Iron and Zinc in Vitro Availability in Pearl Millet Flours (*Pennisetum glaucum*) with Varying Phytate, Tannin, and Fiber Contents

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Simulations of gastro-intestinal digestion, used to estimate in vitro iron and zinc availability, were performed on two kinds of samples: (i) samples with decreased phytate contents from whole pearl millet flour and (ii) nondephytinized or dephytinized samples from two pearl millet grain fractions, a decorticated fraction with low fiber and tannin contents and a bran fraction with high fiber and tannin contents. Iron and zinc in vitro availabilities of whole pearl millet flour were significantly improved by phytate degradation, even if the IP6 were not all degraded. Total dephytinization of decorticated fraction led to a marked increase in iron and zinc in vitro availabilities, but that of bran fraction had no effect on either iron or zinc in vitro availability. Even if phytates are involved in reducing in vitro iron and zinc availability in pearl millet flour, fibers and tannins play an important role by chelating a high proportion of iron and zinc in grain hulls.

INTRODUCTION

In developing countries, because of limited access to animal products (meat, fish, eggs) that provide high intakes of heme-iron and zinc, the main dietary sources of iron and zinc are cereals and legumes. In some countries in Sahelian Africa, millets, and particularly pearl millet (*Pennisetum glaucum*), can represent more than 75% of the total cereal production (*1*). This cereal thus represents an important proportion of dietary intake for millions of people. Unfortunately, the iron and zinc in cereal-based foods are poorly bioavailable due to factors that reduce their intestinal absorption, resulting in high rates of iron and zinc deficiency, especially in infants, children, and women of child-bearing age (*2*). For instance, in Burkina Faso, Mali, and Niger, where millet contributes to more than 20% of the available food energy, prevalence of iron deficiency anaemia is estimated at 83%, 77%, and 57%, respectively, in children under 5 years and around 48% in women of child-bearing age (*3*). Furthermore, mineral absorption is also influenced by the level of mineral contents and by factors that enhance their absorption in the diet, as well as by the physiological status of

the subjects, such as age, disease, or stores of mineral (*4*, *5*). Over the years, many in vivo (*6*−*9*) or in vitro (*10*, *11*) studies have reported the negative effects of phytates, fibers, and tannins on iron or zinc bioavailability or in vitro availability. The overall conclusion of these studies shows that the effects of anti-nutritional factors on mineral availability are highly dependent on the food matrix. Moreover, results of different studies have differed considerably depending on the method used to estimate mineral bioavailability.

Because of their cost and complexity, radio-isotopic measurements of mineral absorption in animals or humans are often impractical and consequently little used. One approach to address this problem has been the development of in vitro or ex vivo measurements of food mineral bioavailability, such as soluble minerals (*12*, *13*) or mineral uptakes in the Caco-2 cell culture model (*11*). These techniques are reproducible, reliable, and sensitive to changes due to processes (*14*). As ex vivo measurements are longer, more complicated, and more expensive than in vitro measurements, for our first approach, we used in vitro simulation of gastro-intestinal digestion to measure iron and zinc bioavailability.

The purpose of the present investigation was to assess the effect of phytates on iron and zinc in vitro availability in pearl millet grain. We first investigated the effect of phytate degradation in pearl millet flour on iron and zinc in vitro availability,

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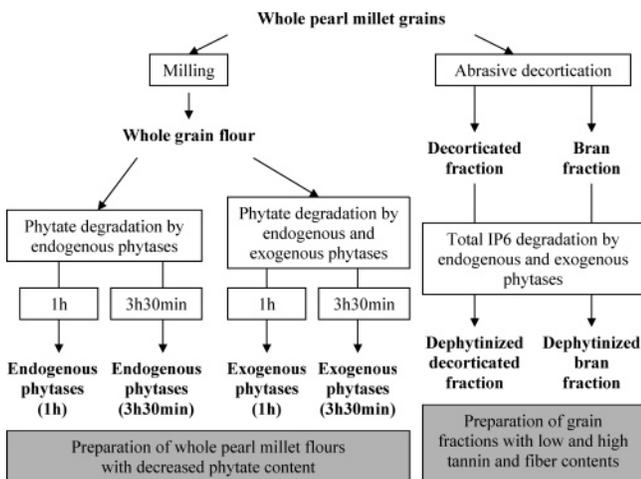*J. Agric. Food Chem.*, Vol. 53, No. 8, 2005 **3241**

Figure 1. Sample preparation and nomenclature.

in some tests only degraded by endogenous phytases and in other tests degraded by both endogenous and exogenous phytases. As fibers and tannins are also known to chelate minerals, their effects on iron and zinc in vitro availability of pearl millet grain fractions with or without dephytinization were subsequently investigated.

