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Fermentation and enzyme treatment of tannin sorghum gruels: effects on phenolic compounds, phytate and in vitro accessible iron

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Abstract

The presence of polyphenols and phytate in cereal products has been shown to interfere with the bioavailability of minerals such as iron. In the present study, we added enzymes (wheat phytase and mushroom polyphenol oxidase) during fermentation of tannin sorghum gruels prepared from flour with or without addition of 5% flour of germinated tannin-free sorghum grains (power flour), and investigated the effects on phenolic compounds, phytate and in vitro accessible iron. Assayable phenolic compounds were significantly reduced by fermentation, with high reductions observed in gruels with added enzymes. Fermentation of the gruels with addition of enzymes reduced (on average) total phenols by 57%, catechols by 59%, galloyls by 70% and resorcinols by 73%. The phytate content was significantly reduced by fermentation (39%), with an even greater effect after addition of power flour (72%). The largest reduction of phytate (88%) was, however, obtained after addition of phytase. The in vitro accessible iron was 1.0% in the sorghum flour and it increased after fermentation with power flour and/or with enzymes. The highest in vitro accessibility of iron (3.1%) was obtained when sorghum was fermented with addition of power flour and incubated with phytase and polyphenol oxidase after the fermentation process.

Keywords: Fermentation; Sorghum; Polyphenols; Condensed tannins; Phytate; In vitro iron accessibility; Polyphenol oxidase; Phytase

1. Introduction

Fermentation of foods involves the action of micro organisms or enzymes that cause desirable biochemical changes and significant modifications in flavour and texture. Lactic-acid fermentation of cereal products, at a household level, resulting in products such as *ogi* (Akingbala, Onochie, Adeyemi, & Oguntimein, 1978), *mahewu* (Bvochora, Reed, Read, & Zvauya, 1999), *uji* (Mbugua, Ledford, & Steinkraus, 1984) and *togwa* (Lorri & Svanberg, 1995), is practised widely in African countries. One advantage of lactic acid-fermented cereal-based gruels is that the growth of different enteropathogenic bacteria is inhibited, rendering the food safe under unhygienic environmental conditions (Kingamkono, Sjögren, Svanberg, & Kaijser, 1995; Nout, Rombouts, & Hautvast, 1989; Svanberg, Sjögren, Lorri, Svennerholm, & Kaijser, 1992). Other benefits include improvement of palatability and acceptability by changes in flavour and texture (Nout et al., 1989), as well as enrichment of nutrients by microbial synthesis (Antony & Chandra, 1998, 1999; Hassan & El Tinay, 1995; Kazanas & Fields, 1981; Svanberg, Lorri, & Sandberg, 1993). Fermentation may also result in reduction of antinutrients, such as phytate and polyphenols (Antony & Chandra, 1998; Reddy & Pierson, 1994).

In the arid and semi-arid areas of Tanzania, tannin varieties of sorghum are the main ingredients used to

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prepare fermented gruels. Traditionally, flour of germinated tannin-free sorghum (power flour) is also added in the fermentation process (Lorri & Svanberg, 1995). The presence of polyphenols and phytate in those foods has been shown to interfere with the availability of minerals such as iron (Svanberg et al., 1993). Low bioavailability of iron from vegetable diets is recognised to significantly contribute to iron deficiency anaemia, the main cause of anaemia in Tanzania (Tatala, Svanberg, & Mduma, 1998). Among phenolic compounds, galloyl and catechol groups are known to have an inhibitory effect on iron availability (Brune, Rossander, & Hallberg, 1989; Glahn, Wortley, South, & Miller, 2002; Matuschek & Svanberg, 2002; Matuschek, Towo, & Svanberg, 2001; Tuntawiroon et al., 1991). Several simple processing methods, such as decortication, soaking, cooking, germination and fermentation, can be used to reduce the amounts of antinutritional factors in those foods (Elmaki, Babiker, & El Tinay, 1999; Mahgoub & Elhag, 1998; Matuschek et al., 2001; Svanberg et al., 1993; Youssef, 1998). Lactic-acid fermentation has been shown to decrease the phenolic content (Hassan & El Tinay, 1995; Obizoba & Atii, 1991), as well as the phytate content (Mahgoub & Elhag, 1998; Svanberg et al., 1993) in sorghum. An extensive reduction (> 90%) of the phytate content in cereal products, that do not contain high amounts of polyphenols, has been shown to significantly improve the iron absorption, in humans, of such foods (Brune, Rossander-Hulthén, Hallberg, Gleerup, & Sandberg, 1992; Hurrell, Reddy, Juillerat, & Cook, 2003).

