Bioaccessibility of selenium from Se rich food grains of seleniferous region of Punjab, India as analyzed by Instrumental and Chemical NAA

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This paper received the IANCAS Best Paper Award (Oral Presentation), at the Fourth International Symposium on Nuclear Analytical Chemistry (NAC-IV), held at BARC, Mumbai, during November 15-19, 2010

Abstract
In the present study, selenium bioaccessibility was measured in rice and maize, cultivated in seleniferous soil of India, using in-vitro gastric digestion (GA) and gastro-intestinal digestion (GI) methods. The concentration of bioaccessible selenium was determined by Instrumental Neutron Activation Analysis (INAA). The total Se was observed to be about 58 mg kg\(^{-1}\) and 29 mg kg\(^{-1}\) in flours of rice and maize respectively. Total Se contents in maize flour sample after GA and GI were approximately 9.5 mg kg\(^{-1}\) and 15 mg kg\(^{-1}\); and in case of rice samples the levels were about 32 mg kg\(^{-1}\) and 38 mg kg\(^{-1}\). The results indicated that the bioaccessibility of GI digestion (51% in maize and 65% in rice) was higher compared to GA digestion (32% and 52% in maize and rice respectively). In addition, the bioaccessible levels of selenium were significantly more in the case of rice compared to maize.

Introduction
Selenium is an essential element for both human beings and animals. Se enters into the body as a dietary supplement through food. But it is also true that after digestion, all mineral contents present in diet may not be completely absorbed and utilized for physiological function of the body. It depends on the efficiency of the digestive system and chemical species of the elements. For example, Se as selenomethionine is more easily absorbed when ingested, than is selenite, selenate or selenocystine. In short, bioaccessibility reflects the efficiency with which the nutrients are absorbed from the alimentary tract and available for further storage and use (Fairweather-Tait, 1992). Most of the cultivated plants possess the ability to absorb, metabolize and store significant amount of Se in their tissue when grown on soil containing available Se. Selenium dominantly enters the food-chain through plants (Rayman, 2008). Accumulation of Se by plants is due to their ability to transform inorganic Se into a variety of organoselenium species, including bioactive compounds,
which has important implications for human nutrition and health. The Tolerable Upper Level (TUL) Se-intake for adults is 400 μg d⁻¹ against the daily requirement of 40 μg (Combs, 2001; Goldhaber, 2003). Bioavailability of Se is determined either by in-vivo administration to similar species to humans, for example murine models or in-vitro methods by simulating digestive system (Kulkarni et al., 2007). In-vitro methods were developed which are rapid and inexpensive (Miller et al., 1981). The results obtained by in-vitro methods are based on the formation of digestive products that are soluble or dialyzable (Ruby et al., 1999).

In the present study, the bioaccessible concentrations of selenium were determined in rice and maize collected from the seleniferous region of Punjab, by submitting the Se-rich samples to in-vitro gastric (GA) and gastrointestinal (GI) digestion. Selenium contents in the raw grains and the GA/GI digests were estimated by INAA.

**Experimental**

Harvested paddy and maize grains were collected from the seleniferous belt of Nawanshahr-Hoshiarpur region of Punjab India. The required amount of grains (about 25g) was washed with distilled water and dried overnight at 40°C. De-husked rice (semi milled) and whole maize grains were ground into fine powder using mortar and pestle. For total Se quantification in raw samples, approximately 100mg of rice and maize flour were taken in triplicate and packed in thin aluminum foils.

The in-vitro gastric digestion protocol was adapted from Kulkarni et al. (2007). Accurately weighed amounts (1.25 g) of rice and maize flour were transferred to 50 mL conical flasks, each containing 12.5 mL of gastric juice solution (6% w/v pepsin in HCl, pH = 1.75). Initially, the mixture was shaken vigorously for 1–2 min. The flasks were then sealed tightly with a parafilm and placed in shaker-incubator set at 37°C and 150 rpm for 3 h. Each sample was digested in triplicate and these digests were then cold centrifuged (Hitachi-RX II) at 4°C for 20 min at 5000 rpm. The supernatant was filtered through Whatman nitrocellulose membrane filters (0.45μm, 25mm Ø).