MATERIALS AND METHODS

Materials. Two kinds of samples were prepared: samples with different levels of phytates, and samples with high and low fiber and tannin contents. **Figure 1** summarizes their preparation as well as their nomenclature.

Millet Flours with Decreased Phytate Content. Grains of pearl millet (*Pennisetum glaucum,* sp. Gampela) were obtained from the "Institut National de l'Environnement et de la Recherche Agronomique" (INERA) of Ouagadougou (Burkina Faso). The Gampela cultivar (yellow in color) is grown locally and consumed in Burkina Faso. The grains were milled using a laboratory mill (IKA M20 Labortechnik, Staufen, Germany) and sieved to pass through a 0.5 mm screen to obtain whole grain flour. Samples with decreased phytate contents were obtained by incubating whole grain flour in 0.1 M acetate buffer pH 5.0 (1/2, p/p), with or without exogenous phytases, for a short or a long period, at 37 °C under low shaking (60 rpm) in an incubator (New Brunswick scientific Co., Inc., Edison, USA). Incubation in acetate buffer allowed moderate phytate degradation by endogenous phytases, while addition of exogenous phytases allowed a greater degree of degradation. Two different exogenous phytases were used, one plant phytase from wheat (Sigma, P-1259) and one microbial phytase from *Aspergillus ficuum* (Sigma, P-9792). These two phytases were used simultaneously to obtain maximal hydrolysis products from two different pathways. The pH and temperature conditions for incubations (pH 5.0, 37 °C) were fixed after confirmation that they permitted to each kind of phytases to be active given their different pH and temperature optima (pH 6.0 and 24−40 °C for pearl millet, pH 5.0 and 55 °C for wheat and pH 2.5 and 37 °C for *Aspergillus ficuum*). The same conditions were used for incubations with both endogenous and exogenous phytases because pH and temperature may influence leaching phenomena. The concentrations of the enzyme preparations were first determined on a sodium phytate solution (Sigma, P-8810) to ensure equivalent activities for both exogenous phytases in the incubating conditions. Phytases were mixed in 0.1 M acetate buffer, pH 5.0 at 144 u/L for phytases from wheat, and at 516 u/L for microbial phytases. Next, millet flour was incubated for a short period (1 h) and for a long period (3.5 h) to obtain samples containing different levels of residual phytates, and in a further case, to achieve total *myo*-inositol 6 phosphate (IP6) degradation in the sample incubated for the long period with exogenous phytases. At the end of the incubation period, the mixtures were cooled to 4 °C and centrifuged at 2600*g* for 15 min. The pellets were then freeze-dried and milled with a laboratory pestle and mortar.

Grain Fractions with Low and High Fiber and Tannin Contents. Two grain fractions were prepared by abrasive decortication to obtain, on one hand, a fraction with low fiber and tannin contents and, on the other hand, a fraction with high fiber and tannin contents. The moisture content of the grains was adjusted to 15% to help decortication as their initial water content was 7.8%. The grains were tempered at 20 °C in closed plastic containers for 16 h in a rotary shaker (Reax 2, Heidolph, Schwabach, Germany) at 30 rpm. Grains were then mechanically sieved to obtain grain samples with homogeneous diameters from 2.0 to 2.5 mm (68% of the grain population). Abrasive decortication was performed on 80 g of the tempered sample with a laboratory huller (TM 050, Satake, Stockport, UK) at 750 rpm. The decorticated grain fraction, composed of endosperm and germ, was obtained after a decortication experiment at a 35% extraction rate. The bran fraction was reconstituted by mixing bran from three decortication experiments, one at a 35% and two at a 12% of extraction rate, which corresponds to the removal of the nonstarch containing part of pearl millet grains. The fractions were freeze-dried and milled using a laboratory mill (IKA M20 Labortechnik, Staufen, Germany) and sieved to pass through a 0.5 mm screen.

Dephytinized Grain Fractions. Thirty grams of each fraction were incubated with the previously described exogenous phytase solution for 3.5 h at 37 °C under low shaking (60 rpm) in an incubator to obtain total IP6 degradation. At the end of the incubation period, the mixtures were cooled to 4 °C and centrifuged at 2600*g*, for 15 min. The pellets were then freeze-dried and milled with a laboratory pestle and mortar.

Analytical Methods. *Acid and neutral detergent fiber* contents were determined according to the gravimetric methods of Van Soest (*15*) and Van Soest and Wine (*16*) using a Dosi-fiber (Selecta, Barcelona, Spain). These measurements correspond approximately to the determination of cellulose and lignin content for the ADF and of cellulose, lignin, and hemicellulose content for the NDF, which are the most likely compounds of fiber to chelate minerals.

