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Iron Contamination during In-Field Milling of Millet and Sorghum

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ABSTRACT: Nutritionally, contaminant iron in foods may lead to overestimation of the satisfaction of iron requirement while iron deficiencies remain a widespread health problem. Iron contamination was measured in millet and sorghum grains after decortication and in-field milling using different equipments in Burkina Faso. Total iron content did not change significantly after decortication, probably due to a balance between losses resulting from the removal of iron-rich peripheral parts and contamination. Total iron contents increased significantly after mechanical milling irrespective of whether iron or corundum grindstones were used. Contamination was highly variable, ranging from 3 to 6 mg iron/100 g DM, and was mainly due to wear of the milling equipment. After in vitro digestion of traditional cereal dishes prepared with iron-contaminated or uncontaminated flours, the contaminant iron was found mainly in the insoluble fraction. Only in sorghum was a small proportion (4%) bioaccessible, showing that contaminant iron has poor nutritional interest.

KEYWORDS: *contamination, decortication, milling, bioavailability, phytate/iron molar ratio, Burkina Faso*

INTRODUCTION

Iron and zinc deficiencies are the most prevalent micronutrient deficiencies worldwide, especially in developing countries.[1] The diet found in these countries is mostly plant-based and then contains mainly nonheme iron, the absorption of which is greatly decreased by dietary factors and by the presence of chelating agents such as phytate or certain phenolic compounds.[2] These compounds also influence the absorption of other divalent cations such as zinc.[3] The bioavailability of iron and zinc is thus lower in plant-based diets than in diets that include foods from both plant and animal sources.

Food processing is known to influence both mineral content and bioavailability.[4] Grain decortication is often part of postharvest processing of cereals in both northern and southern countries. In previous studies, it was shown that during traditional manual or mechanical decortication of millet, iron losses were about 50% and zinc losses ranged from 35 to 46%.[5,6] Mineral losses from sorghum were of the same order of magnitude, whereas phytates and other chelating agents were only partially removed.[5] Analogous results were obtained with rice, with losses reaching almost two-thirds of iron content depending on the whitening technology practices.[7] Other unit operations such as sieving, fermentation, or cooking can lead to iron losses or increases due to contamination.[8]

As reported in a literature review,[9] in developing countries, contaminant iron, also called extrinsic iron, may represent several times the iron content in the staple foods or dishes. This extrinsic iron may originate from food contamination by soil or dust, from iron leaching from iron or steel pots into food during storage and cooking, or from equipment during food processing. As shown in an experimental study in the United States,[10] 70% of the iron in plant samples could originate from soil particulate inclusion by the plant tissues during growth and can be considered as contaminant iron. Despite careful washing of plants or grains during the preparation of most dishes, extrinsic iron is still present in the foods finally consumed. Extrinsic iron may thus considerably increase iron intake. Contaminant iron on the surface of cereals and pulses purchased in a market in India was estimated to comprise between 13 and 47% of total iron.[11] Iron contents up to 20 times higher were found in endogenous Ethiopian teff than in other cereals such as maize and barley and were attributed to soil contamination linked to the traditional method of threshing.[12] A non-negligible part of extrinsic iron in foods can also come from cookware: the iron content of most foods cooked in stainless steel utensils can be higher than that of the same foods cooked in glassware.[13] Similarly, other authors[14] measured from 1.5- to 2-fold more iron in Ethiopian foods cooked in iron pots than in the same foods cooked in aluminum or clay pots. In another study, a significant increase of from 2 to 7 mg/100 g was measured in iron content in the flour after milling, and it was estimated that <2% of this contaminant iron was in absorbable form.[11] In a comparison of the iron contents of rice flour and polished rice from the same Thai market, a difference in iron content of 28.6 mg/100 g was measured, which was attributed to contamination.[15] Using a strong magnet, it was shown that about 60% of this contaminant iron was metal particles originating from the flour mill. More recently, increases in iron content ranging from 43 to 138% were measured between maize grains and flours,[8] which increased with milling intensity. The contaminant iron represented more than intrinsic iron.

