Food Chemistry 138 (2013) 430–436

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Influence of flour blend composition on fermentation kinetics and phytate hydrolysis of sourdough used to make *injera*

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

[a] Center for Food Science and Nutrition, Addis Ababa University, P.O. Box 150201, Addis Ababa, Ethiopia
[b] IRD UMR 204 «Prévention des Malnutritions et des Pathologies Associées» (Nutripass), IRD/Montpellier2/Montpellier1, BP 64501, 34394 Montpellier Cedex 5, France

ARTICLE INFO

Article history:
Received 11 June 2012
Received in revised form 28 August 2012
Accepted 2 October 2012
Available online 10 November 2012

Keywords:
Teff
Sorghum
Wheat
Barley
Phytate
Phytase
Complementary food
α-Galactosides
Cereal fermentation

ABSTRACT

The influence of cereal blends, teff–white sorghum (TwS), barley–wheat (BW) and wheat–red sorghum (WrS), on fermentation kinetics during traditional fermentation of dough to prepare *injera*, an Ethiopian traditional fermented pancake, was investigated in samples collected in households. Barley malt was used with BW and WrS flours. WrS- and BW-*injera* sourdough fermentations were characterised by a transient accumulation of glucose and maltose and a two-step fermentation process: lactic acid fermentation and alcoholic fermentation with ethanol as the main end product. Only transient accumulation of glucose was observed in TwS-*injera,* and equimolar concentrations of lactic acid and ethanol were produced simultaneously. Final α-galactoside concentrations were low in all sourdoughs. Phytic acid (IP6) was completely hydrolyzed in WrS and BW-*injeras* probably due to the combined action of endogenous malt and microbial phytases. Only 28% IP6 hydrolysis was observed in TwS *injera*. Ways to improve IP6 hydrolysis in TwS-*injera* need to be investigated.

1. Introduction

In many developing countries, most foods consumed by young children are cereal based. Cereal based foods contain high amounts of phytic acid (*myo*-inositol 1,2,3,4,5,6 hexakis [dihydrogen phosphate]), which strongly bind minerals like iron and zinc (Lopez, Leenhardt, Coudray, & Rémésy, 2002). Large amounts of these nutrients are required during early life due to accelerated growth (Dallman, 1992), consequently ensuring their bioavailability is critical.

Several studies have documented the beneficial effect of fermentation in improving both the nutrient and sanitary qualities of foods (Nout, 2009; Svanberg & Lorri, 1997). Production of low molecular weight organic acids, such as lactic and acetic acid, reduces pH and may thus limit contamination by foodborne pathogens (Nout & Motarjemi, 1997). Furthermore, fermentation can activate several endogenous enzymes including phytases and may thus result in products with reduced antinutritional factors (Greiner & Konietzny, 2006). The extent to which enzymes like phytases are activated depends on the fermentation kinetics, which in turn, depends on the raw materials used (Hammes et al., 2005).

Several cereal based traditional fermented foods exist in Africa including *kenkey* in Ghana, *togwa* in Tanzania, *mawè* in Benin and *ben-saalga* in Burkina Faso (Guyot, 2010; Nout, 2009). For practical reasons, the fermentation kinetics of traditional fermented foods have usually been characterised based on sample fermentation reproduced in the lab, and may therefore not satisfactorily reproduce fermentation conditions in the field (Tou et al., 2006).

In Ethiopia, the most widely consumed food by young children and adults alike is *injera,* which is a thin, flat, traditional fermented pancake. However, depending on the agro-ecology of the area concerned (highlands versus lowlands), different cereal blends are used to make *injera*. In North Wollo, located in northern Ethiopia, barley–wheat blends (BW) and wheat–red sorghum blends (WrS) are commonly used in the highlands whereas a blend of teff (*Eragrostis tef*) and white sorghum (TwS) is used in the lowlands. Current blending practices may be instrumental for nutrition interventions to help promote food-to-food fortification.

Only few investigations have been made on the traditional fermentation of cereal blends used for the preparation of *injera* (Gedamu, 2008; Yetneberk, Rooney, & Taylor, 2005). These studies mostly focused on the influence of cereal blends on the processing quality and acceptability of *injera* (Yetneberk et al., 2005). To what

* Corresponding author. Tel.: +33 467416285.
E-mail address: jean-pierre.guyot@ird.fr (J.-P. Guyot).

0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2012.10.075

extent such blends influence the fermentation kinetics and the reduction of constituents with antinutritional effects such as IP6 and α-galactosides remains unknown. In this connection, the present study investigated the processing of *injera* made from different flour blends based on field observations. The influence of the blend of flour on fermentation kinetics and its possible implications in phytic acid (IP6) hydrolysis was investigated.

