Food Chemistry 159 (2014) 407–413

Contents lists available at ScienceDirect

Food Chemistry

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

Preconcentration of seleno-amino acids on a XAD resin and determination in regional olive oils by SPE UPLC–ESI-MS/MS

Sabier Torres [a], Soledad Cerutti [a], Julio Raba [a], Pablo Pacheco [a,*], María Fernanda Silva [b,*]

[a] Instituto de Química de San Luis (INQUISAL-CONICET), Chacabuco y Pedernera, CP 5700 San Luis, Argentina
[b] Instituto de Biología Agrícola de Mendoza (IBAM-CONICET), Almirante Brown 500, Chacras de Coria, CP 5505 Mendoza, Argentina

ARTICLE INFO

Article history:
Received 28 August 2013
Received in revised form 26 February 2014
Accepted 9 March 2014
Available online 18 March 2014

Keywords:
Seleno-amino acids
Olive oil
XAD® resin

ABSTRACT

This study describes a method for seleno-amino acids determination in Argentinean olive oils. Preliminary total selenium determination in olive oils probed low concentrations (62.8 ± 1.6 to 117.4 ± 3.0 μg/kg) and the necessity of implementing a preconcentration method. To this end a XAD® resin was employed as sorbent for selenomethionine (Se-Met), selenomethylselenocysteine (Se-MetSeCys), and selenocysteine (Se-Cys) preconcentration. Determinations were performed by UPLC–ESI-MS/MS. Recoveries were between 84% and 97% for the seleno-amino acids studied, reaching a detection limit of 0.09 μg/kg, a precision of 10% (RSD, $n = 6$), and an enhancement factor of 60-fold (6 for the extraction system and 10 for the preconcentration approach). The only detected Se species in the olive oils was Se-MetSeCys in concentrations ranging from 2.0 to 8.3 μg/kg.

1. Introduction

Selenium is a trace mineral essential for health (Goldhaber, 2003; Thomson, 2004). It is a constitutional part of selenoproteins with antioxidant properties, which helps to prevent cell damage caused by free radicals, metabolic products that contribute to the development of diseases such as cancer and coronary heart disease (Combs & Gray, 1998; Goldhaber, 2003). In addition, selenium cooperates in the regulation of the thyroid function and plays an important role in the immune system (Combs & Gray, 1998).

Diet is the major source of this element for humans. Intake depends on the selenium concentration in food and consumption. Its bioavailability varies according to its biochemical nature, being this significantly higher in the form of organic species (Alsing Pedersen & Larsen, 1997; Alzate et al., 2007; Amoako, Uden, & Tyson, 2009; Dumont et al., 2005; Dumont, Vanhaecke, & Cornelis, 2006; Infante, Hearn, & Catterick, 2005).

Epidemiological studies provide evidence that sustained consumption of virgin olive oil contributes to lower incidence of cancer (colon, breast, and skin), coronary heart disease, and aging (Dhur, Galan, & Hercberg, 1990). Healthy properties of olive oil are mainly due to the presence of antioxidant micronutrients, such as, tocopherols and phenolic compounds, important sources of vitamin E. In recent years many studies have reported the biological properties of the antioxidant-radicals scavenging microconstituents of olive oil (Tuck & Hayball, 2002).

Low total selenium concentration in some vegetable oils (Dugo, La Pera, Pollicino, & Saitta, 2003) leads to the need of developing preconcentration steps previous determination. Olive oil contains low protein content (Montealegre et al., 2010) and the presence of seleno-amino acids is unknown. For this reason, the expected seleno-amino acids concentration to be found in olive oil is scarce, but presumable essential and more nutritionally important than the element itself. Among the different techniques of selenium preconcentration recently reviewed (Wake, Bowie, Butler, & Haddad, 2004), solid-phase extraction (SPE) has been the most frequently used. SPE has been generally employed both to preconcentrate and to separate inorganic selenium, selenite, and selenate by fractional elution. However, SPE strategies applied to seleno organic compounds are limited and are devoted to only one compound (Bueno & Potin-Gautier, 2002; Wake et al., 2004). Recently, a stationary phase based on porous graphitic carbon (PGC) has been commercialized as stable HPLC support for seleno-amino acids (Dauthieu, Bueno, Darrouzes, Gilon, & Potin-Gautier, 2006).

Seleno-amino acids determination is usually achieved by hyphenated techniques such as HPLC-ICP MS as well as less expensive methods such as HPLC-HG-AAS. In these hyphenated systems, detection by spectrometric methods has been the most used (Alsing Pedersen & Larsen, 1997; Hill et al., 2003; Lobiński & Szpunar, 1999). Other chromatographic techniques coupled to different mass spectrometers (Carmen Barciela-Alonso,

* Corresponding authors. Tel.: +54 9 02664 46765 (P. Pacheco). Tel.: +54 9 0261 4135010x1228 (M.F. Silva).
E-mail addresses: ppacheco@unsl.edu.ar (P. Pacheco), msilva@fca.uncu.edu.ar (M.F. Silva).

http://dx.doi.org/10.1016/j.foodchem.2014.03.045
0308-8146/

Bermejo-Barrera, & Bermejo-Barrera, 2007) have also been used for seleno-amino acids determination. In addition, these techniques have been applied to selenium speciation analysis in food samples (Gosetti et al., 2007).