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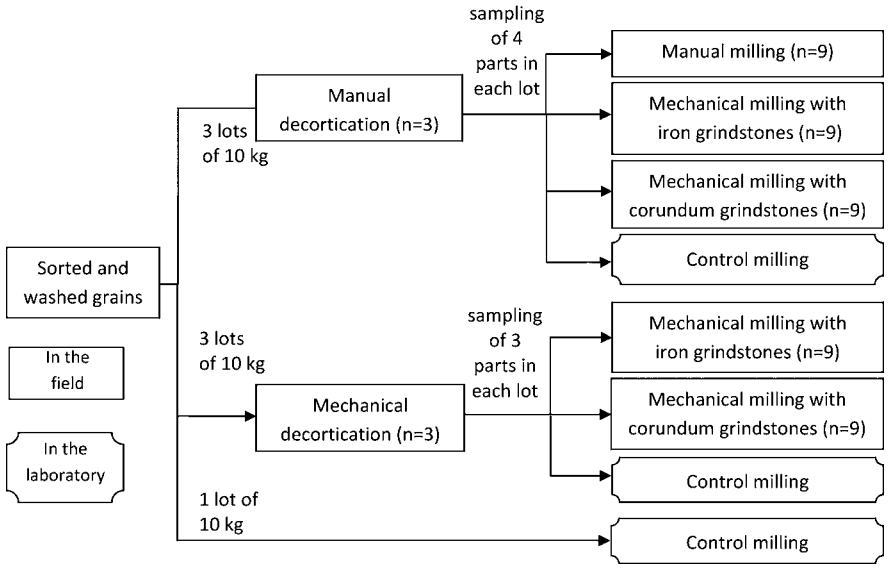

Figure 1. Experimental design for decortication and milling of millet and sorghum.

Beyond its contribution to iron intake, contaminant iron is of nutritional interest only if it improves the iron status. However, this is extremely difficult to measure and has led to controversial results. To be of use, contaminant iron, like intrinsic iron, needs to be exchangeable, that is, soluble in the intestine, to enable it to join the common nonheme pool.[15] There is no simple method to predict the exchangeability of contaminant iron.[12] In a radioiron absorption study to determine soil addition to rice-based Thai meals, it was shown that depending on pH, iron from clay was either partially or not exchangeable.[16] When exchangeable, iron from clay was available for absorption. The most common forms of contaminant iron, ferric oxide and ferric hydroxide, added to maize porridge to simulate contamination, were poorly available, but that addition of ascorbic acid improved the absorption to a level neighboring that of the nonheme dietary iron pool.[17] Lower rates of anemia and better growth were found in children fed from iron pots than in children fed from clay or aluminum pots.[14] In a systematic review, it was also shown that eating foods prepared in iron pots increased the hemoglobin concentration of anemic/iron-deficient individuals.[18]

The main objective of the present work was to measure the iron contamination levels in millet and sorghum grains, the main staple crops in sahelian countries of Africa, from the same seed lots after decortication and milling with different equipment in the field and under controlled laboratory conditions. Zinc contents were determined at the same time. The second objective was to assess the bioaccessibility of contaminant iron using in vitro digestion followed by measurement of dialyzable, soluble, and insoluble iron and, thus, to evaluate its nutritional interest. For the purpose of this study, a cereal-based paste frequently consumed in some African countries was prepared "as eaten" with either highly contaminated or less-contaminated flours.

MATERIALS AND METHODS

Millet and Sorghum. Grains of a yellow local pearl millet (*Pennisetum glaucum*) variety called 'Gampela' and of a local white sorghum (*Sorghum bicolor*) variety called 'Fibmigou' were purchased from local farmers who work for the National Environmental and Agricultural Research Institute (INERA), a research facility in Ouagadougou (Burkina Faso). About 100 kg of each were purchased, well mixed so the same lots of grains have been used throughout the study.

Experimental Design and Processing Methods for Decortication and Milling. The whole experimental design for the preparation of milled samples is presented in Figure 1. The millet and sorghum grains were sorted and washed with tap water in Ouagadougou. Six seed lots of 10 kg of each of the washed millet and sorghum grains were decorticated, three manually by three different women and three mechanically in three different decortication units, and 20 kg of the remaining washed grains were kept as laboratory controls. Manual decortication was performed with a wooden mortar and pestle by women skilled at using this traditional process to decorticate grains at home. Mechanical decortication was performed in small food-processing units using a motorized device of the Engelberg type. In towns, women generally take their grains to this type of unit for decortication. The women at home and the operators in the decortication unit used their know-how to moisten the grains to facilitate decortication (by pouring small quantities of water onto the grains) and for the length of the process. After a sample was removed to measure dry matter content (DMC) (see Analytical Methods), the rest of the lots of decorticated grains were spread out (sheltered from dust) in the air to dry before milling.

