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In vitro bioavailability of total selenium and selenium species from seafood

Jorge Moreda-Piñeiro [a,*], Antonio Moreda-Piñeiro [b], Vanessa Romarís-Hortas [b], Raquel Domínguez-González [b], Elia Alonso-Rodríguez [a], Purificación López-Mahía [a,c], Soledad Muniategui-Lorenzo [a], Darío Prada-Rodríguez [a,c], Pilar Bermejo-Barrera [b]

[a] Department of Analytical Chemistry, Faculty of Sciences, University of A Coruña, Campus da Zapateira, s/n., 15071 A Coruña, Spain
[b] Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry, University of Santiago de Compostela, Avenida das Ciencias, s/n., 15782 Santiago de Compostela, Spain
[c] University Institute of Environment, University of A Coruña, Pazo de Lóngora, Liáns, 15179 Oleiros, Spain

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ABSTRACT

In vitro bioavailability of total selenium and selenium species from different raw seafood has been assessed by using a simulated gastric and intestinal digestion/dialysis method. Inductively coupled plasma-mass spectrometry (ICP-MS) was used to assess total selenium contents after a microwave assisted acid digestion, and also to quantify total selenium in the dialyzable and non-dialyzable fractions. Selenium speciation in the dialyzates was assessed by high performance liquid chromatography (HPLC) coupled with ICP-MS detection. Major Se species (selenium methionine and oxidized selenium methionine) from dialyzate were identified and characterized by HPLC coupled to mass spectrometry (HPLC–MS). Selenocystine was detected at low concentrations while Se-(Methyl)selenocysteine and inorganic selenium species (selenite and selenate) were not detected in the dialyzate. Low bioavailability percentages for total selenium (6.69 ± 3.39 and 5.45 ± 2.44% for fish and mollusk samples, respectively) were obtained. Similar bioavailability percentages was achieved for total selenium as a sum of selenium species (selenocystine plus oxidized selenium methionine and selenium methionine, mainly). HPLC–MS data confirmed SeMet oxidation during the *in vitro* procedure.

1. Introduction

Selenium has been largely recognized as a nutritionally essential element for human life. This is because selenium (mainly the selenocystine species) is a component of certain enzymes (seleno-enzymes) which play important roles in mammals (Gladyshev & Hatfield, 1999). However, the content of selenium in foodstuff in several regions of the world is insufficient to offer the proper protective activity, and this justifies the growing interest in the production of selenium fortified foods and selenium-based nutritional supplements (functional foods) (Finley, 2005; Schrauzer, 2001). The recommended intake of selenium is different in each country and depends on factors like age and sex. However, 55 μg/day is generally regarded as the appropriate amount (NAH, 2000). Brazil nuts, mushrooms and certain vegetables, such as broccoli, contain high levels of selenium, mainly as selenomethionine (SeMet) (Vonderheide et al., 2002). Other foodstuff such as meat (Shi & Spallholz, 1994), seafood and seaweed are able to concentrate selenium (Chapman & Chapman, 1980).

It has been well established that the total content of an essential element present in a foodstuff is not totally available, i.e., it is not totally assimilated by the human body. Bio-availability of essential and toxic elements from foodstuff can be performed by *in vivo* (dosing experimental animals with several concentrations of the target element) or by *in vitro* methods. Testing with animals is expensive, difficult to perform, and ethically controversial, and it provides limited data in each experiment (Hansen, Sandstrom, & Lonnerdal, 1996). Alternative *in vitro* approaches for assessing bio-availability studies are therefore more advantageous (Intawongse & Dean, 2006). Two different *in vitro* experimental approaches can be performed in bio-availability studies. The simplest method, commonly referred as bio-accessibility, indicates the maximum fraction of a substance (trace element) in food that can theoretically be released from the foodstuff in the gastrointestinal (GI) tract (bio-accessible fraction), and becomes then available for intestinal absorption (i.e. enters into the blood stream) (Oomen et al., 2002). A second approach, formally called bio-availability, refers to the fraction of a substance that reaches the systemic circulation (blood) from the GI tract (bio-available fraction), and it is available to promote its action in the exposed organism (Ruby et al., 1999). Experimental differences between both approaches are mainly focused on simulating intestinal

* Corresponding author.
E-mail address: jorge.moreda@udc.es (J. Moreda-Piñeiro).