2. Materials and methods

2.1. Raw materials

Households ($n = 76$) in two villages in North Wollo, northern Ethiopia, one in the highlands (~3500 m above sea level – a.s.l.) and the other in the lowlands (~1500 m a.s.l.), were surveyed to determine the type of cereals and the most common blend proportions used in the preparation of *injera* flours. Accordingly, grains consumed in the lowland (teff and white sorghum), and those consumed in the highlands (barley, wheat and red sorghum), were purchased from local markets serving the two communities. Grains were purchased from the same batch in order to control variability due to grain varietal differences. The processing of the grains into BW- and WrS-flours in the highlands and TwS-flour in the lowlands was conducted by women in the respective villages. Two groups of women (five in the lowlands and six in the highlands) together cleaned the grains, by removing dirt and inedible parts. The grains were then sun dried followed by manual decortication and winnowing, with the exception of *teff* that was not decorticated. After these preliminary steps, the cereals were mixed at a 1:1 ratio (w/w) to make teff–white sorghum (TwS) and barley–wheat (BW) blends and at a 4:1.5 ratio to make wheat–red sorghum (WrS) blends and were then milled in local community milling units that uses mechanical mills.

The resulting TwS flour was subdivided into five equal parts and was distributed to five households to follow TwS *injera* sourdough fermentation. Likewise, BW and WrS *injera* sourdough fermentation were each followed in three households. The different households used the same flour but their own traditional starter culture (*ersho*) to trigger the fermentation.

2.2. Observations and sampling in households

To describe the different processing steps and characterise the fermentation of *injera*, the following measurements were made in five households ($n = 5$) for TwS-*injera* and three households ($n = 3$) for each WrS- and BW-*injeras*: the length of each step was monitored, the raw materials used (flour, water, barley malt and *ersho* starter) were weighed and pH measured. Samples were collected at different intervals during the fermentation of the dough used to make *injera* and were kept at −20 °C until further analysis. To avoid disturbing the households, samples were not collected during the night.

2.3. Dry matter (DM) content

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

2.4. Fermentation kinetics

2.4.1. Change in pH

During fermentation, the pH of the slurry was recorded using a WTW 340i pH meter (Fisher Bioblock Scientific, Illkirch, France). The rate of change in pH ($-\mathrm{dpH}/\mathrm{d}t$) was calculated for each household observation as follows: $-(\mathrm{dpH}/\mathrm{d}t) = \mathrm{pH}_{(t+1)} - \mathrm{pH}_{(t)}/(t_{+1}) - t$, where "$t$" stands for time (hours). The maximal value of $-(\mathrm{dpH}/\mathrm{d}t)$ for each household observation was then averaged to give the maximal rate of change in pH ($-(\mathrm{dpH}/\mathrm{d}t)_{\max}$).

2.4.2. Analysis of mono- and disaccharides and -galactosides

Mono- and disaccharides (glucose, fructose, maltose and sucrose) and α-galactosides (raffinose and stachyose) were extracted by diluting one gramme of fermented paste in 2 ml of milliQ water, the mixture was vortexed, then centrifuged at 4500*g* for 10 min at 4 °C. The supernatants were filtered through 0.20 μm pore size filters and were analysed by HPAEC (high performance anion-exchange chromatography) with a Dionex DX 500 apparatus connected to an amperometric detector Dionex Model ED 40 (Thermo Scientific, Courtaboeuf, France) using a Carbo PA1 column (Dionex S.A., Jouy en Josas, France) after appropriate dilution.

The following conditions were used: mobile phase (eluent) NaOH 90 mM, flow rate 1 ml/min, temperature 35 °C, injection sample extract 25 μl (Haydersah et al., 2012). Results are expressed in mmol/kg of dough.

2.4.3. Analysis of lactic and acetic acid, mannitol and ethanol

Lactic acid, acetic acid, and ethanol were analysed by HPLC using an Aminex HPX-87H, 300 × 7.8 mm column (Biorad, Yvry-sur-seine, France) connected to a refractive index detector (Model Waters 2410; Biorad, France) as previously described in Calderon, Loiseau, & Guyot (2003).

2.5. Analysis of phytate (IP6)

After extraction from 0.2 g of sample in acid solution (10 ml of HCl 0.5 M) at 100 °C for 6 min, IP6 content was determined by measuring myo-inositol hexaphosphate (IP6) content by HPAEC according to Lestienne, Icard-Vernière, Mouquet, Picq, and Trèche (2005), using an AS-11 pre-column and column kit (Dionex, Sunnyvale, USA).

2.6. Phytase activity in flours

Inorganic phosphorus and phytates were removed from flours by ion exchange chromatography as described in Konietzny, Greiner, and Jany (1994). The resulting phytate free supernatant was then incubated in 2.5 mM sodium phytate solution at pH 5.6 and 55 °C for 60 min, and liberated inorganic phosphate was determined using the spectrophotometric method described in Heinonen and Lahti (1981). Phytase activity was calculated as micromoles of inorganic phosphate liberated from sodium phytate per minute per gram (DM) of flour.

2.7. Statistical analyses

All values corresponding to the same type of *injera* (i.e., prepared from the same flour blend in different households) were averaged ($n = 5$ or $n = 3$ depending on *injera* type) and standard deviations are used to estimate the variation.

Data were submitted to analysis of variance (ANOVA), using the general model procedure of SPSS version 15. Statistical differences between means ($P < 0.05$) were tested by Duncan's multiple range test.

3. Results

3.1. Description of the processing of Injera

Injera preparation is a relatively lengthy process, mainly due to its extended fermentation period, which takes 2–3 days (Fig. 1).