Influence of domestic processing on the bioaccessibility of selenium from selected food grains and composite meals

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Revised: 30 September 2015 / Accepted: 22 October 2015 / Published online: 31 October 2015
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Abstract Selenium, an ultra trace element with several health beneficial attributes, should be mainly derived from dietary sources. Since food processing is likely to alter the bioavailability of micronutrients, the influence of such processing such as germination and fermentation on selenium content and bioaccessibility, information on which is lacking, was examined in this study. Bioaccessibility of selenium from four cereal-based composite meals was also studied. Chickpea, green gram and finger millet were employed to study the effect of germination, and for effect of fermentation, batters used in preparation dosa, idli and dhokla were used. Soaking the grains in water as a part of germination and fermentation brought about a decrease in selenium content, while its bioaccessibility was not affected. The information on the loss of selenium during soaking and heat processing of the germinated grains is novel. Fermentation resulted in a further decrease in selenium content, the percent decrease ranging from 26 to 47 in the batters. Similar decreases were seen in the bioaccessible selenium content as a result of soaking and fermentation. Cooking of the fermented batters, however, significantly enhanced the bioaccessibility of selenium from dosa and dhokla by 44 and 71 %, respectively. Selenium content of the four meals ranged from 150 to 228.8 ng/g. Bioaccessible selenium was highest in the finger millet-based meal (32.8 ng/g), followed by sorghum, wheat and rice-based meals. The present investigation thus provides vital and novel information on selenium content and bioaccessibility from foods subjected to processing as is commonly practiced in Indian households.

Keywords Selenium · Bioaccessibility · Germination · Fermentation · composite meal · RDA

Introduction

Selenium is a key trace element required in small amounts for the functioning of a number of enzymes dependent on this mineral such as glutathione peroxidase (GPX) and thioredoxin reductase (Arthur and Beckett 1994). Selenium deficiency has now been recognized as a global problem which needs to be addressed (Valdiglesias et al. 2010). The role of selenium for maintenance of health and the prevention of several diseases is probably more important than it was realised until now (Clark et al. 1996; Ip 1998). Selenium plays an important biological role in humans (Marco et al. 2014), since it functions at the catalytic centre of several selenoproteins (Rayman 2005).

Selenium uptake by the plant–animal–human food chain is dependent on its content in soil (Kabata and Pendias 2001). It varies geographically between and within countries; hence it is important to determine the selenium content in foods consumed widely in each region. Precise determination of selenium content in the food is important because there is a narrow margin of safety between adequate amount and overconsumption (Younju et al. 2009). The Indian Council of Medical Research (ICMR) has recommended an intake of 40 µg/day of selenium as the accepted intake for Indians (ICMR 2010).

It is important to know the bioavailability of selenium present in the diet to establish nutritional selenium status in relation to dietary intake and to intervene promptly in case of its deficiency (Julien et al. 2001).

Food processing methods such as dehulling, cooking, soaking, germination and natural fermentation have been found to influence the digestibility and availability of micronutrients in grain based diets (Jood and Khetarpaul 2005; Pugalenth and

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Vadivel 2005). Processes such as germination and fermentation are reported to increase the physicochemical accessibility of micronutrients, decrease the content of antinutrients such as phytate, or increase the content of enhancers of bioavailability of minerals (Christine and Rosalind 2007). Fermented foods have a special significance in the diets of a predominantly vegetarian population as in India (Ahmad et al. 2008). Despite its biological importance in human health, data on selenium in foods are still very limited, particularly in Asian countries, including India. Information on the influence of fermentation and germination of cereals and pulses on the bioaccessibility of trace minerals such as iron and zinc is adequately reported in literature (Hemalatha et al. 2007), but similar information on the bioavailability of selenium is lacking.

The aim of the present investigation is to generate information on the influence of common domestic food processing methods such as soaking, germination and fermentation of cereals/pulses on the bioaccessibility of selenium. Since food grains are consumed as a part of a composite meal, bioaccessibility of selenium in composite meals prepared by using staple food grains such as rice, wheat, finger millet and sorghum, was also determined in this study. Our study is the first to report the influence of processing methods on the bioaccessibility of selenium from foods. Such information would be useful to formulate dietary strategies to maximize selenium intake for health benefit.