Iron binding phenolic compound content was determined according to the method of Brune et al. (*17*) using a ferric ammonium sulfate reagent that allowed measuring of galloyl (expressed as tannic acid equivalents) and catechol group contents (expressed as catechin equivalents) at two wavelengths (578 and 680 nm, respectively).

Phytate content was estimated by determination of *myo*-inositol hexaphosphate (IP6) content obtained by anion-exchange HPLC separation after phytate extraction according to the method of Talamond et al. (*18*). Separation was achieved using an Ion Pac AS11 anion-exchange column (4 × 250 mm, Dionex) equipped with an Ion Pac AG11 (4 × 50 mm) precolumn and an anion suppressor (AMMS-III 4 mm). The separation was performed by gradient elution using NaOH 0.2 M solution and deionized water as eluents.

Total iron and zinc contents were determined by atomic absorption spectrophotometry (SpectrAA 200, Varian, Victoria, Australia) after dry ashing as described by Laporte et al. (*19*).

Estimation of Iron and Zinc in Vitro Availability. In vitro availability of iron and zinc was estimated by their digestibility under simulated physiological conditions using a method based on the one proposed by Lönnerdal et al. (*20*) with modifications according to other authors (*13*, *21*, *22*). Prior to in vitro digestion, pepsin solution (Sigma, P-7000; 14900 u/m: in 0.1 M HCl) and pancreatin (Sigma, P-1750; 1.85 mg/mL)−bile extract solution (Sigma, B-8631; 11 mg/mL in 0.1 M NaHCO₃) were shaken for 30 min with Chelex-100 resin (Bio-Rad, 142-2842) to remove cations and filtered on ashless Whatman filter n°41 (*21*). About 2 g of dry sample was precisely weighed in an Erlenmeyer and suspended in 20 mL of distilled water. After 10 min of conditioning in a shaking water bath at 37 °C, pH was adjusted to 2.0 with 1 M HCl solution under magnetic stirring. Next, 1.0 mL of the pepsin solution was added, and the mixture was incubated for 1 h. The pH was then increased to about 4.0 with 0.15 mM PIPES (piperazine-*N*,*N*′-bis-[2-ethansulfonic acid]) buffer (Sigma, P-1851), 5.0 mL of the pancreatin−bile extract solution was added, and pH adjusted to 7.0 with the PIPES buffer that allows minimizing pH variation (*22*). The mixture was incubated for 2 h under low magnetically stirring. The suspension was then centrifuged at 10 000*g* for 30 min at 4 °C (*13*). The supernatant was recovered in a silica cap, evaporated on a hot-plate under a range hood, and dry ashed in the
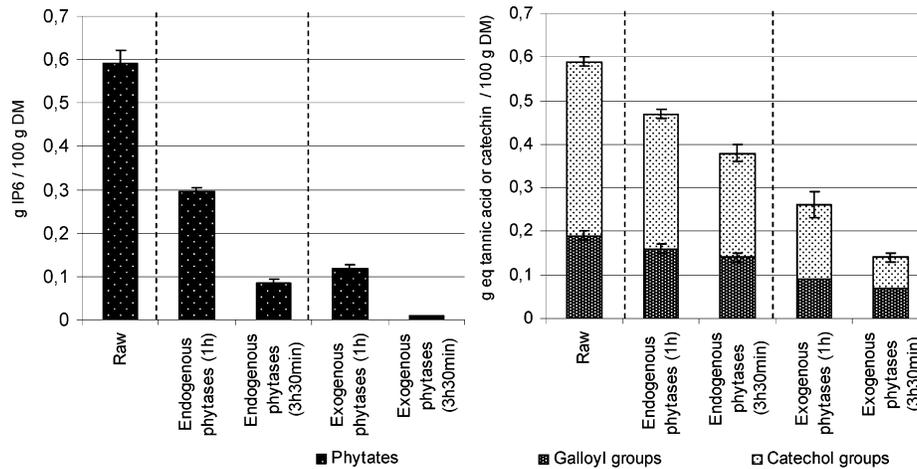
Figure 2. Phytate and iron binding phenolic compound contents (g/100 g DM) of whole pearl millet flours with decreased phytate content. Values are means ± SD of three determinations.
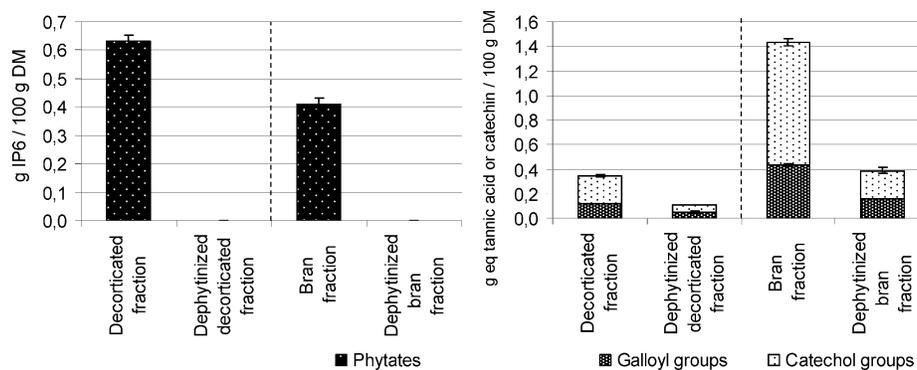

