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Effect of sourdough addition and storage time on *in vitro* starch digestibility and estimated glycemic index of tef bread

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A B S T R A C T

The effect of sourdough amount and storage time on starch digestibility and estimated glycemic index (eGI) of tef bread was investigated. The rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) of 0–30% sourdough fresh tef breads ranged from 49 to 58, 16 to 29 and 20 to 26 g/100 g starch, respectively. Storage of tef breads up to 5 days decreased the RDS by more than 2-fold while SDS and RS increased by 2 and 3 fold, respectively. The eGI for fresh and stored breads ranged from 39 to 89. Addition of sourdough increased the eGI of fresh breads while no uniform pattern was seen in the stored breads. As the storage time increased, all the breads showed a decrease in eGI. *In vivo* study is necessary to further investigate the effect of sourdough on GI of tef bread.

1. Introduction

The global prevalence of diabetes among adults will increase to 8.8% in the year 2035 affecting as much as 592 million adults compared to 8.3% (382 million adults) in the year 2013 (Guariguata et al., 2014). Long term frequent intake of high glycemic index food products produce greater insulin resistance than the low glycemic-index carbohydrates. Lifestyle modification, involving diet and enhanced physical activity, are known to effectively prevent type 2 diabetes mellitus. Owing to this, there is a global shift of consumers from refined white flours to a minimally refined flour or whole meal as consumption of high fiber containing flours are increasingly associated with a lower risk of weight gain, cardiovascular disease and other chronic diseases (Patel, Chandra, Alexander, Soble, & Williams, 2017).

Tef [*Eragrostis tef* (Zucc.) Trotter], an ancient gluten free cereal, is processed into a whole flour and contains high fiber and minerals. This cereal is becoming popular among consumers in Western countries as it is increasingly considered as a healthy and nutritious food. In Ethiopia where tef is highly cultivated, this cereal is used to produce traditional food products mainly *injera* (a fermented flat bread) and thick porridge. Shumoy and Raes (2017a)) reported that the freshly prepared *injera* and porridge food products from different tef varieties exhibited a high GI in the range of 94–137 and 79–99, respectively. Furthermore, Wolter, Hager, Zannini, and Arendt (2013) showed a GI of 74 for a frozen conventional tef bread. The use of tef alone or mixed with wheat flour to prepare bread is becoming more and more popular among Western consumers. There is scarcity of information on GI of conventional tef bread. Tef is gluten free and the manufacture of bread without gluten causes major technological problems for bakers. Indeed, gluten-free breads available on the market are often of poor quality, showing low volume, poor colour and crumbling crumb and mostly with low protein and high fat contents (Segura & Rosell, 2011). However, it has been shown that sourdough could improve the sensory and physical qualities of gluten free breads, *i.e.* among others it can increase the specific volume and lower crumb hardness (Rinaldi, Paciulli, Caligiani, Scazzina, & Chiavaro, 2017). As tef contains high protein content with high digestibility (Shumoy, Pattyn, & Raes, 2018), it could be a good alternative to manufacture a high protein gluten free bread. However, literature regarding tef and the effect of sourdough on the resulting physical quality and starch digestibility of tef bread is scarce. In general, breads, be it at home or in supermarkets, could stay fresh for variable storage times, information pertaining to the freshness level, particularly of tef bread and associated GI is lacking. Therefore, this study was designed to investigate the effect of sourdough addition (10, 20 and 30%) and storage time (1, 2 and 5 days) and fresh breads as a control on *in vitro* starch digestibility and glycemic index of tef bread.

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2. Materials and methods

2.1. Bread production

In this study, flour of unknown varieties of mixed white and brown tef grains were used, as these are commercially available as such on the Ethiopian market. Tef grains of brown and white, 5 kg each, were purchased at a market in Mekelle, Ethiopia and carefully cleaned for impurities by sifting, sieving and winnowing. They were milled at a local disc attrition milling (Mekelle, Ethiopia), packed in polythene pouches, transported to Belgium and stored at −20 °C until further analysis.

Sourdough was prepared according to Lappi et al. (2010) using a commercial starter *Lactobacillus fermentum* (Florapan LA4K; kindly provided by Lallemand, France). Briefly, 1% LA4K starter (based on flour weight), tef flour and 62.6% water (based on dough weight) were mixed manually and fermented in a fermentation cabinet (30 °C, 85% relative humidity (RH)) for 19.5 h until the pH was 3.9–4.1. The titratable acidity was determined by potentiometric titration using 0.1 M NaOH to pH of 8.5 endpoint (Wolter, Hager, Zannini, & Arendt, 2014).

Tef bread in triplicates were baked as described in Hager, Wolter, Czerny et al. (2012) with slight modifications. Tef bread dough was prepared by mixing tef flour, sourdough in different proportions (0, 10, 20 and 30%), 3% yeast, 2% HPMC (hydroxypropylmethylcellulose), 2% salt, 2% sugar and 139% water, all based on dry matter flour weight. The dough was then immediately divided and put into baking pans and allowed to ferment or proof for 45 min in a fermentation cabinet (30 °C, 85% RH) followed by baking (190 °C, 45 min) in a preheated baking oven (MIWE condo, Arnstein, Germany). After cooling the breads for one hour, they were stored in a closed plastic bag and stored at ambient temperature for up to 5 days. Fresh bread (2h after baking) was used as a control. Fresh (2h after baking) white wheat bread was used as a reference material.