Materials and methods

Materials

Finger millet (*Eleusine coracana*), Chickpea (*Cicer arietinum*), green gram (*Phaseolus aureus*) whole and decorticated, black gram decorticated (*Phaseolus mungo*), and rice (*Oryza sativa*), procured from National seed corporation of India Pvt. Ltd., were used for the study, taking care to collect the samples from the same batch. Porcine pancreatin, pepsin and bile extract were from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents used in the experiments were of analytical grade. Milli Q water and acid washed glassware were used throughout the entire study.

Determination of total Selenium concentration in the processed food

Total selenium concentration of the processed food grains was analyzed by using Inductively Coupled Plasma – Atomic Emission Spectroscopy (ICP-AES) (Ultima-II, M/S. Horiba Jobin Ywon). Weighed samples of the grains were subjected to cold acid digestion in the presence of hydrogen peroxide (H_2O_2) and nitric acid (HNO_3 , 68 %) overnight. The digested samples were then boiled for about 30 min and the volume was made up to a suitable volume (Kapolna et al. 2007).

Germanium dioxide was added as internal standard (10 $\mu\text{L/L}$) before analyzing by ICP-AES. Calibration of selenium measurement was carried out by using selenium standard and blank (2 % nitric acid) at a wavelength of 196.02 nm. All the measurements were carried out with the standard plasma operating conditions as recommended by the manufacturer.

Determination of bioaccessibility of Selenium

Bioaccessibility of selenium was determined by the in vitro method of Luten et al. (1996) with suitable modifications. Known amount of samples were subjected to simulated gastric digestion for 2 h in shaking water bath at 110 rpm (Julabo - SW22) by incubation with pepsin (16 g in 100 ml of 0.1 M HCl) at pH 2, at 37 °C. Titratable acidity was determined in a sample of the gastric digesta. Intestinal digestion was carried out at 37 °C with pancreatin–bile extract mixture (4 g porcine pancreatin and 25 g of bile extract (porcine) in 1000 ml of 0.1 M NaHCO_3) using dialysis tubing (molecular mass cut off 10 kDa) containing 25 ml sodium bicarbonate solution being equimolar to the titratable acidity determined. At the end of the intestinal digestion, the dialysates were transferred to sample tubes and stored at –4 °C till the analysis of selenium by ICP-AES as per the standards.

Domestic processing

Germination

Finger millet, chick pea, and green gram whole were soaked for 16 h in milli Q water (1:2.5 w/v), the water was drained off and the soaked grains were allowed to germinate at laboratory condition for 48 h. The germinated seeds were subjected for the analysis of bioaccessible Se; a parallel set of germinated seeds were subjected to heat processing by pressure cooking. The seeds were pressure cooked in 30 ml of milli Q water for 10 min (15 psi). The heat-processed samples were homogenized in a stainless steel Omni mixer and used for the determination of Se content and bioaccessibility. All the analyses were carried out in five replicates. Total Se content and bioaccessible Se was determined as described above.

Fermentation

To study the influence of fermentation on selenium content and bioaccessibility, three commonly consumed fermented foods, namely dosa, idli and dhokla were prepared by using cereal-pulse combinations in different ratios. Rice and black gram decorticated were used in the ratio of 3:1, and 2:1 for the preparation of dosa and idli respectively. Chick pea, green gram decorticated and black gram decorticated were combined in the ratio 2:2:1:1 respectively for the preparation of dhokla. All the ingredients were weighed accurately and soaked in milli Q water (grain: water = 1:2.5 w/v) for 10 h.

The soaked grains were ground with the entire water to a fine batter. The batter was allowed to ferment for 14 h at laboratory temperature without adding any exogenous starter culture. A portion of the batter was steam cooked for 10 min, for the preparation of idli and dhokla, while a portion of dosa batter was pan fried (in a non stick pan without oil).

Total selenium content and bioaccessibility of selenium was determined in non fermented and fermented batter. The bioaccessibility of selenium was analyzed in the cooked fermented products (dosa, idli and dhokla). Determination of total selenium content and its bioaccessibility in these variations of food samples was carried out in five replicates.

Composite meals

Representative meals based on four staple cereals - rice, wheat, finger millet and sorghum along with an appropriate pulse as recommended by the Indian council of Medical Research, India, were used to determine selenium content and bioaccessibility. The composition of the meals was the same as that reported by us earlier (Bhavyashree et al. 2009). The individual items of meals were cooked separately as normally prepared in Indian households, and each meal was homogenized in a stainless steel blender and analysed for total content and bioaccessibility of selenium.