In this work, a method for determination of selenomethionine (Se-Met), selenomethylselenocysteine (Se-MetSeCys), and selenocysteine (Se-Cys) in olive oil is described for the first time. Total selenium concentration was determined by ICPMS following microwave assisted sample digestion. The low concentration of seleno-amino acids in olive oil required a preconcentration step previous determination. To this end, extraction was optimized and a XAD® resin was introduced as sorbent for solid phase extraction. Temporal resolution and determination was achieved by chromatographic separation of the analytes followed by electrospray ionization coupled to a triple quadrupole tandem mass spectrometer (UPLC–ESI-MS/MS). Selection of the related experimental conditions is described and discussed in the following sections.

2. Experimental

2.1. Reagents

Se-Met, Se-MetSeCys, and Se-Cys standards were purchased from Sigma–Aldrich (St. Louis, USA). Water, methanol (MeOH), n-hexane and acetonitrile Optima® LC-MS grade were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Formic acid, 98%, was obtained from Fisher Scientific (Loughborough, UK). Acetic acid (glacial, Trace Metal grade) was obtained from Fisher Chemical (Fisher Scientific Inc., Pittsburgh, Pennsylvania). Ultrapure water (18 MΩ cm) was obtained from EASY pure (RF Barnstead, IA, USA). Sodium hydroxide was used provided by Biopack® (Buenos Aires, Argentina). The Amberlite XAD® resins (XAD-16 and XAD-1180) and hydrochloric acid, 65%, were provided by Sigma–Aldrich (St. Louis, USA).

2.2. Preparation of standard solutions

Amino acid stock solutions were prepared by dissolving respective substances in 0.1 M hydrochloric acid with 20% MeOH, except for Se-Met, which was prepared in 0.5% 2-mercaptoethanol (0.3 mg/g). Stock solutions were prepared once and stored at −20 °C. Dilutions were made with 0.004% (m/v) aqueous solution of 2-mercaptoethanol to avoid oxidation of Se-Met. Working standard solutions were prepared by appropriate dilution with ultrapure water, adjusted to pH with hydrochloric acid or sodium hydroxide. Quantification was achieved by preparing spiked samples with proper amounts of the analytes.

2.3. Sample collection, preparation, and treatment

A total of seven samples of extra virgin olive oil from different Argentinean regions such as Maipu, Pedriel and Alto Agrelo (province of Mendoza), Aimogasta (province of La Rioja), and Cruz del Eje (province of Cordoba) were analyzed. Olive oil characteristics are detailed in Table 2 (in Supplementary material). All samples were produced with the same olive oil manufacturing machinery under similar conditions (equal mill revolutions and kneading time) at a maximum temperature of 24 °C. After collection, the olive oil samples were stored in dark glassy bottles with corks at 8 °C until analysis.

The extraction process was adapted from Pérez-Serradilla et al. studies (Pérez-Serradilla, Japón-Luján, & Luque de Castro, 2007). Thus 30 mL of olive oil were diluted up to 60 mL with 50 mL of n-hexane and 10 mL of 80:20 water:methanol solution and then sonicated for one hour. After that, phase separation was allowed, and the aqueous phase was collected. Excess methanol was eliminated under nitrogen stream at 25 °C. The remaining solution was filtered through a 0.2 μm syringe filter; and diluted up to a 10 mL with ultrapure water prior preconcentration.

The microwave digestion for total Se determination was performed as follows: 0.5 g of olive oil samples were weighed and placed in individual microwave reactors. The aliquots were treated with 7 mL concentrated HNO_3 and 1 mL H_2O_2. Digestion was carried out at a ramp temperature of 10 min up to 200 °C and hold for 10 more minutes. The employed microwave power was up to 1000 W.

2.4. Preconcentration procedure

Preconcentration was optimized by loading 10 mL solution of each selenoaminoacid standard onto the column at a concentration of 50 μg/L.Different pH values were evaluated, by adding either NH_4OH, CH_3COOH or HCl.

Sample preconcentration was performed by loading 10 mL of the olive oil extract on the conical column at a flow rate of 0.5 mL/min by means of Tygon-type tubes with a peristaltic pump. Elution was reached with 1 mL of a 0.5 M formic acid solution at a flow rate of 0.1 mL/min. Finally the eluate was collected into autosampler vials for later determination by UPLC-MSMS.

2.5. Instrumental

Sample digestion was performed with a Milestone Start D microwave system (Sorisole, Italy) and with hermetically sealed 1-cm wall thickness polytetrafluoroethylene reactors (100 mL internal volume).

