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Impact of heat processing on the bioavailability of zinc and iron from cereals and pulses

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Abstract

Influence of heat processing on the bioavailability of zinc and iron from food grains consumed in China was evaluated. Cereals – rice, barley, buckwheat, wheat, and oat, and pulses – chickpea – whole and decorticated, green gram – whole and decorticated, decorticated black gram, decorticated red gram, cowpea, and faba bean were examined for zinc and iron bioavailability by employing an *in vitro* dialysability procedure. Both pressure-cooking and microwave heating were tested for their influence on mineral bioavailability. Zinc bioavailability from food grains was considerably reduced upon pressure-cooking, especially in pulses. Among cereals, pressure-cooking decreased zinc bioavailability by 50.5% and 58.4% in barley and rice, respectively. All the pressure-cooked cereals showed similar percent zinc bioavailability with the exception of barley. Bioavailability of zinc from pulses was generally lower as a result of pressure-cooking or microwave heating. The decrease in bioavailability of zinc caused by microwave heating ranged from 14.3% in chickpea (whole) to 65.8% in cowpea. Decrease in zinc bioavailability was 40.0% in pressure-cooked whole chickpea, 51% and 57% in pressure-cooked or microwave-heated whole green gram, 20.6% and 24.9% in pressure cooked or microwave-heated decorticated green gram, and 46.5% in microwave-heated black gram. Iron bioavailability, on the other hand, was significantly enhanced generally from all the food grains studied upon heat treatment. Thus, heat treatment of grains produced contrasting effect on zinc and iron bioavailability.

Introduction

Marginal zinc deficiency and suboptimal zinc status have been recognized in many population groups in both less-developed and industrialized countries. Despite large-scale intervention programmes, iron deficiency i.e. anaemia remains the most widely prevalent nutritional problem in the world. Iron is an essential trace element whose biological importance arises from its involvement in vital metabolic functions by being an intrinsic component of hemoglobin, myoglobin and cytochromes (Gibson, 1994). Despite large scale intervention programmes, iron-deficiency anaemia remains the most widely prevalent nutritional problem in the world (Sandberg and Andlid, 2002; Hemalath *et al*., 2007). Although many factors are responsible for iron deficiency, the most likely cause of this nutritional problem in developing countries is the poor bioavailability of dietary iron (Gibson *et al.*, 2000). Iron deficiency is especially prevalent among specific population groups, such as infants (Lozoff *et al*., 1996) menstruating and pregnant women, and populations with a high dietary intake of plant-derived proteins (Hallberg, 2001); it can lead to important health problems and retardation in physical and mental development (Beard and Connor, 2003; Hurrell, 2004).

Although many factors including inadequate dietary intake are responsible for the onset of zinc and iron deficiency, the most likely cause is the poor bioavailability of dietary zinc and iron, especially from plant foods (Patterson *et al*., 2010; Hurrell and Egli, 2011). Cereals are the primary sources of zinc in most vegetarian diets, secondary sources being legumes (Lynch, 2011). Besides inherent factors such as phytate, tannin, and fibre negatively influencing the bioavailability of zinc and iron from these food grains, the same may also be influenced by processing, such as cooking, that these food grains undergo. Food processing by heat generally alters the bioavailability of nutrients-both macro and micro. The digestibility and hence absorption of micronutrients such as iron is believed to be improved upon heat processing; with the resultant softening of the food matrix, protein-bound iron is released, thus facilitating its absorption (Hambidge, 2010) In addition, heat processing of food is also likely to alter the inherent factors that inhibit mineral absorption, such as phytate and dietary fibre, especially the insoluble fraction.

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We have recently determined the zinc and iron bioavailability values in cereals and pulses commonly consumed in China, employing an in vitro dialysability procedure and observed differences in the extent of dialysability of zinc and iron from these grains. Zinc bioavailability from the food grains (which was 5.5-21.4% in cereals and 27-56% in pulses) was generally higher than that of iron (1.77-10.2%). A significant negative correlation between inherent phytate and zinc bioavailability value was inferred in the case of pulses, whereas the same was evident for iron in the case of cereals. Calcium had a negative influence on zinc bioavailability in cereals, but not on iron bioavailability.Tannin did not have any significant influence on zinc and iron bioavailability from cereals and pulses. While both insoluble and soluble fractions of the dietary fibre in the food grains generally interfered with zinc bioavailability, the insoluble fraction alone had this effect on iron bioavailability (Luo *et al.*, 2012; Luo *et al*., 2014).