Statistical analysis

All determinations were carried out in five replicates, and the average values are reported. Statistical analysis of data was done applying one way analysis of variance (ANOVA) and the differences between means were determined by Dunnet's test and considered extremely significant when $P < 0.01$ and significant when $P < 0.05$.

Results and discussion

Effect of soaking and germination on selenium content

The influence of soaking and germination of chick pea, green gram and finger millet on the selenium content is presented in Table 1. Among the various legumes consumed in India, green

gram and chickpea are most commonly germinated prior to use in the preparation of specific traditional dishes, especially in southern India, whereas germination of finger millet is a part of the malting process (Hemalatha et al. 2007). Prior to germination, the food grains were soaked in water for 16 h. Soaking brought about a decrease in the selenium content of green gram and finger millet, while such a negative effect was not seen in the case of chickpea. The decrease in the selenium content observed in the soaked grains might be because of leaching of the low molecular weight Se-compounds which are not bound to protein, by passive diffusion as suggested by Slejkovec et al. (2000).

The total selenium content in chick pea, green gram and finger millet, germinated at 48 h ranged from 143 to 224 ng/g (Table 1). Germination of food grains for 48 h did not influence the selenium content of any of the grains examined. A similar absence of effect of germination on selenium content was reported in garden cress and lupin seeds, when these seeds were germinated for up to five days (Frias et al. 2009, 2010). Heat processing of the germinated food grains, however, brought about a significant decrease in the selenium content of all the three grains examined. The percent decrease in the selenium content was 33, 35 and 36 in chickpea, green gram and finger millet, respectively. This decrease in selenium content could probably be attributed to volatilization of selenium. Plants could volatilize selenium as dimethylselenide (DMSe) or dimethyldiselenide (DMDSe) from roots and to a lesser extent, from shoots (Grant et al. 2004). This information on the loss of selenium as a consequence of heat processing of grains is novel and is probably specific to this mineral because of its potential for volatilization.

Effect of germination on selenium bioaccessibility

Effect of germination of the above grains on the bioaccessibility of selenium is presented in Table 2. As in the case of total selenium content, germination did not have any beneficial effect on the bioaccessibility of selenium in any of the grains examined. However, heat processing of the germinated grains brought about a significant decrease in selenium bioaccessibility, the extent of decrease being as high as 59 % in chickpea. The percent decrease in bioaccessible selenium in green gram

Table 1 Influence of soaking and germination on the total selenium content from selected food grains

Food grains	Native (ng/g) (unprocessed)	Soaked (ng/g)	Germinated (ng/g)	
			Raw	Heat processed
Chick pea	171.6 ± 3.4	172.3 ± 11.1 ^{ns}	170.8 ± 3.2 ^{ns}	113.0 ± 8.0**
Green gram	223.2 ± 7.6	207.2 ± 9.5*	224.8 ± 6.3 ^{ns}	145.7 ± 5.0**
Finger millet	141.0 ± 12.2	137.3 ± 8.0 ^{ns}	143.2 ± 3.3 ^{ns}	91.3 ± 6.3**

Values (dry weight basis) are expressed as mean ± SEM of five replicate analyses

ns ($p > 0.05$), * $p < 0.05$, ** $p < 0.01$, significantly different from the native unprocessed food grain

Table 2 Influence of soaking and germination on the bioaccessible selenium from selected food grains

Food grains	Native (ng/g) (unprocessed)	Soaked (ng/g)	Germinated (ng/g)	
			Unprocessed	Heat processed
Chick pea	26.7 ± 2.0 (15.6)	24.3 ± 2.3 ^{ns} (14.1)	25.4 ± 2.2 ^{ns} (14.9)	10.3 ± 0.5** (9.1)
Green gram	39.1 ± 2.6 (17.5)	30.2 ± 1.6 ^{**} (14.6)	35.1 ± 2.6 ^{ns} (15.6)	16.7 ± 0.9** (11.5)
Finger millet	22.7 ± 1.9 (16.1)	19.7 ± 1.1 [*] (14.4)	25.7 ± 1.9 ^{ns} (17.9)	13.2 ± 1.0** (14.5)

Values (fresh weight basis) are expressed as mean ± SEM of five replicate analyses

ns ($p > 0.05$), * $p < 0.05$, ** $p < 0.01$, significantly different from the native unprocessed food grain

Values in parentheses indicate percent bioaccessibility of selenium

and finger millet was 52 and 49, respectively. This decrease in bioaccessible selenium is resultant from a decrease in the total selenium content in heat treated germinated grains Table 3.

