Oxidation of Polyphenols in Phytate-Reduced High-Tannin Cereals: Effect on Different Phenolic Groups and on in Vitro Accessible Iron

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After reduction of phytate with phytase, water slurries of two high-tannin cereal flours were incubated with polyphenol oxidase (mushroom tyrosinase), and the effects on different phenolic groups and on in vitro accessible iron were studied. Enzyme incubation was also performed after cooking, soaking, and germination of the cereals. Phytase incubation significantly decreased the phytate content, and incubation with polyphenol oxidase had a reducing effect on the total phenol content, as well as on the amount of catechol and resorcinol groups. The in vitro accessible iron increased when the cereals were incubated with phytase and polyphenol oxidase, and the highest accessibility of iron was obtained when the germinated samples were incubated. The results from this study imply that oxidation of polyphenols in high-tannin cereals, after reduction of phytate, may be used to increase the bioavailability of iron in foods prepared from these cereals.

Keywords: *Sorghum; finger millet; polyphenols; condensed tannins; tyrosinase; polyphenol oxidase; in vitro iron accessibility; iron availability*

INTRODUCTION

Sorghum and millets are commonly grown for human consumption in Tanzania and in many other arid and semi-arid areas of the world. They are preferred to other cereals primarily because of their resistance to diseases and predators, as well as their tolerance to moisture stress and low soil fertility. For a large proportion of the people in these regions these grains are the principal source of energy and nutrients such as protein, vitamins, and minerals (*1*). Red and brown varieties contain high amounts of condensed tannins, which contribute to the agronomic advantages, but, on the other hand, have negative effects on the nutritional value (*2*−*4*). Of the millet varieties, finger millet is suggested to be the only one containing relatively high amounts of condensed tannins (*5*, *6*). Tannins are known to form insoluble complexes with iron(III) (*7*−*9*) rendering it unavailable for absorption in the gastrointestinal tract (*10*), and the inhibitory effect of sorghum tannins on iron absorption has been shown in a human study (*11*). The complex formation between condensed tannins and iron(III) is probably due to the presence of *ortho*-dihydroxyl (catechol) groups in the phenolic structure (*8*, *9*, *12*). Low availability of iron in the diet, due to inhibitors such as tannins and phytate, is one of the key factors that contribute to the high prevalence of iron deficiency anemia in developing countries (*13*, *14*).

Traditional processing methods such as cooking, soaking, and germination can affect the nutritional value of different foods. Several studies have shown that soaking and germination of high-tannin sorghum can decrease the tannin (*15*−*18*) and the phytate content (*19*, *20*). Germination of finger millet has also been shown to reduce the content of phytate and tannins, with an increased availability of iron as a result (*21*−*23*). The tannin content in high-tannin sorghum was furthermore shown to decrease with cooking (*24*) and steam treatment (*25*).

The effect of different processing methods on cereal tannins has, so far, mostly been studied on the total tannin or phenol content. There are, however, methods available that measure the amounts of different phenolic groups found in food samples. The ferric ammonium sulfate (FAS) method, for example, differentiates between *ortho*-dihydroxyl (catechol) and trihydroxyl (galloyl) groups by the formation of iron complexes with absorbance maxima at two different wavelengths (*12*). The modified vanillin method is, on the other hand, specific for a narrow range of flavanols and dihydrochalcones and it is necessary that a free *meta*-oriented dihydroxyl (resorcinol) group is present to get a positive reaction (*30*−*32*). The total phenol content can be determined using the Prussian blue method, which is based on the reduction of ferric to ferrous iron. This method detects any phenolic hydroxyl group present (*33*). By using a combination of these three methods it may be possible to estimate the contribution of different phenolic groups in food samples and to study how they are affected by different treatments.

The aim of this study was to examine how incubation of two high-tannin cereals with phytase and polyphenol oxidase (tyrosinase) affects the in vitro accessible iron, the total phenol content, the amount of catechol respective resorcinol oriented phenolic groups, as well as the phytate content. The enzyme treatment was performed on untreated, cooked, soaked, and germinated samples of a red variety of sorghum and finger millet.

MATERIALS AND METHODS

Materials. Mushroom tyrosinase (EC 1.18.14.1, T7755), wheat phytase (EC 3.1.3.26, P1259), pepsin (P6887), pancre-

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atin (P1750), bile extract (B8631) and (±)-catechin (C1788) were purchased from Sigma, Sweden. Red sorghum (*Sorghum bicolor* L. Moench), locally called udo, and a red variety of finger millet (*Eleusine coracana*) were purchased at a local market in Dodoma, Tanzania. The grains were sorted and washed several times with tap water, followed by repeated rinsing in deionized water before use.

Soaking and Germination. Soaking was performed on whole grains with H_2O as the soaking medium (1:10 w/v ratio) for 24 h. This was followed by draining of the soaking media and a rinsing step. A portion of the soaked grains was freeze-dried and the remaining part was germinated. Germination was carried out by spreading the soaked grains onto a bloating wet paper that was placed in a dark place at a temperature of 25 °C. The bloating material was kept wet throughout the germination time by spraying H_2O twice a day. The germination was terminated after 72 h by freeze-drying the grains. After freeze-drying the grains were milled in a disc-type mill (Laboratory Mill 3300) to obtain a fine flour (80% passing a 250 μm sieve). All analyses on germinated sorghum udo were performed after removal of the roots and shoots, unless otherwise is stated. The roots and shoots were included in all analyses made on germinated finger millet.

