Microchemical Journal 127 (2016) 120–124

Contents lists available at ScienceDirect

Microchemical Journal

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

Speciation analysis of Se-enriched strawberries (*Fragaria ananassa Duch*) cultivated on hydroponics by HPLC-TR-HG-AFS

Daniel Sánchez-Rodas [a,b,*], Fernanda Mellano [c], Fátima Martínez [d], Pedro Palencia [e], Inmaculada Giráldez [a], Emilio Morales [a]

[a] Department of Chemistry, Faculty of Experimental Sciences, University of Huelva, Campus El Carmen, 21071 Huelva, Spain
[b] Centre for Research in Sustainable Chemistry – CIQSO, Campus El Carmen, 21071 Huelva, Spain
[c] Department of Chemistry, Faculty of Agronomy and Agrosciences, Universidad Nacional de Santiago del Estero, Avda. Belgrano (s) 1912, 4200 Santiago del Estero, Argentina
[d] Department of Agroforestry Sciences, ETSI. University of Huelva, Campus La Rábida, 21071 Huelva, Spain
[e] Department of Organisms and Systems, Polytechnic School of Mieres, University of Oviedo, 33600 Mieres, Spain

ARTICLE INFO

Article history:
Received 7 December 2015
Received in revised form 23 February 2016
Accepted 23 February 2016
Available online 27 February 2016

Keywords:
Selenium
Speciation
Strawberry
HPLC
AFS
Selenoaminoacids

ABSTRACT

High-performance liquid chromatography (HPLC) has been coupled to atomic fluorescence spectroscopy (AFS) for speciation of inorganic Se (selenite Se(IV) and selenate Se(VI)) and selenoaminoacids (Se–methionine SeMet, Se–cystine SeCyst and Se–methylselenocisteine SeMeSeCys). A thermoreduction (TR) step is included before hydride generation (HG) to obtain the volatile hydrides of the selenium species before AFS detection. The instrumental coupling HPLC-TR-HG-AFS has been applied for Se speciation in strawberries collected from plants grown on hydroponics and exposed to inorganic Se (Se(IV) and Se(VI)) and one selenoaminoacid (SeMet). Application of Se was performed by foliar spray and root irrigation at concentrations between 0.5–500 mg L^{-1}. Of the three Se species applied by root irrigation, only Se(VI) was uptaken by the roots, with no significant biotransformation, followed by a sharp disaccumulation after stopping the selenate application. When selenite was applied by foliar spraying at a high concentration, a significant biotransformation was observed, mainly as SeMet. The results indicated that for strawberries, foliar spray is a more suitable route for Se uptake and biotransformation than root irrigation.

1. Introduction

Selenium is a trace and essential element needed in the human diet, present in meat, grain, and vegetables [1–3]. Increased intakes of Se-enriched foods may benefit the human health due to its antioxidant activity [4,5]. Se deficiency in humans is associated to cardiovascular disorder (Keshan disease) and bone degenerative disorder (Kashin–Beck disease) [3,6], whereas elevated Se doses can have toxic effects [7].

There are studies that describe Se-enriched vegetables as a possible supplement of Se in the diet. These biofortification experiments have been performed with rice [8], edible sprouts [9], mushroom [10], carrot [11], and chikpea [12], among others. The uptake of Se by plants differs considering the inorganic or organic species in which Se can be found. Selenate (Se(VI)) is more soluble than selenite (Se(IV)) and it is more easily absorbed by plant roots [13]. Accumulation of Se by plants can be the result of the ability of the plant to transform inorganic Se into organic selenium species, resulting in several selenoaminoacids [14–16].

The speciation of Se in vegetables involves selective and sensitive analytical techniques. Separation of the Se compounds is achieved in most cases either by liquid or gas chromatography (HPLC or GC) coupled to atomic or mass spectrometry. Tandem-mass spectrometry (MS/MS) [17], inductively coupled plasma–mass spectrometry (ICP-MS) [18–22], or atomic fluorescence spectroscopy (AFS) [23–25] has been coupled to HPLC in Se speciation studies. Hydride generation (HG) is employed in combination with atomic spectroscopy detection (AFS or AAS) to convert Se species into volatile hydrides (SeH_2) before detection, as it has been recently reviewed considering both technique development [26] and application to real samples [27]. For GC separations, mass spectrometry is the preferred detector (GC–MS) [28,29]. Sample preparation is also a critical step, as the extraction process should be quantitative, without altering the distribution of the Se species. For biological samples, enzymatic digestion using lipase or protease is widely employed to favour efficient extraction of Se species [30]. It has been reviewed that for Se speciation in plant and biological samples, enzymatic hydrolysis is the preferred extraction method, compared to hot water, acid, or basic extraction procedures [31].