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absorption by means of dialysis membranes during the simulated intestinal digestion in the bio-availability studies (dialysability approach). It must be mentioned that both bio-availability and bio-accessibility tests are affected by the type of food, the composition of food, and also, by the simulated GI conditions used when performing the experiments (Intawongse & Dean, 2006).

Despite the great deal of literature regarding with the assessment of the bio-accessible fraction of essential and toxic elements and organometallic species in foods, data on the bio-available fraction of those targets (including selenium species) is scarce (Intawongse & Dean, 2006; Moreda-Piñeiro et al., 2011). Specifically, selenium speciation studies in the bio-available fraction (dialyzability approach) from seafood are not reported. However, different studies based on bio-accessibility have been addressed for selenium and selenium species in foodstuff containing high selenium concentrations, such as Brazilian nuts (Dumont, De Pauw, Vanhaecke, & Cornelis, 2006). In addition, some studies have been performed in fish, such as swordfish, sardine, tuna and cod (Cabañero, Madrid, & Cámara, 2004, 2007; Crews et al., 1996), in selenium-fortified foodstuff, such as radish (Pedrero, Madrid, & Cámara, 2006), garlic (Dumont, Ogra, Vanhaecke, Suzuki, & Cornelis, 2006), green onion and chives (Kápolna & Fodor, 2007), yeast and yeast-based nutritional supplements (Dumont, Vanhaecke, & Cornelis, 2004; Hinojosa-Reyes, Marchante-Gayón, García-Alonso, & Sanz-Medel, 2006; Hinojosa-Reyes, Ruiz-Encinar, Marchante-Gayón, García-Alonso, & Sanz-Medel, 2006), wheat (Govasmark et al., 2010), chicken eggs and chicken meat (Lipiec, Siara, Bierla, Ouerdane, & Szpunar, 2010).

The objectives of the current work have been the development of an *in vitro* procedure based on dialysability for assessing the bio-availability of total selenium and selenium species from seafood (fish and mollusk). As previously mentioned, bio-availability (dialyzability) studies for total selenium and selenium species from seafood have not yet been addressed.

2. Materials and methods

2.1. Apparatus

Dionex HPLC UltiMate 3000 LC (Dionex, Sunnyvale, CA, USA), equipped with a GP50 gradient pump (Dionex), an AS50 thermal compartment (Dionex) and an AS50 auto-sampler (Dionex). Thermo Finnigan X Series inductively coupled plasma-mass spectrometer (ICP-MS) from Thermo Fisher Scientific Inc., (Waltham, MA, USA). An Accela binary pump (Thermo Scientific) coupled with an Accela autosampler (Thermo Scientific) and an LTQ-Orbitrap Discovey mass spectrometer (Thermo) was used for selenium species identification and characterization. Targets separation by HPLC–ICP-MS experiments was performed by using a 250 × 4.1 mm i.d. Hamilton PRP X-100 anion exchange column with a guard column (25 mm, 2.3 mm i.d. from Hamilton (Reno, NV, USA). HPLC–MS measurements were carried out with a 150 mm × 4.6 mm i.d., 5.0 μm Phenomenex Luna C18(2) reverse phase column from Phenomenex (Torrance, CA, USA). Lab Blender Stomacher 400 (Seward Med. Ltd., London, UK) with Stomacher closure bags 6041/CLR (Seward). LYPH-LOCK 6 litre freeze dry system, model 77530 from Labconco Corporation (Kansas City, MO, USA). Thermostatic oven, model 207, from Selecta (Barcelona, Spain). Vibrating ball mill from Retsch (Haan, Germany) equipped with zircon cups (15 mL) and zircon balls (7 mm diameter). Ethos Plus microwave lab-station (Milestone, Sorisole, Italy) with 100 mL closed Teflon vessels and Teflon covers, HTC adapter plate and HTC safety springs (Milestone). Boxcult incubator situated on a Rotabit orbital-rocking platform shaker (Selecta). Cellu Sep® H1 high grade regenerated cellulose tubular membranes (molecular weight cut-off 10 kDa, 50 cm length, diameter dry 25.5 mm and a volume to length ratio of 5.10 mL cm^{-1}) were from Membrane Filtration Products Inc. (Texas, USA). ORION 720A plus pH-meter with a glass–calomel electrode (ORION, Cambridge, UK). Cellulose acetate syringe filters (0.45 μm) were from Millipore (MA, USA).

