JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Relative Contribution of Phytates, Fibers, and Tannins to Low Iron and Zinc *in Vitro* Solubility in Pearl Millet (*Pennisetum glaucum*) Flour and Grain Fractions

ISABELLE LESTIENNE,† BERTRAND CAPORICCIO,‡ PIERRE BESANÇON,‡ ISABELLE ROCHETTE,† AND SERGE TRÈCHE*,†

Centre IRD de Montpellier, UR 106 "Nutrition, Alimentation, Sociétés", BP 64501, 911, Avenue Agropolis, 34394 Montpellier Cedex 5, France, and Équipe "Nutrition et Aliments", EA 3762, Université Montpellier II, Place Eugène Bataillon, 34095 Montpellier Cedex 05, France

In vitro digestions were performed on pearl millet flours with decreased phytate contents and on two dephytinized or nondephytinized pearl millet grain fractions, a decorticated fraction, and a bran fraction with low and high fiber and tannin contents, respectively. Insoluble residues of these digestions were then incubated with buffer or enzymatic solutions (xylanases and/or phytases), and the quantities of indigestible iron and zinc released by these different treatments were determined. In decorticated pearl millet grain, iron was chelated by phytates and by insoluble fibers, whereas zinc was almost exclusively chelated by phytates. In the bran of pearl millet grain, a high proportion of iron was chelated by iron-binding phenolic compounds, while the rest of iron as well as the majority of zinc were chelated in complexes between phytates and fibers. The low effect of phytase action on iron and zinc solubility of bran of pearl millet grain shows that, in the case of high fiber and tannin contents, the chelating effect of these compounds was higher than that of phytates.

KEYWORDS: Complexes; antinutritional factors; iron; zinc; pearl millet; enzymatic approach

INTRODUCTION

The bioavailability of a mineral is defined as the proportion of the total amount of the mineral in a food, meal, or diet that is actually utilized to ensure normal metabolic functions. To be in the form that is available for absorption by enterocytes, the mineral must first be solubilized in the digestive tract, which is influenced by different dietary and physiological factors. In cereal- and legume-based foods, the availability of iron and zinc for absorption is limited by the presence of antinutritional factors (ANF) in the grains. Phytates, tannins, and certain insoluble fibers are the main compounds that can interact with iron and/or zinc ions (*1*, *2*). The presence of negatively charged groups on these molecules results in the creation of mostly insoluble complexes with divalent cations, which means that they can no longer be absorbed during intestinal digestion. These complexes can be made from two or more compounds, either simple ANF−mineral complexes or complexes involving certain ANF that already interact among themselves or with other compounds of the matrix. For instance, in his review, Reddy reported that most phytates were associated with fibers in cereal grains (*3*). The latest definition of fibers given by the French agency for food sanitary security (AFSSA) includes phytates as one of their constituents (*4*). Thus, there are some complexes between fibers and phytates that are able to chelate minerals and result in fiber−phytate−mineral complexes. In addition, ANF, in particular phytates (*5*) and condensed tannins (*6*), are known for their ability to complex proteins. Phytates are complexed with proteins either directly if the pH is lower than the isoelectric pH of proteins or indirectly by the means of a cation if the pH is higher. Thus, phytates can create different types of complexes depending upon the pH. However, this effect of pH on the nature of complexes has not been highlighted for tannin−protein or tannin−mineral−protein complexes.

Because of (i) the low iron and zinc bioavailability in cereal-based foods and (ii) the importance of pearl millet consumption in Sahelian Africa, we studied the iron and zinc *in vitro* availability in pearl millet flours with varying phytate, tannin, and fiber contents in a previous investigation (*7*). We showed that the total degradation of phytates made it possible to double iron and zinc *in vitro* availability of raw pearl millet flour but did not have any effect on iron and zinc *in vitro* availability of bran of pearl millet grains. Given that hulls of pearl millet grain are particularly rich in tannins, fibers, and minerals, the bioavailability of iron and zinc in bran was probably reduced by the presence of complexes between these compounds.

The aim of the present study was to try to identify the nature of the complexes between antinutritional factors and iron and zinc in pearl millet grain. To this end, we evaluated the effect of the action of fiber- and/or phytate-degrading enzymes on solubilization of iron and zinc from insoluble residues obtained

* To whom correspondence should be addressed. Telephone: (33) 4-67-41-62-95. Fax: (33) 4-67-41-63-30. E-mail: treche@mpl.ird.fr.
† Centre IRD de Montpellier.
‡ Université Montpellier II.

10.1021/jf050741p CCC: $30.25
Published on Web 09/20/2005

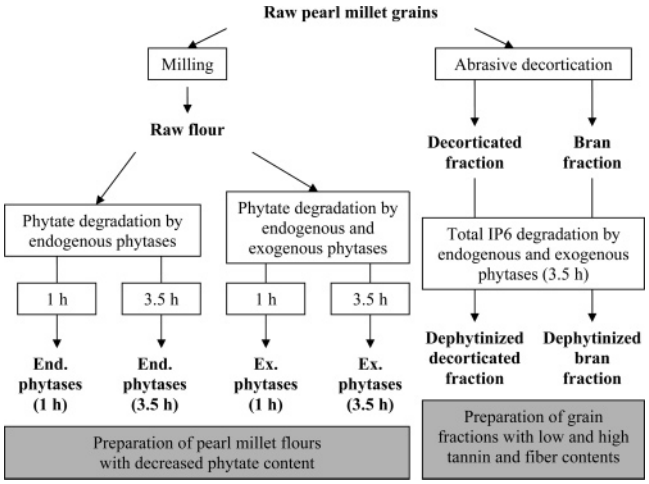

Figure 1. Sample preparation and momenclature.

after *in vitro* digestion (i) of pearl millet flour with decreased phytate contents and (ii) of two pearl millet grain fractions with low or high tannin and fiber contents, after total phytate degradation or not.

