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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 behavior. *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 color 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.

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, Umeta, and Faulks (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, located within the pericarp, varies in thickness 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 such as tannins or polyphenolic compounds (Umeta & 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 et al., 2007). The grain proteins offer an excellent balance among the essential amino acids (Yu et al., 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, Bryant, & Miller, 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 et al., 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 disk attrition mills available in cottage grain mill houses. Injera with much and evenly spread eyes, soft texture, easily rollable and bland

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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 ($p < 0.05$).

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 (Al-Rabadi et al., 2009; Kadan et al., 2008; 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, and 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 disk attrition mill (Mill 2) which is the 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 15,800 × g for 5 min at 4 °C. 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, USA).

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, and Cuq (2008). Bulk density (BD) of the flours was determined according to Kaushal, Kumar, and Sharma (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 and Poshadri (2011) by using 50 mL 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 2° standard observer. The hue (h) and the chroma (C^*) were calculated from the Eqs. (1) and (2) respectively. The spectrophotometer was programmed to report an average of 5 measurements.

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

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

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.