For each type of decortication, the three lots of millet and sorghum grains obtained weighed about 8 kg after decortication. The three lots were then further divided into three to four smaller lots weighing 2 kg to undergo the different types of milling. Each lot was then milled either manually with a wooden mortar and pestle (n = 3 different women) or mechanically with a motorized device equipped with either an iron (n = 3) or a corundum millstone (n = 3). Grains that were decorticated mechanically were not milled manually, as this is never the case in practice. The remaining small seed lots were kept for control milling with a laboratory mill IKA M20 (Labortechnik, Staufen, Germany), sieved to pass through a 0.5 mm screen and stored at 4 °C until analysis.

Standardized Cereal Processing into "Tô". A cereal-based thick paste, locally called "tô", was prepared with a dry matter content of 22−23% according to the traditional recipe from Burkina Faso;[19] quantities were adapted for laboratory use (Figure 2). The initial millet or sorghum flour was divided into two parts, a small one of 15 g and a larger one of 25 g. The 15 g of flour was mixed with 40 g of ultrapure water to obtain a flour suspension, which was then mixed with 140 g of boiling ultrapure water. The resulting "thin gruel" was cooked for 2 min, mixed with the remaining 25 g of flour, and cooked again for 6 min under stirring. The resulting paste was the tô. Optionally, as

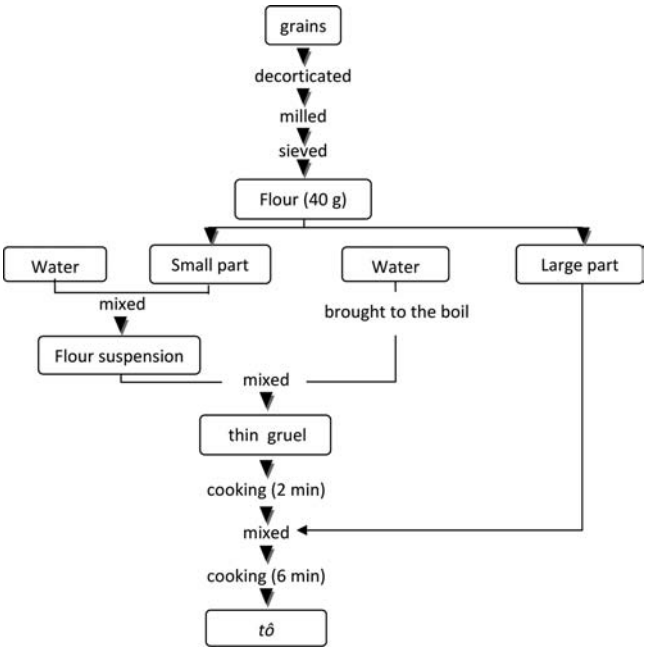

Figure 2. Standardized method for the preparation of tô in the laboratory.

sometimes observed in the field, the pH of the tô was lowered to reach a target value by replacing part of the water with a diluted tamarind decoction. It was checked that the iron content of this decoction was negligible.

Analytical Methods. DM contents were determined by oven-drying at 105 °C to constant weight. Phytate content was determined after extraction in acid solution (0.5 M HCl) at 100 °C for 6 min by measuring the *myo*-inositol hexaphosphate (IP6) content by high-performance anion-exchange chromatography[20,21] and using an AS-11 precolumn and column kit (Dionex, Sunnyvale, CA, USA). Iron and zinc were extracted with a closed-vessel microwave digestion system (ETHOS-1, Milestone, Italy) and analyzed using a Perkin-Elmer AAnalyst 800 atomic absorption spectrometer.[5] Standard reference materials BCR-679 White Cabbage and BCR-191 Brown Bread [IRMM (Institute for Reference Materials and Measurements, European Commission)] were used as controls with iron SpectrAA measurements. The coefficients of variation obtained with these two reference materials were 5.74 and 5.62%, respectively, with distances from the reference value of −1.24% in the case of white cabbage and −4.62% in the case of brown bread.