Figure 3. Phytate and iron binding phenolic compound contents (g/100 g DM) of pearl millet grain fractions. Values are means ± SD of three determinations.

same way as for total mineral contents. The supernatant was analyzed using atomic absorption spectrophotometry (*19*) for in vitro available iron and zinc, including soluble free ionizable iron and zinc and soluble complexes of iron and zinc, which represents iron and zinc that have crossed the first essential, but not sufficient, step to become available for absorption.

Statistical Analysis. Values were calculated per 100 g of dry matter (DM). Chemical analyses (determination of phytate, iron binding phenolic compound, fiber and total iron and zinc content) were carried out in triplicate, and data are presented as means ± standard deviation (SD). In vitro digestions were carried out in triplicate, and in vitro available iron and zinc contents were determined on each digest. Iron and zinc in vitro availabilities are presented as means ± SD of three values calculated from each determination of available mineral content divided by the mean of total mineral content and multiplied by 100.

Data were assessed by analysis of variance (ANOVA) using Duncan's multiple range tests to separate means with significance of differences at 5% level. For samples with decreased phytate contents, two-way analyses of variance were also performed to investigate the effect of added exogenous phytases and of the length of the incubation period, as well as their interaction. For grain fractions, two-way analyses of variance were performed to investigate the effect of dephytinization and of the nature of the fraction, as well as their interaction. All statistical analyses were performed using Statgraphics Plus 5.0 v.

RESULTS AND DISCUSSION

Antinutritional Factors in Samples. Phytate and iron binding phenolic compound (catechol and galloyl group) contents of millet flours are presented in **Figure 2**. In whole pearl millet flour, phytate and iron binding phenolic compound contents were both around 0.60 g/100 g DM, which corresponds to data usually reported for pearl millet (*23, 24*). Fiber contents

were 3.08 and 6.22 g/100 g DM for acid detergent fibers (cellulose and lignin) and neutral detergent fibers (cellulose, lignin, and hemicelluloses), respectively (results not shown).

In the incubated samples, phytases allowed phytate content to be reduced from 50% to 99% depending on the conditions. The reduction in phytate content was significantly improved by an increase in the length of incubation ($P < 0.0001$) and by the addition of exogenous phytases ($P < 0.0001$). The incubation conditions, with or without addition of exogenous enzymes, also led to a decrease in the apparent iron binding phenolic compound content of millet flour. In millet flour incubated without exogenous phytase, the decreases in iron binding phenolic compound content (23% and 39% after 1 and 3.5 h of incubation, respectively) can be attributed to leaching of soluble tannins into the medium. Alonso et al. (*25*) also reported leaching of tannins throughout the soaking stage in legume seeds. In samples incubated with added exogenous phytases, the iron binding phenolic compound content was reduced by 57% and 77% depending on the length of incubation, which cannot be attributed only to leaching. This additional decrease was probably due to complexation phenomena either with proteins added by enzyme preparation (*26*) or with hydrolysis products released by phytases (i.e., phosphate groups, cations). Indeed, some phenolic compounds are known to link proteins and iron because of their ionic groups (*23*), and these interactions could hinder their quantification by colorimetric reagents (*27*).

The phytate, iron binding phenolic compound, and fiber contents of grain fractions are presented in **Figures 3** and **4**. As expected, bran fraction showed much higher iron binding phenolic compound (1.43 vs 0.36 g/100 g DM) and fiber contents (5.16 vs 1.80 g/100 g DM for ADF and 19.05 vs 3.04
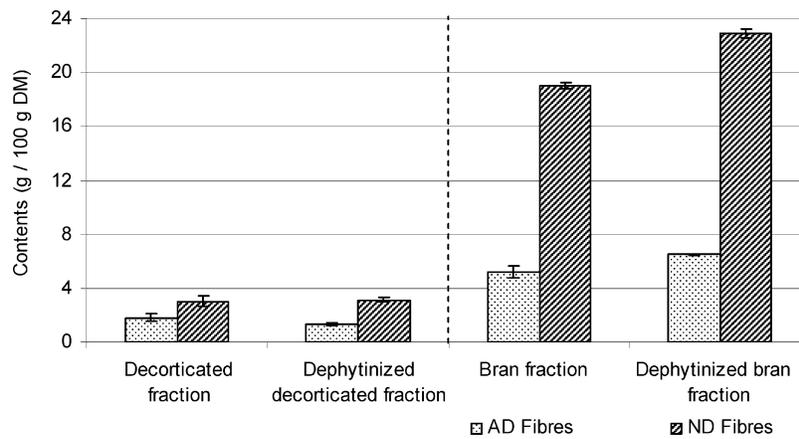
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Figure 4. Fiber contents (g/100 g DM) of pearl millet grain fractions. Values are means ± SD of three determinations.

