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Effects of soaking whole cereal and legume seeds on iron, zinc and phytate contents

Isabelle Lestienne *, Christèle Icard-Vernière, Claire Mouquet, Christian Picq, Serge Trèche

Unitè de Recherche 106 "Nutrition, Alimentation, Sociétés", Centre IRD de Montpellier, BP 64501, 911, Avenue Agropolis, 34394, Montpellier Cedex 5, France

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Abstract

The effects of soaking whole cereal (maize, millet, rice, sorghum) and legume seeds (mung bean, cowpea, soybean) on iron (Fe), zinc (Zn) and phytate (Phy) contents were investigated. In all the above cereals, except millet, the molar ratios of Phy/Fe were above than 14, and ratios of Phy/Zn were above 20 while, in legumes, ratios were lower. Soaking whole seeds for 24 h led to leaching of iron and, to a lesser extent, of zinc ions into the soaking medium. Soaking led to a significant ($P \leqslant 0.05$) reduction in the phytate content of millet, maize, rice and soybean, but did not improve the Phy/Fe molar ratio, while decreasing the Phy/Zn molar ratio only slightly. Soaking on its own was not found to be a good method for improving mineral bioavailability but the results showed that, in combination with other treatments, or with optimized soaking conditions, it could nevertheless prove useful.

Keywords: Phytate; Zinc; Iron; Molar ratios; Soaking

1. Introduction

Cereals and legumes are often rich in fibre-associated anti-nutritional factors (namely phytate, polyphenols, oxalate) (Frölich, 1995) that reduce the bioavailability of minerals. The bioavailability of a nutrient is defined as the proportion of the total nutrient content in a food, meal or diet that is utilized for normal metabolic functions. Many minerals and trace elements are inefficiently and variably absorbed from the diet, for instance iron (<1–30%) and zinc (<15–50%). This phenomenon must be taken into consideration in the preparation of complementary foods in developing countries, where young children often suffer from micro-nutrient deficiencies, such as anemia, caused by iron deficiency (Hurrell, 1997) or decreased growth rate due to zinc deficiency (Gibson & Ferguson, 1998).

Phytate is especially known as a chelating agent that reduces the bioavailability of divalent cations (Weaver & Kannan, 2002). Certain biological or thermal treatments, such as appertisation (Tabekhia & Luh, 1980), allow phytate content to be reduced. The most effective treatments are fermentation (Marfo, Simpson, Idowu, & Oke, 1990) and germination (Honke, Kozlowska, Vidal-Valverde, & Gorecki, 1998) but their application remains limited because of the additional workload they imply or the particular organoleptic properties they induce. Soaking is a simple technological treatment that is often used by mothers to prepare complementary foods at home. Moreover, it can be a simple prolongation of the obligatory washing of the seeds and can also have other advantages, such as facilitating dehulling or swelling of seeds. Previous studies have shown that a long soaking period before fermentation or germination, leads to a reduction in phytate content and to an enhancement of mineral HCl-extractability, used to estimate mineral bioavailability (Duhan, Khetarpaul, & Bishnoi, 2002; Sandberg & Svanberg, 1991).

The inhibitory effect of phytate on zinc absorption can also be predicted in vitro by the molar ratio of phytate to zinc (Phy/Zn). Davies and Olpin (1979)

* Corresponding author.
E-mail address: lestienn@mpl.ird.fr (I. Lestienne).

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showed that molar ratios above 10–15 progressively inhibited zinc absorption and were associated with suboptimal zinc status in rats fed with egg-albumen-based diets with added phytate (0–7.43 g/kg) or zinc (18–144 mg/kg). In the same way, Saha, Weaver, and Mason (1994) showed that absorption of radiolabelled iron in rats decreased significantly when the molar ratios of phytate to iron (Phy/Fe) were above 14 in wheat-flour-based diets containing between 0.19% and 1.85% of phytate.

The objective of this work was to investigate the effects of soaking (for 24 h) whole grains (sorghum, millet, rice, maize) and seeds (soybean, cowpea, mung bean) on iron, zinc and phytate contents in order to evaluate the effectiveness of this treatment for improving the molar ratios of Phy/Fe and Phy/Zn.