These cereals and pulses are mostly consumed in the cooked form, while a few of the legumes are consumed raw, after soaking, in the form of salads. Assessing the bioavailability of zinc and iron from the cooked form of these food grains would therefore be relevant. In the absence of any data for zinc bioavailability from heat-processed food grains, the influence of domestic heat processing on the same has been investigated here. Iron bioavailability as modulated by such heat processing has also been examined to make a comparison.

Materials and Methods

Materials

Cereals-rice, barley, buckbarley, wheat, and oat, and pulses-chickpea-whole and decorticated, green gram-whole and decorticated, decorticated black gram, decorticated red gram, cowpea, and faba bean were procured locally, cleaned and used here. Pepsin, pancreatin, and bile extract, all of porcine origin, iron, and zinc standards were from Sigma Chemical Co., USA. Allother chemicals used here were of analytical grade. Triple distilled water and acid-washed glassware were used throughout the study.

Total zinc and iron

Iron and zinc contents in materials were analysed by atomic absorption spectrophotometry (Varian SpectrAA 200, Victoria, Australia) after dry ashing for 2 h at 530ºC. Depending on the differetmnt treaents, 2-4 g of ash were weighed in a silicon evaporating dish. Next, the ashes were wet-acid digested with nitric acid (65%) on a hot plate and solubilized with 25 ml of 0.5 N HCl.

Bioavailability of zinc and iron

Bioavailability of zinc and iron was defined as the relative amount of iron and zinc that became soluble after enzymatic treatment. Faba bean samples were sequentially digested with enzymes, including amylase, pepsin, pancreatin and bile, under certain conditions following the enzymatic degradation procedure described by Kiers *et al*. (2000). Mixtures were centrifuged at 5,000 x g for 15 min at 4°C. The resulting supernatant was filtered (0.45 µm membrane, FP 030/3, Kaijie, Hangzhou, Zhejiang) and frozen until further analysis. Iron and zinc levels, including soluble free ionizable iron and zinc and soluble complexes of iron and zinc, were analysed by atomic absorption spectrophotometry. Each sample was enzymatically extracted in duplicate. In vitro soluble iron and zinc contents were determinated on three independent digests.

Heat processing of food grains

To examine the influence of heat processing on zinc and iron bioavailability from the food grains, two methods of heat processing - pressure cooking and microwave cooking were employed. Ten gram of the food grains was pressure-cooked in 30mL of triple distilled water for 10 min (15 p.s.i.). In the case of French bean and cowpea, the grains were soaked in triple distilled water overnight and then pressure cooked as above. For microwave cooking, 10 g of the food grains were cooked in 150 mL of triple distilled water at 360W for 30 min in the case of whole grains (pre-soaked overnight) and 20 min for cereals and decorticated pulses (pre-soaked for 4 h). The cooked samples were homogenized in a stainless steel Omni-mixer (Sorvall) and used for the determination of mineral bioavailability as described above.

Statistical analysis

Data were analysed with SPSS (Statistical Package for the Social Sciences) 13.0 for windows. The mean and standard deviation of means were calculated. The data were analysed by one-way analysis of variance (ANOVA). Duncan’s multiple range test was used to separate means. Significance was accepted at a probability $P < 0.05$.

Results and Discussion

All the food grains (both cereals and legumes) studied in this investigation are consumed in the cooked form, while a few of the legumes are also

