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Evaluation of the content and bioaccessibility of iron, zinc, calcium and magnesium from groats, rice, leguminous grains and nuts

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Abstract The objective of this study was to determine the content and the bioaccessibility of minerals (Fe, Zn, Ca and Mg) in commonly consumed food products, such as cereal groats, rice, leguminous grains and nuts purchased from the local market. The contents of Fe, Zn, Ca and Mg in foods were assayed after dry ashing of samples, while the bioaccessibility of these minerals after enzymatic in vitro digestion, was determined by flame atomic absorption spectrometry. A relatively high content of Fe was found in cashew nuts and green lentils, while cashew nuts and buckwheat groats had the highest concentration of Zn. It was found that the highest amount of macro-elements was generally in nuts, in particular: brazil nuts (Ca and Mg), cashews (Mg) and hazelnuts (Ca and Mg). Concerning the mineral bioaccessibility, the highest values for Fe were obtained in cashew nuts and green lentils (2.8 and 1.7 mg/100 g), for Zn in green lentils (2.1 mg/100 g), for Ca in brazil nuts and shelled pea (32.6 and 29.1 mg/100 g), while for Mg in shelled peas and green lentils (43.4 and 33.9 mg/100 g). Generally, the best sources of bioaccessible minerals seem to be leguminous grains and nuts.

Keywords Bioaccessibility \cdot Cereal groats \cdot Minerals \cdot Nuts \cdot Pulses \cdot Rice

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Introduction

Food products of plant origin, especially cereals, leguminous grains and nuts, have always played an important role in human nutrition. Nutritional quality of plant foods depends on many factors, including the content of particular nutrients, their digestibility or bioavailability for an organism, the content of nonnutrients and contaminants, as well as technological processing applied. Many foods of plant origin (raw or processed) have been found to possess functional properties, providing not only nutrients, but also protecting the human organism against some diseases, due to the presence of various phytochemicals (e.g. antioxidants), that exert anticancer, anti-inflammatory, hypocholesterolemic and antidiabetic properties (Djousse et al. 2009; Lee et al. 2010; Kris-Etherton et al. 1999; Tighe et al. 2010).

Cereals and leguminous grains as well as and nuts are considered to be good sources of dietary minerals since they accumulate them during plant growth to be used for further needs (germination, reproduction). However, these plant products also contain oxalic acid, phytates, dietary fibres and polyphenols, that act as mineral binders or chelators, thus reducing bioavailability of minerals, due to the formation of extremely insoluble salts, or very poorly dissociated chelates (Oatway et al. 2001; Sandberg 2002; Fernando et al. 2011; Ou et al. 2011).

Since cereals and leguminous grain products are staple foods for most of populations worldwide, they are also major sources of essential minerals, depending on a particular plant variety, agricultural practices, soil and climatic conditions, as well as technological and culinary practices applied (Afridi et al. 2010). The total contents of Fe, Zn, Ca and Mg in a given food product are not the only criteria determining its nutritional quality, as it also depends on their bioavailability from that product. In order to determine whether a product is a good source of a particular mineral, it is necessary to determine the amount of that mineral released or absorbed in the animal or human organism (Gibson et al. 2010).

There are various *in vitro* and *in vivo* methods to determine bioaccessibility of minerals (Skibniewska et al. 2002). The simplest and low cost *in vitro* method is based on the enzymatic digestion of a product under physiological conditions of the stomach and intestines (Krejpcio et al. 2009; Suliburska et al. 2009).

The objective of this study was to determine the content and to evaluate the bioaccessibility of Fe, Zn, Ca and Mg from cereal, leguminous food products (cereal groats, rice, pulses) and nuts after *in vitro* enzymatic digestion.

Materials and methods

Materials

The experimental materials were food products, such as groats (buckwheat groats, barley groats, corn groats, couscous), rice (white rice and brown rice), leguminous grains (kidney bean, shell pea, green lentils) and nuts (brazil nuts, cashews, hazelnuts and walnuts). All the food products were purchased from the local market (the city of Poznan, 2008/2009).

Each type of food item was taken from five different packages (provided by different producers) purchased from the market. Food samples (2 g) were ground under laboratory conditions with an electrical mill and divided using appropriate sieves into fractions with particles having maximum diameters below 2 mm. Samples were dried at 105°C.

Depending on the package volume, its content was mixed and homogenized in an electric grinder. Finely ground food product was transferred to plastic bags and stored frozen (-20° C) until analyzed.