The use of polyphenol oxidase (PPO) to reduce the content of phenolic compounds in tannin sorghum has been successfully demonstrated (Matuschek et al., 2001). However, the effect of PPO during the lactic-acid fermentation process has not yet been studied. One interesting aspect of inclusion of PPO during lactic-acid fermentation, in combination with phytase to degrade the phytate content, is that the pH changes that occur during the fermentation process might affect the activity of the enzymes, and thereby the effect of the treatment. At the start of the fermentation process, the pH is between 5.0 and 6.0, but it usually drops to around, or below, 4.0 during the process. The optimal pH for wheat phytase is around 5.0 and the optimal pH for PPO is 6.5. In the present study, wheat phytase (EC 3.1.3.26) and mushroom PPO (tyrosinase: EC 1.18.14.1) were added prior to, or after, the fermentation process of tannin sorghum gruels. The effects of these treatments on phenolic compounds, phytate and in vitro accessible iron were then investigated.

2. Materials and methods

2.1. Materials

Mushroom tyrosinase (EC 1.18.14.1, T7755), wheat phytase (EC 3.1.3.26, P1259), pepsin (P6887), pancreatin (P1750), bile extract (B8631), (±)-catechin (C1788) and tannic acid (T0125) were purchased from Sigma–Aldrich, Stockholm, Sweden. Red sorghum (*Sorghum bicolor* L. Moench), locally called udo, was purchased at a local market in Dodoma, Tanzania. The grains were sorted and washed several times with tap water, followed by repeated rinsing in deionised water before use. Dried grains were milled in a disc-type mill (Laboratory mill 3300) to obtain a fine flour (80% passing a 250 μm sieve).

2.2. Soaking, boiling and fermentation

All treatments are illustrated in Fig. 1. Soaking was performed on flour from whole grains of tannin sorghum (U), with H_2O as soaking medium (1:10 w/v ratio), for 12 h. After drying and milling, the flour from the soaked sorghum was boiled for 10 min while stirring to avoid lumps (S + B). After boiling, the gruel was allowed to cool to 45–55 °C, and 5% (w/w) of germinated flour, *power flour*, was added to half of the material, in which liquefaction was allowed to continue for about 30 min. The power flour was prepared by germination of tannin-free sorghum for three days, followed by drying and milling into flour (Svanberg et al., 1993). The cooled sorghum gruels, with or without addition of power flour, were fermented at 30 °C (F) with addition of a local starter culture prepared according to the procedure described by Svanberg et al. (1992), and the fer-

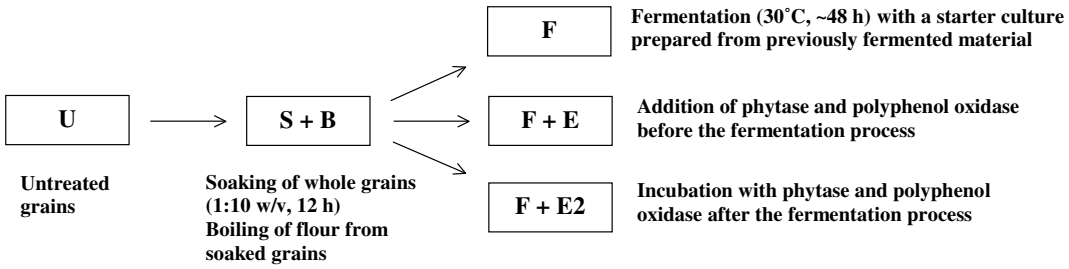

Fig. 1. Flow chart for the preparation of fermented sorghum udo gruels (with or without addition of 5% power flour after the boiling step).

mentation was terminated when the pH reached below 4.0. Fermentation was also carried out with addition of PPO (mushroom tyrosinase, 1000 U/g flour) and wheat phytase (2.0 U/g flour) (F + E). Incubation with enzymes was also performed after the fermentation process (F + E2) according to the following procedure: the pH was raised to 4.8 with NaOH before addition of phytase and incubation at 55 °C for 8 h. After cooling to room temperature, the pH was adjusted to 6.5 with NaOH and PPO was added, followed by incubation at 30 °C for 16 h. All samples were freeze-dried before analysis and all calculations were done on a dry-weight basis.