Table 1. Total and in Vitro Available Iron Contents (mg/100 g DM), Iron in Vitro Availability (%), and Phy/Fe Molar Ratios of Whole Pearl Millet Flours with Decreased Phytate Content[a]

whole pearl millet flours	total Fe[b]	available Fe[c]	% Fe available	Phy/Fe molar ratio
raw	3.15 ± 0.17 a	0.31 ± 0.05 b	9.9 ± 1.4 d	15.9
endogenous phytases (1 h)	2.84 ± 0.19 b	0.63 ± 0.12 a	22.1 ± 4.1 ab	8.8
endogenous phytases (3.5 h)	2.86 ± 0.04 b	0.69 ± 0.02 a	24.1 ± 0.7 a	2.5
exogenous phytases (1 h)	3.07 ± 0.05 ab	0.55 ± 0.05 a	17.7 ± 1.5 c	3.3
exogenous phytases (3.5 h)	3.25 ± 0.04 a	0.70 ± 0.10 a	21.4 ± 2.9 ab	0.2

[a] Values with no common letters in the same column are significantly different ($P \leq 0.05$) as assessed by Duncan's multiple range test. [b] Values are means ± SD of three determinations. [c] Values are means ± SD of three determinations performed on three independent digests.

g/100 g DM for NDF) than decorticated fraction because of the localization of these compounds in the hulls of millet grains. Conversely, the phytate content of bran fraction was lower than that of decorticated fraction (0.409 vs 0.633 g/100 g DM) because phytate of pearl millet is mainly located in the germ (*28*). In both fractions, the treatment with exogenous phytases led to total phytate (IP6) degradation and to a reduction in catechol and galloyl group contents because of their leaching and complexation with the added proteins or hydrolysis products. Fiber contents of grain fractions increased slightly as a result of the treatment. These passive increases can be explained by loss of soluble matter during incubation.

Effect of Phytate Degradation on Iron and Zinc in Vitro Availability of Pearl Millet Flour. *Iron in Vitro Availability.* **Table 1** presents the total and in vitro available iron contents, as well as iron in vitro availability and molar ratios of phytate to iron (Phy/Fe) of whole grain and incubated millet flours.

The treatments without exogenous enzymes significantly decreased ($P \leq 0.05$) the total iron content of millet flour from 3.15 to 2.84 mg/100 g DM because of iron leaching into the medium (*29*). By contrast, when exogenous phytases were added, the total iron content was apparently less affected, which could mean that iron is impounded by the added proteins in the same way as tannins are complexed.

In whole pearl millet flour, 9.9% of iron was found to be available in vitro after gastro-intestinal digestion simulation. This value is quite high related to values of in vitro soluble or accessible iron, from 3.0% to 4.0%, usually found in the

literature for cereals as finger millet, sorghum, rye, or oat after in vitro digestion and centrifugation (*26, 30*). In return, this result is close to values of ionizable iron obtained with the method of Rao and Prabhavathi (*31*) (from 7.1% for pearl millet to 15.0% for rice), which have been shown to correlate highly with percent iron absorption observed in humans.

Each treatment reduced the Phy/Fe molar ratio from 15.9 to 0.2 and significantly increased in vitro iron availability as shown by Duncan's test ($P \leq 0.05$). Phytate hydrolysis may result in the production of lower inositol phosphates. Some of them may inhibit in vitro iron availability (*32*) as well as absorption (*33*), but our results do not allow us to conclude on this subject because these degradation products have not been quantified.