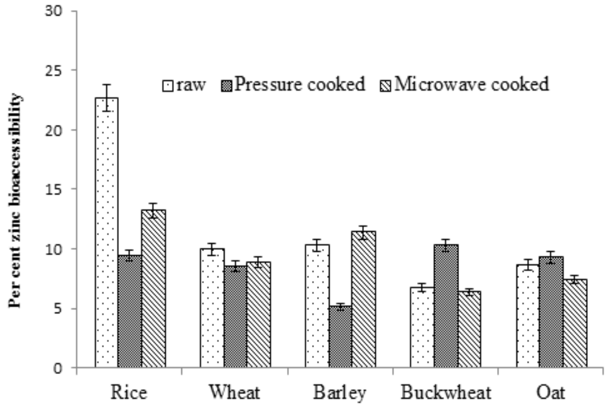

Figure 1. Effect of heat processing on the bioaccessibility of zinc from cereals

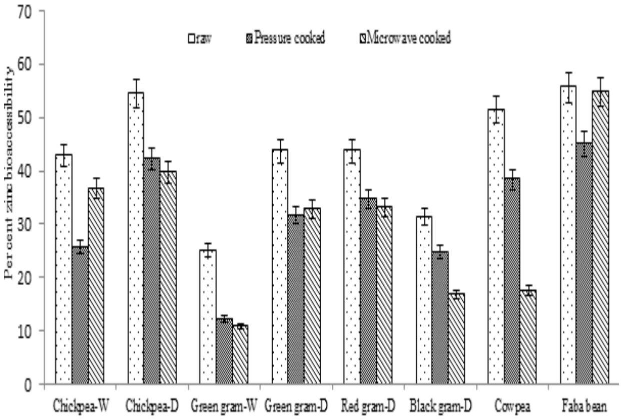

Figure 2. Effect of heat processing on the bioaccessibility of zinc from pulses

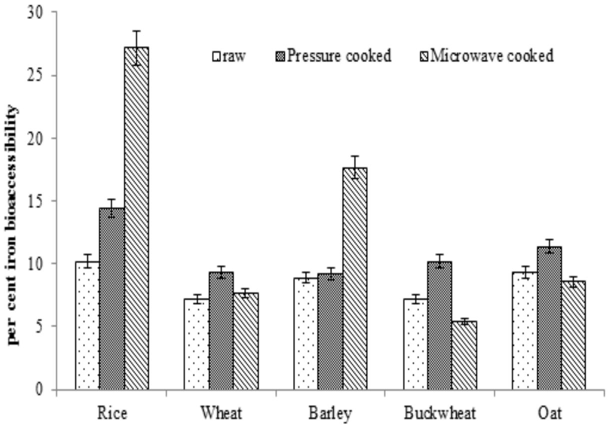

Figure 3. Effect of heat processing on the bioaccessibility of iron from cereals

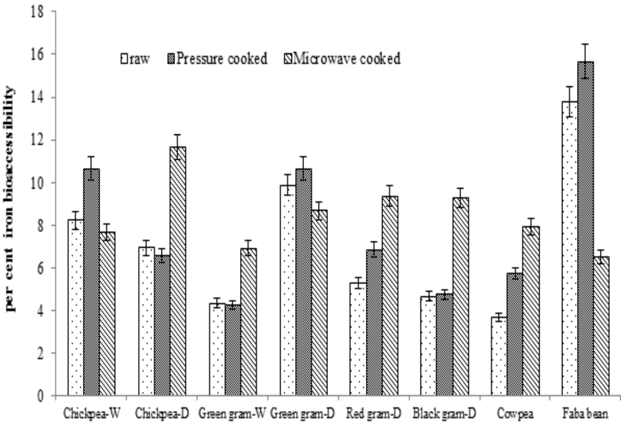

Figure 4. Effect of heat processing on the bioaccessibility of iron from pulses

consumed in raw form after soaking and germination (as salads). Considering the bioavailability of zinc and iron from the cooked form of these grains would, therefore, be more meaningful. Influence of heat processing on the zinc bioavailability from various cereals and pulses is presented in Figures 1 and 2. Heat treatment of the food grains by pressure-cooking generally decreased the bioavailability of zinc, especially in pulses. Among the cereals examined (Figure 1), pressure-cooking decreased the bioavailability of zinc by an extent of 63% and 57% in the case of finger millet and rice, respectively. On the other hand, there was a significant increase (72%) in the bioavailability of zinc from sorghum as a result of pressure-cooking. Microwave cooking also brought about similar decrease in zinc bioavailability in the case of rice (39%) and maize (19%). In the case of wheat, neither of the heat treatment procedures affected the bioavailability of zinc to any significant extent. Thus, all the pressure-cooked cereals had similar percent zinc bioavailability values, with the exception of finger millet, which had a much lower percent zinc bioavailability.

There was generally a significant decrease in the bioavailability of zinc from all the pulses studied, as a result of heat treatment by pressure-cooking or microwave cooking (Figure 2). This decrease ranged from 11.4% in microwave-cooked chickpea (whole) to 63% in microwave cooked cowpea. Decrease in zinc bioavailability was 48% in pressure-cooked whole chickpea, 45% and 55% in heat-processed whole green gram, 32% and 22% in heat-processed decorticated green gram, and 45% in microwave-cooked black gram. As in the case of raw grains, the cooked decorticated pulses had higher concentrations of bioaccessible zinc than their whole counterparts. In general, microwave cooking did not seem to have any advantage over pressure-cooking, with respect to bioavailability of zinc.