For the preconcentration stage, a conical minicolumn was used. It was prepared by placing 60 mg of the XAD® resin into an empty conical tip using the dry packing method. To avoid loss of filling when the sample solution passed through the conical minicolumn, a small amount of quartz wool was placed at both ends of conical minicolumn. The column was then connected to a peristaltic pump with PTFE tubing to form the batch preconcentration system. Tygon-type pump tubings (Ismatec, Cole Parmer, Vernon Hills, IL, USA) and a Minipulse 3 peristaltic pump (Gilson, Villiers-Le-Bell, France) were employed to propel the sample, reagents and eluent.

An inductively coupled plasma mass spectrometer, Perkin-Elmer SCIEX, ELAN DRC-e (Thornhill, Canada) was used for total selenium determinations. The argon gas with a minimum purity of 99.996% was supplied by Praxair (Córdoba, Argentina). An HF-resistant and high performance perfluoracetate (PFA) nebulizer model PFA-ST, coupled to a quartz cyclonic spray chamber with internal baffle and drain lines, cooled with the PC3 system from ESI (Omaha – NE, USA) was used. The instrument conditions were: auto lens mode on, peak hopping measure mode, dwell time of 15 ms, 30 sweeps per reading, 1 reading per replicate, and 3 replicates. Nickel sampler and skimmer cones were used. The conditions for Se determination were: 500 μL/min sample flow rate; 1000 W RF power and 0.75 L/min nebulizer gas flow rate. Digested samples were analyzed using a cross-flow nebulizer, a Scott-type spray chamber, and external calibration with Rh as internal standard.

On the other hand, for seleno-amino acids separation an Acquity™ Ultra High Performance LC system (Waters, Milford) equipped with autosampler injection and pump systems (Waters, Milford) was used. The autosampler vial tray was maintained at 4 °C. The needle was washed with proper mixtures of acetonitrile and water. The separation was performed by injecting a 25 μL sample onto an ACQUITY UPLC® BEH C_8 (Waters, Milford, USA) analytical column with 2.1 mm internal diameter, 50 mm length, and 1.7 μm particle size. The binary mobile phases consisted of water (A) and acetonitrile (B), both containing 0.1% (v/v) formic delivered at 0.15 mL/

Table 1
Concentration values found for total selenium and seleno-amino acids in olive oil samples.

Sample	Total Se (μg/kg)	Se-Met–Se-Cys (μg/kg)
1	↓LOQ[a]	↓LOQ[a]
2	84.2 ± 2.2	8.3 ± 0.7
3	75.7 ± 2.0	2.0 ± 0.2
4	62.8 ± 1.6	4.4 ± 0.4
5	117.4 ± 3.0	6.1 ± 0.5
6	105.0 ± 2.7	2.9 ± 0.2
7	Nd[a]	Nd[a]

[a] Below limit of quantification.

min. The gradient was started at an initial composition of 90% A and 10% B, then 3 min linear gradient to 70% A. A return to the initial conditions was accomplished by a 0.5 min gradient to 90% A, where it was held for 1.5 min. Thus, the total chromatographic run time was 5.0 min. The column was held at a temperature of 25 °C. Under these conditions, no sample contamination or sample-to-sample carryover was observed.

For detection purposes, a Quattro Premier™ XE Micromass MS Technologies triple quadrupole mass spectrometer with a ZSpray™ Electrospray ionization source (Waters, Milford, USA) was used and conditions for the generation and fragmentation of Se-Met, Se-MetSeCys and Se-Cys can be observed in Table 1 (in Supplementary material). The source was operated in the positive electrospray mode ((+)ESI) at 350 °C with N_2 as the nebulizer gas and the source temperature was kept at 150 °C. The capillary voltage was maintained at 3.4 kV and the extractor voltage was set at 2.0 V. Ultrapure nitrogen was used as desolvation gas with a flow of 800 L/h. Argon was used as the collision gas at a flow of 0.18 mL/min, achieving and analyzer pressure of ca. 3×10^{-5} Torr. After optimization, detection was performed in multiple reaction monitoring (MRM) mode of selected ions at the first (Q_1) and third quadrupole (Q_3). To choose the fragmentation patterns of m/z (Q_1) → m/z (Q_3) for the analyte in MRM mode, direct infusions (via syringe pump) into the MS of the seleno-amino acids aqueous standard solutions was performed and the product ion scan mass spectra was recorded. Quantification of Se-Met, Se-MetSeCys, and SeCys was done by measuring the area under the specific peak using MassLynx Mass Spectrometry Software (Waters, Milford, USA).

3. Results and discussion

3.1. Total selenium determination

In order to evaluate the contribution of organic species to the total selenium content in olive oil (Combs & Gray, 1998), the total selenium level was determined. Thus total selenium determination was performed by ICPMS after microwave-assisted digestion and results are depicted in Table 1. Selenium concentrations in olive oil samples ranged from 62.8 ± 1.6 to 117.4 ± 3.0 μg/kg. Only in samples 1 and 7, corresponding to extra virgin olive oil from Maipú (Mendoza) and Aimogasta (La Rioja); respectively, selenium levels were below the limit of quantitation (0.02 μg/kg).