The increase in in vitro iron availability after Phy/Fe molar ratio reduction is in accordance with the results of Saha et al. (*34*) who showed that absorption of radio-labeled iron increased significantly when rats were fed test meals with Phy/Fe molar ratios lower than 14. However, in terms of phytate content, we noticed that a reduction in IP6 content of pearl millet flour from 592 to 296 mg/100 g DM led to a doubled in vitro availability of iron, which was not more improved by stronger IP6 content reductions (up to 8 mg/100 g DM). This is not in agreement with the dose-dependent inhibitory effect of phytate reported by Hallberg et al. (*35*) on human iron absorption by adding 2–250 mg of sodium phytate to wheat rolls. Our results also differ from those of Brune et al. (*36*), as well as Hurrell et al. (*37*), who showed that even low total inositol phosphate amounts strongly inhibit iron absorption in humans fed cereal bread and soy isolates, respectively. Furthermore, a recent study (*38*) showed that reduction of phytate content of a complementary cereal-based food (from 1150 to 660 mg/100 g DM) led to an increase in in vitro iron solubility (from 4.8% to 18.8%) but had no effect on hemoglobin status of infants from 6 to 12 months of age. So, an increase in in vitro iron solubility after incomplete phytate degradation does not guarantee an increase in iron absorption and therefore in iron status of humans consuming phytate containing foods. These discrepancies between in vivo and in vitro studies may be attributed to the absence of simulation of the intestinal absorption and/or its physiologic regulation mechanisms in the in vitro digestion systems. In vitro studies are useful to provide knowledge on mineral and antinutritional factor interactions, or on efficiency of a technological process related to another in improving mineral solubility, but they cannot be substituted for in vivo studies.

Two-way analysis of variance did not show an effect either of the addition of exogenous phytases or of the length of the incubation period on iron in vitro availability of millet flour. This is explained by the slight variation in iron availability at

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Table 2. Total and in Vitro Available Zinc Contents (mg/100 g DM), Zinc in Vitro Availability (%), and Phy/Zn Molar Ratios of Whole Pearl Millet Flours with Decreased Phytate Content[a]

whole pearl millet flours	total Zn[b]	available Zn[c]	% Zn available	Phy/Zn molar ratio
raw	2.51 ± 0.01 a	0.36 ± 0.08 ab	14.2 ± 3.0 c	23.2
endogenous phytases (1 h)	1.96 ± 0.01 b	0.47 ± 0.07 a	23.8 ± 3.7 b	14.9
endogenous phytases (3.5 h)	1.57 ± 0.01 c	0.43 ± 0.04 ab	27.4 ± 2.3 ab	5.3
exogenous phytases (1 h)	1.95 ± 0.01 b	0.32 ± 0.02 b	17.1 ± 0.6 c	6.0
exogenous phytases (3.5)	1.56 ± 0.07 c	0.48 ± 0.07 a	31.0 ± 4.6 a	0.5

[a] Values with no common letters in the same column are significantly different ($P \leq 0.05$) as assessed by Duncan's multiple range test. [b] Values are means ± SD of three determinations. [c] Values are means ± SD of three determinations performed on three independent digests.

Table 3. Total and in Vitro Available Iron Contents (mg/100 g DM), Iron in Vitro Availability (%), and Phy/Fe Molar Ratios of Pearl Millet Grain Fractions[a]

pearl millet grain fractions	total Fe[b]	available Fe[c]	% Fe available	Phy/Fe molar ratio
decorticated fraction	1.91 ± 0.09 c	0.26 ± 0.08 c	13.8 ± 4.2 b	28.1
dephytinized decorticated fraction	1.55 ± 0.12 d	0.57 ± 0.03 b	36.8 ± 1.3 a	0.1
bran fraction	4.64 ± 0.27 b	0.66 ± 0.12 b	14.3 ± 2.7 b	7.5
dephytinized bran fraction	5.72 ± 0.10 a	0.94 ± 0.21 a	16.4 ± 3.7 b	0.0

[a] Values with no common letters in the same column are significantly different ($P \leq 0.05$) as assessed by Duncan's multiple range test. [b] Values are means ± SD of three determinations. [c] Values are means ± SD of three determinations performed on three independent digests.

low phytate contents. Therefore, it seems that the addition of exogenous enzyme preparation led to interactions between phytases, phenolic compounds, and iron. This could be the reason in vitro availability of iron is lower in this case, despite the stronger phytate degradation, than in the case of incubation just with buffer.

Zinc in Vitro Availability. Total and available zinc contents, as well as zinc in vitro availability and molar ratios of phytate to zinc (Phy/Zn) of whole and incubated millet flours, are presented in **Table 2**.

Both treatments (with or without exogenous phytases) had the same effect on the total zinc content of millet flour, which was reduced by 22% and 38% after a short and long incubation period, respectively. Hence, contrary to what was observed for iron, addition of exogenous enzymes had no effect on total zinc content, whereas the longer incubation period significantly reduced it ($P < 0.0001$) because of gradual zinc leaching into the medium (*29*).