Kaur and Kawatra (2002) have reported a higher retention of zinc in rats fed diets based on dehusked, soaked, and pressure-cooked rice bean. Soaking and dehusking of rice bean followed by pressure-cooking resulted in a greater percentage of soluble zinc. The same was observed in rice bean that was sprouted and pressure-cooked. This higher solubility was attributed to a decrease in antinutritional factors such as phytic acid and dietary fibre inherently present

Table 1. Bioaccessible zinc and iron from pressure-cooked cereals and pulses

Food grain	Bioaccessible zinc		Bioaccessible iron	
	Raw	Pressure-cooked	raw	Pressure-cooked
Cereals				
Rice	0.331±0.008	0.211±0.012[b]	0.214±0.008	0.287±0.003[a]
Wheat	0.187±0.006	0.176±0.013	0.389±0.006	0.458±0.005
Barley	0.186±0.006	0.095±0.005[b]	0.256±0.006	0.268±0.008
Buckwheat	0.165±0.006	0.312±0.023[a]	0.632±0.007	0.876±0.012[a]
Oat	0.152±0.004	0.182±0.012	0.584±0.008	0.723±0.023[a]
Pulse				
Chickpea-W	1.211±0.038	0.654±0.013[b]	0.683±0.008	0.794±0.009
Chickpea-D	1.724±0.058	1.345±0.069[b]	0.512±0.006	0.356±0.004[b]
Green gram-W	0.854±0.039	0.432±0.008[b]	0.234±0.007	0.212±0.003
Green gram-D	1.121±0.084	0.965±0.016	0.589±0.004	0.765±0.008
Red gram-D	1.264±0.087	1.076±0.043[b]	0.342±0.007	0.456±0.009
Black gram-D	0.946±0.076	0.746±0.009[b]	0.356±0.005	0.312±0.002
Cowpea	1.574±0.023	1.127±0.023[b]	0.145±0.004	0.367±0.004[a]
Faba bean	1.368±0.029	1.129±0.034[b]	1.234±0.032	1.478±0.034[a]

Values (mg/100 g) are expressed as average±SEM of five independent determinations.
[W]: whole; D: decorticated.
[a]: Significant increase.
[b]: Significant decrease.

in this legume, as a result of dehusking/soaking/sprouting. In the present study, consistently lower concentrations of bioaccessible zinc were observed in the heat-treated food grains. This trend was evident even in whole legumes, and those soaked overnight prior to heat treatment.

While there was a decrease in the bioavailability of zinc as a result of heat processing of the grains, the same generally increased the bioavailability of iron from the food grains studied, with a few exceptions (Figures 3 and 4). The bioavailability of iron from pressure-cooked cereals ranged from 7% in wheat to 12% in the case of rice. These iron bioavailability values were significantly higher than those of corresponding raw cereals except finger millet. Increase in the percent iron bioavailability was still greater when rice or finger millet were microwave-cooked (Figure 3). The iron bioavailability values of pressure-cooked pulses were significantly higher than those of corresponding raw pulses except in decorticated chickpea, whole green gram, and black gram (Figure 4). Microwave heating generally produced even higher increase in iron bioavailability from pulses-decorticated chickpea, whole green gram, red gram, black gram, and cowpea. Microwave cooking is decreased iron bioavailability in sorghum, maize, whole chickpea, and French bean by 44, 12, 23, and 60%, respectively. Incidentally, the percent bioavailability of both zinc and iron from pressure-cooked cereals was similar.

Between these two heat-processing methods employed here, pressure-cooking is the commonly adopted practice in Indian households. As evident from Table 1, the actual bioaccessible zinc content (mg/100 g) of pressurecooked cereals ranged from 0.05 (finger millet) to 0.21 (sorghum), while that of iron ranged from 0.16 (rice and finger millet) to 0.47 (sorghum). Although the percent bioavailability of iron from pressure-cooked rice is the highest, it still cannot be considered as the best provider of this mineral among cereals, because of the low inherent iron content. Sorghum, which is the staple of a majority of the rural population, appears to be the best provider of both zinc and iron. The bioaccessible zinc content (mg/100 g) from pressure-cooked pulses ranged from 0.36 in whole green gram to 1.18 in decorticated chickpea, whereas bioaccessible iron from these pressure-cooked pulses ranged from 0.11 (whole green gram) to 0.70 (French beans). Thus, each one of these pulses seems to be better providers of zinc than of iron, despite consistently higher inherent iron content. Among the pulses examined, decorticated chickpea, cowpea, French bean, and red gram are among the best sources of bioaccessible zinc. French bean stands out among pulses as far as iron bioavailability is concerned, whereas therest of the pulses have bioaccessible iron content comparable to cereals.

