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Speciation of Se in *Bertholletia excelsa* (Brazil nut): A hard nut to crack?

Emmie Dumont *, Liesbet De Pauw, Frank Vanhaecke, Rita Cornelis

Department of Analytical Chemistry, Ghent University, Proeftuinstraat 86, B-9000 Gent, Belgium

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Abstract

A separation method based on ion-pairing liquid chromatography was combined with both elemental (inductively coupled plasma mass spectrometry (ICP-MS)) and molecular (electrospray tandem mass spectrometry (ES-MS-MS)) mass spectrometry in order to unravel the identity of the Se-species present in the complex matrix of Brazil nuts rich in Se. Via enzymatic digestion, Se-species were released from the matrix. Subsequently the species were separated and the Se was monitored on-line by ICP-MS. By HPLC–ES-MS-MS, the species were identified based on their molecular mass and their specific product ions. The main compound was identified as Se-Methionine. Another compound was identified as Se-Cystine, partly on the basis of the isotopic pattern of Se. This research was further extended to the analyses of in vitro gastrointestinal digests of the Brazil nuts. These digests were analyzed for their Se-content and screened for the presence of the different Se-species by HPLC–ICP-MS. In both the gastric and the intestinal digests, we were able to identify the Se-species as Se-Methionine and Se-Cystine by HPLC–ES-MS-MS. By coupling HPLC to both elemental and molecular mass spectrometry, the species present in Brazil nuts and supposedly extractable by our body were fully characterized.

Keywords: Brazil nuts; Se; HPLC; ICP-MS; ES-MS-MS; Gastrointestinal digestion

1. Introduction

Being aware of the favourable influence Se may have on our organism, it is important to know which dietary sources of this element are most beneficial. Although there is an ample choice of Se-supplements, we focus on natural sources of Se.

A study by Ip and Lisk (1994) demonstrated that cancer mortality was reduced by 50% by supplementing people with Se-enriched Brewer's yeast (*Saccharomyces cerevisiae*). Many studies have been devoted to the speciation of Se in this Se-enriched supplement (Dumont et al., 2004a; McSheehy & Mester, 2003; Encinar, Sliwka-Kaszynska, Polatajka, Vacchina, & Szpunar, 2003). The main compound present in this matrix was identified as Se-Methionine (Dumont et al., 2005; Yoshida et al., 2002). Nearly every item of our diet has been screened for its total Se-content. Fruits and vegetables contain less than 0.01 μSe g^{-1}. The Se content in grain products varies between 0.02 and 0.4 μg Se g^{-1}. Meat is known to be an even more important source of Se: 0.1–0.4 μg Se g^{-1} (Ip, 1998). Researchers started to measure Se-species in different natural products containing high amounts of Se. Brazil nuts (*Bertholletia excelsa*) are known to be one of the products with the highest Se content. These nuts are the fruits from the Brazil nut tree, which grows in the Amazon

* Corresponding author. Tel.: +32 92646603; fax: +32 92646699.
E-mail address: emmie.dumont@ugent.be (E. Dumont).

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River basin. The soil on which the tree grows is of great importance, since the Se-content of the nuts is highly dependent on the amount of Se present in the soil. It was shown that Brazil nuts originating from the central part of Brazil contained up to ten times more Se than the nuts exploited from the Western part of Brazil (Chang, Gutenmann, Reid, & Lisk, 1995). Some attempts have been made to identify the species present in Brazil nuts. A study by Chunhieng et al., concentrated on the distribution of Se in Brazil nuts among the different protein fractions (Chunhieng et al., 2004a; Chunhieng et al., 2004b). The nuts were investigated in search for a candidate reference material for the speciation of Se-compounds. Se-Methionine was demonstrated to be present be it only on the basis of HPLC–ICP-MS, lacking molecular information to prove this (Bodo et al., 2003). In the studies by Wrobel, Kannamkumarath, Wrobel, and Caruso (2003) the distribution of Se in different nut types, amongst which Brazil nuts, was examined by HPLC–ICP-MS. The identity of one peak was verified as Se-Methionine by spiking with the corresponding selenium standard. To our knowledge, only one research group attempted to characterize the Se-species by obtaining molecular information. In that study the enzymatic hydrolysate of the Brazil nut was examined by ion-pairing chromatography combined with ICP-MS. Fractions with the Se-containing species were pooled and further examined by ES-MS. On the basis of the results thus obtained a peptide structure was proposed (Vonderheide et al., 2002).