MATERIALS AND METHODS

Materials. Two kinds of samples were used (i) samples with different levels of phytates obtained after incubation of raw pearl millet flour with buffer (action of endogenous phytases) or with exogenous phytase preparation (action of endogenous and exogenous phytases) and (ii) dephytinized or nondephytinized samples with low and high fiber and tannin contents: a decorticated grain fraction (low fiber and tannin contents) and a bran fraction made of peripheral parts of grain (high fiber and tannin contents). **Figure 1** summarizes their preparation as well as their nomenclature.

Samples with decreased phytate contents were obtained by incubating raw pearl millet flour in 0.1 M acetate buffer at pH 5.0 (1/2, p/p), with or without exogenous phytases, for a short (1 h) or long (3.5 h) period, at 37 °C under low shaking (60 rpm) in an incubator (New Brunswick Scientific Co., Inc., Edison, NJ). Two different exogenous phytases were used, one plant phytase from wheat (Sigma, P-1259) at 144 units/L (1.1 units/g of pearl millet flour or grain fractions) and one microbial phytase from *Aspergillus ficuum* (Sigma, P-9792) at 516 units/L (3.9 units/g of pearl millet flour or grain fractions). 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.

A more detailed description of the sample preparation as well as total iron and zinc contents of the samples and their iron and zinc *in vitro* digestibility are given in a previous paper (*7*). The antinutritional factor contents of these samples are listed in **Table 1**.

Analytical Methods. *Acid and Neutral Detergent Fiber.* Acid and neutral detergent fiber contents were determined according to the gravimetric methods of van Soest (*8*) and van Soest and Wine (*9*) using a Dosi-fiber (Selecta, Barcelona, Spain). These measurements correspond approximately to the determination of cellulose and lignin content for ADF and of cellulose, lignin, and hemicellulose content for NDF, which are the most likely compounds of fiber to chelate minerals.

Iron-Binding Phenolic Compound. Iron-binding phenolic compound content was determined according to the method of Brune et al. (*10*) using a ferric ammonium sulfate reagent that enabled the measurement of galloyl (expressed as tannic acid equivalents) and catechol group contents (expressed as catechin equivalents) at two wavelengths (578 and 680 nm, respectively).

Phytate. Phytate content was estimated by determination of *myo*-inositol hexaphosphate (IP6) content obtained by anion-exchange HPLC separation using the method described by Lestienne et al. (*7*), after phytate extraction according to the method of Talamond et al. (*11*).

Total Iron and Zinc. 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. (*12*).

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. (*13*) with modifications. Gastric digestion (pepsin, HCl) was performed at pH 2 for 1 h followed by intestinal digestion (pancreatin and bile extract) at pH 7 for 2 h, and the mixtures were then centrifuged at 10000*g* for 30 min at 4 °C (*7*). The supernatants were analyzed using atomic absorption spectrophotometry (*12*) for *in vitro* available iron and zinc, including soluble-free ionizable iron and zinc and soluble complexes of iron and zinc, i.e., iron and zinc that have undergone the first essential but not sufficient step to become available for absorption.

Treatments of the Insoluble Residues of *in Vitro* Digestion. To identify the factors responsible for inhibiting intestinal absorption of iron and zinc, the insoluble *in vitro* digestion residues were incubated with phytate- and/or fiber-degrading enzymes. This method was inspired by the study of Hocquellet and L'Hotelier (*14*) with several modifications.

Table 1. Phytate (IP6, g/100 g of DM), Tannin (Iron-Binding Phenolic Compounds, Galloyl and Catechol Groups, g of Tannic Acid, and Catechin eq/100 g of DM) and Fiber (ADF and NDF, g/100 g of DM) Contents[a] of Pearl Millet Flours and Grain Fractions

pearl millet flours and grain fractions	phytates[b]	tannins[b]	ADF fibers	NDF fibers
raw	0.592 ± 0.032 b	0.602 ± 0.011 b	3.08 ± 0.28 c	6.22 ± 0.17 f
endogenous phytases (1 h)	0.296 ± 0.006 d (–50)	0.464 ± 0.010 c (–23)	2.51 ± 0.23 de	7.48 ± 0.08 d
endogenous phytases (3.5 h)	0.084 ± 0.006 f (–86)	0.368 ± 0.008 e (–39)	2.72 ± 0.04 d	8.36 ± 0.36 c
exogenous phytases (1 h)	0.118 ± 0.008 e (–80)	0.258 ± 0.022 f (–57)	2.23 ± 0.06 e	6.82 ± 0.04 e
exogenous phytases (3.5 h)	0.008 ± 0.000 g (–99)	0.140 ± 0.013 g (–77)	2.18 ± 0.07 e	6.86 ± 0.15 e
decorticated fraction	0.633 ± 0.021 a	0.361 ± 0.01 e	1.80 ± 0.30 f	3.04 ± 0.39 g
dephytinized decorticated fraction	0.002 ± 0.000 g (–100)	0.117 ± 0.01 h (–70)	1.31 ± 0.11 g	3.14 ± 0.13 g
bran fraction	0.409 ± 0.019 c	1.427 ± 0.03 a	5.16 ± 0.39 b	19.05 ± 0.21 b
dephytinized bran fraction	0.000 ± 0.000 g (–100)	0.392 ± 0.01 d (–73)	6.54 ± 0.07 a	22.91 ± 0.34 a

[a] Values are means ± SD of three measurements. 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 in parentheses are the percentages of reduction in phytate or tannin contents because of treatments with endogenous or endogenous and exogenous phytases.