Cooking. Cooking was performed by addition of cereal flour to boiling H_2O, followed by cooking for 5 min to get a thick porridge. The porridge was freeze-dried and ground in a mortar before further treatment and analysis.

Enzyme Incubation. A 1-g sample of cereal flour was suspended in 10 mL of H_2O and the pH was adjusted to 4.8 with HCl before addition of lyophilized phytase (0.8 or 2.0 units (u)). The mixture was incubated in a shaking water bath at 55 °C for 8 h. In some samples the pH was adjusted to 6.5 with NaOH followed by addition of tyrosinase (900 or 1500 u from a solution of 900 or 1500 u/mL, respectively) and incubation at 30 °C for 16 h. Combinations of low (0.8 u phytase and 900 u tyrosinase) or high (2.0 u phytase and 1500 u tyrosinase) amounts of enzymes were used in all incubations. Samples used for phenolic analyses were freeze-dried before extraction, and samples for determination of in vitro iron accessibility or phytate content were used directly after the enzyme incubation. Control samples were made by incubation of untreated sorghum udo at 55 and 30 °C without enzymes or with inactivated (boiled) enzymes.

Determination of Iron Accessibility. Iron accessibility was measured as iron solubility at physiological conditions according to the method of Svanberg et al. (*34*) with some minor modifications. A 1-g sample of cereal flour was suspended in 10 mL of H_2O followed by addition of 10 mL of a pepsin solution (0.3% pepsin in 0.1 mol/L HCl). The pepsin solution was added directly to the samples that had been incubated with enzymes. In addition to pepsin the solution also contained physiological amounts of Na (49 mmol/L as NaCl), K (12 mmol/L as KCl), Ca (10 mmol/L as $CaCl_2 \cdot 2H_2O$), Mg (2.4 mmol/L as $MgCl_2 \cdot 6\ H_2O$), and phosphate (3.5 mmol/L as KH_2PO_4) (*35*). The mixture was incubated in a shaking water bath at 37 °C for 90 min. The pH was then adjusted to 2.0 with NaOH, and 3 mL of a pancreatin and bile solution (0.012 g pancreatin and 0.075 g bile extract in 0.1 mol/L $NaHCO_3$) was 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 5000*g* for 20 min. The supernatant was filtered through a 45-μm filter and analyzed for soluble iron, including free soluble complexes of iron, with atomic absorption spectrometry at 248.3 nm on a Philips PU9100X spectrometer (Pye Unicam, Sweden). The amount of soluble iron in the filtrate was expressed as a percentage of the total amount of iron in the sample.

Determination of Iron Content. The total amount of iron in the cereal samples was determined by wet acid digestion in an Ethos Plus microwave system (Milestone, Bergamo, Italy). To 0.3 g of cereal flour were added 3 mL of H_2O, 0.15 mL of 37% HCl, and 0.75 mL of 65% HNO_3, which was digested by a temperature program reaching 180 °C in 15 min and holding that temperature for 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 Carlsson et al. (*36*).

Table 1. Total Iron Content in Sorghum Udo and Finger Millet[a]

	Iron content (mg/100 g dry weight)	
	sorghum udo	finger millet
untreated	7.87 ± 0.16[a]	12.9 ± 0.57[a]
cooked	8.65 ± 0.93[a]	14.8 ± 0.56[a,b]
soaked	6.53 ± 0.36[a,b]	16.4 ± 0.83[b]
germinated	5.01 ± 0.03[b]	15.4 ± 2.88[a,b]

[a] Mean values ± SD of duplicate samples are shown. Figures in the same column followed by different letters are significantly different ($p < 0.05$).

Determination of Phytate Content. A 0.5-g sample of cereal flour was extracted with 10 mL of 0.5 mol/L HCl overnight followed by centrifugation at 3500*g* for 10 min and filtration. Higher-concentration HCl was added to the samples that had been incubated with phytase to get a final concentration of 0.5 mol/L before extraction. The clear supernatant was analyzed for total phytate content with HPIC as described by Carlsson et al. (*37*).

Determination of Phenolic Compounds. The extractions and measurements of phenolic compounds were performed at 25 °C. The total phenol content was determined by the Prussian blue method (*33*), and the amount of resorcinol groups was determined by the modified vanillin method (*32*). Acidified methanol (1% HCl (37%) in methanol) was used as extraction solvent for these determinations. The amount of catechol groups was determined by the modified ferric ammonium sulfate (FAS) method (*12*) using 50% dimethylformamide in acetate buffer (pH 4.4) as extraction solvent. All extractions were carried out according to the following procedure: Cereal flour (100−200 mg) was mixed with 5 mL of extraction solvent and extracted for 1 h with shaking every 10 min. This was followed by centrifugation at 5000*g* for 10 min. The clear supernatant was collected, and the extraction was repeated once. The supernatants from the two extractions were pooled and analyzed spectrophotometrically by the methods mentioned before. Catechin was used as standard and results are expressed as mg of catechin equivalents (CE) per g of dry weight.