The Se bioavailability is dependent on the digestibility of the different Se-containing proteins (Combs, 2001). The matrix of Brazil nuts is quite complex (66–67% fat, 14% proteins and 13% of carbohydrates). It is surely interesting to examine the behavior of the Se-species in our gastric and intestinal digestive tract.

In the present paper a method, previously developed in our laboratory, and already shown to be successful for the speciation of Se in different types of Se-supplements was applied to identify the Se-species present in Brazil nuts (Dumont et al., 2004a; Dumont et al., 2005). The complexity of the matrix of the Brazil nuts and the lower Se-concentration hampered the application of this method. The Se-species were extracted after appropriate sample clean-up. The method is based on the combination of an efficient HPLC-method with both elemental and molecular mass spectrometry. The Se-species were characterized based on retention time matching and on-line detection of the molecular ions and their product ions. When molecular information is lacking, the isotopic pattern of Se was used in the identification. The research was further extended to the analysis of in vitro gastrointestinal digests of the nuts.

2. Materials and methods

2.1. Instrumentation

The microwave digester was a Milestone mls 1200 mega from Analis, Namur, Belgium. The Inductively Coupled Plasma Mass Spectrometer (ICP-MS) was a quadrupole based Perkin–Elmer SCIEX Elan 5000 (Glendale, Ontario, Canada). The electrospray tandem mass spectrometer (ES-MS-MS) was a Quattro Micro system (Micromass, Manchester, UK), equipped with a Z-spray source. High Performance Liquid Chromatography (HPLC) experiments with hyphenation to the ES-MS-MS were done on a Waters Alliance 2690 model equipped with an autosampler. For hyphenation to the ICP-MS an HPLC pump, model 625 from Alltech (Deerfield, IL, USA), equipped with a 6-way injection valve model 7161 from Rheodyne (Cotati, CA, USA) and a 10 μl loop, was applied. The mobile phase was degassed and flushed with argon prior to analysis. Two XTerra columns with appropriate guard columns were used for separation: an analytical column (*L*: 250 mm, i.d. 4.6 mm, 5 μm particles) with guard column (*L* 20 mm, i.d. 3.9 mm, 5 μm particles), and a narrowbore column with the same packing material (*L*: 250 mm, i.d. 2.1 mm, 5 μm particles) with guard column (*L*: 20 mm, i.d. 2.1 mm, 5 μm particles) for HPLC–ICP-MS and HPLC–ES-MS-MS, respectively. All columns were from Waters Corporation, (Milford, MA, USA).

2.2. Reagents and materials

All chemicals used were of analytical grade purity. Ultrapure Milli Q water was produced in the laboratory by using a Millipore system (Bedford, MA, USA). The Se-standards Se-Methionine (Se-Met) and Se-Cystine (Se-$(Cys)_2$), pepsin from porcine gastric mucosa, pancreatin from porcine pancreas, protease XIV from *Streptomyces griseus* and tetraethylammoniumchloride (TEACl) were from Sigma (Bornem, Belgium). Methanol, KH_2PO_4 and formic acid were purchased from Vel (Leuven, Belgium). NaOH was bought from Carlo Erba (Milan, Italy). H_2O_2 (30%) was from Merck (Darmstadt, Germany). $CHCl_3$ and CH_2Cl_2 were from UCB (Brussels, Belgium). Nitric acid (14 M) was purified by sub-boiling distillation in quartz equipment. The 5 ml polypropylene test tubes used in the simulation of the digestion are from Falcon, Becton Dickinson labware (Meylan, France). The 0.22 μm pore PVDF syringe filters to filter the samples prior to analysis were from Millipore (Bedford, MA, USA). The PEEK tubing for all couplings had an i.d. of 0.1 mm. The length of the tubing was limited to 40 cm in order to reduce peak broadening due to diffusion of the separated species in the tubing.