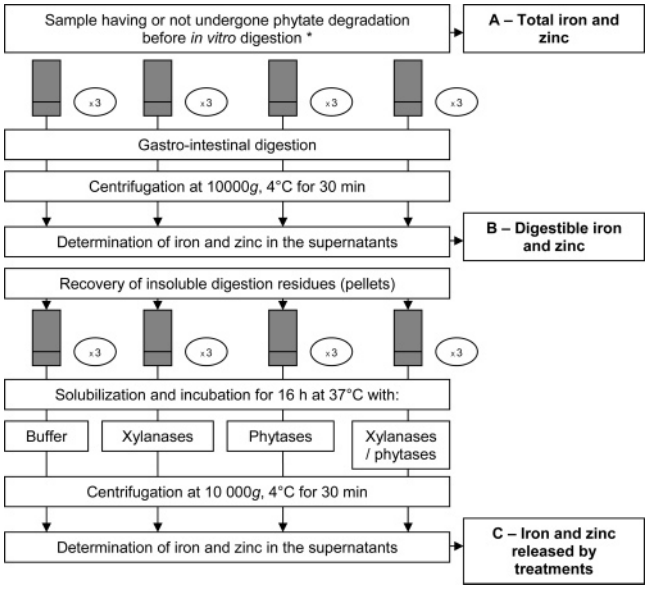

Figure 2. Experimental protocol. *For the totally dephytinized samples, only the treatments with buffer and xylanase solution were carried out.

Solution of xylanases (Fluka, 95595) at 12000 units/L (120 units/g of sample), solution of phytases from *Aspergillus ficuum* (Sigma, P 9792) and from wheat (Sigma, P 1259) at 516 units/L (5.16 units/g of sample) and 144 units/L (1.44 units/g of sample), respectively, and solution of xylanases and phytases (containing the same quantities of enzyme as the preceding enzyme solutions) were prepared in 0.1 M acetate buffer at pH 5.0. These solutions were prepared immediately before use and were agitated with resin Chelex-100 (Bio-Rad, 142-2842) to remove cations and filtered on ashless Whatman filter number 41. All of the experiments were performed in a period of 3 months to limit the loss of enzyme activity because of the storage. The different treatments applied to the insoluble *in vitro* digestion residues of a sample are summarized in **Figure 2**.

A total of 12 independent gastrointestinal digestions were carried out per sample to obtain 12 insoluble residues on which three replications of each of the four different treatments were carried out: incubation in 0.1 M acetate buffer at pH 5.0 without the enzyme, in xylanase solution, phytase solution, or xylanase and phytase solution. Each residue, obtained from 2 g of sample, was solubilized in 20 mL of solution. After homogenization, the mixtures were incubated under magnetic stirring (100 rpm) in a shaking water bath at 37 °C for 16 h. Suspensions were then centrifuged at 10000*g* for 30 min at 4 °C. The supernatants were recovered in silica caps to determine the quantities of iron and zinc released by each treatment using atomic absorption spectrophotometry (*12*).

Expression of Results and Statistical Analysis. The quantities of iron and zinc: total (A), digestible (B), and released by the different treatments (C) were expressed as percentages of dry matter (**Figure 2**). The data presented are means of solubility ± standard deviation (SD) determined after three replications of each treatment and calculated from the quantities of released iron or zinc.

The results are presented in two ways: (i) histograms representing iron or zinc solubility of insoluble residues after the different treatments and (ii) tables giving iron or zinc solubility after the cumulated effect of *in vitro* gastrointestinal digestion and treatment of the insoluble residues.

The solubility rates shown in the histograms, taking into account the effect of each treatment applied to insoluble residues, were calculated according to the following equation:

$$\text{solubility H (\%)} = \frac{\text{quantity of iron (or zinc) released by a treatment after } \textit{in vitro} \text{ digestion of 2 g of sample (C)}}{\text{total quantity of iron (or zinc) in 2 g of sample (A)} - \text{quantity of iron (or zinc) solubilized from 2 g of sample during } \textit{in vitro} \text{ digestion (B)}} \times 100$$

H solubilities are shown in the histograms.

The solubility rates shown in the tables, taking into account the effect of the treatment applied to the residue as well as that of the *in vitro* gastrointestinal digestion that preceded it, were calculated according to the following equation:

$$\text{solubility T (\%)} = \frac{\text{quantity of iron (or zinc) solubilized from 2 g of sample during } \textit{in vitro} \text{ digestion (B)} + \text{quantity of iron (or zinc) released by a treatment after } \textit{in vitro} \text{ digestion of 2 g of sample (C)}}{\text{total quantity of iron (or zinc) in 2 g of sample (A)}} \times 100$$

T solubilities are shown in the tables.

Analyses of variance (one-way ANOVA) were carried out with Statgraphics Plus version 5.0 using Duncan's multiple range test to separate means of solubility presented in the tables with the significance of differences at the 5% level.