3.2. Extraction of seleno-amino acids

The proposed extraction methodology was chosen after testing two alternatives. Initially, an extraction procedure based on precipitation with cold acetone was assayed (Concha-Herrera, Lerma-García, Herrero-Martínez, & Simó-Alfonso, 2010), but low recoveries (9.2–16.9%) were observed. A second method was tested involving mixing olive oil with hexane followed by methanol:water extraction (80:20) in ultrasonic bath (Pérez-Serradilla, Priego-Capote, & Luque De Castro, 2007). This second approach allowed obtaining recoveries close to 100%. Se-MetSeCys and SeCys are volatile species at temperatures above 40 °C and the use of high sample preparation temperatures are the main causes of selenium losses (Dugo et al., 2003), thus five ultrasound cycles of 1 min each were applied to avoid analytes' losses. Additionally, several methanol:water ratios were evaluated. Based on the results obtained from spiked samples, high recoveries ranging between 84 and 98% was obtained with the mentioned 80:20 (v/v) methanol:water mixture. Finally, methanol excess was eliminated under nitrogen stream at room temperature. This stage was critical because the presence of methanol interfered the subsequent preconcentration of the seleno-amino acids.

3.3. Preconcentration of seleno-amino acids

Unfortunately, whatever the kind of separation, resolution of Se species in food samples remains quite poor and a single separation procedure is often unable to clearly distinguish more than three or four species in a complex matrix. To quantify more species, or to validate the obtained results, it becomes necessary to combine successively two or more kinds of separation steps (Szpunar, 2000).

XAD resins are able to separate hydrophobic (adsorbed onto XAD) and hydrophilic (not adsorbed onto XAD) acids, bases, and neutrals. The selectivity of XAD is affected by factors such as solvent type, pH, and ionic strength. Amino acids, such as D,L-phenylalanine have been adsorbed onto polymeric materials like Amberlite XAD-4 and XAD-16 (Díez, Leitão, Ferreira, & Rodrigues, 1998). In addition, a XAD-8 resin has been used to estimate organic Se content in natural waters (Fio & Fujii, 1990).

From the mentioned above, two polymeric resins were tested: XAD-16 and XAD-1180. Results are shown in Fig. 1. As a case mode, Se-MetSeCys retention on different resins was tested at different pH values. Both resins showed the best retention at pH 1.5, with 28% and 48% respectively, providing XAD-1180 the best performance. This material was chosen for further studies.

As follows, different parameters affecting the retention and elution of the analytes were optimized.

3.3.1. pH optimization

Since seleno-amino acids average charge depends on pH (Dauthieu et al., 2006), optimization of this parameter regard adsorption on the polymeric resin becomes relevant. Results can be observed in Fig. 2. Seleno-amino acids retention decreased as the pH increased. Se-Met showed retention values closed to 100% at pH 1.5. Similarly, Se-MeSeCys and Se-Cys showed the highest retention percentage at this pH value, which was close to a 70%. At pH 1.5, seleno-amino acids charge is positive due to protonation according to their pKas values; decreasing with increasing pH (Dauthieu et al., 2006). As result, a pH value of 1.5 was chosen for further experiments.

3.3.2. Sample loading flow rate effect

In order to optimize seleno-amino acids retention onto the XAD-1180 resin, the sample loading flow rate was studied (Fig. 3a). This variable was ranged from 0.5 to 2 mL/min, higher flow rates were not tested due to the excessive back pressure generated. When loading flow rate decreased adsorption equilibria was facilitated thanks to an increased contact between the analyte and the resin. For this reason a sample loading flow rate of 0.5 mL/min was chosen.

3.3.3. Optimization of the eluting conditions

Organic selenium species desorption from XAD resins can be carried out with acids (Dacheng, Alfthan, & Aro, 1994). In this study, formic acid was chosen according to Dauthieu et al. studies. Fig. 3b shows Se-MetSeCys recovery *vs.* the eluent concentration.

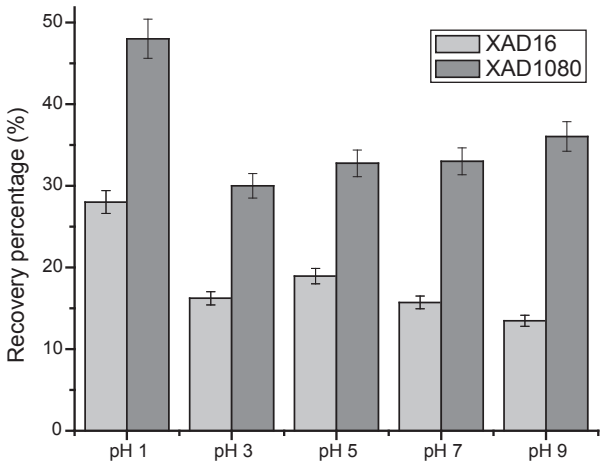

Fig. 1. Recovery percentage of selenocysteine on XAD 1180 and XAD 16 at different pH values (concentration of selenocysteine: 50 μg/L; loading flow rate, 1 mL/min; elution flow rate, 0.5 mL/min; eluent, 0.5 M formic acid).