2.3. Phytate content

A 0.5 g sample of flour was extracted with 10 ml of 0.5 M HCl during stirring for 3 h, followed by centrifugation at 2100*g* for 10 min and filtration. The clear supernatant was analyzed for total phytate content, with HPIC, as described by Carlsson, Bergman, Skoglund, Hasselblad, and Sandberg (2001).

2.4. Total iron content

The total amounts of iron in the untreated flour and in the freeze-dried processed samples were determined by wet acid digestion in an Ethos Plus microwave system (Milestone, Bergamo, Italy). To a weighed sample of 0.3 g were added 3 ml of H_2O, 0.15 ml of 37% HCl and 0.75 ml of 65% HNO_3, which was followed by digestion with a temperature programme reaching 180 °C for 15 min and holding that temperature in 20 min. After cooling to room temperature the samples were transferred to test tubes and diluted to 10 ml with H_2O. The following day, 0.1 ml of ascorbic acid solution (20 g/l) was added to 0.9 ml of sample and this solution was analyzed for total iron with HPIC, as described by Fredrikson, Carlsson, Almgren, and Sandberg (2002).

2.5. In vitro accessible iron

In vitro iron accessibility was measured as iron solubility under physiological conditions according to the method of Svanberg et al. (1993) with some minor modifications. A 0.5 g sample of flour was suspended in 5 ml H_2O, followed by addition of 5 ml of a pepsin solution (0.3% pepsin in 0.1 M HCl). In addition to pepsin, the solution also contained physiological amounts of Na (49 mM as NaCl), K (12 mM as KCl), Ca (10 mM as $CaCl_2 \cdot 2H_2O$), Mg (2.4 mM as $MgCl_2 \cdot 6H_2O$) and phosphate (3.5 mM as KH_2PO_4) (Diem & Lentner, 1975). The pH was adjusted to 2.0 with NaOH and the mixture was incubated in a shaking water bath at 37 °C for 90 min. After incubation, 1.5 ml of a pancreatin and bile solution (6 mg of pancreatin and 37.5 mg of bile extract in 0.1 M $NaHCO_3$) were added. The pH was adjusted to 5.0 with NaOH and the mixture was incubated for an additional 30 min. After adjustment of pH to 6.0 with NaOH, the mixture was centrifuged at 4300*g* for 20 min and the supernatant was filtered through a 45 μm filter. The pH in the filtrate was lowered by addition of 0.1 ml of HCl (0.5 M) to 0.8 ml sample, followed by addition of 0.1 ml of ascorbic acid solution (20 g/l). After 10 min, the samples were centrifuged (10,000*g*, 4 min) and the clear solution obtained was analyzed for soluble iron, including free soluble complexes of iron, with HPIC, as described by Fredrikson et al. (2002). The amount of soluble iron in the filtrate was expressed as a percentage of the total amount of iron in the sample.