Iron bioaccessibility was determined by enzymatic in vitro digestion according to previously described methods[8,22] including first the determination of the titratable acidity of samples.[23] For gastric digestion, tô samples were first diluted with ultrapure water and homogenized to obtain a dry matter content neighboring 9−10% in sealed glass flasks and brought to 37 °C in a water bath. α-Amylase (20 μL) from *Bacillus licheniformis* (Sigma A-3403-1MU) was added to the samples, which were then incubated at 37 °C for 5 min. The pH was adjusted to 2.0 with 1 M HCl; 1 mL of pepsin solution was then added (Sigma, P-7000, 14900 u/mL in 0.1 M HCl), and the samples were incubated horizontally for 1 h at 37 °C in a shaking water bath. Aliquots (40 g) of each pepsin-digested sample were then transferred in separate large tubes for the intestinal digestion. To gradually increase the pH to mimic intestinal digestion, a dialysis bag (Spectra/por I dialysis tubing, MWCO 12−14 kDa) containing 20 mL of the piperazine-*N*,*N*′-bis-[2-ethanesulfonic acid] sodium salt (PIPES) buffer (Sigma, P-3768) of the previously determined molarity from titratable acidity results was introduced into each large tube and incubated at 37 °C for 30 min to reach pH 6.7. Five milliliters of enzyme solution, containing pancreatin (Sigma, P1750, 1.85 mg/mL) and bile extract solution (Sigma, B8631, 11 mg/mL in 0.1 M $NaHCO_3$), were then added, and the samples were incubated horizontally for 2 h at 37 °C in a shaking water bath. During incubation, dialyzable iron was diffused into the dialysis bag so that the same concentration was reached on both sides of the membrane.

The dialysis bags were then removed and washed with pure water. Their contents (the dialysates) were weighed. The digestion mixtures remaining in the tubes were centrifuged at 10000*g* for 15 min at 4 °C to separate the insoluble and soluble iron fractions, respectively, in the pellet and supernatant. Thus, the sum of dialyzed, soluble nondialyzed, and insoluble fractions should be equal to the total amount of mineral in the sample before digestion.

The iron contents of the dialysates, supernatants, and pellets were analyzed as described above. The iron contents of the pellets corresponded to the insoluble iron fraction after enzymatic digestion. Dialyzable and soluble nondialyzable (soluble ND) Fe percentages were calculated as

$$\text{dialyzable Fe \%} = C_D(W_D + W_S)/(C_D W_D + C_S W_S + C_I W_I) \times 100$$

and

$$\text{soluble ND Fe \%} = W_S(C_S - C_D)/(C_D W_D + C_S W_S + C_I W_I) \times 100$$

where C_D, C_S, and C_I are iron concentrations in the dialysate, supernatant, and pellet fractions in μg/100 g and W_D, W_S, and W_I are the weights (g) of dialysate, supernatant, and pellet fractions. All samples were analyzed at least in triplicate.

Statistical Analysis. Biochemical analyses were performed in triplicate. Values were averaged. Data were subjected to analysis of variance (ANOVA), and Fischer's least significant difference tests were used to compare means at the 5% significance level, using the software Statgraphics Plus version 5.1. A multifactorial ANOVA analysis was also performed to identify the factors influencing iron content in the decorticated and milled cereal grains, using the same software.

RESULTS AND DISCUSSION

Effect of Decortication and Milling Techniques on Iron and Zinc Content in Millet and Sorghum. The iron contents of the millet and sorghum samples resulting from the different combinations of decortication and milling were determined (Figure 3). Zinc contents were also determined, because of its nutritional interest and also as a negative control of contamination, due to the very low concentration of zinc found in soil and dust or in iron or corundum grindstones. In the Earth's crust, iron is the fourth most abundant element and represents about 5% of its weight, but with huge variations depending on the soil type, whereas zinc in soils is 300−1000 times less abundant.[24] Iron content in soils in Burkina Faso is indeed very high because soils are mainly ferruginous.[25]

The iron contents of manually and mechanically decorticated millet grains milled with the control mill were very close, and about 40−50% lower than the iron content of the whole grains milled in the same way. This result is in good agreement with previous results[5] and corresponds to losses of the iron located in the peripheral parts of the grains, that is, the pericarp and part of the aleurone layer that are removed during decortication. The iron content in the grains decorticated manually and milled with a mortar and pestle was slightly higher, but the difference was not significant. This may be due to dust contamination during milling, which takes place outside in a windy environment, and the soil in Burkina Faso is known to be rich in iron. The same manually decorticated grains milled with either corundum or iron grindstones had higher iron contents than the original iron content of the grains, confirming contamination by exogenous iron. The standard deviations of