Zinc in vitro availability of whole grain millet flour was 14.2% with a Phy/Zn molar ratio of 23.2. Concerning phytate degradation by endogenous phytases, zinc availability was increased to 23.8% and 27.4% after incubation for 1 and 3.5 h, respectively. These results agree with those of Davies and Olpin (*39*) or Saha et al. (*34*) who showed in rat that a decrease in the molar ratio Phy/Zn to values below 15 improved zinc absorption. Nävert et al. (*40*) also observed an increase in zinc absorption from 9.6% to 19.8% in their study on human subjects fed wheat rolls containing phytate contents of 0.66−0.15% (decreasing Phy/Zn molar ratios from 17 to 4). However, in millet flours treated with exogenous phytases, a decrease in the Phy/Zn molar ratio from 23.2 to 6.0 did not allow an improvement in zinc in vitro availability. This result was confirmed by the significant interaction ($P < 0.05$) found between the addition of exogenous phytases and the length of the incubation period according to results of two-way analysis of variance. Thus, as for iron, it appears that the added proteins interact with the zinc in the millet flour and prevent its solubilization despite phytate degradation. This can be explained by the fact that zinc is known to catalyze a lot of enzymatic activities, among which the activity of many phosphatases (*41*). A review by Matsui (*42*) already reported that zinc bioavailability was not increased by addition of exogenous phytases in a number of animal studies. Conversely, after the longer incubation period with exogenous phytases, an improvement in zinc in vitro availability (from 17.1% to 31.0%) was observed. This can be explained by the

fact that, in this case, despite the presence of exogenous proteins, all of the inositol phosphates (inositol hexaphosphate but also the lower inositol phosphates) were degraded, as it could be noted from the chromatographic profile which did not present any peak. Some of the hydrolysis products of IP6, particularly IP5 and maybe IP4 and IP3, also participate indeed in the inhibition of zinc availability (*43−45*). Furthermore, it is interesting to note that this improvement was not significantly higher than that obtained after phytate degradation by endogenous phytases (27.4%). Hence, our results agree with the conclusions of Hurrell (*46*) that incomplete degradation of phytate (IP6) is an effective way to double the in vitro availability of zinc.

Effect of Total Phytate Degradation on Iron and Zinc in Vitro Availability of Pearl Millet Fractions with Low and High Tannin and Fiber Contents. *Iron in Vitro Availability.* **Table 3** presents total and in vitro available iron contents, as well as iron in vitro availability and molar ratios of phytate to iron (Phy/Fe) of nondephytinized and dephytinized grain fractions.

Because of the high iron content of the hulls of pearl millet grains, total iron content of bran fraction was significantly higher than that of decorticated fraction (4.64 vs 1.91 mg/100 g DM). After incubation with exogenous phytases, this difference was still greater as decorticated fraction total iron content decreased to 1.55 mg/100 g DM, while that of bran fraction increased to 5.72 mg/100 g DM. Consequently, part of the iron of decorticated fraction must leach into the medium during incubation, while iron of bran fraction seems to be impounded in the material, which leads to an apparent increase in the total iron content because of the concomitant loss in soluble components. This result agrees with the fact that the total iron content of whole grain millet flour was not decreased by leaching when exogenous phytases were added for the incubation. As bran fraction displayed high catechol and galloyl group and fiber contents, these components are potential candidates for such iron impoundment.

This assumption was confirmed by studies of iron in vitro availability of nondephytinized and dephytinized fractions. Although the molar ratios of Phy/Fe of nondephytinized fractions were very different (28.1 and 7.5 for decorticated and bran fraction, respectively), no difference was observed in their iron availability, which was around 14%. Moreover, dephytinization of grain fractions led to a considerable increase in iron in vitro availability of decorticated fraction (from 13.8% to 36.8%), while that of bran fraction was not significantly changed

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Table 4. Total and in Vitro Available Zinc Contents (mg/100 g DM), Zinc in Vitro Availability (%), and Phy/Zn Molar Ratios of Pearl Millet Grain Fractions[a]

pearl millet grain fractions	total Zn[b]	available Zn[c]	% Zn available	Phy/Zn molar ratio
decorticated fraction	1.99 ± 0.03 b	0.33 ± 0.06 c	16.4 ± 2.8 c	31.3
dephytinized decorticated fraction	1.02 ± 0.02 c	0.46 ± 0.04 b	45.2 ± 3.0 a	0.1
bran fraction	2.95 ± 0.05 a	0.66 ± 0.07 a	22.5 ± 2.4 b	13.7
dephytinized bran fraction	1.99 ± 0.06 b	0.45 ± 0.10 bc	22.7 ± 4.9 b	0.0

[a] Values with no common letters in the same column are significantly different ($P \leq 0.05$) as assessed by Duncan's multiple range test. [b] Values are means ± SD of three determinations. [c] Values are means ± SD of three determinations performed on three independent digests.