Statistical Analysis. Differences in mean values of total iron content, in vitro accessible iron, phenolic compounds, and phytate content were tested by analysis of variance (ANOVA) and significance levels were obtained with Tukey's HSD multiple range test (*38*). A significance level of <0.05 was used.

RESULTS

Incubation of the cereals with phytase and tyrosinase was performed in enzyme combinations at low amounts (0.8 u phytase and 900 u tyrosinase) or at high amounts (2.0 u phytase and 1500 u tyrosinase). The samples were never incubated with tyrosinase without previous incubation with phytase. All analyses on germinated sorghum udo were performed after removal of the roots and shoots, unless otherwise is stated. The mass losses during the soaking and germination processes were small (≤4%) and did not have any significant effect on the reported results.

Total Iron Content. The total iron content was 7.87 ± 0.16 mg/100 g dry weight in sorghum udo and 12.9 ± 0.57 mg/100 g dry weight in finger millet. The iron content in sorghum udo was lower after soaking and germination, but significantly ($p < 0.05$) only in the germinated sample (Table 1). The iron content in soaked finger millet was significantly higher than in the untreated sample.

Table 2. Effect of Cooking, Soaking, and Germination on in Vitro Accessible Iron and Phytate Content in Sorghum Udo and Finger Millet[a]

	in vitro accessible iron (mg/100 g dry weight) (% soluble iron is shown in parentheses)		phytate (μmol/g dry weight)	
	sorghum udo	finger millet	sorghum udo	finger millet
untreated	0.27 ± 0.02^{a} (3.47)	0.39 ± 0.06^{a} (3.03)	9.33 ± 0.11^{a}	11.6 ± 0.19^{a}
cooked	0.17 ± 0.05^{b} (1.93)	0.35 ± 0.01^{a} (2.37)	8.93 ± 0.33^{a}	12.8 ± 0.07^{b}
soaked	0.17 ± 0.01^{b} (2.61)	0.31 ± 0.05^{a} (1.92)	10.6 ± 0.18^{b}	10.8 ± 0.01^{c}
germinated	0.10 ± 0.03^{b} (2.00)	0.39 ± 0.07^{a} (2.54)	7.67 ± 0.14^{c}	8.50 ± 0.04^{d}

[a] Mean values ± SD of duplicate samples are shown. Figures in the same column followed by different letters are significantly different ($p < 0.05$).

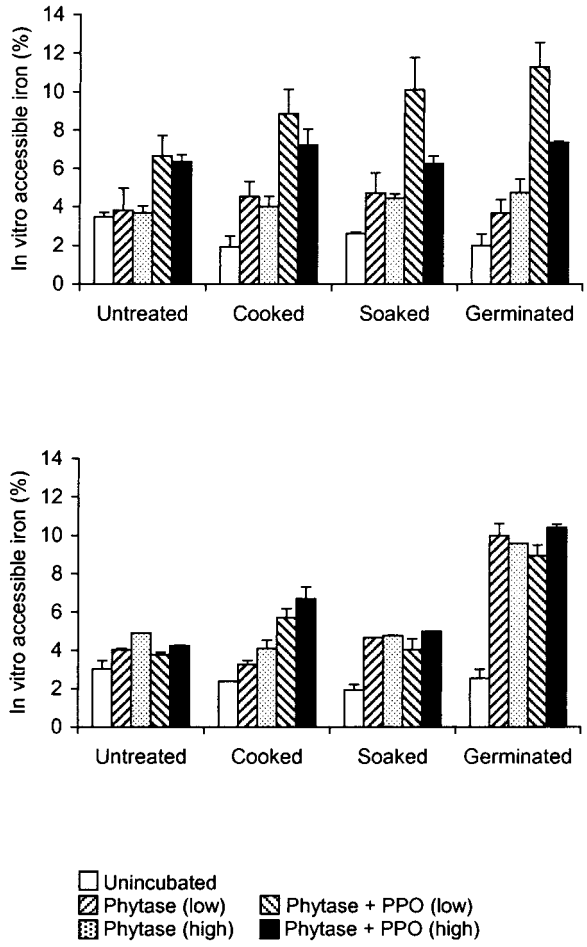

Figure 1. Effect of incubation with phytase and tyrosinase (PPO) on in vitro accessible iron in sorghum udo (top) and finger millet (bottom). Mean values ± SD of duplicate samples are shown.

In Vitro Accessible Iron. The in vitro accessibility of iron in sorghum udo was significantly ($p < 0.05$) reduced by cooking, soaking, and germination, but no significant effect was obtained in finger millet (Table 2). Incubation with phytase and tyrosinase, on the other hand, increased the accessible iron in sorghum udo and finger millet (Figure 1a and b). Incubation of sorghum udo without enzymes or with inactivated (boiled) enzymes confirmed that this effect was mainly due to the activities of the enzymes (data not shown). It could also be seen visually that colored reaction products were formed during the incubation with active tyrosinase.

Effect of Enzyme Incubation in Untreated Samples. The in vitro accessible iron was significantly higher after incubation with phytase and tyrosinase in the untreated samples of sorghum udo (low and high amounts, respectively, $p < 0.002$) and finger millet (high amounts, $p < 0.03$) compared with the unincubated samples (Figure 1a and b). Incubation with high amounts of enzymes increased the in vitro accessible iron, with 83 and 40% in sorghum udo and finger millet, respectively.