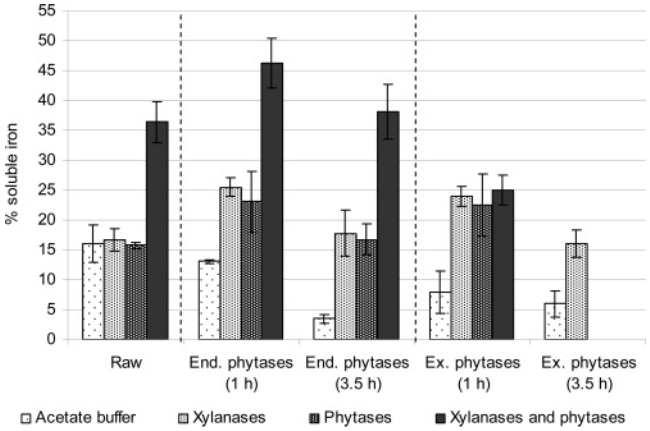

Figure 3. Solubility of indigestible iron (%) of pearl millet flours after treatment of insoluble digestion residues with acetate buffer, xylanases, phytases, or xylanases in combination with phytases. The flours had decreased phytate contents because of the action of endogenous or exogenous phytases. Values are means of solubility ± SD calculated from digestible and released quantities of iron determined after three replications of each treatment.

RESULTS AND DISCUSSION

Effects of the Different Antinutritional Factors on Iron Solubility. *Effects of Phytates.* In insoluble residues of raw pearl millet flour, degradation of fibers or phytates did not release more iron than the treatment with buffer (**Figure 3**). On the other hand, simultaneous action of xylanases and phytases made it possible to release approximately 20% units more iron than that released with the treatment without enzymes. Thus, only the simultaneous degradation of fibers and phytates allowed the solubilization of some iron, which indicates that part of the indigestible iron is linked to fiber−phytate complexes.

In samples with decreased phytate contents after the action of endogenous phytases (endogenous phytases), degradation of fibers or phytates allowed solubilization of about 12% units more iron than that solubilized by treatment with buffer, while

Table 2. Solubility of Iron (%) after Gastrointestinal Digestion and Treatment of Insoluble Residues of Pearl Millet Flours with Decreased Phytate Contents[a]

pearl millet flours	digestion + buffer	digestion + xylanases	digestion + phytases	digestion + xylanases and phytases
raw	24.4 ± 1.6 b	24.9 ± 2.4 c	24.2 ± 0.8 b	42.7 ± 2.3 c
endogenous phytases (1 h)	32.3 ± 3.7 a	42.0 ± 2.4 a	40.2 ± 2.2 a	58.2 ± 2.5 a
endogenous phytases (3.5 h)	26.8 ± 0.7 b	37.6 ± 2.3 b	36.8 ± 1.4 a	53.1 ± 3.1 b
exogenous phytases (1 h)	24.4 ± 1.7 b	37.6 ± 0.7 b	36.4 ± 3.1 a	38.4 ± 2.8 c
exogenous phytases (3.5 h)	26.2 ± 4.2 b	34.2 ± 1.2 b		

[a] Values are means of solubility ± SD calculated from the sum of quantities of digestible (three digestions) and released iron (three replications of each treatment applied to insoluble digestion residues). Values with no common letters in the same column are significantly different ($p \leq 0.05$) as assessed by Duncan's multiple range test.

simultaneous action of the two enzymes led to the release of 20% units more iron. The degradation of phytates before *in vitro* digestion consequently facilitated iron solubilization by phytases and/or xylanases.

With regard to samples with reduced phytate contents after the action of endogenous and exogenous phytases (exogenous phytases), degradation of fibers or phytates allowed solubilization of a larger quantity of iron than treatment with buffer, but simultaneous action of the two enzymes did not allow the release of additional iron. Thus, it is possible that exogenous phytases are involved in the formation of complexes with iron, which would then not be released by either action of xylanases or phytases.

Results of the total solubility of iron after the cumulated effect of *in vitro* digestion (B) and treatment of insoluble residues (C) also showed inhibition of iron solubilization in samples treated with exogenous phytases before *in vitro* digestion (**Table 2**). Indeed, the proportions of iron solubilized after digestion and treatment with xylanases were lower in samples treated with exogenous phytases (38 and 34% for samples incubated for 1 and 3.5 h, respectively) than for the other pearl millet flours, after *in vitro* digestion and treatment with xylanases and phytases (between 43 and 58%).

In short, about 43% of iron in raw pearl millet flour was solubilized after *in vitro* digestion and simultaneous action of xylanases and phytases, against about 24% after action of only one of the two enzymes. Thus, a high proportion of iron in pearl millet flour seems to be linked to fiber−phytate complexes. Partial phytate degradation by action of endogenous phytases before *in vitro* digestion increased the proportion of iron that can be solubilized. On the other hand, treatment of pearl millet flour by addition of exogenous phytases before *in vitro* digestion decreased the proportion of iron that can be solubilized, probably because of interactions between added proteins and other compounds of the matrix likely to chelate iron.

Effects of Fibers and Tannins. Action of xylanases or phytases on insoluble digestion residues of the decorticated fraction (**Figure 4**) caused more iron to be released than treatment without enzymes (approximately 16% units of additional soluble iron). Concerning residues of the bran fraction, there was also a significant increase in iron solubility ($p \leq 0.05$) after action of xylanases or phytases but to a lesser extent (less than 3% units). Simultaneous action of the two enzymes led to the release of more than 20 and 15% units of additional iron for residues of decorticated and bran fractions, respectively.

These results are comparable with those obtained on residues of dephytinized fractions (**Figure 5**), because, in both fractions, the quantities of iron released by treatment with buffer were very low, whereas the action of xylanases led to the release of more than 40 and 20% units of iron for the decorticated and bran fractions, respectively.

