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

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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.
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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)

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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 × 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

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