Food Chemistry 174 (2015) 60–67

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Enzymatic degradation of phytate, polyphenols and dietary fibers in Ethiopian *injera* flours: Effect on iron bioaccessibility

Kaleab Baye [a], Jean-Pierre Guyot [b], Christèle Icard-Vernière [b], Isabelle Rochette [b], Claire Mouquet-Rivier [b,*]

[a] Center for Food Science and Nutrition, Addis Ababa University, P.O. Box 150201, Addis Ababa, Ethiopia
[b] IRD UMR 204 Nutripass, IRD/UM1/UM2, BP 64501, 34394 Montpellier Cedex 5, France

ARTICLE INFO

Article history:
Received 22 August 2013
Received in revised form 1 August 2014
Accepted 3 November 2014
Available online 8 November 2014

Keywords:
Xylanase
Cellulase
Phytase
Polyphenol oxidase
Iron bioaccessibility
Teff
Sorghum
Wheat

ABSTRACT

The effect of removing phytate (IP6), iron-binding polyphenols, and dietary fibers on iron bioaccessibility in wheat-red sorghum (WrS) and teff-white sorghum (TwS) flour blends used in Ethiopia to make *injera*, a fermented pancake, was evaluated through the application of exogenous enzymes.

Phytase treatment led to >90% reduction in IP6 and to an IP6:Fe molar ratio <1, but iron bioaccessibility was not improved ($P > 0.05$). Phytase + xylanase + cellulase (P + X + C) treatment increased iron bioaccessibility in TwS (non-detectable to 1.6%) and WrS (1.9–3.2%), whereas phytase + polyphenol oxidase (P + PPO) treatment only showed improvement in the TwS blend. P + X + C + PPO treatment of the WrS blend increased the soluble non-dialysable iron fraction (6.7%) more than P + PPO treatment (3.9%).

Although responses to enzyme treatments and iron bioaccessibility were matrix dependent, a positive effect of dietary fiber hydrolysis with X + C was obtained, irrespective of the blend. Dietary fibers had a negative effect on iron bioaccessibility independent of phytates.

1. Introduction

Iron deficiency affects about two billion people worldwide, making it the most prevalent mineral deficiency (WHO, 2009). Although it has several causes, iron deficiency in developing countries has been associated with the predominantly plant-based nature of diets, which contain relatively low amounts of bioavailable iron (Hurrell & Egli, 2010).

The bioavailability of iron in plant-based foods is determined by their contents of absorption enhancers and inhibitors (Hurrell & Egli, 2010). Among absorption inhibitors, phytate and polyphenols have been found to be the most important determinant factors. However, the effect of fibers on iron bioavailability remains controversial as some authors have linked this inhibition with fiber-associated phytates and not with the fiber *per se* (Frölich, 1995). Part of the controversy may also be due to different types of fiber (i.e. fermentable vs poorly/non-fermentable).

Several food processing techniques, including fermentation and germination, have been shown to hydrolyse phytic acid and iron-binding phenolics (Kayodè et al., 2013; Matuschek, Towo, & Svanberg, 2001). Although dephytinisation alone has been shown to improve iron bioavailability, several studies showed that the effect was limited, especially in the presence of large amounts of polyphenols (Hurrell, Reddy, Juillerat, & Cook, 2003; Lestienne, Caporiccio, Besançon, Rochette, & Trèche, 2005).

In Ethiopia, the most commonly consumed food by children and adults alike is *injera*, a fermented pancake that can be prepared from different cereal blends including teff (*Eragrostis tef*), white or red sorghum (*Sorghum bicolor*), barley (*Hordeum vulgare*), and wheat (*Triticum aestivum*) (Baye, Guyot, Icard-Vernière, & Mouquet-Rivier, 2013). Fermentation of teff-white sorghum-based *injera* has been shown to cause only 28% degradation of phytate and no changes in iron-binding polyphenols (Baye, Mouquet-Rivier, Icard-Vernière, Rochette, & Guyot, 2013). In contrast, fermentation of wheat-red sorghum-based *injera* led to complete hydrolysis of phytate and partial removal of iron-binding phenolic compounds. However, the degradation did not improve iron in vitro bioaccessibility (Baye, Mouquet-Rivier, Icard-Vernière, Picq, & Guyot, 2014), suggesting that either more iron-binding phenolic compounds need to be removed or that other factors, such as fiber, were responsible for the low bioaccessibility of iron in vitro. Applying exogenous enzymes to disrupt the food matrix could be an effective way of assessing the relative effects of different iron absorption inhibitors, which would make it possible to better target the inhibitors and to increase their degradation (Lestienne et al., 2005; Matuschek et al., 2001; Wang, Cheng, Ou, Lin, & Liang, 2008). Although the effects of enzyme treatments on iron bioaccessibility have already been

* Corresponding author. Tel.: +33 467416295.
E-mail address: claire.mouquet@ird.fr (C. Mouquet-Rivier).

http://dx.doi.org/10.1016/j.foodchem.2014.11.012
0308-8146/

reported in previous studies, little or no information was provided on the extent of the degradation of the targeted absorption inhibitors. This is unfortunate, since iron bioaccessibility does not only depend on the type but also on the amount of absorption inhibitors that remain in the foods. In addition, the combined effect of enzymes that target fibers, phytate, and iron-binding phenolic compounds on iron bioaccessibility has not yet been assessed.

The main objective of this study was thus to evaluate to what extent iron can be released from the food matrix and become bioaccessible by reducing or eliminating major mineral absorption inhibitors. To this end, we evaluated the effects of several enzymatic treatments that targeted phytates, iron-binding polyphenols, and non-digestible carbohydrates.

