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

Kaleab Baye a, b, Claire Mouquet-Riviere 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 

IRD UMR 204 “Prévention des Malnutritions et des Pathologies Associées” (Nutripass), IRD/Montpellier2/Montpellier1, BP 64501, 34394 Montpellier Cedex 5, France

A R T I C L E I N F O

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

A B S T R A C T

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.

© 2012 Elsevier Ltd. All rights reserved.

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 & Motarjem, 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 fermentations 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
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) altogether 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) flour 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 fermentations 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 BW- and WRs-injerias: 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 (−(dpH/dt)) was calculated for each household observation as follows: −(dpH/dt) = pH_{t+1} − pH_{t}/(t_{t+1} − t), where “t” stands for time (hours). The maximal value of −(dpH/dt) for each household observation was then averaged to give the maximal rate of change in pH (−(dpH/dt)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 4500g 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 (eluents) 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, Loisau, & 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 (2003), 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).
During household observations, the flours were first mixed with water and a small amount of barley malt (BM) was added in the preparation of BW- and WrS-injera (Fig. 1). Fermentation was triggered by inoculating the resulting dough with ersho, a backslop starter obtained from previous fermentations. Processing is practically the same irrespective of the cereals used, apart from the length of the dough fermentation step, which took up to 45 h for WrS- and BW-injera but only 33 h for TwS-injera.

3.2. Fermentation kinetics

3.2.1. Changes in pH

The kinetics of the decrease in pH followed the same pattern in the three types of injeras (Fig. 2). However, in that of TWs, the mean pH ± SD at the start of the fermentation was lower (5.4 ± 0.4) than that of BW (6.0 ± 0.4) and WRs (6.3 ± 0.4).

During the first 10 h of fermentation, pH decreased on average from 5.4 to 4.0 in TWs-injera, and from, respectively, 6.0 to 4.4 and 6.3 to 4.5 in BW and WRs-injera, with no time lag. The maximum rate of pH decrease was similar in the three fermentations but pH 4.5 was reached more rapidly in TWs-injera (Table 1). A second phase characterised by a slower rate of pH decrease that extended from 10 to 33 h in TWs and from 10 to 45 h in BW and WRs-injera was observed. The pH of the TWs-injera was lower throughout the fermentation period. The final pH of TWs-injera was 3.6, of WRs 4.0 and of BW-injera 3.9.

3.2.2. Kinetics of substrate consumption and product formation

At the beginning of the fermentation of BW and WRs-injera, the dominant sugar was maltose followed by glucose (Fig. 3A). The concentration of maltose at the start of fermentation was 33.1 mmol/kg in BW and 32.2 mmol/kg in WRs-injera. An increase in maltose concentration was observed in BW and WRs in the first 2 h of fermentation, after which it started to decrease to reach final values of 4.4 and 0.6 mmol/kg, respectively. The initial glucose concentration was around 20 mmol/kg in both blends and followed a similar pattern to that of maltose.

In TWs-injera, glucose was the main sugar followed by maltose (Fig. 3A). The glucose concentration increased sharply from an initial value of 36–67 mmol/kg in the first 6 h of fermentation. This was followed by a decrease to reach a final concentration of ~4 mmol/kg.
Sucrose and fructose concentrations were relatively low in all three injeras and decreased to trace concentrations during the first 10 h of fermentation.

Trends in the production of lactate, acetate, ethanol and mannitol were similar in WrS- and BW-injeras. The concentrations of lactic acid, acetic acid and mannitol increased and levelled off after 10 h of fermentation in BW- and WrS-injeras (Fig. 3B). Lactic and acetic acid accumulation was consistent with the drop in pH observed during this period. However, ethanol was the main end product and its final concentration was nearly twice that of lactic acid by the end of dough fermentation (Fig. 3B).

TwS-injera displayed a distinctive pattern, with ethanol and lactic acid produced at equimolar concentrations and according to the same trend throughout fermentation (Fig. 3B). Very low concentrations of acetic acid and mannitol were detected.