2.2. Physicochemical properties of tef flour

Flour particle size distribution was measured by a laser diffraction particle size distribution analyzer (Beckman coulter, LS 13 320 Series, USA) based on the instrument manual.

The falling number (FN) was determined according to AACC (1999) method No. 56-81b using 7 g flour sample and 25 ml distilled water.

The pasting property of tef flour was determined using a Rheometer MCR 102 (Anton Paar GmbH, Graz, Austria.) according to Hellemans et al. (2017) with slight modification, flour in water suspension 14% (2.8 g of flour in 20 ml of water). The pasting temperature (PTem), peak temperature (PeakT), peak time (PT), initial viscosity (IV), peak viscosity (PV), holding viscosity (HV), final viscosity (FV), breakdown (BD), setback (SV) were recorded. The viscosity was expressed in mPa.s.

Protein content of tef flour was determined by Kjeldahl method (AOAC, 1995) with 5.4 as nitrogen to protein conversion factor.

Apparent amylose content of tef flour was determined by using Megazyme kit K-AMYL and the amylose (%) was calculated according to:

$$\text{Amylose (\%)} = \frac{\text{Absorbance(Con A supernatant)}}{\text{Absorbance(total starch aliquot)}} \times \frac{6.15}{9.2} \times 100$$

where: 6.15 and 9.2 are dilution factors for Con A supernatant and total starch aliquot respectively.

2.3. Bread physical properties

Specific volume (SV) of the breads was measured using a 3D Volscan Profiler (Stable Micro Systems Volscan Profiler 600, UK) following instrument manual. The crumb texture (hardness, springiness, cohesiveness, chewiness and resilience) was measured using a texture analyzer (TA.XTplus, Stable Micro Systems) on uniform slices of 25-mm thickness according to Debonne et al. (2018).

2.4. Free glucose, and starch fractions of tef flour and bread

Free glucose (FG) was measured according to Englyst, Kingman, and Cummings (1992) using an assay kit GOPOD-format K-GLUC 09/14 (Megazyme International Ireland Ltd) and the glucose % was calculated as:

$$\%\text{glucose} = \frac{\text{At} \times \text{Vt} \times \text{C} \times \text{D}}{\text{As} \times \text{Wt}} \times 100$$

where:

At: absorbance of test solutions, **Vt**: total volume of test solutions (Vt = 25.2 plus 1 ml per gram wet weight of samples used), **C**: concentration (C = 0.394 mg glucose/ml) of standard, which may be corrected for moisture content, **D**: dilution factor = 18.

The TS, RDS, SDS and RS contents were determined according to Englyst et al. (1992) using an assay kit GOPOD-format K-GLUC 09/14 (Megazyme International Ireland Ltd) and were calculated as:

*TS = (TG - FG) × 0.9

**RDS = (G20−FG) × 0.9

**SDS = (G120−G20) × 0.9

**RS = TS - (SDS + RDS)

Values were expressed as g/100 g dm of (flour)* and (starch)**.

Where; G120: Glucose content after 120 min of digestion.

G20: Glucose content after 20 min of digestion.

TG: Total glucose.

0.9: Glucose to starch conversion factor.

2.5. In vitro glycemic index of tef bread

The rate of *in vitro* starch hydrolysis was analyzed following the method recommended by Goni, Garcia-Alonso and Saura-Calixto (1997). The area under the hydrolysis curve (AUC) was calculated using the equation:

$$\text{AUC} = C\infty(t\infty - to) - (C\infty/k)[1 - \exp[-k(t\infty - to)]]$$

where C∞ corresponds to the equilibrium percentage of starch hydrolyzed after 180 min, **t∞** is the final time (180 min), to is the initial time (0 min) and k is the kinetic constant.

The hydrolysis index (HI) was calculated as AUC of a sample as percentage of the corresponding AUC of fresh white bread (Goni et al., 1997; Granfeldt, Bjorck, Drews, & Tovar, 1992). The white bread used as reference had a dry matter content of 56 g/100 g and a total starch content of 68 g/100 g dm. Bread crumb, taken from the center of the bread was sampled. The estimated glycemic index (eGI) was calculated according to equations suggested by both:

Goni et al. (1997): $eGI_G = (0.549 \times HI) + 39.71$, and

Granfeldt et al. (1992): $eGI_{Gr} = (0.862 \times HI) + 8.198$

2.6. In vitro protein digestibility of tef flour and bread

The *in vitro* protein digestibility (IVPD) was analysed according to Hsu, Satterlee, and Miller (1977). The IVPD was calculated as:

$$\text{IVPD (\%)} = 65.66 + 18.1\Delta pH_{10\ min}$$

where $\Delta pH_{10\ min}$ is the pH difference of initial and after 10 min digestion in bread suspensions.

2.7. Statistical analysis

To assess differences among tef varieties and fermentation times, two-way analysis of variance (ANOVA) was performed. If ANOVA showed significant ($p < 0.05$) interaction between the main factors,