Effect of Enzyme Incubation in Cooked Samples. In the cooked samples of sorghum udo and finger millet, the in vitro accessible iron was significantly higher after incubation with phytase and tyrosinase in both low and high amounts (Figure 1a and b). Compared with the unincubated samples, the in vitro iron accessibility was about 3 times higher in sorghum udo and about 1.5 times higher in finger millet. Compared with phytase incubation, the accessibility was approximately 95 and 76% higher, respectively ($p < 0.01$).

Effect of Enzyme Incubation in Soaked Samples. Incubation of soaked sorghum udo with phytase and tyrosinase in low amounts resulted in an approximately 3-fold increase in accessible iron compared with that of the unincubated sample (Figure 1a). Incubation with high amounts resulted in an in vitro accessible iron that was about 1.5 times higher. In soaked finger millet, the in vitro accessible iron increased approximately 1.5 times after incubation with phytase compared with the unincubated sample, but there was no additional effect after tyrosinase incubation (Figure 1b).

Effect of Enzyme Incubation in Germinated Samples. Incubation of germinated sorghum udo with both phytase and tyrosinase had a large increasing effect on in vitro accessible iron, similar to the one seen in soaked sorghum udo (Figure 1a). Compared with the unincubated sample, the accessible iron was approximately 4.5 times higher with low amounts of enzymes and approximately 2.5 times higher with high amounts. Incubation of germinated finger millet with phytase alone or with phytase and tyrosinase resulted in an in vitro accessibility of iron that was about 3 times higher than that in the unincubated sample (Figure 1b).

Phytate Content. Cooking, soaking, and germination of sorghum udo and finger millet had a diverse effect on the phytate content (Table 2). A significant decrease ($p < 0.01$) compared with the untreated sample could, however, be seen in germinated sorghum udo and in soaked and germinated finger millet. Addition of phytase significantly decreased the phytate content in both cereals (Figure 2a and b). Phytate was degraded by 72% when sorghum udo was incubated with 0.8 u phytase and by 87% when it was incubated with 2.0 u phytase. The effect was even larger in the cooked and the soaked samples (88 and 90% reduction with 2.0 u phytase, respectively), and the phytate was almost totally degraded in the germinated sample after incubation with phytase. The reduction of phytate in finger millet after incubation with phytase was, in similarity to sorghum udo, most pronounced in the germinated sample. No difference could, however, be seen if the

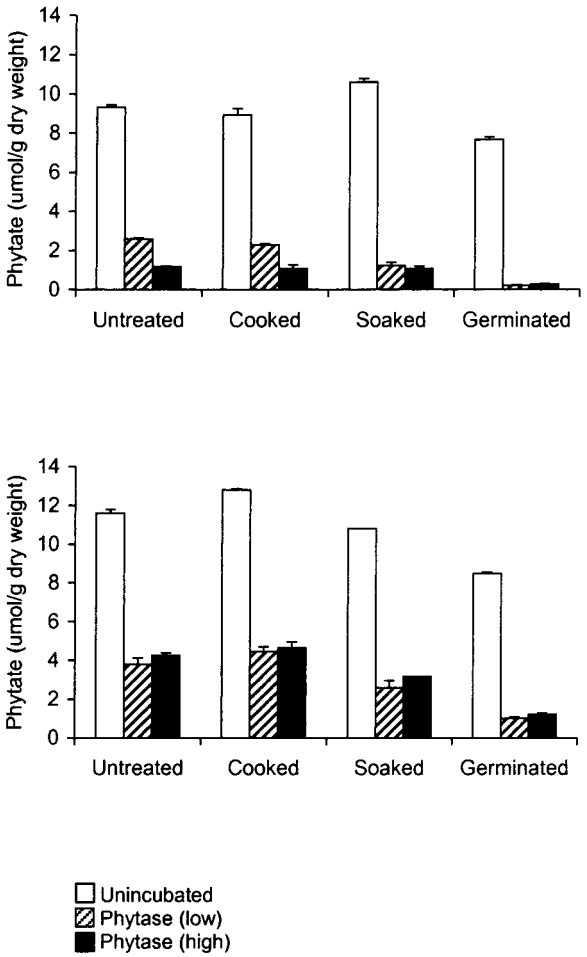

Figure 2. Effect of incubation with phytase on phytate content in sorghum udo (top) and finger millet (bottom). Mean values ± SD of duplicate samples are shown.

samples were incubated with low or high amounts of phytase, which was true also for the untreated, cooked, and soaked samples of finger millet. Phytase incubation reduced the phytate with about 65% in untreated and cooked finger millet and with 74 and 87% in soaked and germinated samples, respectively.

Phenolic Compounds. The content of phenolic compounds was several times higher in sorghum udo than in finger millet (Table 3). The total phenol content in sorghum udo and finger millet was 28.5 and 3.39 mg CE/g dry weight, respectively; the amount of catechol groups was 11.9 and 2.49 mg CE/g dry weight, respectively; and the amount of resorcinol groups was 9.93 and 1.48 mg CE/g dry weight, respectively. Incubation of sorghum udo without enzymes or with inactivated (boiled) enzymes decreased the amount of phenolic compounds as compared with that of the unincubated sample. Incubation with active tyrosinase did, however, result in the lowest amounts of phenolic compounds (Table 4).