2.6. Phenolic compounds

The extractions and measurements of phenolic compounds were performed at 25 °C. The amounts of phenolic compounds reported in this paper refer to amounts that were extractable and assayable. The total phenol content was determined by the Prussian blue method (Price & Butler, 1977), which is based on the reducing power of phenolic hydroxyl groups and thereby detects all phenolic compounds. The amount of resorcinol phenolic groups was determined by the modified vanillin method (Price, van Scoyoc, & Butler, 1978), which specifically measures phenolic compounds with a *meta*-dihydroxyl (resorcinol) group, eg flavonoid structures, including condensed tannins. For total phenols and resorcinol groups, acidified methanol (1% HCl (37%) in methanol) was used as extraction solvent, and (±)-catechin was used as standard. The amount of iron-binding phenolic groups, i.e., catechol (*ortho*-dihydroxyl) and galloyl (trihydroxyl) groups, were determined by the modified ferric ammonium sulphate (FAS) method (Brune, Hallberg, & Skanberg, 1991) using 50% dimethylformamide in acetate buffer (pH 4.4) as extraction solvent. The modified FAS method is based on the ability of catechol and galloyl groups to form iron complexes with different absorbance maxima. By measuring the absorption at 578 and 680 nm, the contributions of catechol and galloyl groups, respectively, can be calculated against standard curves of (±)-catechin and tannic acid, respectively, at both wavelengths. All extractions were carried out according to the following procedure: an amount of 100 mg of cereal flour was mixed with 5 ml extraction solvent and extracted for 1 h with shaking every 10 min. This was followed by centrifugation at 4300*g* for 10 min. The clear supernatant was collected and the extraction repeated once. The supernatants from the two extractions were pooled and analyzed spectrophotometrically by the methods mentioned above. Total phenols, catechols and resorcinols were expressed as mg catechin equivalents

(CE)/g and galloyls are expressed as mg tannic acid equivalents (TAE)/g.

2.7. Statistical analyses

Differences in mean values of duplicate samples ($p < 0.05$) of total iron content, in vitro accessible iron, phenolic compounds and phytate were tested by analysis of variance (ANOVA) and significance levels were obtained with Tukey's HSD multiple range test (Wilkinson, 1990). Simple regression analysis was performed on in vitro accessible iron and total phenols, catechols, galloyls and resorcinols, respectively.

3. Results and discussion

3.1. Fermentation acidification

The pH values in the sorghum gruels after addition of the local starter culture ranged from 5.31 to 5.40. Addition of power flour or enzymes (phytase and PPO) did not affect the initial pH values, and the pH was similar in all samples after fermentation (~3.75). Product acidification is usually caused by lactic-acid bacteria, and it is a general characteristic of the fermentation process (Bvochora et al., 1999; Nout et al., 1989). Inoculation with a recycled culture from a previous fermented product has been shown to accelerate and stabilise the acidification, resulting in a final pH between 3.6 and 4.0 (Antony & Chandra, 1999; Nout et al., 1989), which was also obtained in the present study.

3.2. Effect of fermentation on the phenolic content

The amount of total assayable phenols was lower ($p < 0.05$) after soaking and boiling (~18.0 mg CE/g) compared with the untreated sorghum flour (~26.4 mg CE/g), see Table 1. Fermentation of sorghum gruels (F), with or without power flour, significantly ($p < 0.05$) reduced the amount of total phenols to about 13.8 mg CE/g (Table 1). A reduction was also observed for the different phenolic groups, namely, catechol (*ortho*-dihydroxyl), galloyl (trihydroxyl), and resorcinol (*meta*-dihydroxyl) groups. Compared with the untreated sorghum flour, fermentation without and with addition of power flour reduced the catechol groups by 32% and 43%, respectively. Galloyl phenolic groups were affected in a similar manner to catechols, but the initial amounts were lower, 6.1 mg TAE/g versus 14.1 mg CE/g (Table 1). Fermentation of sorghum gruels reduced the amount of galloyl groups to 3.2 mg TAE/g, and the decreasing effect was enhanced by addition of power flour (2.58 mg TAE/g). Resorcinol groups also decreased with fermentation, but addition of power flour resulted in slightly higher amounts (Table 1). The amounts of galloyl groups found in the present study were similar to amounts reported by Brune et al. (1989) and Towo, Svanberg, and Ndossi (2003). Hydrolysable tannins, such as tannic acid, are not present in sorghum grains, but galloyl groups may nevertheless be present in simple phenolic structures. Condensed tannins containing galloyl groups (prodelphinidins) have, furthermore, been found in cereals and legumes (Brandon, Foo, Porter, & Meredith, 1982; Helsper, Kolodziej, Hoogendijk, & van Norel, 1993). However, the galloyl–iron complexes formed in the present study had low absorbance, which might result in an overestimation of these structures when assayed by the FAS method.