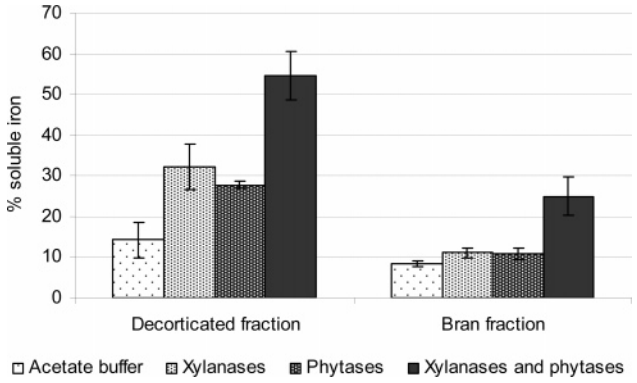

Figure 4. Solubility of indigestible iron (%) of pearl millet grain fractions with low (decorticated fraction) and high (bran fraction) fiber and tannin contents after treatment of insoluble digestion residues. Values are means of solubility ± SD calculated from digestible and released quantities of iron determined after three replications of each treatment.

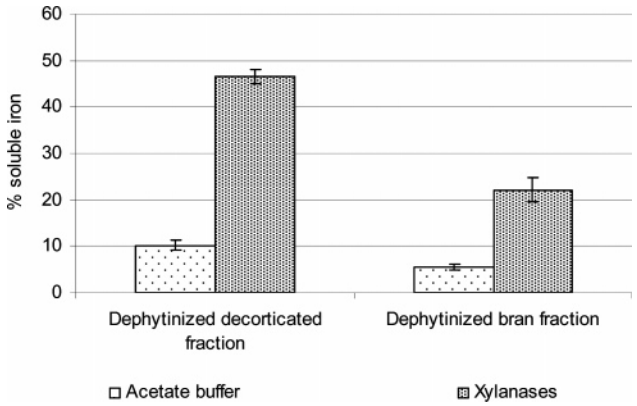

Figure 5. Solubility of indigestible iron (%) of dephytinized pearl millet grain fractions after treatment of insoluble digestion residues. Values are means of solubility ± SD calculated from digestible and released quantities of iron determined after three replications of each treatment.

Thus, it appears that there is no great difference in the nature of the iron-containing complexes present in pearl millet decorticated grains and bran, because, in both fractions, iron mainly appears to be rendered insoluble by complexes in which both phytates and fibers play a role.

When looking at the sum of digestible iron (B) and iron released from insoluble residues (C), simultaneous action of xylanases and phytases allowed solubilization of 61 and 36% of iron for the decorticated and bran fractions, respectively (**Table 3**). These results are similar to those obtained after treatment of dephytinized decorticated and bran fractions with xylanases (66 and 35%, respectively). The marked difference in iron solubility between the decorticated and bran fraction after these treatments is probably due to the fact that the iron-

Table 3. Solubility of Iron (%) after Gastro-intestinal Digestion and Treatment of Insoluble Residues of Nondephytinized and Dephytinized Pearl Millet Grain Fractions[a]

pearl millet grain fractions	digestion + buffer	digestion + xylanases	digestion + phytases	digestion + xylanases and phytases
decorticated fraction	26.1 ± 2.3 b	41.6 ± 2.5 b	37.6 ± 3.7 a	60.8 ± 5.5 a
bran fraction	21.4 ± 3.0 b	23.8 ± 1.8 d	23.5 ± 1.6 b	35.7 ± 3.3 b
dephytinized decorticated fraction	43.2 ± 1.7 a	66.2 ± 0.4 a		
dephytinized bran fraction	21.0 ± 3.8 b	34.9 ± 1.4 c		

[a] Values are means of solubility ± SD calculated from the sum of quantities of digestible (three digestions) and released iron (three replications of each treatment applied to insoluble digestion residues). Values with no common letters in the same column are significantly different ($p \leq 0.05$) as assessed by Duncan's multiple range test.

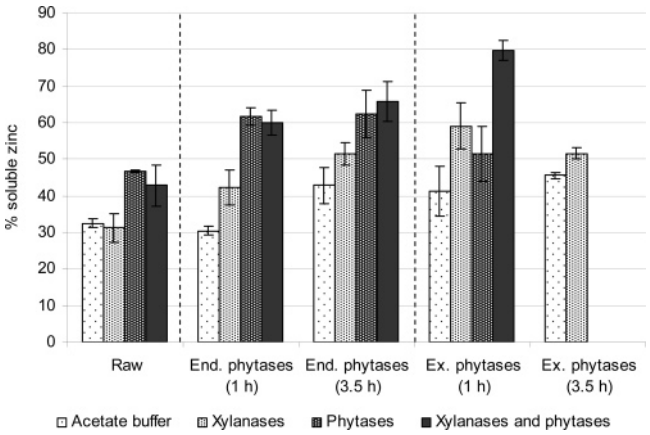

Figure 6. Solubility of indigestible zinc (%) of pearl millet flours after treatment of insoluble digestion residues with acetate buffer, xylanases, phytases, or xylanases in combination with phytases. The flours had decreased phytate contents because of the action of endogenous or exogenous phytases. Values are means of solubility ± SD calculated from digestible and released quantities of zinc determined after three replications of each treatment.

binding phenolic compound content is much greater in the bran fraction than in the decorticated fraction (1.43 versus 0.36 g/100 g of DM). Indeed, some studies have already shown that some phenolic compounds were involved in the reduction of iron solubility (*15*, *16*, *17*, *18*) and that their inhibiting effect was dose-dependent (*19*, *20*).

Enzymatic degradation of phytates and fibers enabled solubilization of around 63 and 35% of iron contained in decorticated and bran fractions, respectively, against only approximately $^2/_3$ after action of one of the two enzymes (around 40 and 23% for decorticated and bran fractions, respectively). The results of the study of these fractions tend to confirm the presence of fiber−phytate−iron complexes in pearl millet grain and also indicate the presence of tannin−iron complexes that render insoluble the majority of iron contained in the bran of pearl millet grains.