Enzymatic digestion

Enzymatic digestion *in vitro* was performed according to the method developed by Skibniewska et al. (2002). Samples (approx. 2 g) of a finely ground food product were weighed in conical flasks and treated with deionised water (20 ml) and shaken for 10 min. In order to create suitable conditions for pepsin action, pH was brought to 2 using 0.1 M HCl aqueous solution (Suprapure, Merck), then pepsin solution (0.5 ml/100 ml) was added to the homogenate. Subsequently samples were placed in a thermostat shaker (37°C) for 2 h. During the incubation process, pH was assured or corrected by an addition of 6 M HCl aqueous solution, when necessary. After 2 h digested samples were treated with 6% NaHCO₃ aqueous solution (Extrapure, Merck) to bring pH to 6.8-7.0, and subjected to pancreatin solution (10 ml/40 ml of homogenate), and placed in a thermostatic shaker (37° C) for 4 h. Afterwards, digested samples were centrifuged for 10 min (3.800 rpm/min), and clear solution was quantitatively transferred to quartz crucibles, and treated with a mixture of concentrated nitric (65% w/w) and perchloric (70% w/w) acids (2:1 v/v) (Suprapure, Merck). Samples were placed in a thermostatic block and heated until complete mineralization.

In order to determine the total content of minerals in native products, food samples (2 g) were ashed in a muffle furnace at 450°C until complete mineralization and then dissolved in 1 N nitric acid. Each product was analysed in triplicate.

Determination of minerals

The content of minerals in native and *in vitro* digested food products was determined by atomic absorption spectrometry (AAS-3, Zeiss spectrometer), after an appropriate dilution with deionized water (for Fe, Zn) or with LaCl₃ (0.3% solution, for Ca and Mg) using the airacetylene flame. The methods were validated by a simultaneous analysis of the reference material (*Soya Bean Flour, INCT-SBF-4*), with the accuracy for Ca, Mg, Fe and Zn of 98,2%, 92,6%, 96.7% and 94.2%, respectively. The content of minerals in food products was expressed in mg/100 g dry mass, while the degree of a mineral released (bioaccessibility) was expressed as the amount of mineral (mg) liberated during the enzymatic digestion *in vitro* from 100 g of product and a percentage of a mineral released vs. its total content.

Deionised water and acid-washed glassware were used in this study.

Statistical analysis

The experimental results were given as mean \pm SD of three parallel measurements. The statistical analysis was carried out using the STATISTICA 7.0 software and the ANOVA test at the significance level α =0.05.

Results and discussion

Table 1 shows the total content of minerals in the analysed food products. As it can be seen, the content of Fe, Zn, Ca

Food product	Fe	Zn	Ca	Mg
Cereal groats				
Buckwheat groat (Fagopyrum esculentum Moench)	$2.9{\pm}0.03^{b}$	$2.8{\pm}0.03^{b}$	63.2 ± 1.44^{b}	128.3±4.41°
Barley groat (Hordeum L.)	$2.7{\pm}0.04^{b}$	$2.0{\pm}0.04^{\mathrm{b}}$	$78.4{\pm}2.13^{b}$	$73.8 {\pm} 2.53^{b}$
Maize groat (Zea mays)	$0.75{\pm}0.02^{\rm a}$	$0.76{\pm}0.01^{a}$	$14.5 {\pm} 0.90^{a}$	$27.5{\pm}1.44^{a}$
Couscous (wheat groat) (Triticum L.)	$2.0 {\pm} 0.02^{b}$	$1.7{\pm}0.01^{\rm b}$	86.5 ± 2.81^{b}	$57.4 {\pm} 1.80^{b}$
Rice (Oryza L)				
White rice	$0.67{\pm}0.04^{a}$	$1.6 {\pm} 0.02$	$23.6{\pm}0.92^{a}$	$30.7{\pm}1.73^{a}$
Brown rice	$1.0 {\pm} 0.05^{b}$	$1.8 {\pm} 0.01$	37.2 ± 1.32^{b}	100.1 ± 2.61^{b}
Pulses				
Red kidney bean (Phaseolus Vulgaris)	$3.5{\pm}0.18^{a}$	1.9 ± 0.15	$30.5 {\pm} 0.30^{a}$	95.9±1.32
Shelled pea (Pisum sativum)	$3.8{\pm}0.08^{a}$	$1.8 {\pm} 0.06$	69.3 ± 1.72^{b}	87.5±2.33
Green lentils (Lens culinaris)	$5.3 {\pm} 0.12^{b}$	2.3 ± 0.04	$48.4{\pm}1.21^{ab}$	86.5±1.75
Nuts				
Brazil nuts (Bertholletia Excela)	$2.2{\pm}0.09^{a}$	$2.4{\pm}0.11^{ab}$	$170.3 \pm 4.61^{\circ}$	221.2 ± 6.40^{b}
Cashews (Anacardium occidental)	$5.4 {\pm} 0.12^{b}$	$3.0{\pm}0.24^{b}$	25.1 ± 1.85^{a}	$195.7{\pm}3.74^{ab}$
Hazelnuts (Corylus avellana)	$2.5{\pm}0.14^{a}$	$1.5{\pm}0.02^{a}$	$132.4 \pm 4.32^{\circ}$	140.0 ± 5.33^{a}
Walnuts (Juglans regia)	$2.1 {\pm} 0.07^{a}$	$1.8{\pm}0.03^{ab}$	73.1 ± 3.22^{b}	140.1 ± 4.17^{a}