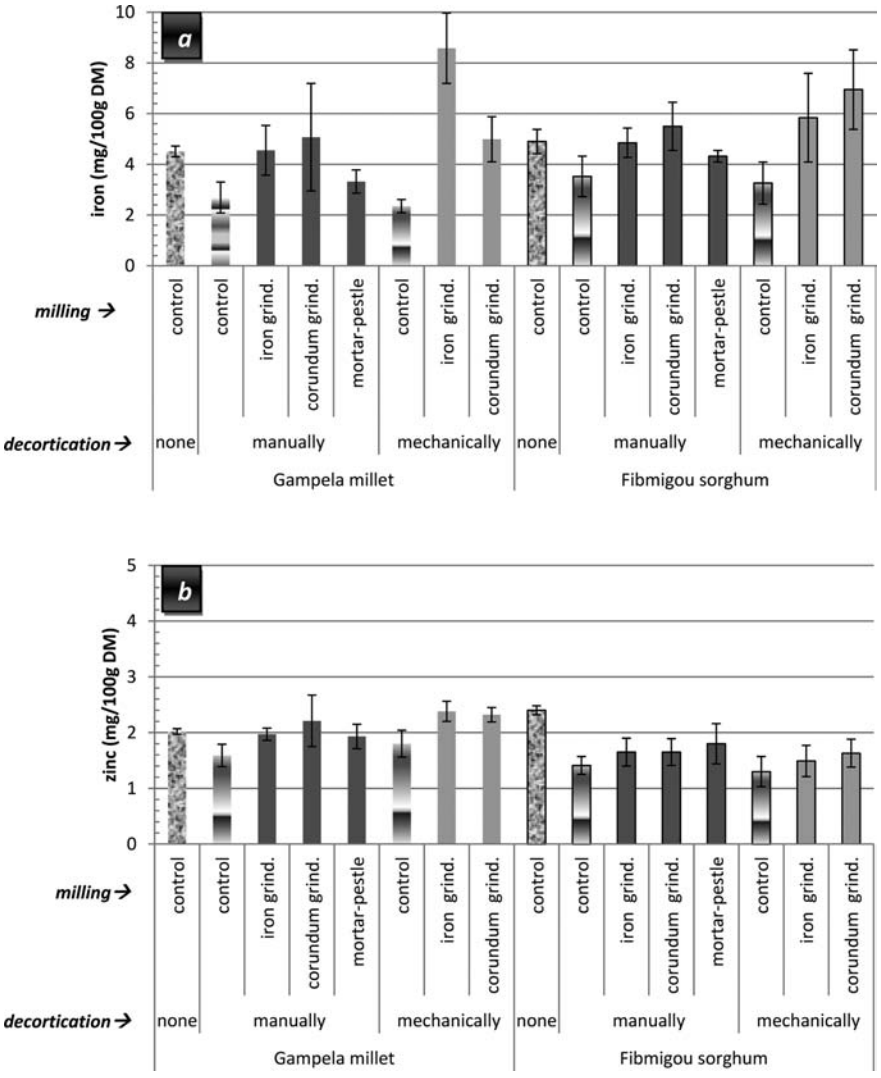

Figure 3. Iron (a) and zinc (b) contents of Gampela millet and Fibmigou sorghum after decortication in the field (or not) and milling in the field and comparison with control processes performed in the laboratory. In the field milling was performed either with a mortar and pestle or with a device equipped with an iron or corundum grindstone. Values are the means of at least six to nine repetitions; error bars represent standard deviation.

all the results for samples obtained in the field were high, especially samples mechanically milled using corundum grindstones, confirming that the contamination probably has different origins. Compared to controls milled in the laboratory, the increase in iron content after mechanical milling, whatever the type of grindstone used, ranged from 60 to 80%, reaching a final iron content of 8.5 mg/100 g DM. A 3−4-fold increase in iron content was measured in grains milled mechanically with iron grindstones, and at least a 2-fold increase was obtained with the corundum grindstones. This confirms our previous hypothesis that iron contamination is probably due to the wear of the mill grindstones, but could also come from other metal parts of the mill. These results are in good agreement with those of other studies[8,11] which also reported iron contamination attributable to milling equipment. Contamination could also be partly due to the soil even if the level of contamination was much higher than that measured in maize flour in Benin,[8] where the total iron content was about 4800 μg/100 g DM. It can be assumed that due to local climate and soil conditions, iron contamination by dust is higher in Burkina Faso than in Benin. The equipment used to mill the grains was also different in the two studies, with greater wear of metal parts in Burkina Faso.