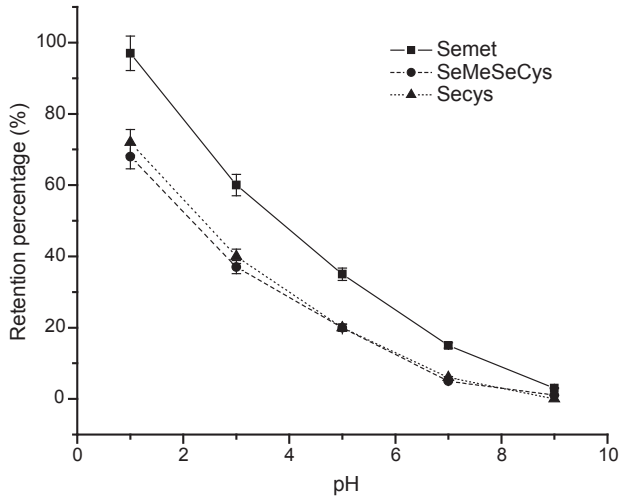

Fig. 2. Percentage recovery values of seleno-amino acids on XAD 1180 at different pH values (concentration of seleno-amino acids: 30 μg/L; loading flow rate, 0.5 mL/min; elution flow rate, 0.1 mL/min; eluent, 0.5 M formic acid).

Quantitative elution was achieved with 0.5 M formic acid. Additionally to the concentration, the eluent flow rate was studied between 0.1 and 2 mL/min. As observed in Fig. 3c, an elution flow rate of 0.1 mL/min was selected as optimum.

3.4. Chromatographic separation and determination of seleno-amino acids

As mentioned, mass spectrometric parameters were optimized by direct injection of the standards into the mass spectrometer. Ionization of selenium species was reached in positive mode, which was in good agreement with previous studies (Lindemann & Hintelmann, 2002b). The proposed fragmentation patterns of the studied selenospecies are showed in Fig. 1 (in Supplementary material). Fragmentations included the lost of ammonia ($m/z = 17$) for Se-Met and Se-Cys molecules. When the Se-Se bond is present, like in Se-MetSeCys, the cleavage of the bond next to the selenium atoms is preferred, keeping the Se-Se bond intact.

Selenium species separation is usually achieved in reversed phase mode employing a C_{18} column, many examples can be found