2.2. Reagents

Ultra-pure water of resistance 18 MΩ cm^{-1} was obtained from a Milli-Q purification device (Millipore Co.). Methanol (gradient grade) and formic acid (98–100%) were from Merck (Poole, UK). Citric acid 99% (m/m), ammonia 25% (m/v) and selenite (Se(IV)) stock standard solution, 1000 mg L^{-1}, were from Panreac (Barcelona, Spain). Standard solutions of selenate (Se(VI)), $SeCys_2$, SeMeCys and SeMet (1000 g L^{-1}) were prepared by dissolving the appropriate amounts of sodium selenate (Na_2SeO_4) and SeMeCys (Se-(Methyl)selenocysteine hydrochloride 95%) from Aldrich (Milwaukee, WI, USA); and $SeCys_2$ (seleno-L-cystine 95%) and SeMet (seleno-D,L-methionine 99%) from Sigma Chemicals (St Louis, MO, USA). Digestive enzymes (porcine pepsin, p-7000, porcine pancreatin, P-1750), bile salts (approx. 50% sodium cholate and 50% sodium deoxycholate) and piperazine-NN-bis(2-ethane-sulfonic acid) di-sodium salt (PIPES), were obtained from Sigma Chemicals. Sodium hydrogen carbonate was from Merck. AnalaR nitric acid 69% (m/m), hydrochloric acid 37% (m/m) and hydrogen peroxide 33% (m/v) were from Panreac. DORM-2 (dog–fish muscle) certified reference material (CRM) was from the National Research Council of Canada (Ottawa, Canada).

2.3. Seafood samples

Mollusk, white fish and cold water fish samples were obtained from a local supermarket. Samples were pre-treated as was indicated in a previous paper (Moreda-Piñeiro et al., 2012). All samples were preserved in pre-cleaned polyethylene bottles.

2.4. Microwave assisted acid digestion of samples and dialyzate extracts

Powdered samples (0.5 g) and dialyzates (1.0 mL) were subjected to a microwave assisted acid digestion procedure in triplicate under optimized conditions reported elsewhere (Moreda-Piñeiro et al., 2012). Before ICP-MS measurements, acid digests were filtered through 0.45 μm cellulose acetate syringe filters.

2.5. In vitro digestion and dialysis procedure

The *in vitro* digestion was performed in triplicate by weighing 0.5 g of powdered samples into 100 mL Erlemeyer flasks according to the procedure described elsewhere (Moreda-Piñeiro et al., 2012). Reagent blanks were also obtained to control possible contamination. Dialyzates (~17 mL) were freeze dried and then dissolved in 5.0 mL of mobile phase (20.0 mM of citric acid plus 2.0% (v/v) methanol (HPLC–ICP-MS). The freeze dried residue was however dissolved with 5.0 mL of 1.0% (v/v) formic acid solution plus 10% (v/v) methanol when performing HPLC–MS experiments. Both dialyzate and the residual fractions were kept at −20 °C before measurements.

2.6. ICP-MS measurements

Total selenium in the acid digests and dialyzates was measured by ICP-MS under operating conditions listed in Supplementary Table S1. Determinations were performed by using aqueous standard solutions in 2.0 M nitric acid covering selenium concentrations from 0 to 1000 μg L^{-1}. Germanium at a concentration of