3.3. Changes in components with antinutritional effects

3.3.1. Changes in α-galactosides

The initial concentration of raffinose was the highest in BW-injera (1.2 mmol/kg) followed by WrS-injera (0.6 mmol/kg) and TwS-injera (0.2 mmol/kg). A large proportion of raffinose was degraded in all injeras during the first 10 h of fermentation (Fig. 4). Stachyose was detected as traces in WrS- and BW-injeras, whereas in TwS-injera, it was found at a similar concentration (0.24 mmol/kg) to raffinose, but fermented more slowly (Fig. 4).

3.3.2. Kinetics of IP6 degradation

The pattern of IP6 degradation during dough fermentation differed with the type of injera. In injeras made from TwS and WrS, IP6 degradation was already nearly complete after 10 h of fermentation. Whereas in injera made from TwS, only 28% of the IP6 was degraded, and degradation occurred at a very slow rate compared to the other types of injera (Fig. 4). It is worth noting that the level of IP6 at the beginning of fermentation was higher in TwS-injera than in the other two types. In BW- and WrS-injeras, degradation of IP6 mainly occurred between 2 and 6 h, corresponding to a pH of between 5.9 (at 2 h) and 4.9 (at 6 h), and in TwS-injera between 2 and 10 h, corresponding to pH of between 5.1 (at 2 h) and 4.0 (at 10 h).

3.4. Phytase activity

Significantly higher ($P < 0.05$) phytase activity was observed in BW- and WrS-flours than in TwS-flour (Table 2). Since barley malt was used to prepare BW- and WrS-injeras, phytase activity was also measured in this ingredient and in barley flour. Malted barley had higher ($P < 0.05$) phytase activity than raw barley. However, the effect of malting was highly variable as evidenced by the high standard deviations. Nevertheless, no significant difference was observed between barley malt and BW-injera flour.

4. Discussion

Due to agro-ecological conditions governing the cultivation of cereals in North Wollo (northern Ethiopia), injera is mainly made from barley and wheat in the highlands and from teff at lower altitudes. Different mixtures of these cereals with red or white sorghum are used in households. Barley malt is added to the wheat/barley or wheat/sorghum blends. A common characteristic among the different doughs is a rapid drop in pH likely due to backslipping (Nout, Rombouts, & Havelaar, 1989). The pH rapidly reached values below 4.5, promoting better hygienic conditions (Kingamkono, Sjogren, Sanverberg, & Kajiser, 1994; Nout et al., 1989). Nevertheless, in the three types of sourdough, maximum rates of pH decrease (−dpH/dt)max were nearly twice lower than those reported for the lactic acid fermentation of pearl millet (Songréné-Ouattara et al., 2009) and rice/soybean slurries (Nguyen, Guyot, Icard-Vernière, Rochette, & Loiseau, 2007).

Different fermentation patterns occurred depending on the cereal flour blend. Maltose and glucose were the main fermentable sugars in the BW- and WrS-injeras with transient accumulation of maltose. In TwS-injera, glucose was the main sugar that accumulated transiently and no transient accumulation of maltose was observed. In BW- and WrS-injeras, fermentation patterns suggest a two-step fermentation process: lactic acid fermentation and alcoholic fermentation, suggesting combined action of lactic acid bacteria (LAB) and yeasts. The production of mannitol during the lactic acid fermentation step of BW- and WrS-injeras strongly suggests the presence of heterolactic LAB due to their ability to use free fructose or fructose bound in sucrose as electron acceptors to produce mannitol (Calderon et al., 2003; Vrancken, Rimaux, De Vuyst, & Leroy, 2008; Wisselink, Weusthuis, Eggink, Hugenholz, & Grobben, 2002). The fact that lactic acid production stopped in the early stage of fermentation, whereas ethanol production continued until the end of fermentation, may be due to inhibition of LAB by ethanol and/or by efficient competition of yeasts for substrates. Indeed, like in traditional African brewing processes (Jepsersen,
the addition of barley malt early in the process most probably triggered the formation of maltose and its consumption by yeasts to produce ethanol. In an attempt to modify the traditional African process to produce energy-dense fermented pearl millet gruels, Tou et al. (2007) showed that addition of barley malt changed the fermentation pattern by inducing maltose accumulation. However, contrary to what was observed in BW- and WrS-injeras fermentation, lactic acid production remained higher than that of ethanol. Such differences between BW- and WrS-injeras and pearl millet fermentation may not only be explained by the difference in cereals used, but also by the fermentation conditions, i.e., solid state fermentation and a lower rate of pH decrease for the injera sourdough and submerged fermentation with a more rapid drop in pH in pearl millet slurries that could affect diffusion of the substrates and hence their accessibility and rate of consumption by LAB or yeasts.