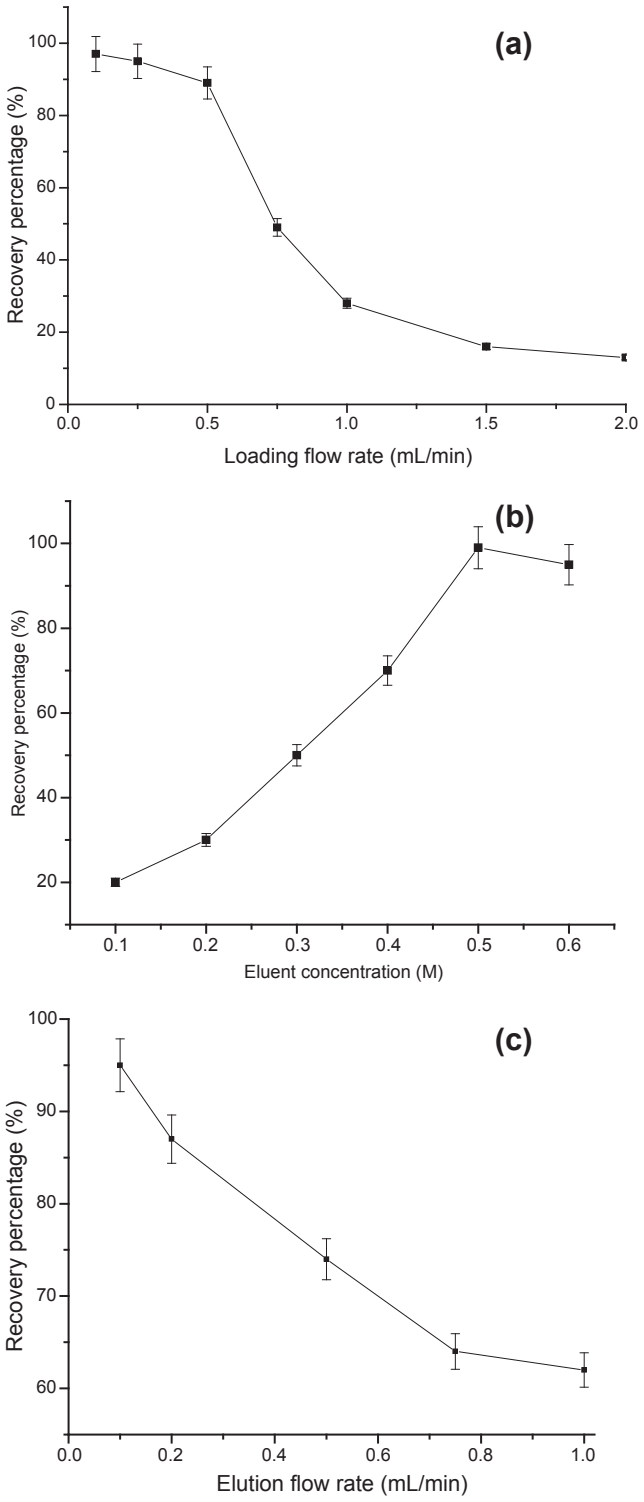

Fig. 3. (a) Percentage recovery values of Se-MeSeCys according to loading flow rate variations on XAD 1180 (concentration of Se-MeSeCys: 30 μg/L; pH, 1.5; elution flow rate, 0.1 mL/min; eluent, 0.5 M formic acid). (b) Percentage recovery values of Se-MeSeCys according to the eluent concentration after preconcentration on XAD 1180 (concentration of Se-MeSeCys: 30 μg/L; pH, 1.5; loading flow rate, 0.5 mL/min; elution flow rate, 0.1 mL/min). (c) Percentage recovery values of Se-MeSeCys according to the eluent flow rate after preconcentration on XAD 1180 (concentration of Se-MeSeCys: 30 μg/L; pH, 1.5; loading flow rate, 0.5 mL/min; eluent, 0.5 M formic acid).

in the literature (Lindemann & Hintelmann, 2002a). However, in this study, best chromatographic resolution of the studied Se-compounds was reached with a C_8 column, examples of the

employment of this column for Se speciation can also be found (Carmen Barciela-Alonso et al., 2007). A chromatogram of the investigated species is shown in Fig. 4. A mobile phase gradient was used in order to achieve a separation of the compounds within 3 min.

3.5. Analytical performance

The proposed methodology allowed obtaining a detection limit of 0.01 μg/kg and a quantification limit of 0.09 μg/kg calculated according to 3σ and 10σ definitions; respectively. The preconcentration system was linear up to at least 200 μg/kg ($r = 0.997$). Precision, expressed as relative standard deviation (RSD%; $n = 6$), was approximately 10%. Considering the extraction procedure starting with 30 mL of olive oil, the 10 mL of sample loaded onto the preconcentration system and the 1 mL of final eluate volume, an enhancement factor of 60-fold (6 for the extraction and 10 for preconcentration system) compared to the seleno-amino acids determination by UPLC–ESI-MS/MS without preconcentration was obtained.

3.6. Recovery study for the preconcentration procedure

Due to the absence of olive oil reference materials with certified concentrations of seleno-amino acids, a recovery study was performed. To this end, the studied organic selenium species were added to aliquots of three samples of extra virgin olive oil at three concentration levels, which were afterwards extracted,