2. Materials and methods

2.1. Cereals and legumes

The whole cereal grains of millet (*Pennisetum glaucum*) and sorghum (*Sorghum bicolor*) were purchased at a local market in Ouagadougou (Burkina Faso), while maize (*Zea mays*) and rice (*Oriza sativa*) came from France, and whole legume seeds from China (soybean, *Glycine hyspida*), Senegal (cowpea, *Vigna unguiculata*) and Madagascar (mung bean, *Vigna radiata*).

2.2. Soaking process

Hundred grams of dry whole seeds were cleaned by soaking for 15 min in 300 ml of 1% bleaching-water solution to ensure that the bacteriological quality of each species was constant. The cereal seeds were then soaked in 300 ml and the legume seeds in 500 ml of mineral water because of the difference between their water absorbance capacities. Seeds were soaked for 24 h at 30 °C with slow shaking (60 rpm) in an incubator (New Brunswick scientific Co., Inc., Edison, USA). After draining, the soaking waters were stored at 4 °C before chemical analysis, which was carried out on the same day. Soaked seeds were freeze-dried and ground (IKA M20 Labortechnik, Staufen, Germany) to pass through a 0.5 mm screen. Soaking of each different species of seed was carried out in duplicate.

2.3. Determination of phytate content

Phytate content was estimated by determination of *myo*-inositol hexaphosphate content obtained by anion-exchange HPLC separation, according to the method of Talamond, Gallon, and Trèche (1998) with slight modifications. Phytate was extracted from 0.2 g of flour treated with 10 ml of HCl (0.5 M). The mixture was heated with stirring, for 6 min, by immersing the vial in boiling water, and then centrifuged for 20 min at 5000*g*, at 4 °C. The supernatant was recovered and 1.5 ml of HCl (12 N) was added. The resulting solution was then shaken and evaporated to dryness with a centrifugal evaporator (JOUAN RC 10-10, Saint Herblain, France). The vial was stored at 4 °C until analysis. Ten minutes before injection, the residue was diluted with 2 ml of deionized water and filtered through a 0.2 μm disposable filter tip-syringe assembly. The filtrate was then diluted in deionized water (1/50, v/v) and 50 μl were injected into an Omniac Pax-100 anion-exchange column (25 cm $\times$ 4 mm I.D. Dionex) equipped with an Omniac Pax-100 (8 μm) pre-column and an anion suppressor (ASRS-I 4 mm). The separation was performed by gradient elution using three solvents: 0.2 M NaOH solution, deionized water–isopropanol (1/1, v/v), and deionized water.

2.4. Determination of total Fe and Zn contents

Total Fe and Zn contents were determined by atomic absorption spectrophotometry (Varian SpectrAA 200, Victoria, Australia) after dry mineralization for 2 h at 530 °C. Depending on the botanical origin of the seeds, 2–4 g of flour were weighed in a silicon evaporating dish. Next, the ashes were wet-acid digested with nitric acid on a hot plate and solubilized with 25 ml of 0.5 N HCl.

2.5. Statistical analysis

Values were calculated per 100 g dry matter (DM) of raw seeds used for soaking. Each sample was analyzed in triplicate and values were then averaged. Thus, mineral or phytate contents are the means of three values for raw seeds and of six values for soaked seeds (soaking carried out in duplicate). Data were assessed by analysis of variance (ANOVA). Duncan's multiple range test was used to separate means. Significance was accepted at probability $P \leqslant 0.05$ (Duncan, 1955).

3. Results and discussion

3.1. Effect of soaking on total iron and zinc contents

As far as mineral contents of raw seeds are concerned, the iron content of millet were very high (11.1 mg/100 g DM) while other cereals contained only 1.7–3.7 mg/100 g DM (Table 1). The iron contents of the legumes were generally higher, from 6.6 to 7.3 mg/100 g DM. Zinc contents showed nearly the same profile, with low values for cereals, between 1.6 mg/100 g DM for sorghum and 3.7 mg/100 g DM for millet, and higher values for legumes, from 2.8 to 3.8 mg/100 g DM.