2. Materials and methods

2.1. Materials

Wheat, red sorghum, teff, and white sorghum were purchased from local markets in North Wollo, Ethiopia. Two flour blends commonly used to make *injera*, wheat-red sorghum (WrS) and teff-white sorghum (TwS), were used in the present investigation (Baye, Guyot et al., 2013). Based on observations of traditional methods of preparation in North Wollo, Ethiopia, teff and white sorghum grains were mixed at a 1:1 ratio (w/w) to make TwS blends and wheat and red sorghum were mixed at a 4:1.5 ratio to make WrS blends. The blends were then milled in local community milling units that use mechanical mills. The flour blends used in this study came from a single batch and were sieved to pass through a 0.5 mm screen.

The enzymes used were mushroom tyrosinase/polyphenol oxidase (Sigma–Aldrich, Lyon, France EC 1.14.18.1, T7755, activity >1000 U/mg solid), xylanase from *Trichoderma viride* (Fluka, Sigma–Aldrich, Lyon, France EC 3.2.1.8, 95595; 12,000 μ/l), cellulase from *Trichoderma reesei* (Celluclast 1.5 L, Novozymes-Biologicals, Le Pecq, France, E.C. 3.2.1.4, activity 0.7 endo-glucanase unit (EGU)/mg), and phytase from *Aspergillus niger* (DSM, Basel, Switzerland, EC 3.1.3.8, 20 Phytase unit (PU)/mg).

2.2. Experimental design

We measured the bioaccessibility of iron in TwS and WrS flours treated with phytase (P), phytase + xylanase + cellulase (P + X + C), phytase + polyphenol oxidase (P + PPO), or phytase + xylanase + cellulase + polyphenol oxidase (P + X + C + PPO) (Fig. 1).

2.3. Enzymatic treatments

To prevent fermentation and to inactivate endogenous enzymes, the flours were dry heat sterilised at 190 °C for 6 min prior to enzyme treatment (Darmady, Hughes, Jones, Prince, & Tuke, 1961). After enzyme treatment, flours were lyophilised prior to in vitro bioaccessibility tests.

For treatment with phytase (P), cereal flours (6 g) were suspended in 0.1 mol/l acetate buffer (pH 5.6) in a 1:3 (w/v) flour:buffer mix. For every gram of flour, 0.009 g (180 PU) of phytase was added, and the mixture was incubated in a shaking water bath at 35 °C for 30 min. The length of the incubation period, the ratio of flour to buffer and the amount of phytase added was that shown in preliminary assays to allow complete degradation of phytate.

For treatment with xylanase + cellulase (X + C), 0.014 g (~50 U) of xylanase and 8 μl of cellulase (6.8 EGU) were added to the dephytinised flour, and the mixture was incubated in a shaking water bath at 35 °C for 3 h. The amount of cellulase to be added was based on the optimisation of Wang et al. (2008).

For treatment of dephytinised flour with polyphenol oxidase (PPO), NaOH was added until the pH reached pH 6.5, the optimum for PPO activity, and 0.1 mol/l MES (2-(*N*-morpholino) ethanesulphonic acid-M8250) buffer was added to achieve a final flour:buffer ratio of 1:10 (w/v). The proportion of enzyme added (1000 U) was based on the works of Matuschek et al. (2001). Incubation was carried in a shaking water bath at 35 °C for 16 h in the dark.

Treatment of flours with phytase, xylanase, cellulase and polyphenol oxidase (P + X + C + PPO) were performed sequentially: P, X + C, then PPO, as described above.

2.4. Dry matter (DM) content

DM contents were determined by oven drying at 105 °C to constant weight.

2.5. Iron determination

Total iron was analysed by atomic absorption spectrophotometry after extraction by wet mineralisation. About 0.4 g of the sample flour was placed in a closed-vessel microwave digestion system

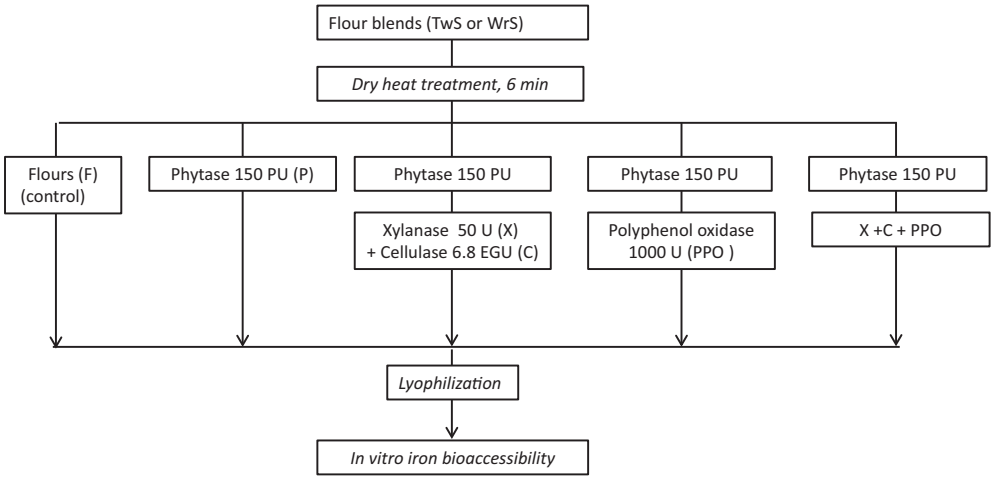

Fig. 1. Experimental design of enzymatic treatments (with concentrations) applied to teff-white Sorghum (TwS) and wheat-red Sorghum (WrS) blends used to prepare Ethiopian *injera*. Measurements of in vitro iron bioaccessibility were performed in quadruplicate.