A reduction of phenolic compounds during lactic-acid fermentation of cereals has previously been shown by other authors (Antony & Chandra, 1998). It has been suggested that PPO activity, either from the cereal or the micro flora, contributes to some extent (Dhankher &

Table 1
Effect of different treatments on total phenols and different phenolic groups in (a) sorghum udo and (b) sorghum udo + 5% power flour[a]

	Total phenols (mg CE/g)	Phenolic groups		
		Catechols (mg CE/g)	Galloyls (mg TAE/g)	Resorcinols (mg CE/g)
(a)				
U	26.6 ± 0.27^{A}	14.1 ± 0.16^{A}	6.10 ± 0.29^{A}	13.9 ± 0.54^{A}
S + B	17.7 ± 0.41^{B}	12.4 ± 0.17^{B}	4.33 ± 0.45^{B}	6.13 ± 0.24^{B}
F	13.8 ± 0.03^{C}	9.60 ± 0.47^{C}	3.20 ± 0.05^{C}	5.15 ± 0.02^{B}
F + E	12.6 ± 0.65^{D}	6.63 ± 0.10^{D}	2.32 ± 0.04^{D}	3.77 ± 0.17^{C}
F + E2	9.9 ± 0.44^{E}	5.37 ± 0.32^{E}	1.69 ± 0.01^{E}	2.60 ± 0.01^{D}
(b)				
U	26.1 ± 0.30^{A}	13.7 ± 0.42^{A}	5.55 ± 0.49^{A}	13.7 ± 0.19^{A}
S + B	18.3 ± 0.99^{B}	11.4 ± 0.55^{B}	3.89 ± 0.11^{B}	7.17 ± 0.16^{B}
F	13.7 ± 0.29^{C}	7.85 ± 0.01^{C}	2.58 ± 0.14^{C}	6.26 ± 0.01^{C}
F + E	12.7 ± 0.66^{D}	5.73 ± 0.42^{D}	1.83 ± 0.15^{D}	3.23 ± 0.01^{D}
F + E2	10.7 ± 0.54^{E}	4.78 ± 0.21^{E}	1.12 ± 0.07^{D}	2.85 ± 0.23^{E}

[a] Mean values ± SD of two replicates analyzed in duplicate are shown. Values within the same column followed by different letters are significantly different ($p < 0.05$). For abbreviations, see Fig. 1.

Chauhan, 1987). The decrease in phenolic compounds during fermentation could also be due to the acidic environment that may result in abstraction of hydride ions and rearrangement of the phenolic structures (Chen, Zhu, Tsang, & Huang, 2001; Porter, Hrstich, & Chan, 1986). Acidic treatment, has furthermore, been shown to cause cleavage of proanthocyanidins into flavan-3-ols, which thereafter are oxidised to quinones (Chen et al., 2001; Kennedy, Munro, Powell, Porter, & Foo, 1984; Porter et al., 1986). It is, however, also possible that fermentation, or simply the addition of water, results in a reduced extractability of the phenolic compounds, which may be due to self polymerisation and/or interaction with macromolecules such as proteins (Beta, Rooney, Marovatsanga, & Taylor, 2000).

3.3. Effect of enzyme incubation on the phenolic content

Addition of phytase and PPO before fermentation (F + E) resulted in a further significant reduction of total assayable phenols to about 12.7 mg CE/g. Furthermore, there was an even more pronounced decreasing effect on catechol, galloyl, and resorcinol groups, respectively, after inclusion of enzymes (Table 1). The optimal pH for the PPO used in the present study is 6.5, but addition of the local starter culture reduced the pH to about 5.4 in the sorghum gruels. Enzyme incubation was therefore also performed after the fermentation process, with pH values and temperatures optimal for both phytase and PPO. This incubation (F + E2) resulted in lower amounts of total phenols, catechols, galloyls and resorcinols, respectively, compared with when the enzyme was present during the fermentation (F + E) (Table 1). The total content of assayable phenols decreased by 63%, and the amounts of catechol, galloyl, and resorcinol groups by 62%, 72%, and 81%, respectively. For catechol and galloyl groups, there was a larger effect when power flour also was present during the fermentation period.