The iron content of soaked seeds was significantly ($P \leqslant 0.05$) lower than unsoaked seeds in all species except

Table 1
Changes in iron and zinc contents (mg/100g dry matter)[A] after soaking whole seeds for 24 h

Varieties	Treatment	Iron content	Zinc content
Millet	Unsoaked	11.1 ± (0.68)a	3.66 ± (0.08)a
	Soaked	7.33 ± (0.34)b	3.39 ± (0.09)b
Maize	Unsoaked	1.97 ± (0.12)a	1.93 ± (0.04)a
	Soaked	1.85 ± (0.10)a	1.72 ± (0.11)b
Sorghum	Unsoaked	3.73 ± (0.09)a	1.57 ± (0.04)a
	Soaked	2.22 ± (0.09)b	1.55 ± (0.07)a
Rice	Unsoaked	1.66 ± (0.38)a	2.94 ± (0.11)a
	Soaked	0.67 ± (0.06)b	2.07 ± (0.25)b
Soybean	Unsoaked	7.31 ± (0.26)a	3.64 ± (0.19)a
	Soaked	6.16 ± (0.19)b	3.50 ± (0.14)a
Cowpea	Unsoaked	6.60 ± (0.26)a	3.78 ± (0.12)a
	Soaked	5.68 ± (0.17)b	4.10 ± (0.24)a
Mung bean	Unsoaked	7.17 ± (0.28)a	2.81 ± (0.10)a
	Soaked	5.17 ± (0.15)b	2.71 ± (0.20)a

[A] Values are means ± (SD). Means for the same species in the same column that do not have the same letter are significantly different at $P \leqslant 0.05$, as assessed by Duncan's multiple range test.

maize. The biggest reduction in iron content occurred in rice grains (60%). Zinc content also decreased significantly ($P \leqslant 0.05$) in millet, maize and rice grains, but the reduction did not exceed 30%. Reduction after soaking may be attributed to leaching of iron and zinc ions into the soaking medium (Saharan, Khetarpaul, & Bishnoi, 2001). The leaching of zinc was lower than iron and this phenomenon may be due to the fact that zinc and iron are not located in the same place in the seeds nor are they linked with the same molecules. Indeed, zinc is found in a large number of enzymes and other proteins, where it plays an important structural role. Table 1 shows that the zinc in legume seeds did not leach and, indeed, legume seed envelopes do not contain any protein, whereas zinc did leach from cereal grains whose hulls contain protein bodies (aleurone layer) (Pernollet, 1978).

3.2. Effect of soaking on phytate content

The phytate contents of the different raw materials, shown in Table 2 varied from 236 to 1084 mg/100 g DM. These values are close to those reviewed by Reddy (2002), except for soybean and mung bean, which appear to be rather low in our study (878 mg/100 g compared to 1000–2200 mg/100 g for soybean in the literature and 236 mg/100 g against 590 to 1100 mg/100 g for mung bean). In legumes, phytates are associated with protein bodies (Reddy, 2002) and Maga (1982) pointed out that phytate levels should increase with increasing protein content. We also observed this correlation in our study, since analyzed seeds showed rather low protein contents for soybean and mung bean seeds (37.7 and 20.3 g/100 g DM, respectively), as well as low levels of phytate.

Depending on the botanical origin of the seeds, a significant reduction ($P \leqslant 0.05$) in phytate content (between 17% and 28%) was obtained by soaking whole seeds for 24 h at 30 °C. Two groups were distinguished: Millet, maize, rice and soybean which showed a significant reduction in phytate content, and sorghum, cowpea and mung bean which showed no significant reduction. As regards the phytate content of the soaking water,

Table 2
Changes in phytate content (mg/100g dry matter)[A] after soaking whole seeds for 24 h