according to Duncan's test ($P \leq 0.05$). This was confirmed by two-way analysis of variance that revealed a significant interaction between the nature of the fraction and dephytinization ($P < 0.0005$). These results can be attributed to the negative effects of iron binding phenolic compounds or fibers present in large amounts in bran fraction. In their study on human subjects fed low- and high-tannin sorghum porridges, Hurrell et al. (7) showed that, after dephytinization, iron absorption was increased almost 2-fold in comparison with low-tannin porridge, while no significant change was noted in high-tannin porridge. Indeed, as seen in the first part of this work and as reported by other studies, phenolic compounds are able to link with metal cations through their carboxyl and hydroxyl groups (17, 47) and have an inhibitory effect on iron bioavailability (9, 48). Concerning fibers, Olivares et al. (10) reported their significant effect on in vitro iron bioavailability in complementary foods, which was low when total fiber content was high. However, several other studies rather attribute the observed effect of fibers on mineral absorption to phytates, which occur together with fibers in fiber-rich diets (36, 49).

Our results showed that iron in vitro availability of bran fraction was not only limited by phytate but also by the other anti-nutritional factors, and both iron binding phenolic compounds and fibers could be responsible for the inhibition of iron in vitro availability. This also pointed out that the molar ratio of Phy/Fe was not a sufficient indicator of iron bioavailability if the levels of anti-nutritional factors other than phytate were high.

Zinc in Vitro Availability. Total and available zinc contents, as well as zinc in vitro availability and molar ratios of phytate to zinc (Phy/Zn) of nondephytinized and dephytinized grain fractions, are presented in **Table 4**.

As for total iron content, the total zinc content of bran fraction was higher than that of decorticated fraction but to a lesser extent (2.95 and 1.99 mg/100 g DM, respectively), as zinc is distributed more uniformly than iron in pearl millet grain. For both fractions, treatment with phytases led to significant decreases in total zinc contents by leaching into the medium, as has already been observed in millet flours.

In nondephytinized grain fractions, zinc in vitro availability of bran fraction was significantly higher ($P \leq 0.05$) than that of decorticated fraction with 22.5% and 16.4%, respectively. This difference may be related to the high molar ratio of Phy/Zn of decorticated fraction (31.3) as compared to that of bran fraction, which is slightly lower than 15. This result indicates that phytates play an important role in reducing zinc bioavail-

ability in the core of pearl millet grain, which was confirmed by the increase from 16.4% to 45.2% in zinc in vitro availability observed after dephytinization of decorticated fraction. As for iron, a significant interaction between the nature of the fraction and dephytinization ($P < 0.0001$) was observed as the zinc in vitro availability of bran fraction was not significantly changed after dephytinization. Few studies have investigated the effect of tannins on zinc absorption, but they appear to have little influence (50). On the other hand, the inhibitory effect of certain fibers such as hemicelluloses, cellulose, or wheat bran has been reported (8, 51), although there is no consensus about the effect of fibers. Indeed, a beneficial effect on zinc absorption was found by Hara et al. (52) after the addition of fibers from cornhusk in rat diets.

Thus, in peripheral parts of pearl millet grain, zinc in vitro availability also appears limited by compounds other than phytate, and the effect of fibers and iron binding phenolic compounds on zinc in vitro availability should be further examined.

In summary, phytate degradation occurring during incubation allows iron and zinc in vitro availabilities of pearl millet flour to be doubled. The addition of exogenous phytases did not lead to additional improvement in iron and zinc in vitro availability as compared to that obtained after action of endogenous phytases. A reduction of iron and zinc in vitro availabilities was even observed in some cases, indicating that this protein addition may affect mineral accessibility by changing molecule interactions in the food matrix. Furthermore, phytate are not the only components that reduce iron and zinc bioavailability in pearl millet grain. A large proportion of iron and zinc located in the core of the millet grain are chelated to phytate, but iron and zinc located in the hulls appear to be more closely linked to fibers and/or to iron binding phenolic compounds. To further investigate the effects of antinutritional factors on iron and zinc bioavailability, in vitro studies using a more specific enzymatic approach should be undertaken and will likely provide interesting results about mineral and chelator interactions. In the longer term, a study taking into account the complex physiological conditions of gastric digestion, followed by the simulation of intestinal absorption using Caco-2 cell culture model, could be performed to confirm these antinutritional factor and mineral interactions.

ABBREVIATIONS USED

ADF, acid detergent fiber; DM, dry matter; IP6, IP5, ..., *myo*-inositol 6-phosphate, *myo*-inositol 5-phosphate, ...; NDF, neutral detergent fiber; SD, standard deviation.

ACKNOWLEDGMENT

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