The fermentation pattern of the TwS-injera dough was more conventional since only one fermentation step was identified, and was comparable with other cereal fermentations in the absence of malt (Blandino, Al-Aseeri, Pandiella, Cantero, & Webb, 2003; Guyot, 2012). Simultaneous and equimolar production of both lactic acid and ethanol is typical of heterolactic fermentation and suggest the dominance of heterofermentative LAB.

Regarding raffinose and stachyose, which can cause gastric distress, their removal during fermentation of injera dough is consis-
Phytase activities of flours used to prepare injera.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Phytase activity (PU/g DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>0.36 ± 0.01</td>
</tr>
<tr>
<td>Barley malt</td>
<td>0.59 ± 0.15</td>
</tr>
<tr>
<td>BW-flour</td>
<td>0.49 ± 0.01</td>
</tr>
<tr>
<td>WrS-flour</td>
<td>0.43 ± 0.07</td>
</tr>
<tr>
<td>TwS-flour</td>
<td>0.27 ± 0.00</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviations. Different superscripts represent statistically significant difference (P < 0.05).

The type of cereals and fermentation conditions, the efficiency of IP6 degradation can vary. Indeed, IP6 degradation was higher in BW and WrS-injeras than in pearl-millet fermentation (Tou et al., 2006), whereas in TwS-injera, phytate degradation was surprisingly low. Many factors are known to play a role in the rate and extent to which IP6 is degraded, among which the endogenous phytase activities of the raw materials and the processing conditions like pH, which is known to modulate the activities of both plant and microbial phytases (Greiner & Konietzny, 2006). The higher endogenous phytase activity of flour blends containing barley and wheat is consistent with the results of previous studies showing higher activities for these cereals (Egli, Davidson, Jiillerat, Barclay, & Hurrell, 2002; Reale et al., 2007) and IP6 degradation under the range of optimal pH values observed for barley and wheat phytases (Greiner & Konietzny, 2006). Moreover, the addition of malt in BW- and WrS-injeras possibly helps create a favourable environment for yeasts with phytase activities, indeed some strains of yeast species like Saccharomyces cerevisiae are known to display phytase activity (Vats & Banerjee, 2004). The low efficiency of IP6 degradation during TwS-fermentation may be due to the low endogenous phytase content of the grains and to poor microbial activity. Based on the results obtained in BW- and WrS-injeras, degradation of phytate in TwS-injera could be promoted by the addition of malt.

5. Conclusion

Differences in the composition of flour blends used to prepare injera influenced fermentation patterns and hence the final composition of the sourdough. More striking was the difference in IP6 degradation patterns. Given the nearly complete degradation of phytate in BW and WrS-injeras, mineral bioavailability in these injeras is less likely to be hampered by IP6. Whether such differences in IP6 hydrolysis will result in products with different mineral bioavailability is currently under investigation. On the other hand, particular attention will have to be paid to identifying methods to improve IP6 degradation in TwS-injera.

Acknowledgements

This research was made possible by the support provided by the IRD and the French Embassy in Ethiopia.

References


Figure 4. Changes in α-galactosides and IP6 during injera sourdough fermentation. TwS, teff–white sorghum; WrS, wheat–red sorghum; BW, barley–wheat. Error bars represent the standard deviation of means.

Figure 5. RI and the French Embassy in Ethiopia. Kassel University Press.