Similar results were obtained for sorghum, except for the mechanical milling of mechanically decorticated grains: whatever the type of grindstone used, the iron content almost doubled after milling compared to control milling. In both cases, high standard deviations were obtained, probably due to the heterogeneous wear of the metal parts of the mill, which varied with the milling units.

The zinc contents of decorticated millet and sorghum grains remained low after milling even if there was a slight increase after in-field milling, whatever the method used. Zinc contents ranged from 1.6 to 2.4 mg/100 g DM. This did not correspond to consistent changes. The main difference between millet and sorghum was that more zinc was lost from sorghum during decortication, as shown by comparing the zinc content of decorticated grains with undecorticated controls. Similar results were previously obtained[5] with zinc losses of, respectively, 55 and 59% after manual and mechanical decortication in sorghum, whereas zinc losses were 46 and 35% in millet, mainly due to the removal of the germ. These results also

confirm our hypothesis that no significant zinc contamination occurs during the first steps of cereal processing.

Factors Influencing Iron Content in Decorticated and Milled Cereal Grains. After milling, with and without previous decortication, all results concerning millet and sorghum grain iron contents were subjected to multifactorial ANOVA, with the type of cereal, the method of decortication, and the method of milling as parameters (Table 1) to identify the factors influencing iron content. The parameter "cereal" was significant. Iron contents were quite high in both millet and sorghum, respectively, 4 and 5 mg/100 g DM, but sorghum had significantly higher iron contents. Manual decortication led to a small but nonsignificant decrease in iron content, whereas there was no change in iron content after mechanical decortication. This is surprising, as abrasive decortication has been shown to cause 50% loss of iron when 10% of the DM of the whole grain was removed,[5] and we could have expected a significant reduction in iron content. We hypothesize that losses resulting from the removal of the iron-rich peripheral parts of the seed are counterbalanced by iron contamination from the decortication device. In contrast, the effect of milling was highly significant, showing that this processing technique has a major influence on iron content. Iron contents were higher after milling with a mortar and pestle than after control milling, but the difference was not significant. Iron contents were also much higher after mechanical milling with either iron or corundum grindstones than after milling with a mortar and pestle or control milling, and the difference was significant. Iron contamination was thus confirmed, probably from different sources, mainly from the equipment but also from dust. No significant differences were observed between samples milled with iron grindstones and those milled with corundum grindstones. Contamination due to mechanical milling could mainly be due to contact with the metal parts of the equipment and not specifically the grindstones. Similarly, a significant increase of from 2 to 7 mg/100 g was measured in iron content in the flour after milling, and it was estimated that <2% of this contaminant iron was in absorbable form.[11] Finally, the final iron content in flours resulted from several iron gains and losses during decortication and milling. It appeared then to be difficult to estimate the share between extrinsic and intrinsic forms. In a comparison of the iron contents of rice flour and polished rice from the same Thai market, a difference in iron content of 28.6 mg/100 g was measured, which they attributed to contamination.[15] Using a strong magnet, they showed that about 60% of this contaminant iron was metal particles originating from the flour mill. More recently, in a study of African traditional processing of maize, increases in iron content ranging from 43 to 138% between grains and flours were also measured, which increased with milling intensity.[8] The contaminant iron represented 1−3.3 mg/100 g DM, that is, more than intrinsic iron.

Table 1. Effects of Processing Factors on Iron Content after Decortication and Milling (ANOVA Analysis)

			iron (mg/100 g DM)		
			n	mean[a]	SD
cereal	$P = 0.0147$	Gampela millet	63	3.99a	2.02
		Fibmigou sorghum	44	4.89b	1.55
decortication	$P = 0.1196$	mortar-pestle	56	4.01	1.33
		mechanical	43	4.78	2.50
		without	8	4.61	0.31
milling	$P = 0.0000$	control	48	3.09a	0.95
		mortar-pestle	12	3.82a	0.63
		iron grindstones	24	5.96b	2.00
		corundum grindstones	23	5.65b	1.61

[a]Values in the same column with different letters are significantly different ($p < 0.05$).