Effects of the Different Antinutritional Factors on Zinc Solubility. *Effects of Phytates.* In insoluble residues of raw pearl millet flour (**Figure 6**), degradation of fibers did not allow the release of more zinc than the treatment with buffer. On the other hand, phytate degradation allowed the release of about 14% units more zinc than that released by the treatment with buffer, while simultaneous action of xylanases and phytases did not lead to an additional release of zinc compared to the action of the single phytases. It thus appears that phytates are responsible for the inhibition of zinc solubilization.

The study of pearl millet flours that underwent a reduction in phytate content before *in vitro* digestion confirmed this first result. In samples whose phytate contents were reduced by action of endogenous phytases, the greater the degree of phytate degradation, the greater the quantities of zinc released by the different treatments. This can be attributed to the stronger hydrolysis of phytate molecules that decreased from 6 or 5 phosphates to less than 5 phosphates (IP4, IP3, IP2, and IP1), which, according to Lönnerdal et al. (*21*), does not appear to have any negative effect on zinc bioavailability.

Furthermore, in samples with decreased phytate contents (endogenous and exogenous phytases), treatment with xylanases allowed slightly more zinc to be solubilized than the treatment with buffer. It thus seems that fibers play a role in the chelation of zinc in pearl millet flour and that this minor role was detectable only when the majority of phytates was degraded.

The values for total zinc solubility after the combined action of *in vitro* digestion and treatment of insoluble residues (**Table 4**) allow the effects of phytates related to fibers to be put into perspective. Thus, when phytates were partially degraded by endogenous phytases before *in vitro* digestion, the percentages of soluble zinc after digestion and incubation with buffer increased from 42 to 47 and 59% for samples containing 0.59, 0.30, and 0.08 g of IP6/100 g of DM, respectively (**Table 1**). Furthermore, after the action of xylanases, the solubility of zinc in samples with decreased phytate contents because of the action of endogenous phytases before *in vitro* digestion (endogenous phytases) increased from 47 to 56% for flour incubated for 1 h and from 59 to 65% for flour incubated for 3.5 h, revealing the slightly negative effect of fibers on zinc solubility.

Effects of Fibers and Tannins. Separate action of xylanases and phytases on insoluble digestion residues of decorticated fraction with low fiber and tannin contents led to a greater release of zinc (**Figure 7**) than treatment without enzymes (+10 and +30% units, respectively). The simultaneous action of the two enzymes did not allow more zinc to be released than the action of single phytases. Concerning the bran fraction with high fiber and tannin contents, the action of xylanases did not lead to additional zinc solubilization compared to the treatment without enzymes. On the other hand, the action of phytases and the simultaneous action of the two enzymes allowed about 8 and 25% units of additional zinc to be solubilized, respectively, in comparison with treatment without enzymes. These results indicate that the nature of the complexes between zinc and antinutritional factors is probably not the same in decorticated and bran fractions of pearl millet grains. Zinc from decorticated grains is probably mainly chelated by phytates and only slightly by fibers, whereas that of bran is probably mainly linked to fiber−phytate complexes.

These conclusions on the nature of the complexes were also obtained in the dephytinized fraction (**Figure 8**), with a majority of phytate−zinc complexes and a low proportion of fiber−zinc complexes, although the quantities of solubilized zinc were different. Indeed, in the dephytinized decorticated fraction, all of the zinc was released by the action of phytases followed by that of xylanases, whereas about 40% of the zinc remained insoluble in the case of simultaneous action of the two enzymes

Table 4. Solubility of Zinc (%) after Gastrointestinal Digestion and Treatment of Insoluble Residues of Pearl Millet Flours with Decreased Phytate Contents[a]

pearl millet flours	digestion + buffer	digestion + xylanases	digestion + phytases	digestion + xylanases and phytases
raw	42.2 ± 1.4 c	41.1 ± 1.7 c	54.3 ± 1.4 b	51.1 ± 3.3 d
endogenous phytases (1 h)	47.0 ± 3.4 bc	56.0 ± 1.3 b	70.8 ± 2.2 a	69.6 ± 1.5 c
endogenous phytases (3.5 h)	58.5 ± 2.5 a	64.8 ± 2.8 a	72.7 ± 4.2 a	75.2 ± 3.5 b
exogenous phytases (1 h)	51.2 ± 5.3 b	65.9 ± 4.9 a	64.7 ± 6.9 a	83.2 ± 2.1 a
exogenous phytases (3.5 h)	62.5 ± 2.1 a	66.6 ± 2.4 a		

[a] Values are means of solubility ± SD calculated from the sum of quantities of digestible (three digestions) and released zinc (three replications of each treatment applied to insoluble digestion residues). Values with no common letters in the same column are significantly different ($p \leq 0.05$) as assessed by Duncan's multiple range test.

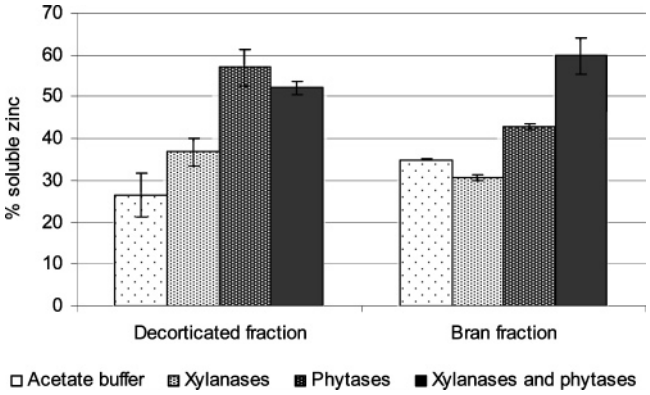

Figure 7. Solubility of indigestible zinc (%) of pearl millet grain fractions with low (decorticated fraction) and high (bran fraction) fiber and tannin contents after treatment of insoluble digestion residues. Values are means of solubility ± SD calculated from digestible and released quantities of zinc determined after three replications of each treatment.

