Food Chemistry 181 (2015) 304–309

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Does boiling affect the bioaccessibility of selenium from cabbage?

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ARTICLE INFO

Article history:
Received 10 October 2014
Received in revised form 10 February 2015
Accepted 11 February 2015
Available online 16 February 2015

Keywords:
Selenium species
Bioaccessibility
PBET
Enzymes
Cabbage
Boiling

ABSTRACT

The bioaccessible selenium species from cabbage were studied using an *in vitro* physiologically-based extraction test (PBET) which establishes conditions that simulate the gastric and gastrointestinal phases of human digestion.

Samples of cabbage (*Brassica oleracea*) grown in peat fortified with different concentrations of Se(IV) and Se(VI) were analysed, and several enzymes (pepsin, pancreatin and amylase) were used in the PBET. The effect of boiling before extraction was also assayed. Selenium speciation in the PBET extracts was determined using anionic exchange and LC–ICP/MS. The selenocompounds in the extracts were Se(IV), SeMet and, mostly, Se(VI) species. The results show that the activity of the enzymes increased the concentration of the selenocompounds slightly, although the use of amylase had no effect on the results. The PBET showed the concentration of inorganic selenium in the extracts from boiled cabbage decreased as much as 4-fold while the release of SeMet and its concentration increased (up to 6-fold), with respect to raw cabbage.

1. Introduction

Selenium is considered one of the most important trace elements as it plays an important role in human and animal nutrition (Hartikainen, 2005; Zeng & Combs, 2008). The activity of selenoproteins provides antioxidant protection and is involved in thyroid hormone metabolism (Rayman, 2000; Reilly, 2006). Selenomethionine (SeMet) is an essential selenoamino acid which is the main nutritional source of Se for animals, and it is known to be easily bioavailable (Schrauzer, 2003). Some plants can accumulate high concentrations of selenium and their tolerance to it could be attributed to the synthesis of non-protein selenoamino acids, such as γ-glutamyl-selenomethyl-selenocysteine (γ-glu-SeMeSeCys) and selenomethylselenocysteine (SeMeSeCys) (Terry, Zayed, de Souza, & Tarun, 2000). The reviews concerning the studies carried out to characterise Se species produced by different Se species have been found in the literature (Pedrero & Madrid, 2009; Pyrzynska, 2009).

One of the main ways of incorporating selenium into the body is by oral ingestion. Depending on the type and nature of the food, the amount and the frequency of ingestion, both the concentration of Se and its chemical forms in the body can vary. Therefore, given the effects that different Se species can have on the organism, it is necessary to establish a method to study the bioaccessibility of this element, as a preliminary step before acquiring further knowledge of its bioavailability. To this end several approaches have been developed to assess the bioaccessibility in Se-enriched vegetables (Dumont, De Pauw, Vanhaecke, & Cornelis, 2006; Dumont, Ogra, Vanhaecke, Suzuki, & Cornelis, 2006; Thiry et al., 2012). Of the *in vitro* methods reported in the literature, the physiologically-based extraction test (PBET) seems to be the easiest and most widely used (Ruby, Davis, Schoof, Eberle, & Sellstone, 1996) The method, initially applied to soils, uses a two-stage enzymolysis procedure to assess the potential for metals to be released into the digestion tract and absorbed into the blood stream (Funes-Collado, Rubio, & López-Sánchez, 2011).

Various studies assess Se bioaccessibility in food (such as cereals, fish, shellfish, meat, mushrooms and vegetables). It has been reported that vegetable consumption (i.e., cabbage) provides an important source of Se in most human nutrition and seems to decrease cancer risk (Silva-Dias, 2012). Fresh vegetables are very common and widely consumed, especially in central Europe, although several diets typically subject vegetables to some cooking process, such as boiling (Agudo et al., 2002). Therefore, several studies have focused on the assessment of both bioaccessible and bioavailable Se species in raw and cooked vegetable products (radish, onion, chives and garlic) (Kápolna & Fodor, 2007;

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http://dx.doi.org/10.1016/j.foodchem.2015.02.052
0308-8146/

Pedrero, Madrid, & Camara, 2006) and nuts (Dumont, De Pauw, Vanhaecke, et al., 2006; Dumont, Ogra, Vanhaecke, et al., 2006); raw and cooked fish (Crews et al., 1996; Cabañero, Madrid, & Cámara, 2004, 2007); yeast and yeast-based nutritional supplements (Dumont, Vanhaecke, & Cornelis, 2004; Hinojosa, Ruiz-Encinar, Marchante-Gayón, García Alonso, & Sanz-Medel, 2006) and wheat (Govasmark et al., 2010). The use of proteolytic digestion provides information on Se speciation in the original sample; however, detailed information on the identity and content of the different species released after gastrointestinal digestion is needed to evaluate the bioaccessibility of Se from selenized edible vegetables.

Here we study Se bioaccessibility in cabbage (*Brassica oleracea*) using the PBET and the effect of boiling on the PBET results. Then Se species from raw and boiled cabbage are determined and compared. The influence of enzymes during the extraction is also studied.

2. Materials and methods

2.1. Plant culture

Commercial peat provided by Plantaflor Humus Verkaufs-GMbH was used. The peat (composed of perlite and vermiculite) contains more than 90% organic matter (dry matter), 1% N (dry matter), and 60% moisture. It is free from Cl^- and its conductivity is less than 175 μS/cm. Cabbage seeds were sown in multi-pots (depth 6 cm) and cultivated in a plant growth chamber (Ibercex, Spain) in a walk-in configuration, for 3 weeks, under controlled environmental conditions of 70% relative humidity, 22 °C and a 16 h photoperiod (110 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR)). Then, in a greenhouse (temperature range 18–30 °C), individual plants were transplanted into individual pots (14 cm upper diameter, 9.5 cm lower diameter, 16 cm height) of 2 L volume, filled with peat, and the pots were placed on a tray to collect irrigation water. All the plants were irrigated on the basis of their water demands.