pH Effect on Iron Bioaccessibility in Tô Prepared with Iron Contaminated Flour. In Burkina Faso, tô is sometimes acidified during its preparation to improve the taste. Several authors have already shown that a low pH obtained by fermentation can improve the bioaccessibility of micronutrients in food.[8,26] Iron bioaccessibility was consequently measured in tô prepared at different pH values ranging from 4.3 to 6.5, corresponding to the range encountered in the field, and using millet flour highly contaminated with iron (Table 2). No significant effect of the pH of the tô was found on dialyzable, soluble ND or insoluble iron expressed as percentages. At the three pH levels, dialyzable iron was about 3% of total iron, whereas soluble nondialyzable and insoluble iron represented, respectively, about 20 and 75% of total iron. In all cases, it was shown that iron bioaccessibility was low and that contaminant iron probably did not join the nonheme iron exchangeable pool of the food. In contrast, a partial isotopic exchange between clay iron and intrinsic iron in in vitro studies using radioiron with an acid pH was reported,[16] but this was not the case at neutral pH. In studies to identify possible factors in sorghum and maize beer that promote Fe absorption, dialysis and ultrafiltration of the beer were used,[17] and it was shown that a large proportion of soluble iron was in an ionic form or complexed with low-molecular-weight compounds. But at neutral pH, little or no chelator was obtained that could stabilize iron in a soluble form and consequently enhance its absorption. In the present study on tô, none of these pH effects were obtained, maybe due to a strong matrix effect with highly stable iron complexes or due to the fact that the differences in

Table 2. Phytate/Fe Molar Ratio ([Phy/Fe]) and Iron Bioaccessibility in Millet Tô Prepared at Different pH Values with the Same Iron-Contaminated Flour[a]

				dialyzable iron[b]			
tô pH	total iron (μg)	phytate (g)	[Phy/Fe]	μg	%	soluble ND iron[b] (%)	insoluble iron[b] (%)
4.3				320a ± 130	3.8a ± 1.4	20.8a ± 3.2	75.4a ± 4.4
5.3	8579 ± 641	0.30	3.0	249a ± 83	2.6a ± 0.9	19.0a ± 2.4	78.4a ± 3.0
6.5				236a ± 90	2.8a ± 1.2	25.0a ± 9.0	72.3a ± 10.0

[a]Contents are expressed for 100 g DM. [b]Values are the mean ± SD. Values in the same column with different letters are significantly different ($p < 0.05$).

Table 3. Phytate/Fe Molar Ratio ([Phy/Fe]) and Iron Bioaccessibility in Millet and Sorghum Tô Prepared from Flours Highly or Slightly Contaminated with Iron[a]

					dialyzable iron[b]		soluble ND iron[b]		insoluble iron[b]	
sample	iron contamination	total iron (μg)	phytate (g)	[Phy/Fe]	μg	%	μg	%	μg	%
millet	high	8766	0.30	2.9	207a ± 61	2.4a ± 0.8	1895a ± 181	21.5a ± 3.2	6777a ± 790	76.1a ± 3.8
	low	3668	0.25	5.8	423a ± 210	11.2b ± 4.4	974b ± 194	27.5a ± 9.1	2268b ± 531	61.2b ± 6.3
sorghum	high	8043	0.71	7.5	372a ± 40	4.5a ± 0.2	1899a ± 116	23.0a ± 0.8	5991a ± 615	72.5a ± 0.8
	low	3835	0.59	13.1	200b ± 40	5.2a ± 0.7	1005b ± 76	26.4a ± 2.4	2631b ± 437	68.4b ± 2.0

[a]Contents are expressed for 100 g DM. [b]Values are the mean ± SD. Values in the same column with different letters are significantly different ($p < 0.05$).

pH tested, from 4.3 to 6.5, were not large enough. For further analyses, tô was then prepared without acidification, at a pH of around 6.5.

Bioaccessibility of Intrinsic and Contaminant Iron in Millet and Sorghum-Based Tô. To measure the bioaccessibility of contaminant iron, two samples of millet and sorghum flours with high and low iron contamination levels were chosen. The total iron contents were at least 2-fold higher in the iron-contaminated flour, that is, with more extrinsic than intrinsic iron, than in the less-contaminated flour. Millet and sorghum flours with analogous total iron content were selected, irrespective of their level of contamination (Table 3). Iron contents of the less-contaminated flours were around 3500−4000 μg/100 g DM and of iron-contaminated flour, 8000−9000 μg/100 g DM. Similarly to previous results,[5] phytate content in sorghum was >2-fold higher than in millet. The phytate/iron molar ratios of the iron-contaminated millet and sorghum flours were also about twice less than in the less-contaminated flours, suggesting an improvement in iron bioavailability. However, in all cases they were >1, the threshold value under which the intestinal iron absorption from cereal-based food is improved.[2]

