16a	5.37±0.09bc	5.89±0.17a
DZ-01-99	2	8.0±0.0a	37.5±17.8	1.06±0.02e	0.87±0.04cd	2.15±0.31a	3.05±0.36cd	6.18±0.25bc	6.15±0.41d	6.58±0.24bc
DZ-Cr-37	1	7.0±1.4a	43.8±8.8	1.05±0.02de	0.81±0.01a	2.02±0.27a	2.91±0.2c	5.42±0.08a	4.65±0.08a	5.69±0.09a
DZ-Cr-37	2	9.0±2.8a	49.4±31.2	1.02±0.02cd	0.82±0.02ab	2.31±0.11a	3.19±0.23d	5.96±0.27b	4.95±0.32ab	6.27±0.27b
DZ-Cr-387	1	9.5±2.1a	40.3±31.3	0.96±0.01b	0.87±0.01bcd	2.10±0.16a	3.06± 0.01cd	6.13±0.13b	5.60±0.07c	6.49±0.13b
DZ-Cr-387	2	9.5±0.7a	42.2±3.1	0.99±0.01bc	0.89±0.01d	2.65±0.07b	3.50±0.05e	6.46±0.13c	6.40±0.32d	6.70±0.13c
Wheat		14.0±1.4	28.7±2.9	0.70±0.01	0.85±0.01	1.50±0.12	2.27±0.11	6.38±0.09	4.41±0.07	7.34±0.07
Rice		4.5±2.1	0.0±0.0	1.1±0.01	0.84±0.01	1.78±0.05	2.58±0.13	7.21±0.07	1.70±0.09	6.67±0.10
Variety		ns	ns	**	**	*	ns	**	**	**
Mill		ns	ns	**	*	**	*	**	**	**
Variety X Mill		ns	ns	**	ns	ns	ns	ns	ns	ns

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

*, ** and ns indicate the level of significance in the effects of tef variety, mill and their interaction. * $p<0.05$, ** $p<0.01$ and ns= not significant ($p>0.05$).

FC = foaming capacity, FS = Foaming stability after 60’, WAC = water absorption capacity, OAC = oil absorption capacity, WHC = water holding capacity, SV = swelling volume, WAI = water absorption index, WSI = water solubility index and SP = swelling power..

Table 4. Pasting properties of hydrated flours.

Variety	Mill	PV (mPas)	TV (mPas)	BV (mPas)	FV (mPas)	SV (mPas)	Peak time (min)	PT (ºC)
DZ-99-01	1	1336 ± 23a	858 ± 73b	478 ± 71a	1767 ±155a	908 ± 83ab	8.51 ± 0.10b	75.2 ± 0.8b
DZ-99-01	2	1344 ± 8a	829 ± 72ab	515 ± 70ab	1690 ± 128a	861 ± 56a	8.47 ± 0.11a	74.9 ± 1.2b
DZ-Cr-37	1	1304 ± 37a	844 ± 12b	461 ± 34a	1957 ± 22b	1113 ± 23c	8.73 ± 0.07c	83.1 ± 0.9d
DZ-Cr-37	2	1317 ± 49a	744 ± 44a	574 ± 10b	1713 ± 46a	969 ± 7b	8.51 ± 0.03b	79.4 ± 1.0c
DZ-Cr-387	1	1618 ± 59b	883 ± 35b	735 ± 24c	1840 ± 45ab	956 ± 15b	8.49 ± 0.03b	73.1 ± 0.6a
DZ-Cr-387	2	1676 ± 67b	823 ± 43ab	853 ± 26d	1701 ± 47a	878 ± 17a	8.33 ± 0.01a	71.8 ± 0.2a
Wheat		2060 ± 19	1192 ± 17	868 ± 6	2512 ± 30	1319 ± 13	9.25 ± 0.04	84.9 ± 0.3
Rice		4023 ± 83	1495 ± 95	2528 ± 139	3569 ± 56	2075 ± 129	9.07 ± 0.01	75.3 ± 0.2
Variety		**	ns	**	ns	**	**	**
Mill		ns	*	**	**	**	**	**
Variety x Mill		ns	ns	ns	ns	ns	ns	*

Data are the mean ± standard deviation. Values with a letter in common in the same column are not significantly different (p>0.05)
*, ** and ns indicate the level of significance in the effects of tef variety, mill and their interaction. * p<0.05, ** p<0.01 and ns= not significant (p>0.05).
PV= pasting viscosity, TV = trough viscosity, BV = breakdown viscosity, FV = final viscosity, SV = set back viscosity, and PT = pasting temperature.

Table 5. Starch fractions, FSG, RAG and SDRI expressed in % referred to dry matter

Variety	Mill	FSG (%)	RAG (%)	RDS (%)	SDS (%)	RS (%)	TS (%)	SDRI (%)
DZ-01-99	1	1.48 ± 0.08b	34.3 ± 0.9ab	29.5 ±0.8ab	38.5 ±2.2bc	7.7±1.0bc	75.7±1.0a	39.0±1.0bc
DZ-01-99	2	1.60 ± 0.06b	34.8 ± 0.8abc	29.9 ±0.7ab	36.2±2.2abc	8.0±1.1bcd	74.1±1.1a	40.7±1.5bcd
DZ-Cr-37	1	1.18 ± 0.06a	34.0 ± 2.4ab	29.5 ±2.2ab	39.5±2.5bc	6.5±1.1ab	75.6±1.1a	39.7±1.6ab
DZ-Cr-37	2	1.86 ± 0.08c	38.5 ± 1.6c	33.0 ±1.5b	33.0±2.9ab	8.9±2.3cd	74.9±2.3a	44.4±1.6cd
DZ-Cr-387	1	1.43 ± 0.01b	33.8 ± 2.6a	29.1 ±2.3a	40.8±2.5c	5.7±1.6a	75.7±1.6a	38.5±1.6a
DZ-Cr-387	2	1.49 ± 0.31b	38.0 ± 1.0bc	32.9 ±0.9b	31.1±2.2a	10.5±1.5d	74.5±1.5a	44.1±1.6d
Wheat		0.46 ± 0.02	39.6 ± 2.2	35.2 ±2.0	44.1±2.5	2.3±1.2	79.0±1.2	41.9±1.3
Rice		0.20 ± 0.01	47.4 ± 1.9	42.4 ±1.7	37.4±2.9	8.2±2.9	88.0±2.9	48.3±1.3
Variety		ns	ns	ns	ns	ns	ns	ns
Mill		**	*	*	**	**	ns	*
Variety x Mill		**	ns	ns	ns	*	ns	ns

Data are on dry basis and the mean ± standard deviation. Values with a letter in common in the same column are not significantly different ($p>0.05$)

*, ** and ns indicate the level of significance in the effects of tef variety. mill and their interaction. * $p<0.05$, ** $p<0.01$ and ns= not significant ($p>0.05$).

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