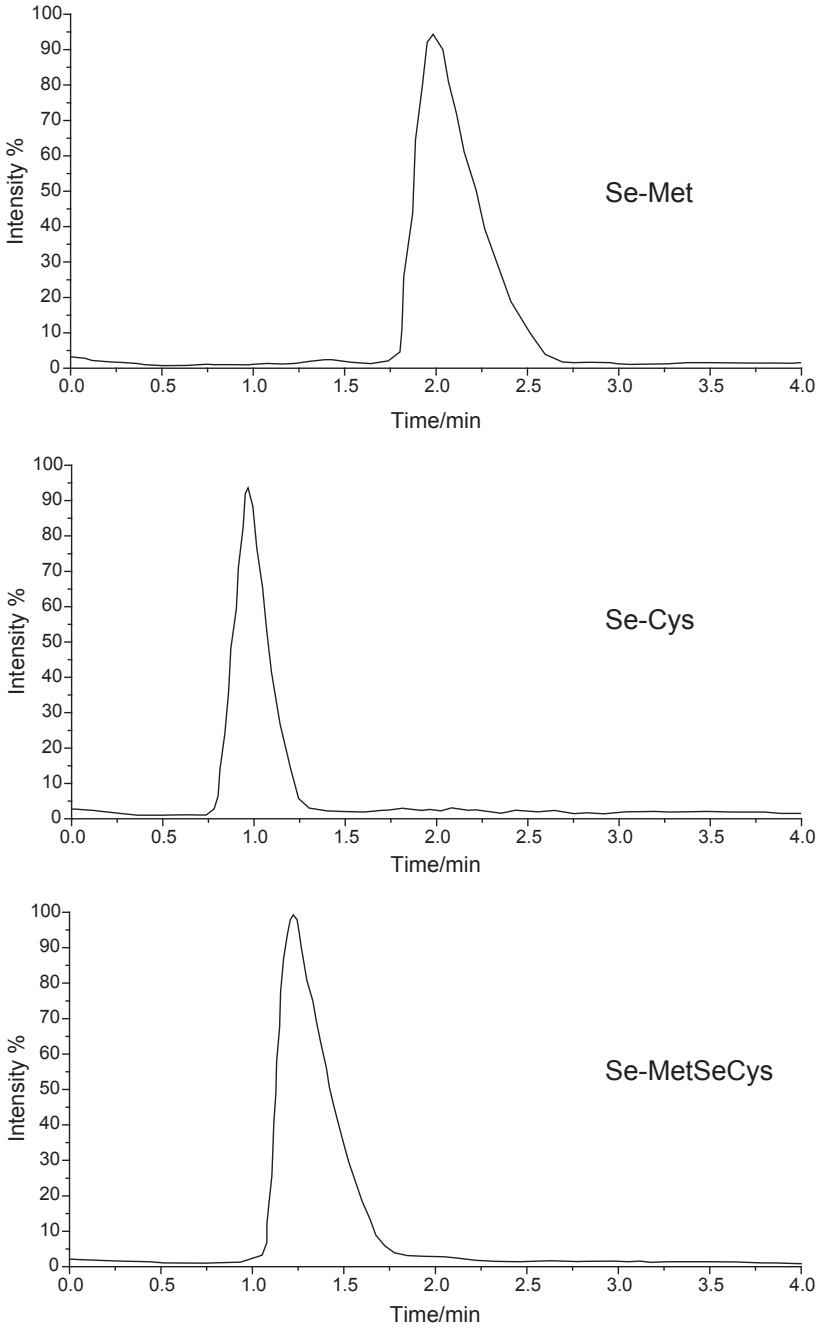

Fig. 4. Seleno-amino acids extracted ion chromatograms obtained by UPLC-MS/MS (MRM mode). This figure corresponds to a single run of a mixture containing the three studied seleno-amino acids (Seleno-amino acids concentrations: 50 μg/L).

Table 2
Recovery study.

Added (μg/kg)	Base (μg/kg)	Found (μg/kg)	Recovery (%)[a]
Se-MetSeCys			
10	2.0 ± 0.2	11.3 ± 0.9	93 [a]
25	2.0 ± 0.2	24.8 ± 2.0	91 [a]
50	2.0 ± 0.2	50,4 ± 2.2	97 [a]
Se-Met			
10	–	8.4 ± 0.7	84 [a]
25	–	23.0 ± 2.0	92 [a]
50	–	47.2 ± 4	94 [a]
Se-Cys			
10	–	9.6 ± 0.8	96 [a]
25	–	23.9 ± 1.9	95 [a]
50	–	49.1 ± 4.0	98 [a]

[a] 100* [(found-base)/added].

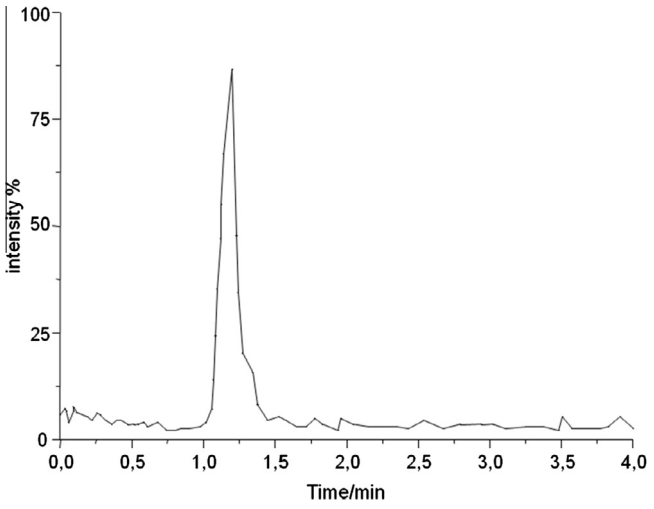

Fig. 5. Extracted ion chromatograms corresponding to an olive oil sample (extra virgin; from Pedriel, Mendoza) where a single peak corresponding to Se-MetSeCys can be observed.

preconcentrated, separated, and determined following the procedures described above. Results are shown in Table 2. A good precision of the method can be observed.

3.7. Seleno-amino acids determination in olive oil

Determination of organic species of selenium was carried out after extraction, preconcentration onto XAD-1180, separation, and determination by UPLC–ESI-MS/MS. Individual characteristics of each olive oil samples used in the study are shown in Table 2 (in Supplementary material) and the determined seleno-amino acids concentrations are shown in Table 1. The only detected Se species was Se-MetSeCys, as it can be observed in Fig. 5, in concentrations from 2.0 to 8.3 μg/kg, which has also been reported as one of the most abundant Se species in some plant material. In addition, studies carried out in plants have shown that the content of some seleno-species shows no significant differences before and after enzymatic hydrolysis suggesting that these species are not part of proteins (Montes-Bayón, Molet, González, & Sanz-Medel, 2006). In samples corresponding to extra virgin olive oil from Maipu (Mendoza) and Aimogasta (La Rioja); respectively, no seleno-amino acids were detected.

4. Conclusion

For the first time, a method for extraction, preconcentration, and determination of Se-Met, Se-MetSeCys, and SeCys in olive oil samples by UPLC–ESI-MS/MS has been developed. Since olive oil is a complex matrix, a selective extraction approach was set up, which was properly validated. Due to the expected low content of Se-amino acids in olive oils, a preconcentration procedure employing a XAD-1180 resin as solid sorbent was successfully developed, which allowed an enhancement of the detection limit turning the methodology compatible with seleno-amino acids concentrations in the samples.