Total Phenols. Cooking, soaking and germination reduced the total phenol content in the cereals (Table 3). The reduction was significant ($p < 0.05$) in cooked and germinated sorghum udo and in soaked finger millet. Incubation of the different samples of sorghum udo and finger millet with both phytase and tyrosinase caused a further decrease in the total phenol content (Figure 3a−d and 4a−d, respectively). The decrease was generally significant ($p < 0.05$) when compared with that of samples that had been incubated with phytase alone and with unincubated samples. Incubation of sorghum udo with phytase and tyrosinase in high amounts decreased the total phenol content by 59, 29, 62, and 42% in untreated, cooked, soaked, and germinated samples, respectively. The same treatments on finger millet resulted in 20, 40, 26, and 32% decreases, respectively.

Catechol Groups. The amount of catechol groups was reduced by germination in sorghum udo and by soaking and germination in finger millet (Table 3). No significant decrease could be seen when the cereals were cooked. Incubation of sorghum udo with phytase and tyrosinase decreased the amount of catechol groups about 30% compared with that of phytase incubation in untreated,

Table 3. Effect of Cooking, Soaking, and Germination on Total Phenols and the Amount of Catechol and Resorcinol Groups in Sorghum Udo and Finger Millet[a]

	sorghum udo (mg catechin equivalents/g dry weight)			finger millet (mg catechin equivalents/g dry weight)		
	total phenols	catechol groups	resorcinol groups	total phenols	catechol groups	resorcinol groups
untreated	28.5 ± 3.03[a]	11.9 ± 0.74[a]	9.93 ± 0.70[a]	3.39 ± 0.15[a]	2.49 ± 0.01[a]	1.48 ± 0.10[a]
cooked	18.2 ± 2.10[b]	11.2 ± 0.28[a]	7.89 ± 0.29[b]	3.12 ± 0.11[a,b]	2.29 ± 0.01[a]	0.96 ± 0.05[b]
soaked	24.3 ± 0.95[a,b]	10.5 ± 0.28[a]	7.32 ± 0.62[b,c]	2.96 ± 0.06[b]	1.68 ± 0.18[b]	0.97 ± 0.01[b]
germinated	12.6 ± 0.18[b,c]	5.89 ± 0.08[b]	5.79 ± 0.03[c]	3.02 ± 0.03[a,b]	1.62.± 0.08[b]	0.83 ± 0.01[b]

[a] Mean values ± SD of duplicate samples are shown. Figures in the same column followed by different letters are significantly different ($p < 0.05$).

Table 4. Incubation of Sorghum Udo with Active or Inactive Enzymes and the Effect on the Phenolic Content[a]

	total phenols		catechol groups		resorcinol groups	
	active	inactive	active	inactive	active	inactive
phytase (0.8 u)	22.0 ± 1.34	18.9 ± 1.20	8.84 ± 0.31	11.6 ± 0.03 *	6.96 ± 0.57	8.17 ± 0.30
phytase (2.0 u)	21.3 ± 1.86	19.3 ± 0.90	7.49 ± 0.22	10.3 ± 0.02 *	6.36 ± 0.04	9.17 ± 0.57 *
phytase + PPO (0.8 u + 900 u)	13.5 ± 1.08	16.5 ± 1.20	6.46 ± 0.53	10.6 ± 0.09 *	4.28 ± 0.17	6.93 ± 0.57 *
phytase + PPO (2.0 u + 1500 u)	11.6 ± 0.14	18.6 ± 0.77 *	4.84 ± 0.68	10.5 ± 0.19 *	3.81 ± 0.07	6.96 ± 0.09 *
incubation without enzymes	23.2 ± 1.48		11.7 ± 0.54		10.3 ± 0.42	

[a] Mean values ± SD of duplicate samples are shown. Results are expressed as mg catechin equivalents/g dry weight. Figures followed by an asterisk are significantly different from those for incubation with active enzymes.