Strawberry (*Fragaria ananassa Duch*) cultivation is of great importance in Spain, one of the main producers of this fruit in Europe. The main producing area is located in the province of Huelva, in the

* Corresponding author at: Centre for Research in Sustainable Chemistry–CIQSO, Campus El Carmen, 21071 Huelva, Spain.
E-mail address: rodas@uhu.es (D. Sánchez-Rodas).

http://dx.doi.org/10.1016/j.microc.2016.02.017
0026-265X/

southwest of the country, representing an important agricultural and economical activity. At present, there are no studies about Se biofortification with strawberries. The aim of the present study is to perform a speciation study of Se-enriched strawberries cultivated on hydroponic with HPLC coupled to AFS detection. Strawberry plants were exposed to Se(IV), Se(VI), and Se–methionine (SeMet), considering foliar spray and irrigation, in order to evaluate the possible accumulation and biotransformation of Se in the fruits.

2. Materials and methods

2.1. Materials and chemicals

Sodium selenite ($NaSeO_3$), sodium selenate (Na_2SeO_4), Se–methionine z(SeMet), Se–methylselenocysteine (SeMetSeCys), and Se–cystine (SeCyst) (Sigma–Aldrich) were employed to prepare stock solutions of 500 or 1000 mg Se L^{-1}, which were stored in the fridge at 4 °C during 6 months. Calibration solutions were daily prepared by appropriate dilution of the stock solutions. Protease XIV (*Streptomyce griseus*) and Lipase VII (*Candida rugosa*) for enzymatic hydrolysis were also purchased from Sigma–Aldrich. The mobile phase for liquid chromatography consisted of aqueous solutions of KH_2PO_4 and K_2HPO_4 (Merck). Thermoreduction and hydride generation were achieved using KBr, HCl (Panreac), and $NaBH_4$ (Sigma–Aldrich) aqueous solutions. The different solutions were prepared with Ultra Pure Milli-Q water from an Elix 3 system (Millipore).

2.2. Instrumentation

Se speciation analysis was carried out coupling high-performance liquid chromatography (HPLC), followed by thermoreduction (TR), hydride generation (HG), and atomic fluorescence spectroscopy (AFS) detection of the volatile hydrides. The working conditions for this instrumental coupling are summarized in Table 1. The chromatographic separation of the selenium species takes place in an anion exchange column using a potassium phosphate buffer as mobile phase. The outlet of the column was connected on-line to a speciation heated coil (PS Analytical, Great Britain) for thermoreduction (TR) of Se(VI) and the selenoaminoacids at 150 °C with the aid of a KBr solution prepared in HCl. Hydride generation of volatile Se hydrides is performed adding a $NaBH_4$ solution with the peristaltic pump of a Millenium Excalibur AFS detector (PS Analytical, Great Britain). A detailed description of the HPLC-TR-HG-AFS instrumentation and the optimization of the parameters involved in the analysis of Se species have been previously reported for biological samples (algae and yeast) [25].

Table 1
Optimized HPLC-TR-HG-AFS operation conditions for Se speciation analysis.

HPLC	
Column	Hamilton PRP- X100 (250 mm × 4.1 mm × 10 μm)
Mobile phase	80 mM KH_2PO_4/K_2HPO_4 buffer, pH 6.0
Flow rate	1.0 mL min^{-1}
Injection volume	200 μL
Thermoreduction (TR)	
Heating block temperature	150 °C
Reaction coil	3 m, i.d. 0.5 mm
Reductant	5% (*w*/*v*) KBr in 6 M HCl
Reductant flow rate	1.0 mL min^{-1}
Hydride generation (HG)	
Reductant	1.2% (*w*/*v*) $NaBH_4$ in 0.4% (*w*/*v*) NaOH
Reductant flow rate	1.0 mL min^{-1}
Purging Ar gas flow rate	400 ml min^{-1}
Counter-current air flow rate	2 L min^{-1}
Atomic fluorescence spectroscopy (AFS)	
Radiation source	Selenium Super Lamp (Photron)
Primary and boosted currents	25 mA (both)
Gain setting	100
Detection wavelengths	190–210 nm
H_2 flow rate	50 mL min^{-1}