3.4. Effect on the phytate content

The phytate content in the sorghum flour, about 12.1 μmol/g, was significantly ($p < 0.05$) reduced in all processed samples (Fig. 2), eg soaking and boiling (9.3 μmol/g) and fermentation (7.4 μmol/g). Addition of power flour, followed by fermentation, resulted in amounts that were markedly lower (3.4 μmol/g) than in the samples without power flour. A decreasing effect of lactic-acid fermentation on the phytate content in sorghum has been demonstrated earlier (Svanberg et al., 1993). Both cereal and microbial phytases can contribute to a reduction in phytate during the fermentation process (Dhankher & Chauhan, 1987; Reddy & Pierson, 1994). That addition of power flour contributed to a further decrease in phytate (Fig. 2) is probably due to the

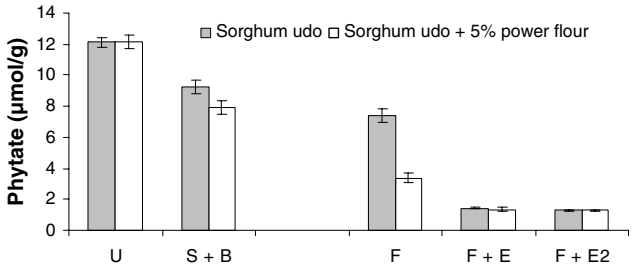

Fig. 2. Changes in phytate content with different treatments of sorghum udo, with or without addition of 5% power flour. Mean values ± SD of two replicates analyzed in duplicate are shown.

phytase activity of the germinated seeds (Bartnik & Szafranska, 1987; Greiner, Jany, & Alminger, 2000).

Effective phytate hydrolysis in cereals can also be achieved by the use of exogenous phytase (Hurrell et al., 2003; Matuschek et al., 2001; Sandberg & Svanberg, 1991; Türk & Sandberg, 1992). In the present study, addition of wheat phytase before fermentation (F + E) resulted in a significantly lower phytate content than with fermentation alone (F) (Fig. 2). There was, however, no further reduction in the phytate content when the gruel that was incubated with enzymes after the fermentation process (F + E2). Cereal phytases often have pH optima around 5.0 (Greiner et al., 2000; Sandberg & Svanberg, 1991), and both the wheat phytase added and the phytase in the power flour will therefore have a high activity during the fermentation process. It can also be concluded that raising the temperature to 55 °C, which is optimal for the added wheat phytase, did not have any further effect on the phytate hydrolysis, making this enzyme suitable for use during the normal lactic-acid fermentation processes.

3.5. Effects on iron content and in vitro accessible iron

The total iron content in the untreated flour and freeze-dried processed samples ranged from 62.0 to 83.6 mg/kg. The variation in iron content between the samples is most likely due to contamination iron in the sorghum grains that were obtained at a local market in Tanzania (Tatala et al., 1998). However, processing of the gruel samples had no significant effect ($p > 0.05$) on the iron content (data not shown).

The in vitro accessible iron was 1.0% in the sorghum flour, and it did not change after preparation to a gruel or fermentation of the gruel. In the sorghum gruels fermented with addition of enzymes but without power flour, the in vitro accessible iron increased to 2.0% (Fig. 3). With addition of power flour in the sorghum gruels, the in vitro accessible iron significantly increased to 1.9% ($p < 0.05$), even after fermentation without enzymes, and slightly more, to 2.4%, after fermentation with enzymes (Fig. 3). Addition of enzymes, after the

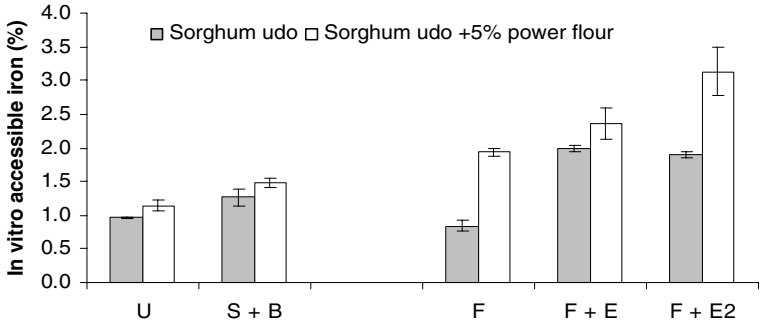

Fig. 3. Effect of different treatments on in vitro accessible iron in sorghum udo with or without addition of 5% power flour. Mean values ± SD of two replicates analyzed in duplicate are shown.