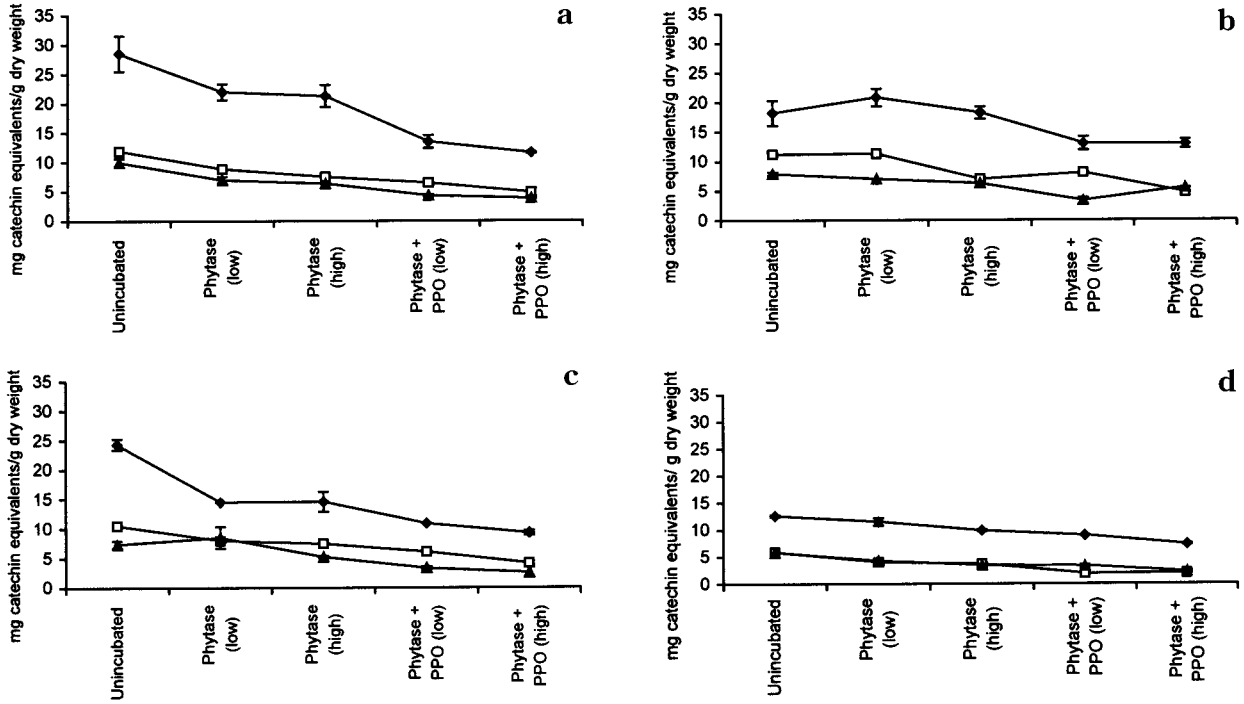

Figure 3. Effect of incubation with phytase and tyrosinase (PPO) on phenolic compounds in (a) untreated, (b) cooked, (c) soaked, and (d) germinated sorghum udo. Mean values ± SD of duplicate samples are shown. Total phenols, ♦; catechol groups, □; resorcinol groups, ▲.

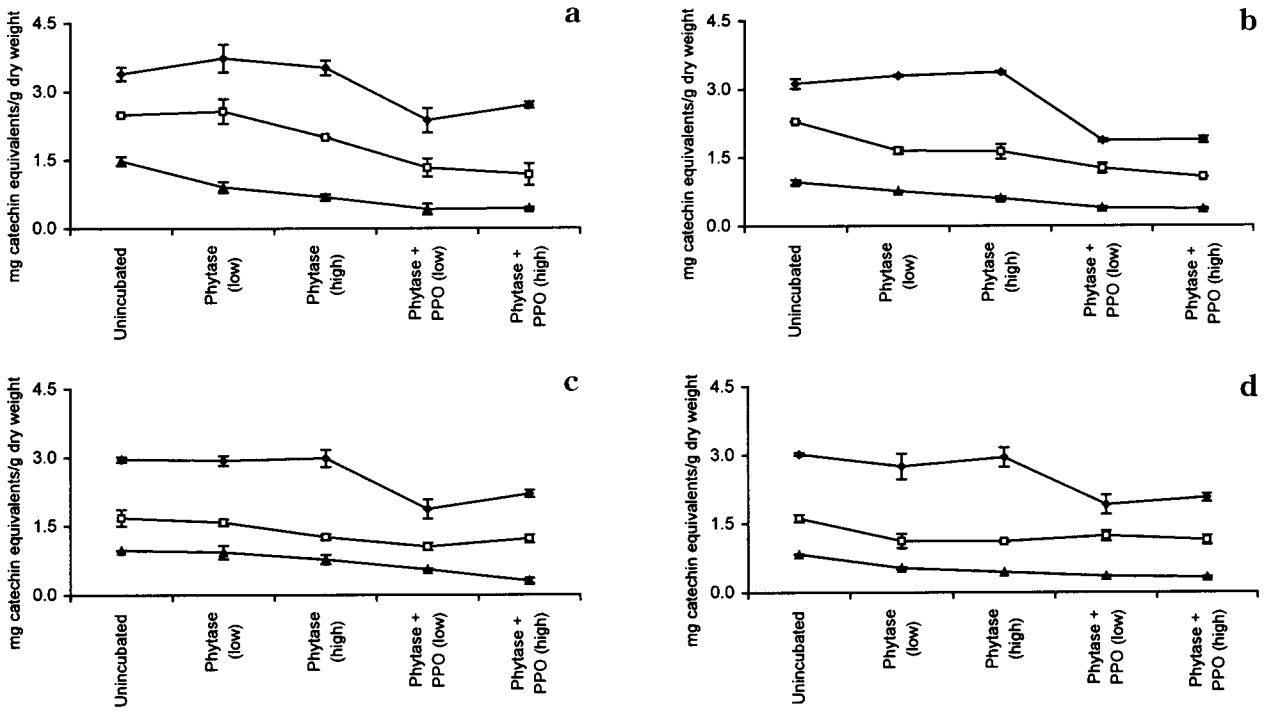

Figure 4. Effect of incubation with phytase and tyrosinase (PPO) on phenolic compounds in (a) untreated, (b) cooked, (c) soaked, and (d) germinated finger millet. Mean values ± SD of duplicate samples are shown. Total phenols, ♦; catechol groups, □; resorcinol groups, ▲.

cooked, and soaked samples ($p < 0.05$, Figure 3a−c). The decrease in germinated sorghum udo was almost 50% (Figure 3d). The catechol groups in untreated, cooked, and soaked finger millet were also significantly reduced after incubation with phytase and tyrosinase in low amounts compared to those incubated with phytase (Figure 4a−d).