number of replicates for each food product: n=3

^{a, b, c}—values in columns sharing the same superscript letters are not statistically different at p < 0.05

and Mg varied considerably among the types of foods. Generally the highest contents of these minerals were found in nuts, followed by leguminous grains, cereal groats, while they were lowest in rice. Also within each food group, representing cereal grains, leguminous grains and nuts, the concentration of Fe, Zn, Ca and Mg fell within a wide range of values, reflecting the specific plant genetic capacity to accumulate minerals, their anatomical role and function, as well as the environmental and processing conditions applied during the production of these food items. Taking into account specific minerals, the highest concentrations of Fe were found in cashews, green lentils and other leguminous grains, while white rice and maize groats had relatively low contents of this mineral. The content of Zn ranged from 0.8 mg/100 g in maize groats to 3.0 mg/100 g in cashews. Relatively high contents of this element were found in buckwheat groats, brazil nuts and green lentils, while in the other food products the concentration of Zn did not exceed 2 mg/100 g. The highest content of Ca was determined in brazil nuts and hazelnuts, while the lowest in maize groats and white rice. Concerning Mg, the highest concentration of this mineral was in nuts, i.e. brazil nuts, cashews, hazelnuts and walnuts. Buckwheat groats and brown rice were also good source of Mg, whereas white rice and maize groats had a significantly lower content of this element. The content of divalent and trivalent minerals is usually higher in the outer layer of grains (bran), where they are bound with phytates, polyphenols and fibres. This trend is reflected in e.g. brown and white rice, where the whole grain (brown rice) contains significantly higher amounts of Ca (~1.6 fold), Mg (~3.3 fold) and Fe (~1.6 fold), but not Zn. Similar differences in the content of minerals in brown and white rice were reported by Grembecka and Shefer (2009) who found higher amounts of Mg in brown rice (above 100 mg/ 100 g) than in white rice (7–29 mg/100 g).

Grembecka et al. (2006) analyzed the concentrations of Mg, Zn, Fe and P in different types of groats purchased on the local market (Poland). The levels of Mg, Fe and Zn in buckwheat, barley and maize groats, except for couscous (wheat groats), were similar to the values obtained in this study. Our results are also similar to the contents of Mg, Fe and Zn in buckwheat groats reported earlier by Amarowicz and Fornal (1987).

Pulses may be defined as dried edible seeds of cultivated legumes. They belong to the family of peas, beans and lentils. Pulses contain an average of 20–40% protein for dry substances, including amino acids essential in human and animal diets (lysine, triptophan, cystine, methionine, valine), carbohydrates and fats (especially soybeans and peanuts). They are also fairly good sources of vitamins (thiamin, niacin) and minerals (Ca, Mg, P, Fe, Zn, Cu). Cabrera et al. (2003) reported that in leguminous grains the levels of microelements (in mg/100 g) ranged from 0.15–0.5 for Cu, 1.88–8.24 for Fe and 3.26–7.02 for Zn. Anwar et al. (2007)

determined the chemical composition of different cultivars of mung bean (*Vigna radiata L*.). The contents of Fe, Mg, Ca, and Zn (in mg/ 100 g) ranged as follows: Fe 10.58–19.9, Mg 4.86–5.17, Ca 35.92–48.29, and Zn 2.49–4.72.

Nuts are nutrient-dense foods with complex matrices rich in unsaturated fatty acids and other bioactive compounds, such as L-arginine, dietary fiber, minerals, tocopherols, phytosterols and polyphenols. By virtue of their unique composition, nuts are likely to beneficially impact heart health and reduce the incidence of diabetes. Epidemiologic studies have associated nut consumption with a reduced incidence of coronary heart disease in both genders and diabetes in women (Kendall et al. 2010; Ros et al. 2010). Brazil nuts were found to contain highly variable and often very high concentrations of Se, Ba and Sr (Caglarirmak 2003).