Varieties	Phytate content		% Phytate loss
	Unsoaked	Soaked	
Millet	762 ± (66)a	550 ± (18)b	28
Maize	908 ± (97)a	721 ± (16)b	21
Sorghum	925 ± (81)a	882 ± (44)a	4
Rice	1084 ± (12)a	904 ± (81)b	17
Soybean	878 ± (130)a	678 ± (32)b	23
Cowpea	559 ± (44)a	624 ± (42)a	Apparent increase
Mung bean	236 ± (36)a	225 ± (12)a	8

[A] Values are means ± (SD). Means with a different letter in the same row are significantly different at $P \leqslant 0.05$, as assessed by Duncan's multiple range test.

after soaking for 24 h, no *myo*-inositol hexaphosphate was found (results not shown). This means that phytate was hydrolyzed by phytases either directly in seeds or in the water after leaching into the soaking medium.

3.3. Effect of soaking on Phy/Fe and Phy/Zn molar ratios

The Phy/Fe molar ratios of raw materials (Table 3), which are associated with iron absorption capacity, were generally consistent with those reported by Allen and Ahluwalia (1997). They were below 14 for millet, soybean, cowpea and mung bean, which consequently may have an acceptable level of iron bioavailability. However, for soybean, these authors mention a Phy/Fe molar ratio of 70 (against 10 in our study) which is more in agreement with the low absorption of iron reported in in vivo studies using soybean-based diets. Our result can be explained by the low phytate content measured here in soybean seeds. The Phy/Zn molar ratios of maize, sorghum and rice were above 30 and close to 20 for millet and soybean. These results reinforce previous results (Adeyeye, Arogundade, Akintayo, Aisida, & Alao, 2000; Ferguson, Gibson, Thompson, Ounpuu, & Berry, 1988) showing that the bioavailability of zinc in cereals and legumes would be lower than that in vegetables and in some roots and tubers whose Phy/Zn molar ratios are generally less than 20.

Soaking of whole seeds for 24 h did not decrease Phy/Fe molar ratios, which remained above 14 in maize, sorghum and rice. In fact there was an increase in these molar ratios after soaking, especially in sorghum and rice, because of the decrease in the iron content. After soaking, the Phy/Zn molar ratios decreased slightly in almost all seed species but they nevertheless remained much higher than the limiting value generally recognized for improvement of zinc absorption in maize, sorghum, rice and soybean. The Phy/Zn molar ratio of these species is above 20, which hence predicts a low rate of zinc absorption for these seeds.

Table 3
Influence of soaking whole seeds for 24 h on Phy/Fe and Phy/Zn molar ratios

		Phy/Fe molar ratio	Phy/Zn molar ratio
Millet	Unsoaked	5.3	18.5
	Soaked	6.5	16.4
Maize	Unsoaked	34.4	40.6
	Soaked	33.3	41.4
Sorghum	Unsoaked	22.8	62.8
	Soaked	35.2	58.5
Rice	Unsoaked	55.5	36.3
	Soaked	123	45.8
Soybean	Unsoaked	10.1	23.5
	Soaked	9.8	20.0
Cowpea	Unsoaked	7.8	15.8
	Soaked	9.7	15.7
Mung bean	Unsoaked	2.8	8.2
	Soaked	3.6	8.0

4. Conclusion

Based on the molar ratios of phytate to mineral of analyzed raw grains and seeds, iron and zinc bioavailability is insufficient in the cereals studied, except for millet, and legumes have only low zinc bioavailability. Soaking under the chosen conditions (related to those applicable in households in developing countries) does not adequately improve the molar ratios of phytate to iron and phytate to zinc in the analyzed seeds, and hence probably does not improve the bioavailability of these minerals. Nevertheless, soaking can be used to increase the bioavailability of zinc, estimated by the Phy/Zn molar ratio, because it leads to a slight reduction in the phytate content. On the other hand, soaking is not effective for iron owing to the leaching of iron ions into the soaking medium. Finally, since no phytate remains in the soaking medium, in order to limit mineral loss, the water used for soaking can also be used for cooking whole seeds of rice, cowpea and mung bean, which absorb a considerable amount of cooking water and, in this way, the leached minerals may be to a certain extent recovered.

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