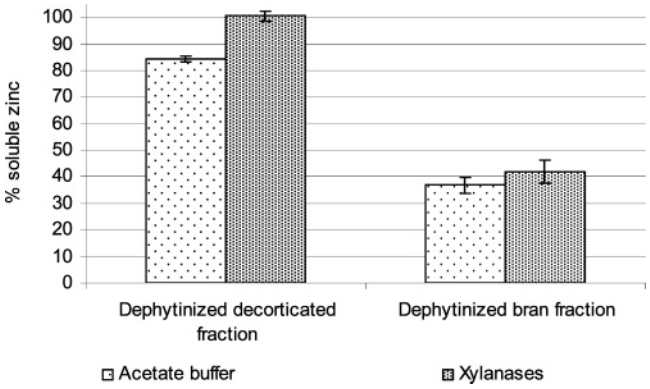

Figure 8. Solubility of indigestible zinc (%) of dephytinized pearl millet grain fractions after treatment of insoluble digestion residues. Values are means of solubility ± SD calculated from digestible and released quantities of zinc determined after three replications of each treatment.

on the dephytinized fraction. This result is similar to the results previously obtained on pearl millet flours (**Figure 6**), showing that the greater the degree of phytate degradation before *in vitro* digestion, the greater the quantity of zinc released by the different treatments. This could be explained by the major role of IP5 and possibly of IP4 in zinc complexation by phytates (*22*, *23*). Conversely, in the bran fraction, the proportion of soluble zinc after the action of xylanases on residues of the dephytinized fraction was lower than with the simultaneous action by xylanases and phytases on those of the nondephytinized fraction (about 40 versus 60%). This indicates that, like iron, zinc in the bran of pearl millet grain is probably linked to fiber−phytate complexes whose hydrolysis necessitates combined action of xylanases and phytases.

If we consider the data in **Table 5**, which shows total solubility after *in vitro* digestion and treatment of insoluble residues, we can also assume the presence of other complexes in the bran of pearl millet grains. Indeed, a maximum of only 69% of zinc from bran fractions was released by the different treatments, against 100% from the dephytinized decorticated fractions. This could be due to tannin−zinc or protein−zinc complexes in the bran of pearl millet grains. To our knowledge, these kinds of complexes have not yet been mentioned in the literature, but our results tend to prove their existence. The lack of information on these possible complexes can be explained by the fact that the particularly strong phytate contribution to low zinc bioavailability probably masks these interactions between zinc and other chelator compounds in food matrixes.

Thus, single enzymatic degradation of phytates from decorticated pearl millet grains allowed solubilization from 64 to 92% of zinc depending upon the treatment, which confirms the major contribution of phytates to low zinc bioavailability in cereal-based food. However, the study of the bran fraction revealed that single phytate degradation did not enable an increase in zinc solubility, which indicates the presence of other complexes. Zinc in pearl millet grain hulls is thus probably partly linked to fiber−phytate complexes, and it is also possible that zinc is partially chelated by some tannins or proteins of the matrix, which requires further study.

In summary, the iron in pearl millet flour was mainly chelated by complexes involving both fibers and phytates, so that this

Table 5. Solubility of Zinc (%) after Gastrointestinal Digestion and Treatment of Insoluble Residues of Nondephytinized and Dephytinized Pearl Millet Grain Fractions[a]

pearl millet grain fractions	digestion + buffer	digestion + xylanases	digestion + phytases	digestion + xylanases and phytases
decorticated fraction	38.7 ± 2.3 c	47.2 ± 1.8 c	64.2 ± 2.5 a	60.0 ± 0.3 b
bran fraction	49.6 ± 1.4 b	46.2 ± 2.2 c	55.7 ± 1.0 b	68.9 ± 2.5 a
dephytinized decorticated fraction	91.5 ± 1.0 a	100.3 ± 1.0 a		
dephytinized bran fraction	51.2 ± 1.5 b	55.2 ± 2.4 b		

[a] Values are means of solubility ± SD calculated from the sum of quantities of digestible (three digestions) and released zinc (three replications of each treatment applied to insoluble digestion residues). Values with no common letters in the same column are significantly different ($p \leq 0.05$) as assessed by Duncan's multiple range test.

iron was solubilized by simultaneous action of xylanases and phytases. However, in the bran of pearl millet grains that contain high fiber, tannin, and also iron contents, the majority of iron appeared to be chelated by tannins (iron-binding phenolic compounds). Thus, phytates, fibers, and tannins alike decrease the bioavailability of iron in pearl millet flour.

Concerning the zinc of pearl millet flour, it was mainly linked to phytates and the greater the degree of phytate degradation before *in vitro* digestion, the greater the quantity of zinc released. Furthermore, fibers and probably also tannins or some proteins could be involved in the formation of complexes with zinc in the bran of pearl millet grains. Some lower inositol phosphate resulting from phytate hydrolysis (IP5 and IP4) appears to contribute to a considerable extent to low zinc bioavailability in pearl millet flour. It would be useful to verify this hypothesis by determining the residual contents in these degradation compounds at the different steps of our experimental protocol.

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

The authors thank the INERA station of Kamboincé (CNRST, Burkina Faso) for providing pearl millet grains. Our thanks also goes to J. P. Guyot, C. Icard-Vernière, and V. Lullien-Péllerin (INRA, Montpellier) for their help.