Germination is reported to produce contrasting effects on the bioaccessibility of zinc and iron from food grains (Hemalatha et al. 2007). While there was a significant decrease in zinc bioaccessibility as a result of germination of finger millet and green gram for 48 h, bioaccessibility of iron was significantly enhanced by the process of germination in these grains. These authors also reported a significant reduction in the tannin content of the food grains as a result of germination.

Influence of fermentation on selenium content

Fermented foods such as idli, dosa and dhokla are popular breakfast items in Indian culinary. Cereal-pulse combinations as required for the preparation of these foods were examined for selenium content and its bioaccessibility. As in the case of germination, soaking of the food grains prior to fermentation brought about a significant decrease in the total selenium content. The percent decrease observed was 20, 24 and 18 in the unfermented batter of dosa, idli and dhokla respectively when compared to raw grains. Fermentation resulted in a further decrease in

the selenium content, the percent decrease being 26, 29 and 47 for dosa, idli and dhokla, respectively. The selenium content of the fermented foods examined here ranged from 0.015–0.019 mg/100 g. Similar values of selenium in fermented rice (0.02–0.03 mg/100 g) have been reported (Praveen et al. 2012).

The optimum time required for the activity of micro flora to increase the selenium content has been reported to be 72 h (Odumodu 2007). However, the time allowed for fermentation in the present study was 12 h, as is commonly practised at the household level in India. This could be the reason for the lack of a beneficial effect of fermentation on selenium content of the foods studied.

Influence of fermentation on bioaccessible selenium

Effect of fermentation on selenium bioaccessibility from foods such as dosa, idli, and dhokla is reported in Table 4. Similar to its effect on total selenium, fermentation significantly decreased the bioaccessibility of selenium from the cereal-pulse combinations examined. The process of soaking prior to fermentation itself caused significant decreases in selenium bioaccessibility. There was a 43 % decrease in bioaccessible selenium in the batter ground from the soaked grains

Table 3 Effect of Soaking and fermentation on the total selenium content in selected foods

Fermented Foods	Native (ng/g)	Soaked (ng/g)	Fermented batter (ng/g)
Dosa	261.2 ± 2.6	207.6 ± 5.0**	192.1 ± 3.1a**
Rice: black gram (D) (3:1)			
Idli	280.6 ± 4.1	212.5 ± 4.1**	199.2 ± 6.1a**
Rice: black gram (D) (2:1)			
Dhokla	289.9 ± 5.3	236.1 ± 4.3**	153.0 ± 4.9a**
Chickpea: green gram(D):black gram (D):rice (2:2:1:1)			

Values are expressed (dry weight basis) as mean ± SEM of five replicate analyses

ns (not significant) $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, significantly different from the raw grains

a-Significant decrease from soaking,

D: Decorticated

Table 4 Effect of Soaking and fermentation on bioaccessible selenium from selected foods

Foods	Native (ng/g)	Unfermented batter (ng/g)	Fermented batter (ng/g)	Fermented cooked batter (ng/g)
Dosa	40.5 ± 1.0	23.1 ± 1.5**	20.6 ± 0.9**	29.7 ± 0.8**
Rice: black gram (D) (3:1)	(15.5)	(11.2)	(7.9)	(11.4)
Idli	43.2 ± 1.3	18.0 ± 1.0***	30.0 ± 1.2**	31.7 ± 0.9**
Rice: black gram (D) (2:1)	(15.4)	(8.5)	(10.7)	(11.3)
Dhokla	44.3 ± 0.9	27.6 ± 0.7**	20.8 ± 0.5**	35.6 ± 1.3**
Chickpea: green gram(D):black gram (D):rice (2:2:1:1)	(15.3)	(11.7)	(7.2)	(12.3)

Values are expressed (fresh weight basis) as mean ± SEM of five replicate analyses

ns (not significant) $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, significantly different from the raw grains

D: Decorticated

Values in parentheses indicate the percent bioaccessible Se

for the preparation of dosa, while the same was 58 and 38 % in the unfermented batters of idli and dhokla, respectively. Fermentation of these batters brought further significant decrease in the bioaccessible selenium, the percent decrease ranging from 30 to 53.

Cooking of the fermented batters to their respective products, however, resulted in a significant increase in the bioaccessibility of selenium from dosa (44 % increase) and dhokla (71 % increase). Steaming of the fermented batter as in the preparation of idli and dhokla thus seems to beneficially improve the bioaccessibility of selenium, while pan-frying applied for the preparation of dosa did not have a similar effect.