fermentation process in the gruel with added power flour resulted in the highest in vitro iron accessibility (3.1%). The in vitro accessible iron was significantly higher after all treatments in the gruels containing power flour compared with the gruels containing only sorghum udo ($p < 0.01$).

A high content of phytate and polyphenolic compounds contributes strongly to a low availability of iron (Brune et al., 1989; Glahn et al., 2002; Matuschek et al., 2001), and the sorghum variety used in the present study contained large amounts of those antinutrients. The percentage in vitro iron accessibility from the tannin sorghum obtained in this study (1.0%) corresponds with 0.94% absorption from tannin sorghum in a human study conducted by Hurrell et al. (2003). We have shown, in earlier studies, that degradation of phytate in tannin sorghum did not significantly increase in vitro iron availability (Matuschek et al., 2001), whereas it had an increasing effect in tannin-free sorghum (Svanberg et al., 1993). Similarly, Hurrell et al. (2003) showed that iron absorption in humans was improved after dephytinisation of tannin-free sorghum gruels but not in tannin sorghum gruels, which appeared to be due to the strong inhibitory effect of polyphenolic compounds. In the present study, we also investigated the effect of reduction of the phenolic content without reduction of phytate. Incubation with PPO alone did not increase the in vitro accessibility of iron in the sorghum gruels (<1.0% accessible iron before and after incubation). However, sufficient reduction of both phytate and phenolic compounds in tannin sorghum slurries has been shown to increase the accessibility of iron in vitro (Matuschek et al., 2001). The treatments resulting in the lowest levels of phytate and phenolic compounds in the present study, i.e., samples that had been incubated with enzymes, also had the highest in vitro accessibility of iron. The phytate content was equally reduced when the enzyme incubation was performed during or after the fermentation process, while oxidation of iron-binding phenolic compounds was higher when the enzyme incubation was performed after the fermentation process. The in vitro accessibility of iron was also significantly higher in those samples (Fig. 3).

The affinity for iron differs between the different phenolic groups, and catechol and galloyl groups are considered to be the ones responsible for the iron-binding capacity of polyphenolic compounds (Brune et al., 1989; Jovanovic, Simic, Steenken, & Hara, 1998). Catechol groups are, however, believed to be more common in cereal foods (Santos-Buelga & Scalbert, 2000). Catechol and galloyl groups, respectively, were shown to have a higher correlation ($r = -0.78$) with in vitro accessible iron than total phenols and resorcinol groups, respectively ($r = -0.59$). In addition to terminal phenolic groups, the type and size of the compounds formed during processing will also affect the availability of iron. Tabera, Frias, Estrella, Villa, and Vidal-Valverde (1995) showed that natural fermentation of lentils resulted in a lower tannin/catechin ratio, which indicates a lower degree of polymerisation of the phenolic compounds in the fermented product. This may have a positive effect on the iron availability, since small and simple phenolic compounds generally form soluble iron complexes. It is also possible that lactic acid, or other organic acids, formed during the fermentation process, have an effect on the availability of iron. There are, however, studies that show both positive and negative effects of lactic acid on iron availability (Derman et al., 1980; Salovaara, Sandberg, & Andlid, 2002).

4. Conclusions

Fermentation can reduce the amount of phytate and phenolic compounds and increase the in vitro accessible iron in tannin sorghum gruels. The effect is enhanced by incubation with phytase and PPO during, or after, the fermentation process, especially in combination with a small amount of flour from germinated seeds, power flour. Fermented and germinated foods are already extensively consumed in Tanzania, which may offer an opportunity to stimulate a more widespread consump-

tion of fermented foods with an improved iron nutritional value.

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