2.3. Cultivation of Se-enriched strawberries

Strawberries were grown on coconut fibre, a hydroponic inert substrate. Cultivation was performed at the greenhouse of the Department of Agroforestry Sciences of the University of Huelva. 10 strawberry plants were placed in each 100 L sack of inert substrate. For each Se-enrichment experiment, 3 sacks with a total of 30 plants were employed. Each cultivation campaign lasted from December to April. Plants were watered every 3 days with fertilizer containing nitrate, ammonium, phosphorous, and potassium.

Two complete cultivation campaigns were performed in successive years, each lasting 26 weeks (December—May). Strawberries plants were planted the 1st week. During the first cultivation campaign, foliar spray of Se was performed during 6 weeks, starting the 19th week until the 24th week. Foliar spray of 100 ml of Se solution to each group of 10 plants was performed once a week. Four Se solutions were employed for foliar spray: 0.5 and 5 mg L^{-1} of Se(IV), and 0.5 and 5 mg Se L^{-1} of Se(VI). All the spray solutions were prepared in 0.2 mg L^{-1} of detergent (Triton X-100, Sigma).

During the second cultivation campaign performed the following year, foliar spray experiments were repeated, using only the most concentrated solutions of 5 mg L^{-1} of Se(IV) and 5 mg L^{-1} of Se(VI). During the second campaign, application of Se via root irrigation was also tried, considering 5 mg L^{-1} of Se(IV), 5 ml L^{-1} of Se(VI) and 2 mg L^{-1} of SeMet.

Also, a third short experience was conducted during 1 week of the second year by: i) daily foliar spray at a high concentration of 500 mg L^{-1} of Se(IV), and ii) root irrigation of 100 mg L^{-1} of Se(IV). Strawberries were collected at the end of this experience, treated and analysed in a similar way as previously described.

2.4. Sampling, sample preparation, and enzymatic hydrolysis

For each sampling campaign, strawberries were collected once a week during a total of 7 weeks (between March and May), once the plants started to bear fruits. For each Se-enrichment experiment, 3 strawberries were taken at random from the plants. The strawberries were frozen in liquid N_2 and freeze dried during 48 h in a lyophilizer (Telstar LyoAlfa). Hereafter, the strawberries were crushed in an agate mortar and homogenized to obtain a composite sample. 50 mg of the composite sample were submitted to enzymatic hydrolysis, adding 15 mg of a mixture of protease XVI: lipase (1:2) and 3 ml of distilled water. The solution was sonicated during 2 min with a sonication probe at 50% of power (Bandelin Sonopulse HD2200, Germany). After hydrolysis, the solution was centrifuged at 5000 rpm during 15 min. The supernatant was separated for Se speciation analysis by HPLC-TR-HG-AFS.

3. Results and discussion

3.1. Instrumental coupling HPLC-TR-HG-AFS for Se speciation

The HPLC separation of two inorganic oxyanions (Se(IV), Se(VI)) and three selenoaminoacids is achieved in a strong anion exchange column with a 80 mM phosphate buffer mobile phase in ca. 20 min. The eleution order is SeCyst, SeMetSeCys, Se(IV), SeMet, and Se(VI). After chromatographic separation, the selenium species are converted on-line into volatile hydrides thanks to a thermoreduction step (addition of a KBr solution prepared in HCl at 150 °C) followed by hydride generation (addition of a $NaBH_4$ solution). An argon flow at a glass liquid separator carries the volatile hydrides to an H_2 diffusion flame of an AFS detector. The instrumental coupling HPLC-TR-HG-AFS is suitable for selenium speciation of sample extracts with Se concentration in the μg L^{-1} range. The optimized parameters are summarized in Table 1, and a chromatogram of the Se species is represented in Fig. 1. Limits of detection calculated conserving a signal-to-noise ratio of three, as defined by the ISO Norm 12530:1997 [32] were as follows: Se(IV) 0.4 μg L^{-1}, Se(VI)