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Traditional fermentation of tef injera: Impact on *in vitro* iron and zinc dialysability

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ABSTRACT

Tef [*Eragrostis tef* (Zucc.) Trotter], an ancient cereal mainly produced in Ethiopia, is increasingly getting higher acceptance in the global market because it is gluten free and has high iron content. The aim of this study was to evaluate the *in vitro* dialysability of Fe and Zn in a backslop fermented gluten free flat bread known as injera. The traditional fermentation caused up to 49–66% reduction of phytic acid (PA). Molar ratios of PA:Fe and PA:Zn decreased from 14 to 1 and from 63 to 19, respectively, after 120 h of fermentation. The total soluble fractions of Fe and Zn ranged between 11 and 38% and between 11 and 29%, respectively, after 120 h of fermentation. The dialyzable Fe content of the white varieties ranged between 3 and 9% after 120 h fermentation while no effect was observed for the brown varieties. The dialyzable Zn ranged between 2 and 11%, with only a clear effect of fermentation in one white variety. Consumption of tef could be a good source of Fe and Zn, but may not provide the absolute recommended daily Fe and Zn intakes.

1. Introduction

Iron and zinc deficiencies are highly prevalent in the world, *i.e.* ranked 9th and 11th, respectively, in the list of the major risk factors for global burden of disease and they predominantly occur in developing countries (Lachat et al., 2006; Raes, Knockaert, Struijs, & Van Camp, 2014). In Ethiopia, an estimated prevalence risk of about 14% and 81% was reported for Fe and Zn deficiency, respectively. These values are among the highest on the African continent (Joy et al., 2014). Deficiency of Fe principally causes anemia and diseases of the immune system, whereas that of Zn causes growth retardation, impaired cognitive and immune system development (Humer & Schedle, 2016).

Increasing the efficiency of the release of minerals during gastrointestinal digestion (Raes et al., 2014) and fortifying food (Paganini, Uyoga, & Zimmermann, 2016) were suggested as potential strategies to improve the Fe and Zn status of individuals. However, due to the non-existence of governmental regulations to fortify major food sources in Ethiopia, dietary food remained as the sole source of Fe and Zn. Dialysability/bioavailability of Fe in animal-based food products ranges between 15 and 35%, while it is only about 10% in plant-based food products (Zimmermann, Chaouki, & Hurrell, 2005). Dialysability of non-heme Fe and Zn in plant-based food is mainly inhibited by phytic acid (PA), phenolic compounds and calcium (Humer & Schedle, 2016). Different and/or combinations of food processing techniques, *e.g.*, sprouting, malting, fermentation and heat treatment, have been reported as effective strategies for elimination and/or degradation of many of the mineral inhibitors (Humer & Schedle, 2016; Platel & Srinivasan, 2016; Raes et al., 2014).

In Ethiopia, consumption of plant-based food complemented with almost no animal-based food prevails due to the poor economic background and religious–inspired dietary habits. About 44% of the Ethiopian population are orthodox religion followers (CSA (Central Statistic Agency), 2007). This religion strongly prohibits consumption of any animal based food products for roughly 215 days of the year which forces majority of the population to be exclusively dependent on cereal grains and legume based foods. Tef [*Eragrostis tef* (Zucc.) Trotter], an ancient cereal mainly produced in Ethiopia, recently became a highly valued cereal. Nowadays, it is consumed in different forms of food, mainly as injera, porridge, gluten free cakes and breads. Injera, a fermented soft and porous pancake made of different cereals, preferably from tef, occupies the traditional Ethiopian food staple. Different reports indicate a wide range of Fe levels (5–150 mg/100 g

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dm) and a moderate range of Zn levels (2–4 mg/100 g dm) in tef (Abebe et al., 2007; Baye, Mouquet-Rivier, Icard-Verniere, Picq, & Guyot, 2014; Mamo & Parsons, 1987). Although tef could be a good source of Fe due to its unusually high Fe levels, the co-existence of high contents of inhibitors, *e.g.*, phytic acid, tannins and phenolic compounds, might impair its dialysability (Raes et al., 2014). The inhibitory effect of these anti-nutrients could even be exacerbated by consumption of tef as a whole grain.

Previous studies on the efficiency of traditional fermentation for improving the dialysability of Fe and Zn in tef injera were merely based on PA:mineral molar ratios (Abebe et al., 2007; Umeta, West, & Fufa, 2005; Urga & Narasimha, 1997). However, this molar ratio method was claimed to be not reliable for predicting physiological dialysability of minerals in both white and brown tef varieties, if other mineral-binding anti-nutrients are involved (Baye et al., 2014). White and brown tef varieties do not have different physical properties apart from their seed color difference, however, it has been repeatedly reported that brown tef varieties contained higher contents of Fe, phenolic compounds and condensed tannins (Bultosa, 2007; Shumoy & Raes, 2016). Information on the effect of the traditional fermentation on the reduction of inhibitory compounds and on the possible improvement of the dialysability of Fe and Zn in injeras made of pure white and brown tef varieties is limited. Hence, the objective of this study was the investigation of the effect of the Ethiopian traditional fermentation on the *in vitro* dialysability of Fe and Zn in tef injera using known tef varieties of brown and white colored seed coats.