2.2. Exposure of plants to selenium

Cabbage seeds were also grown separately in pots containing peat fortified with selenium sodium salts (Se(IV):Se(VI); 1:9) at three levels of supplementation: 6 mg Se kg^{-1} (low level), 21 mg Se kg^{-1} (medium level) and 169 mg Se kg^{-1} (high level).

In order to improve growth, 1 g of an NPK fertilizer (which contains equal amounts of NO_3^-, P_2O_5 and K_2O in the same ratio (15% w/w)) was added to all the growth media 3 times every 2 months. Cabbages were harvested at around 4 months. After collection, vegetable samples were cleaned with deionized water and stored at −20 °C. To determine the total Se content, part of the cabbage samples was dried at 40 °C, milled with a glass mortar, transferred to an HDPE bottle, and stored at room temperature until analysis.

2.3. Analytical methods

Inorganic Se stock solutions were prepared to a concentration of 1000 mg L^{-1} from selenite 99% Na_2SeO_3 (*Aldrich*, Milwaukee, WI, USA) and selenate 99% Na_2SeO_4 (*Aldrich*). Organic Se stock solutions were also prepared to a concentration of 1000 mg L^{-1} from selenocystine ($SeCys_2$) and selenomethionine (SeMet) (*Aldrich*) with HCl 0.5%.

2.3.1. Acidic microwave digestion of cabbage

0.2 g samples of vegetable (previously dried at 40 °C) were weighed in PTFE vessels containing 8 mL of HNO_3 (Panreac Hyperpur) and 2 mL of H_2O_2 (*Prolab*). The resulting mixture was digested using a microwave system (Milestone Ethos Touch Control, 1000 W) via the following programme: 10 min ramp from room temperature to 90 °C; 5 min at 90 °C; 10 min ramp from 90 to 120 °C; 10 min ramp from 120 to 190 °C; and 10 min at 190 °C. After digestion, the samples were filtered (Whatman 40) and diluted to 20 mL with double deionized water, transferred to an HDPE bottle and stored at 4 °C until analysis.

2.3.2. Extraction of Se by enzymatic physiologically-based extraction test

The PBET is based on two sequential extraction steps (gastric and gastrointestinal). For the gastric step, 3.5 g samples of fresh raw cabbage were introduced into 100 mL stoppered glass flasks (DIN NS 29/32, *Lenz*) with 50 mL of gastric solution consisting of 1 L double deionized water, 1.25 g pepsin (CAS 9001-75-06) (*Panreac*), 0.50 g citric acid (*Fluka*), 0.50 g 99% maleic acid (*Aldrich*), 210 μL DL-lactic acid (*Aldrich*) and 250 μL 100% acetic acid (*Merck pro-analysis*). The pH of the resulting solution was adjusted to 1.3 with 37% hydrochloric acid (*Panreac Hyperpur*). The flasks containing the resulting solutions were left in a thermo-agitator water bath (*Clifton NE5-28D*) (37 °C) for 1 h. Then, an aliquot of 3 mL from the resulting solution was taken to be analysed later. To maintain the volume constant, 3 mL of gastric solution was then added. For the gastrointestinal step, 11 mL of saturated sodium hydrogen carbonate solution (*Merck*) was added to the flasks to raise the pH to 7. Then, 1.5 g L^{-1} of porcine bile salts (CAS 8008-63-7) (*Sigma–Aldrich*), 0.4 g L^{-1} of pancreatin (CAS 8049-47-6) (*Sigma–Aldrich*) and 0.1 g L^{-1} of alpha-amylase from *Bacillus subtilis* (CAS 9000-90-2) (*Sigma–Aldrich*) were added. After 3 h in the thermo-agitator water bath, an aliquot of 3 mL was taken from the resulting solution. Finally, the gastric and gastrointestinal extracts were filtered through 0.45 and 0.20 μm nylon membranes and stored at 4 °C for approximately 16 h before analysis.

Table 1
Selenium speciation in cabbage using PBET (a) with enzymes (pepsin, pancreatin and amylase) and (b) without any enzymes. Total Se in cabbage: 952 ± 16 mg Se kg^{-1}.

Extraction system applied	Se speciation (mg kg^{-1}) (n = 3) LC–ICP/MS							
	Gastric extract				Gastrointestinal extract			
	$SeCys_2$	Se (IV)	SeMet	Se (VI)	$SeCys_2$	Se (IV)	SeMet	Se (VI)
(a)	2.8 ± 0.1	2.0 ± 0.6	2.5 ± 1.3	307 ± 45	3.3 ± 0.6	27 ± 1	13 ± 1	347 ± 12
(b)	0.7 ± 0.2	12 ± 1	8.6 ± 1.4	154 ± 17	0.4 ± 0.1	50 ± 15	9.1 ± 1.1	220 ± 32
	Gastric step				Gastrointestinal step			
	$SeCys_2$	Se(IV)	SeMet	Se(VI)	$SeCys_2$	Se(IV)	SeMet	Se(VI)
LOD/(mg kg^{-1})	0.08	0.04	0.08	0.04	0.09	0.04	0.08	0.05
LOQ/(mg kg^{-1})	0.28	0.12	0.25	0.14	0.31	0.13	0.28	0.15