The total elemental concentrations of five different tree nuts, i.e. almond (*Prunus dulcus*), brazil nut (*Bertholletia excelsa*), pecan (*Carya pecan*), macadamia (*Macadamia integrifolia*) and walnut (*Juglans nigra*) that are consumed in South African households were investigated by Moodley et al. (2007). Cabrera et al. (2003) reported that the levels of Cu, Fe and Zn in nuts (in mg/ 100 g) were as follows: 0.4–2.56, 0.73–7.56 and 2.56–6.90, respectively. Caglarirmak (2003) determined the concentrations of minerals in some walnut genotypes (*Juglans regia L.*) commonly grown in Turkey. He found that the average contents of Ca, Mg, Zn and Fe were as follows (in mg/100 g): 85.0, 90.0, 2.01 and 2.90, respectively.

The content of minerals in food products of plant origin depends on genetic factors (e.g. plant variety) and environmental factors (e.g. type of soil, fertilization, moisture, climatic conditions), as well as the processing applied (e.g. milling, grinding, dehulling). For these reasons levels of minerals in cereal grains, leguminous grains and nuts may differ significantly from source to source, country to country, climatic and agricultural practices, processing conditions, etc. The results obtained in this study showed variable contents of minerals (Fe, Zn, Ca and Mg), depending on the type of foods (raw vs. processed, cereals, leguminous grains, nuts).

The content of minerals in a particular food item has got only an informative value when comparing with various food sources. Another important factor characterizing nutritional quality of a food product as a source of minerals is their bioaccessibility. Table 2 shows the amount of minerals (mg) released from 100 g of products and the percentage of release for minerals during in vitro enzymatic digestion of the analyzed food products. Plant foods are generally considered as rather poor sources of bioaccessible Fe, since it is bound with various organic ligands in the forms of insoluble complexes with phytates, oxalates and tannins. The highest values were determined for cashews and green lentils, whereas the lowest for hazelnuts and walnuts. Similarly, the bioaccessibility values for Zn were very low, being highest for green lentils and cashews.

Table 2 The content released minerals (% of release) during in vitro enzymatic digestion of food products (mean ± SD; values in mg/100 g d.m.)

Food product	Fe	Zn	Ca	Mg
Cereal groats				
Buckwheat groat (Fagopyrum esculentum Moench)	$0.48 \pm 0.02^{b} (14.6)$	1.0±0.03 ^b (35.2)	8.1±0.81 ^a 12.5)	14.1±1.02 ^b (11.0)
Barley groat (Hordeum L.)	0.16 ± 0.01^{a} (5.8)	0.59 ± 0.02^{ab} (29.9)	23.8±1.01 ^b (31.7)	1.7±0.24 ^a (2.2)
Maize groat (Zea mays)	$0.24{\pm}0.02^{a}$ (32.1)	$0.63 \pm 0.01^{ab} \ (85.5)$	7.5 ± 0.42^{a} (52.8)	1.6±0.21 ^a (5.9)
Couscous (wheat groat) (Triticum L.)	0.28 ± 0.01^{a} (14.4)	0.27±0.01 ^a (16.0)	$16.6 \pm 0.88^{ab} (18.8)$	3.3±0.31 ^a (5.5)
Rice (Oryza L)				
White rice	0.16±0.01 (21.4)	0.48 ± 0.01^{a} (31.4)	14.7±0.79 ^b (63.6)	5.3±0.46 (17.1)
Brown rice	0.15±0.01 (14.2)	0.73 ± 0.02^{b} (39.7)	10.3±0.99 ^a (27.6)	5.0±0.52 (5.0)
Pulses				
Red kidney bean (Phaseolus Vulgaris)	0.33 ± 0.01^{a} (9.4)	1.0 ± 0.01^{a} (52.0)	8.4 ± 0.22^{a} (27.5)	11.9±0.73 ^a (12.4)
Shelled pea (Pisum sativum)	0.96 ± 0.06^{b} (25.0)	1.3 ± 0.02^{ab} (69.7)	29.1±0.39 ^b (42.0)	43.4±0.14 ^b (49.6)
Green lentils (Lens culinaris)	1.7±0.15 ^b (32.6)	2.1±0.02 ^b (92.2)	28.5±0.26 ^b (59.0)	33.9±0.18 ^b (39.2)
Nuts				
Brazil nuts (Bertholletia Excela)	$0.64{\pm}0.02^{b}$ (28.8)	$0.50 \pm 0.02^{b} (20.6)$	32.6±0.88 ^b (19.1)	20.6±0.79 ^b (9.3)
Cashews (Anacardium occidental)	2.8±0.11° (51.1)	1.6±0.08 ^c (52.4)	6.9 ± 0.97^{a} (27.5)	27.9±0.69 ^b (14.3)
Hazelnuts (Corylus avellana)	0.05 ± 0.00^{a} (2.0)	0.03 ± 0.00^{a} (1.9)	15.3±1.01 ^{ab} (11.6)	13.5±0.41 ^a (9.6)
Walnuts (Juglans regia)	0.06 ± 0.00^{a} (2.9)	0.21±0.01 ^{ab} (11.4)	5.5±0.55 ^a (7.5)	10.5±0.32 ^a (7.5)