LITERATURE CITED

(1) Gilloly, M.; Bothwell, T. H.; Charlton, R. W.; Torrance, J. D.; Bezwoda, W. R.; MacPhail, A. P.; Derman, D. P.; Novelli, L.; Morral, D.; Mayet, F. Factors affecting the absorption of iron from cereals. *Br. J. Nutr*. **1984**, *51*, 37−46.

(2) Camara, E.; Amaro, M. A. Nutritional aspect of zinc availability. *Int. J. Food Sci. Nutr*. **2003**, *54*, 143−151.

(3) Reddy, N. R. Occurrence, distribution, content, and dietary intake of phytate. In *Food Phytates*; Reddy, N. R., Sathe, S. K., Eds.; CRC Press: Boca Raton, FL, **2002**; pp 25−51.

(4) Champ, M. Les fibres alimentaires: Définitions et aspects analytiques. *La Lettre Scientifique de l'IFN*. **2003**, 91.

(5) Wise, A. Phytate and zinc bioavailability. *Int. J. Food Sci. Nutr*. **1995**, *46*, 53−63.

(6) Bravo, L. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. *Nutr. Rev.* **1998**, *56*, 317−333.

(7) Lestienne, I.; Besançon, P.; Caporiccio, B.; Lullien-Péllerin, V.; Trèche, S. Iron and zinc *in vitro* availability in pearl millet flours (*Pennisetum glaucum*) with varying phytate, tannin, and fiber contents. *J. Agric. Food Chem.* **2005**, *53*, 3240−3247.

(8) van Soest, P. S. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. *J. Assoc. Off. Anal. Chem.* **1963**, *46*, 829−835.

(9) van Soest, P. S.; Wine, R. H. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. *J. Assoc. Off. Anal. Chem.* **1967**, *50*, 50−55.

(10) Brune, M.; Rossander, L.; Hallberg, L. Iron absorption and phenolic compounds: Importance of different phenolic structures. *Eur. J. Clin. Nutr*. **1989**, *43*, 547−557.

(11) Talamond, P.; Doulbeau, S.; Rochette, I.; Guyot, J. P.; Trèche, S. Anion-exchange high-performance liquid chromatography with conductivity detection for the analysis of phytic acid in food. *J. Chromatogr., A* **2000**, *871*, 7−12.

(12) Laporte, J.; Kovacsik, G.; Pinta, M. Milieux végétaux. In *Spectrométrie d'absorption atomique*, 2nd ed.; Pinta, M., Ed.; Masson ORSTOM: Paris, France, 1980; Vol. 2, pp 378−421.

(13) Lönnerdal, B.; Yuen, M.; Glazier, C.; Litov, R. Magnesium bioavailability from human milk, cow milk, and infant formula in suckling rat pups. *Am. J. Clin. Nutr.* **1993**, *58*, 392−397.

(14) Hocquellet, P.; L'Hotellier, M. D. Bioavailability and speciation of mineral micronutrients: The enzymolysis approach. *J. AOAC Int.* **1997**, *80*, 920−927.

(15) Svanberg, U.; Lorri, W.; Sandberg, A. S. Lactic fermentation of non-tannin and high-tannin cereals: Effects of *in vitro* estimation of iron availability and phytate hydrolysis. *J. Food Sci*. **1993**, *58*, 408−412.

(16) Matuschek, E.; Towo, E.; Svanberg, U. Oxidation of polyphenols in phytate-reduced high-tannin cereals: Effect on different phenolic groups and on *in vitro* accessible iron. *J. Agric. Food Chem.* **2001**, *49*, 5630−5638.

(17) Matuschek, E.; Svanberg, U. Oxidation of polyphenols and the effect on *in vitro* iron accessibility in a model food system. *J. Food Sci*. **2002**, *67*, 420−424.

(18) Matuschek, E.; Svanberg, U. The effect of fruit extracts with polyphenol oxidase (PPO) activity on the *in vitro* accessibility of iron in high-tannin sorghum. *Food Chem.* **2005**, *90*, 765−771.

(19) Siegenberg, D.; Baynes, R. D.; Bothwell, T. H.; Macfarlane, B. J.; Lamparelli, R. D.; Car, N. G.; Macphail, P.; Schmidt, U.; Tal, A.; Mayet, F. Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. *Am. J. Clin. Nutr*. **1991**, *53*, 537−541.

(20) Glahn, R. P.; Wortley, G. M.; South, P. K.; Miller, D. D. Inhibition of iron uptake by phytic acid, tannic acid, and ZnCl2: Studies using an *in vitro* digestion/Caco-2 cell model. *J. Agric. Food Chem.* **2002**, *50*, 390−395.

(21) Lönnerdal, B.; Sandberg, A. S.; Sandström, B.; Kunz, C. Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. *J. Nutr*. **1989**, *119*, 211−214.

(22) Sandström, B.; Sandberg, A. S. Inhibitory effects of inositol phosphates on zinc absorption in humans. *J. Trace Elem. Med. Biol*. **1992**, *6*, 99−103.

(23) Han, O.; Failla, M. L.; Hill, A. D.; Morris, E. R.; Smith, J. C. Inositol phosphates inhibit uptake and transport of iron and zinc by a human intestinal cell line. *J. Nutr*. **1994**, *124*, 580−587.

Received for review April 1, 2005. Revised manuscript received August 5, 2005. Accepted August 8, 2005. This work is part of the Cerefer project (www.mpl.ird.fr/cerefer) funded by the European Commission contract number ICA4-CT-2002-10047.

JF050741P