For gastro-intestinal digestion, the pH of the each solution obtained after gastric digestion was adjusted up to pH 7, by drop-wise addition of a saturated solution of NH₄HCO₃. To this mixture, 10 mL of pancreatic digestion solution (mixture of 2% w/v Pancreatin and 0.2% w/v bile salts) was added and shaken vigorously for 1 min. The flasks were incubated at 37°C, 150 rpm for 4 h in a shaker incubator. The digests were then centrifuged at 4°C and filtered, following a procedure similar to the gastric digest. Clear supernatant of both gastric and gastro-intestinal digestion was taken for analysis. 200μL of either gastric or gastro-intestinal digests were carefully transferred onto a Whatman filter paper (no. 41, 42.5mm Ø) with the help of micropipette and then air dried. This step was repeated 10 times so as to transfer a total solution of 2 mL. Each filter paper was folded 3-4 times to make it square shaped and packed in thin aluminum foil.

For Se quantification in raw samples and in GA and GI digests, all packed samples were co-irradiated in the self-serve position of the CIRUS reactor for 7 h duration at a neutron flux of ~10¹³ cm⁻² s⁻¹ with Se ICP liquid standard (Spex) containing known amount of Se (5-25 μg), fused in pure amorphous silica powder and two reference materials namely CRM DOLT-1 (Dogfish Liver) from National Research Council of Canada (NRCC) and SL-1 (Lake Sediment) from IAEA. The samples were allowed to cool for about 9 days, and then the radioactive assay of the samples was -ray spectrometry using carried out by HPGe detector for 1–10 h, depending on Se concentration in samples. The peak areas were determined using peak-fit software PHAST. The peak areas were used for the calculation of the selenium concentration by relative method of NAA.

**Results and Discussion**

A typical gamma ray spectrum generated using data obtained from HPGe detector of the gamma-ray spectrometer with Compton suppressed detection system is presented in Fig. 1. The figure indicates gamma rays (136, 264.7 and 279 keV) that typically signify the presence of ⁷⁵Se(120d).
The selenium concentrations determined in two reference materials, IAEA SL-1 and NRCC CRM DOLT-1 were 2.82 ± 0.08 mg kg⁻¹ and 7.43 ± 0.18 mg kg⁻¹ respectively as against the reported values of 2.9 and 7.34 ± 0.42 mg kg⁻¹ respectively. The percent deviations from certified/information values are within ± 3%. The selenium concentration in raw flour, GA and GI hydrolysates (digests) are given in Table 1. The uncertainties quoted in Table 1 are the standard deviations at ±1σ confidence limits obtained from three independent sample analyses and the % relative standard deviations are in the range of 1.5-5%. INAA quantification of selenium in cereal grains, as reported by this group (Sharma et al. 2009) was found to agree with quantification by ICP-MS in the case of wheat (Cubadda et al., 2010).

Total Se concentrations in maize and rice flour were 29.05 ± 3.5 mg kg⁻¹ and 58.2 ± 5.9 mg kg⁻¹ respectively. In view of maize being taken as whole grain flour and rice as semi-milled (unpolished) in rural Punjab, daily consumption of 100 g of maize flour or semi-milled rice would possibly result in an approximate intake of Se between 7.5 to 15.0 fold, the tolerable upper intake level (TUL) for adults (400 μg Se d⁻¹). With reference to the estimates of bioaccessibility of selenium, the present study indicated that the bioaccessibility obtained by gastrointestinal digestion was higher compared to gastric digestion in both the samples investigated. In addition, the concentration of selenium in terms of its bioaccessible levels was significantly higher in rice as compared to maize. This is due to the fact that the materials undergo pancreatic digestion in the gastrointestinal tract at neutral pH, resulting in higher accessibility of the bioavailable forms of the element. Pancreatin, which is a major enzymatic component, added in the GI, is a mixture of many enzymes that break complex nutrients into simple molecules, making various bioavailable forms of selenium more bioaccessible. The Se concentrations of the samples investigated in this study appeared to be very high in cereal grains for human consumption similar to wheat reported earlier by our group (Cubadda et al., 2010; Sharma et al., 2009). These levels of intake might lead to chronic toxic effects of selenium accumulation in humans through Se-rich food grains and livestock, fed on rice and maize straw as fodder. As an alternative to the social, economic and health impact caused by this Se-rich crop product in the region, exploring the opportunities of using...
locally grown grains for fortification of low-Se grain batches or production of naturally enriched products as Se supplements for human and animal nutrition, is an alternative that is worth considering. Further studies are in progress to investigate the bioaccessible species of Se in products derived from high-Se grains grown in the study area.

References