number of replicates for each food product: n=3

^{a, b, c}—values in columns sharing the same superscript letters are not statistically different at p < 0.05

It was found that the highest bioaccessibility values for Ca were recorded for brazil nuts and green lentils, while the lowest for walnuts, cashews and maize groats. Shelled peas and green lentils turned out to be the best sources of bioaccessible Mg, while the poorest sources were maize and barley groats.

As it can be seen from Table 2, the degree of release of minerals from food products can vary considerably, depending on the type of mineral, food item and its matrix composition. The degree of Fe release ranged from 2.0% (hazelnuts) to 51.1% (cashews). The release for Zn ranged from 1.9% (hazelnuts) to 92.2% (green lentils). The release values for Ca ranged from 7.5% (walnuts) to 59.0% (green lentils). The release for Mg also fell within a wide range of values from 2.2% (barley groats) to 49.6% (shelled peas).

Hemalatha et al. (2007a) found lower biaccessibility of iron and zinc from cereals (Fe: 4.13-8.05%; Zn: 5.5-21.4%) and pulses (Fe: 1.77-10.2%; Zn: 27-56%) consumed in India in compared to products analysed in this experiment. In this study the release of minerals (expressed as the percentage of total content) varied considerably depending on the type of mineral and a particular food matrix. It is known that the availability of minerals depends on the contents of inhibitors, such as phytic acid, oxalates and polyphenols. It was shown that the degradation of phytic acid in cereal porridges can improve Fe absorption by humans (Hurrell 2003). Oatway et al. (2001) reported that buckwheat grains contain relatively high levels of phytates (1.08%) in comparison with other grains or pulses (except for soybean). Nuts also contain significant amounts of mineral binding compounds such as tannins (0.01–0.88%), phytates (0.15–0.35%) and fiber (7%) (Kris-Etherton et al. 1999; Venkatachalam and Sathe 2006). According to Sandberg (2002), higher contents of phytates in kidney beans (in comparison with peas and lentils) may be associated with lower percentages of release of minerals.

Another factor influencing mineral bioaccessibility is dietary fibre, that can also change the activity of digestive enzymes. Amarowicz et al. (2006) are of the opinion that dietary fiber components may interact with amphoteric moieties of digestive enzymes and a lower enzyme activity. Moreover, they reported that some hydrated fibre fractions may reduce the rate of interaction between enzymes and food, which could also be a factor responsible for poor digestibility of nutrients from leguminous and cereal grains. The study of Karamac et al. (2007) showed that tannins present in buckwheat groats are strong chelators of Cu, Fe and Zn.

Hemalatha et al. (2007a) showed that tannin in cereals and pulses consumed in India did not have any significant influence on zinc and iron biaccessibility. Moreover, mentioned authors found significant negative correlation between phytate content and zinc biaccessibility in pulses, while phytic acid content had a negative influence on iron release from cereals.

Technological processes also influence on the bioaccessibility of minerals. It was found that fermentation and extrusion increase iron and zinc release from lupine grains (Krejpcio et al. 2009), while pressure-cooking and microwave heating reduced biaccessibility of zinc and enhanced iron from cereals and pulses (Hemalatha et al. 2007b).

Conclusions

The concentrations of minerals (Fe, Zn, Ca and Mg) depended on the type of food product. The highest concentrations of Fe were found in cashew nuts and green lentils, Zn in cashew nuts and buckwheat groats, Ca and Mg in nuts (brazil nuts, cashew nuts and hazelnuts), while the lowest contents of these minerals were determined in white (dehulled) rice. The bioaccessibility of minerals *in vitro* also varied considerably, depending on the mineral and the type of the food matrix. Generally, the best sources of bioaccessible Fe, Zn, Ca and Mg were found to be pulses and nuts, in particular cashews (Fe), lentils (Zn), brazil nuts (Ca) and shelled peas (Mg).

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