Resorcinol Groups. Cooking and germination of sorghum udo, and cooking, soaking, and germination of finger millet, significantly decreased the resorcinol groups ($p < 0.05$, Table 3) and incubation of the different samples of sorghum udo and finger millet with phytase and tyrosinase resulted in a further reduction (Figure 3a−d and 4a−d, respectively). The amount of resorcinol groups was about 50% lower after incubation with phytase and tyrosinase compared with amounts after incubation with phytase alone.

Effect of Removal of the Roots and Shoots in Germinated Sorghum Udo. The content of phenolic compounds in germinated sorghum udo was much lower after

Table 5. Phenolic Content in Germinated Sorghum Udo Before and After Removal of the Roots and Shoots[a]

	roots and shoots included			roots and shoots removed		
	total phenols	catechol groups	resorcinol groups	total phenols	catechol groups	resorcinol groups
unincubated	20.8 ± 0.56[a]	8.43 ± 0.03[a]	8.28 ± 0.00[a]	12.6 ± 0.18[a]	5.89 ± 0.08[a]	5.79 ± 0.03[a]
phytase (0.8 u)	16.4 ± 0.17[b]	7.02 ± 0.28[a,b]	7.49 ± 1.46[a]	11.5 ± 0.69[a]	4.01 ± 0.29[b]	4.30 ± 0.06[b]
phytase (2.0 u)	16.4 ± 0.18[b]	7.32 ± 0.54[a,b]	8.25 ± 1.64[a]	9.88 ± 0.14[b]	3.69 ± 0.04[b]	3.41 ± 0.51[b]
phytase + PPO (0.8 u + 900 u)	15.6 ± 0.91[b]	5.81 ± 0.82[b]	4.30 ± 1.60[a]	8.95 ± 0.06[b]	1.88 ± 0.25[c]	3.30 ± 0.25[b,c]
phytase + PPO (2.0 u + 1500 u)	15.8 ± 0.41[b]	6.09 ± 0.63[b]	3.84 ± 0.76[a]	7.32 ± 0.15[c]	1.97 ± 0.00[c]	2.25 ± 0.26[c]

[a] Mean values ± SD of duplicate samples are shown. Figures in the same column followed by different letters are significantly different ($p < 0.05$). Units are expressed as mg catechin equivalents/g dry weight.

removal of the roots and shoots (Table 5). The decrease was observed on the total phenol content, as well as on the amounts of catechol and resorcinol groups. Incubation with phytase and tyrosinase generally resulted in a larger percentage reduction of the phenolic content when the roots and shoots were removed.

DISCUSSION

In this paper we report of enzymatic process methods that significantly reduce the amount of phytate and polyphenols (tannins) and increase the in vitro accessibility of iron in colored varieties of sorghum and finger millet.

Effect on Phytate. The phytate content decreased when sorghum udo and finger millet were incubated with phytase, and the greatest effect was seen in the germinated samples (Figure 2a and b). Moreover, the germination process itself significantly reduced the phytate content in finger millet (Table 2), which may be due to activation of endogenous phytase. Activation of phytase during germination has been shown in several cereals (*39*−*41*), as well as in legumes (*42*).

Effect on Phenolic Compounds. Catechol groups are considered to be the phenolic constituents mainly responsible for the iron binding capacity of condensed tannins (*8*). However, galloyl groups have also been reported to have strong iron binding properties (*10*, *12*), but sorghum is not known to contain any polyphenols with galloyl groups (*2*). Brandon et al. (*43*) nevertheless found minor amounts of trihydroxyl (galloyl) groups in one high-tannin variety of sorghum. With the FAS method we also obtained low levels of galloyl groups, expressed as tannic acid equivalents, but because tannic acid structures have not been reported in sorghum these values are not reported.