Significant increases in the bioaccessibility of iron and zinc have been reported upon fermentation of batters of idli and dosa, which were retained even when the batters were cooked to their respective products. However, such a beneficial effect was not observed in the case of the fermented batter of dhokla. While the phytate and tannin content of the batters was significantly reduced by fermentation, such a decrease in these anti-nutritional factors was not seen in the batter of dhokla. This was attributed to the presence of chickpea and green gram in the batter of dhokla, in addition to rice and black gram. In the present investigation, however, there was no beneficial effect of fermentation as such on selenium bioaccessibility, but cooking of the fermented batters beneficially enhanced the same.

Content and bioaccessibility of selenium from composite meals

Food grains are invariably consumed as a part of composite meals containing various other ingredients. Hence it would be more meaningful to determine selenium content and bioaccessibility from composite meals. Therefore, the present investigation also aimed at determining the selenium content and bioaccessibility from independent composite meals based on four staple cereals, viz.; rice, wheat, finger millet and sorghum.

The total and bioaccessible selenium content in the four meals is presented in Table 5. Selenium content of the four meals ranged from 150 ng/g in the rice-based meal to 229 ng/g in the finger millet-based meal. Selenium content of the wheat and sorghum-based meals was 217 and 170 ng/g, respectively. Thus, the selenium content of the four meals based on different staple cereals was somewhat similar.

Bioaccessible selenium ranged from 27 to 33 ng/g in the composite meals (Table 5). Finger millet-based meal provided the highest content of bioaccessible selenium (33 ng/g), followed by sorghum, wheat and rice-based meals. Bioaccessibility of selenium from the composite meals seems to be dependent on their total selenium content. While finger millet had the highest total selenium (229 ng/g), rice had the least (150 ng/g). Considering the fresh weight of the whole meal and the bioaccessible selenium content, the rice, wheat, finger millet and sorghum-based meals would provide approximately 18, 15,

Table 5 Selenium content in composite meals prepared based on rice, wheat, finger millet and sorghum

Composite meal	Total Se content (ng/g dry weight)	Bioaccessible Se (%)	Bioaccessible Se (ng/g fresh weight)
Rice based	150.0 ± 13.7	18.0 ± 0.9	26.9 ± 1.0
Wheat based	216.6 ± 5.3	12.7 ± 1.1	27.6 ± 0.9
Finger millet based	228.8 ± 14.3	14.3 ± 1.2	32.8 ± 1.3
Sorghum based	170.0 ± 10.0	16.6 ± 0.9	28.2 ± 0.9

Values are expressed as mean ± SEM of five replicate analyses

20 and 17 µg selenium, respectively, which would meet around one-third the suggested intake for selenium of 45 µg/day, as prescribed by the ICMR for Indians. Consumption of two such meals per day would meet almost the entire recommended amount for this vital trace element. This information assumes importance in making recommendations and evolving dietary strategies for maximizing the intake of selenium, thus exploiting its health beneficial potential.

Conclusions

The present investigation thus provides vital information on selenium content and bioaccessibility from foods that have undergone processing as is commonly practiced in Indian households. Soaking in water as a part of germination brought about a decrease in selenium content, while its bioaccessibility was not affected. The information on the loss of selenium during soaking and heat processing of the germinated grains is novel. Soaking of the food grains prior to fermentation and the process of fermentation itself significantly decreased the total selenium content. Similar decreases were seen in the bioaccessible selenium content as a result of soaking and fermentation. On the other hand, cooking of the fermented batters significantly enhanced the bioaccessibility of selenium from dosa and dhokla. Selenium content and bioaccessibility of the composite meals prepared from four staple cereals was somewhat similar. The four meals would meet one-third of the suggested intake for selenium, based on their bioaccessible selenium content. This novel information on the influence of domestic processing methods on the content and bioaccessibility of selenium goes a long way in forming dietary strategies to derive this trace element maximally.

Acknowledgments The first author acknowledges the Indian Council of Medical Research, New Delhi, India, for the award of fellowship. The authors gratefully acknowledge Dr.K.Srinivasan, Chief Scientist, Department of Biochemistry & Nutrition, CSIR-CFTRI, for his very useful and constructive suggestions throughout the study. This work was financially supported by the 12th Five-year plan project of the Institute, WELFO (BSC 0202).

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