2. Materials and methods

2.1. Chemicals and reagents

α-Amylase from porcine pancreas (Type VI-B, > 10 units/mg solid), pepsin from porcine gastric mucosa (3200–4500 units/mg protein), pancreatin from porcine pancreas (8xUSP, P7545), bile from porcine bile extract (P1001879903), dialysis membranes (MMCO 12,400 Da, 99.99% retention, width 32 mm, height 30 m, D0530-100 FT), gallic acid, catechin, Folin-Ciocalteue's reagent, 2,2-bipyridine, thioglycolic acid (TGA), phytic acid sodium salt and vanillin were purchased from Sigma-Aldrich (Belgium). Technical grade CH_3OH, $FeCl_3$, NaOH, HCl, KCl, NaCl, KH_2PO_4, $NaHCO_3$, $CaCl_2(H_2O)_2$, $MgCl_2(H_2O)_6$, NH_4Cl and HNO_3 were acquired from VWR Chemicals (VWR international, Leuven, Belgium). ICP multi-element standard solution IV was purchased from Inorganic Ventures, the Netherlands.

2.2. Samples and sample preparation

Four tef varieties, *i.e.*, Quncho (DZ-Cr-387), Tsedey (DZ-Cr-37), Zagurey (local) and Zezew (local), harvested in 2013, were obtained from Axum Agricultural Research Centre (Tigray, Ethiopia). They were manually cleaned and milled by disc attrition at a local tef miller in Ethiopia. The flours were then packed in plastic bags and brought to Ghent University (Belgium) and stored at − 20 °C until further analysis. The moisture contents of the flours were in the range of 7.9–8.4 g/100 g flour with no significant differences among the varieties ($p > 0.05$). The particle size distribution of the flour was measured using a test sieve shaker (Endecott, LTD, London SW, England), providing the following results: 100% < 850 μm, 99–100% < 425 μm, 96–99% < 300 μm, 78–85% < 212 μm and 66–77% < 150 μm.

Fermented tef injera was prepared following the procedure as shown in Fig. 1. The fermentation of tef dough was done microaerophilic under static conditions at 25 °C. Previously prepared (five days fermented) thin tef dough was used as a backslop to start the fermentation. The backslop fermentation was initiated naturally (without addition of starter culture). Injera from each tef variety was prepared from three independent replicate fermentations. Prior to mineral and phytic acid analysis, the injeras were oven-dried (105 °C) 24 h, until a constant moisture level was obtained (Abebe et al., 2007). Subsequently, the injeras were ground into fine flour using a porcelain

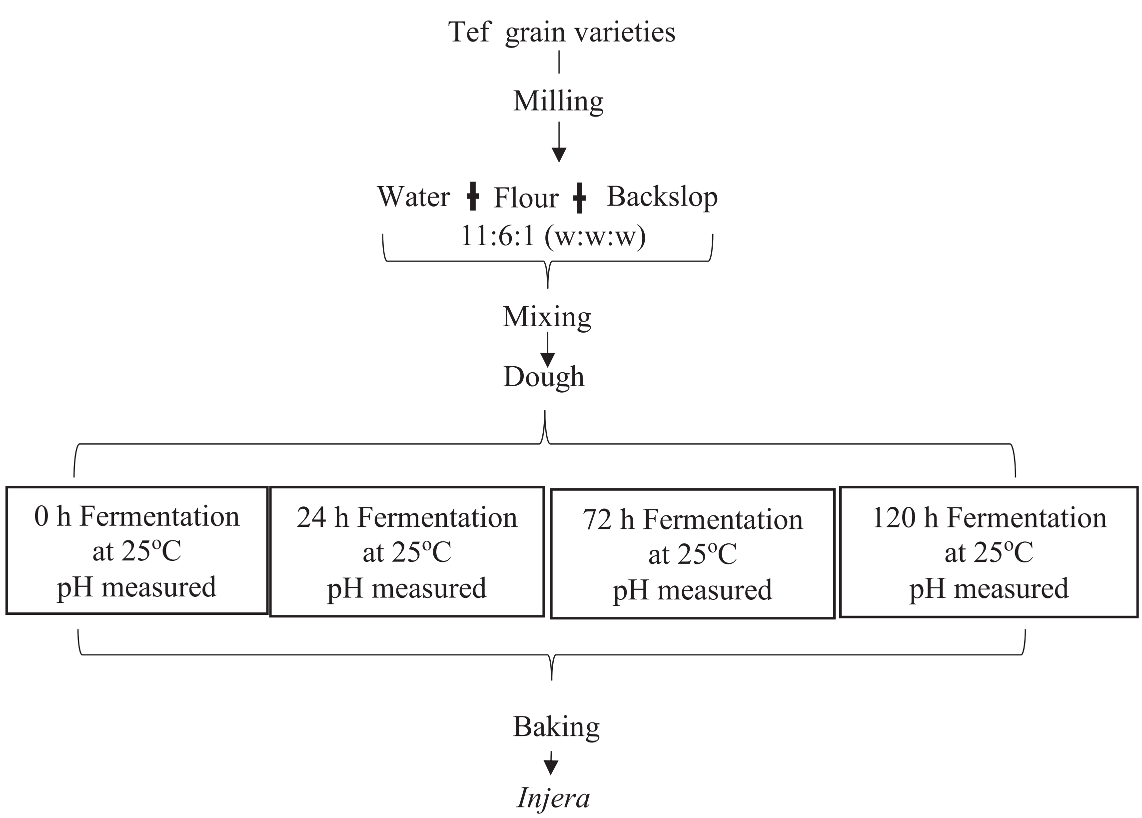

Fig. 1. Flowchart of tef injera preparation (Urga & Narasimha, 1997).