Cooking, Soaking, and Germination. The reduced amount of measurable phenolic compounds as a result of soaking and germination (Table 3) may be attributed to a number of factors. Leaching of phenolic compounds into the soaking media, as well as to the moistened covering tissue surrounding the germinating seeds, could have contributed significantly to the reduction of phenolic groups in the grains. It is also likely that part of the phenolic compounds might have entered the grain endosperm with imbibed water during the soaking and germination processes (*18*, *44*). These phenols may form strong complexes with macromolecules, such as endosperm proteins, making them more difficult to extract. Activation of polyphenol oxidase has also been suggested to contribute to the decrease in phenolic compounds during soaking and germination (*45*, *46*). However, no significant inverse relationship between the decrease in tannins and the increase in polyphenol oxidase activity was found during germination of legume seeds (*47*). These authors suggested that it is not likely for the enzyme and the substrate to react in whole seeds, because they are located in different compartments. Furthermore, it is known that the enzyme and the phenolic substrates are separated at the cellular level (*48*). The decrease in phenolic compounds as a result of cooking (Table 3) could be due to thermal degradation, changes in the chemical reactivity, or formation of insoluble complexes with food components such as proteins (*25*, *49*−*51*). It is also possible that condensation of phenolic compounds might have contributed to a reduction in phenolic groups, as well as to the large reduction in resorcinol groups observed predominantly in enzyme-treated finger millet (Figure 4a-d). Bishop and Nagel (*52*) showed that condensation between the C-ring of malvidin-3,5-diglucoside and the A-ring of catechin resulted in a product with a decreased number of resorcinol groups. The content of total phenols in germinated sorghum udo was higher when the roots and shoots were included: 20.8 mg CE/g dry weight as compared with 12.6 without roots and shoots (Table 5). Both values are lower than that in the untreated sorghum udo (28.5 mg CE/g dry weight). Obviously, a substantial amount of the phenols are either transferred from the seeds into the roots and shoots during the germination process or there is a *de novo* synthesis of simple phenolic compounds in these compartments. Formation of nontannin phenolic compounds in the roots and shoots of sorghum during germination has been shown earlier (*53*, *54*). Thus, germination is a complex process that may contribute to a reduction of phenolic compounds, as well as to formation of new simple phenols. The variable effect on the phenolic contents due to the different treatments might have been due to the complexity of the phenols, the different structural configuration of these compounds, and the existence of other phenol-related compounds, which might have interfered with the methods used to determine the phenolic content.

Enzyme Incubation. The decrease in phenolic compounds as a result of phytase incubation (Figures 3a−d and 4a−d) might have been caused by complex formation between the enzyme and the phenolic compounds, making the phenolics less assayable. It is a well-known fact that tannins easily form complexes with macromolecules such as proteins (*55*). However, incubation without addition of phytase decreased the phenolic content to about the same extent (Table 4). The decrease in phenolic compounds after addition of polyphenol oxidase (tyrosinase) may be a combination of the effect of

addition of protein and the actual oxidation of phenolic compounds. It is probable, though, that polyphenol oxidase has low affinity for the condensed tannins in sorghum udo and finger millet. Udayasekhara and Deosthale (*47*) suggested that tannins can be oxidized by polyphenol oxidase, but that the oxidation occurs slowly, and Cheynier et al. (*56*) found that polyphenol oxidase from grapes had low activity against endogenous procyanidin dimers. Oxidation of complex polyphenolic structures at a higher rate has, however, been shown to take place in the presence of a simple phenolic compound that can be used as an initial substrate by polyphenol oxidase (*57*,

Effect on in Vitro Accessible Iron. The in vitro accessibility of iron in the varieties of sorghum and finger millet used in this study was very low (Table 2), which is mostly due to their high content of phytate and phenolic compounds. A negative correlation between human iron absorption and the phenolic content of cereal meals has been shown by other authors (*10*, *11*). Incubation of phytate-reduced sorghum udo and finger millet with polyphenol oxidase (tyrosinase) had a positive effect on the in vitro accessible iron in most samples (Figure 1a and b). The increase in accessible iron was accompanied with a decrease in total phenols, as well as in the amount of catechol and resorcinol groups (Figures 3a−d and 4a−d). A decrease in phenolic compounds was also observed in soaked and germinated finger millet, but there was no effect on in vitro accessible iron. The accessible iron in soaked and germinated finger millet did, however, increase with phytase incubation. The phytate content in these samples was reduced with approximately 74 and 87%, respectively, which may have an effect on in vitro accessible iron because of the comparatively low content of phenolic compounds in finger millet. Incubation of the different samples of sorghum udo with phytase had, on the other hand, no effect on in vitro accessible iron. A similar effect has been shown in a previous study by Svanberg et al. (*34*), where high- and low-tannin sorghum were incubated with phytase. The enzyme incubation resulted in a lower increase of in vitro accessible iron in the high-tannin variety compared with that in the low-tannin variety, which was explained by the high content of iron-binding phenolics in the former.

Total Iron Content. The iron content in sorghum udo (Table 1) was similar to or a little higher than when compared with that of other determinations (*34*, *59*, *60*). The iron content in finger millet was higher than expected because values from 4 to 6 mg/100 g have been reported earlier (*21*, *22*, *61*). A possible explanation is that there might be contamination iron left after the washing procedure. This theory was supported by the fact that the iron content in the finger millet sample was about 25% lower after washing the grains in diluted HCl (0.5 mol/L) compared with the level after washing in deionized water. Removal of contamination iron in cereals with diluted HCl has been demonstrated before (*62*). The presence of contamination iron may also explain the higher iron content after some treatments compared with those of the untreated samples (Table 1). Cooking, soaking, and germination were made on different batches of the grains, and the content of contamination iron after the washing procedure may therefore differ in those samples.

CONCLUSION

Treatment of high-tannin cereal preparations with phytase and polyphenol oxidase increased the in vitro accessibility of iron in those foods. Germination of the cereals enhanced the effect of the enzymes, i.e., significantly more phytate and polyphenols were reduced, respectively, and the in vitro accessible iron was also highest in the enzyme-treated germinated samples. The method may contribute to an increased bioavailability of iron in foods prepared from high-tannin cereals, which in turn can affect the prevalence of iron deficiency anemia in areas where those are consumed as staples.

ABBREVIATIONS USED

CE, catechin equivalents; FAS, ferric ammonium sulfate; HPIC, high-performance ion chromatography.

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