Iron bioaccessibility in millet and sorghum was measured in "as-eaten" foods, that is, in samples of tô, in this case prepared with the previously mentioned flours (Table 3). In the following text, these samples are referred to as "iron-contaminated tô" and "less-contaminated tô". In iron-contaminated tô prepared from millet, the content and the percentage of dialyzable iron were very low, showing poor bioaccessibility. The percentage of dialyzable iron did not exceed 2.4%. Expressed as a percentage, the dialyzable iron fractions were lower in iron-contaminated tô than in the less-contaminated tô. This shows very low bioaccessibility of the contaminant iron, lower than that of the intrinsic iron. However, whereas in millet the dialyzable iron content did not differ with the level of contamination, in sorghum, the dialyzable iron content was significantly higher in the iron-contaminated tô than in the less-contaminated tô. This suggests that a small part of contaminant iron could be available for absorption.

The soluble ND iron fractions represent the proportion of total iron that is potentially absorbable, but is most probably bound in macromolecular complexes. It can be assumed that this soluble iron could be released and dialyzed if fewer chelating factors were present in the tô or if the tô were eaten simultaneously with food containing some absorption enhancers such as ascorbic acid. In both millet and sorghum tô, these soluble ND fractions did not significantly differ with the level of contamination, which ranged from 21 to 27%. Consequently, the corresponding soluble ND iron contents of both millet and sorghum were significantly higher in iron-contaminated tô than in the less-contaminated tô. This shows that part of contaminant iron is soluble and, thus, a higher quantity of iron could be available for absorption in iron-contaminated tô.

Finally, insoluble iron represented the far largest fraction in millet and sorghum tô, iron-contaminated or not. Nevertheless, the percentages of insoluble iron (around 65% of total iron in less-contaminated millet and sorghum tô) were significantly lower than in the contaminated tô (74% of total iron). This was even more striking when insoluble iron was expressed as contents. As expected, the majority of contaminant iron joined the insoluble iron pool.

To better understand the fate of extrinsic iron and its distribution between the fractions during in vitro digestion, differences in total, dialyzable, soluble, and insoluble iron were calculated from data expressed in micrograms per 100 g DM in iron-contaminated or less-contaminated flour or tô. In millet, extrinsic iron was about 5100 μg/100 g DM and was divided into soluble and insoluble fractions of, respectively, a fifth and four-fifths. The distribution was slightly different in sorghum: about 4% of the extrinsic iron, which represented 4200 μg/100 g DM, joined the dialyzable fraction, about 20% joined the soluble ND fraction, and the rest was in the insoluble fraction. It thus appears that the majority of extrinsic iron was not bioaccessible because it was insoluble. The soluble fraction could be considered as potentially absorbable, depending on the compounds with which it interacts.

In conclusion, traditional decortication led to no apparent iron contamination. Conversely, high iron contamination was observed after mechanical milling, which doubled (or even more) the total iron content of the sorghum or millet flours. The share between extrinsic and intrinsic iron is difficult to estimate in the final product.

Phytate/iron molar ratios were lower in iron-contaminated flours, suggesting an improvement in iron bioavailability. However, the measurement of bioaccessibility in iron-contaminated tô showed that contaminant iron was mainly insoluble and thus not available for absorption. These results highlight a limitation in the use of phytate/iron molar ratios in such contaminated products and show that the main part of the extrinsic iron brought by the milling process presents a poor nutritional interest. Nevertheless, a small proportion of contaminant iron joined the soluble fraction after in vitro digestion and may thus be potentially absorbable. It would be interesting to develop methods to distinguish extrinsic from intrinsic iron in foods, to better evaluate the quantity of bioaccessible iron to be brought by other foods of the diet or by

fortification to meet iron requirements. Meanwhile, with the aim of increasing the quantity of iron absorbed from this type of iron-contaminated cereal-based foods, it is recommended to eat simultaneously vegetable sauces rich in ascorbic acid or including small pieces of animal foods that are able to enhance iron absorption.

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ABBREVIATIONS USED

IP6, inositol-6-phosphate or phytate; DM, dry matter

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