It is generally believed that heat processing improves the digestibility and bioavailability of both macro- and micronutrients. However, this trend was not evident in the case of zinc bioavailability from a majority of food grains in the present study. The differences between zinc and iron dialysabilities from food grains can be attributed to the different chemical forms of these metals (valence states) (Karakaya, 2004), different physicochemicalenvironment, and their possible different localizations in the grains. These minerals are linked or complexed to other different constituents, possibly proteins (Luo *et al.*, 2013). The types of linkages and associated constituents could be different for zinc and iron.

The decrease in zinc bioavailability on heat treatment of the tested food grains in the present study could be attributed to interactions of zinc with proteins, and/or other food components thereby hindering its absorption. Although the zinc bioavailability from raw pulseswas higher relative to cereals, the negative effect of cooking was also higher in the case of pulses. Whether this observation that the decrease in zinc bioavailability was more prominent in the case of pulses upon heat possessing is attributable to the higher amounts of protein present in them remains to be evidenced. Cooking of food grains, which generally improves protein digestibility, has not resulted in improved absorbability of zinc, unlike that of iron. Carbonaro *et al.* (1995) have reported that iron bioavailability was compromised as a result of impaired protein digestibility, while that of zinc did not seem to be affected. There was no difference in zinc dialyzability from two globulins, with different digestibility extracted from white beans. The authors inferred that the main determinant of zinc dialyzability was probably amino acid composition of the protein, rather than its digestibility. This suggested that interaction of zinc with protein was more specific compared to that of iron. Differences, if any, in the nature of interaction of the two metals with food proteins upon heat processing, remain to be understood.

Cooking presumably modifies the seed composition, in turn influencing the zinc and iron dialysability. Cooking has been reported to result in phytate reduction in food grains. The changes in zinc and iron dialysability caused by cooking as evidenced here cannot be fully explained by the changes occurring in any single constituent. Interactions with the protein matrix may play an important role as far as the potential miner alavailability is concerned. Further studies are necessary to better understand the changes in food matrix induced by cooking which mostly influence zinc and iron availability. The differences in in vitro availability that exist between zinc and iron present in food grains could be due to their different chemical forms, differences in their association with other grain constituents, and differences in their localizations within the grain.

The *in vitro* method employed here for the estimation of mineral (Zn and Fe) availability is based on simulation of gastro-intestinal digestion and estimation of the proportion of the nutrient convertible to an absorbable form in the digestive tract, by measuring the fraction that dialyses through a membrane. This method has been well standardized by Kiers *et al*. (2000) and internationally accepted. The dialyzability of a mineral gives a fair estimate of its availability for absorption *in vivo*. Such in vitro methods are rapid, simple, and inexpensive. In vitro methods provide relative rather than absolute estimates of mineral absorbability since they are not subjected to the physiological factors that can affect bioavailability (Theil, 2004). However, such relative estimates of the mineral bioavailability in terms of in vitro zinc and iron dialysability from heatprocessed food grains obtained in the present investigation are still valid and suffice to form a strategy to derive maximum mineral availability. The duration of heat processing employed here is based on what is generally encountered in traditional cooking process in Indian households.

Conclusion

The present study has evidenced that zinc bioavailability from food grains was considerably reduced upon pressure-cooking, especially in pulses. Among the cereals, pressure-cooking decreased zinc bioavailability by as much as 63% and 57% in finger millet and rice, respectively. Nevertheless all the pressure-cooked cereals showed similar percent zinc bioavailability values, with the exception of finger millet, which had a much lower percent zinc bioavailability. The decrease in bioavailability of zinc caused by microwave heating ranged from 11.4% in chickpea (whole) to 63% in cowpea. Iron bioavailability, on the other hand, was significantly enhanced generally from all the food grains studied upon heat treatment. Thus, heat treatment of grains produced contrasting effect on zinc and iron bioavailability. Considering the bioaccessible zinc values from pressure-cooked food grains observed in the present study, legumes provide significantly higher amounts of zinc than cereals. Consumption of liberal amounts of legumes through the diet could probably contribute to prevent zinc deficiency. On the other hand, our observation on the bioavailability of iron from pressure-cooked food grains suggests that pulses generally do not score over cereals with respect to deriving iron.

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