The methodology was successfully applied to the analysis of several olive oils from different regions and implantation modes. The only species found was Se-MetSeCys, which adds nutritional value to olive oil based on seleniuṁs characteristics as essential element.

Acknowledgements

The authors wish to acknowledge the financial support received by the National Council of Scientific and Technical Research (CONICET), the National Agency for Promotion of Science and Technology and the National University of San Luis (INQUISAL).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.03.045.

References

Alsing Pedersen, G., & Larsen, E. H. (1997). Speciation of four selenium compounds using high performance liquid chromatography with on-line detection by inductively coupled plasma mass spectrometry or flame atomic absorption spectrometry. *Fresenius' Journal of Analytical Chemistry, 358*(5), 591–598.

Alzate, A., Cañas, B., Pérez-Munguía, S., Hernández-Mendoza, H., Pérez-Conde, C., Gutiérrez, A. M., et al. (2007). Evaluation of the inorganic selenium biotransformation in selenium-enriched yogurt by HPLC-ICP-MS. *Journal of Agricultural and Food Chemistry, 55*(24), 9776–9783.

Amoako, P. O., Uden, P. C., & Tyson, J. F. (2009). Speciation of selenium dietary supplements; formation of S-(methylseleno)cysteine and other selenium compounds. *Analytica Chimica Acta, 652*(1–2), 315–323.

Carmen Barciela-Alonso, M., Bermejo-Barrera, A., & Bermejo-Barrera, P. (2007). Separation and determination of Se-compounds by liquid chromatography coupled with electrospray mass spectrometry. *Journal of Trace Elements in Medicine and Biology, 21*(Suppl. 1(0)), 23–25.

Bueno, M. t., & Potin-Gautier, M. (2002). Solid-phase extraction for the simultaneous preconcentration of organic (selenocystine) and inorganic [Se(IV), Se(VI)] selenium in natural waters. *Journal of Chromatography A, 963*(1–2), 185–193.

Combs, G. F., Jr, & Gray, W. P. (1998). Chemopreventive agents: Selenium. *Pharmacology and Therapeutics, 79*(3), 179–192.

Concha-Herrera, V., Lerma-García, M. J., Herrero-Martínez, J. M., & Simó-Alfonso, E. F. (2010). Classification of vegetable oils according to their botanical origin using amino acid profiles established by high performance liquid chromatography with UV–vis detection: A first approach. *Food Chemistry, 120*(4), 1149–1154.

Dacheng, W., Alfthan, G., & Aro, A. (1994). Determination of total selenium and dissolved selenium species in natural waters by fluorometry. *Environmental Science and Technology, 28*(3), 383–387.

Dauthieu, M., Bueno, M., Darrouzes, J., Gilon, N., & Potin-Gautier, M. (2006). Evaluation of porous graphitic carbon stationary phase for simultaneous preconcentration and separation of organic and inorganic selenium species in "clean" water systems. *Journal of Chromatography A, 1114*(1), 34–39.

Dhur, A., Galan, P., & Hercberg, S. (1990). Relationship between selenium, immunity and resistance against infection. *Comparative Biochemistry and Physiology – C Pharmacology Toxicology and Endocrinology, 96*(2), 271–280.

Díez, S., Leitão, A., Ferreira, L., & Rodrigues, A. (1998). Adsorption of phenylalanine onto polymeric resins: Equilibrium, kinetics and operation of a parametric pumping unit. *Separation and Purification Technology, 13*(1), 25–35.

Dugo, G., La Pera, L., Pollicino, D., & Saitta, M. (2003). Determination of selenium content in different types of seed oils by cathodic stripping potentiometry (CSP). *Journal of Agricultural and Food Chemistry, 51*(19), 5598–5601.

Dumont, E., Cremer, K. D., Hulle, M. V., Chéry, C. C., Vanhaecke, F., & Cornelis, R. (2005). Identification of the major selenium compound, Se-Methionine, in three yeast (*Saccharomyces cerevisiae*) dietary supplements by on-line narrowbore liquid chromatography–electrospray tandem mass spectrometry. *Journal of Chromatography A, 1071*(1–2), 191–196.

Dumont, E., Vanhaecke, F., & Cornelis, R. (2006). Selenium speciation from food source to metabolites: A critical review. *Analytical and Bioanalytical Chemistry, 385*(7), 1304–1323.

Fio, J. L., & Fujii, R. (1990). Selenium speciation methods and application to soil saturation extracts from San Joaquin Valley, California. *Soil Science Society of Americal Journal, 54*(2), 363–369.

Goldhaber, S. B. (2003). Trace element risk assessment: Essentiality vs. toxicity. *Regulatory Toxicology and Pharmacology, 38*(2), 232–242.

Gosetti, F., Frascarolo, P., Polati, S., Medana, C., Gianotti, V., Palma, P., et al. (2007). Speciation of selenium in diet supplements by HPLC–MS/MS methods. *Food Chemistry, 105*(4), 1738–1747.

Hill, S. J., Arowolo, T. A., Butler, O. T., Cook, J. M., Cresser, M. S., Harrington, C., et al. (2003). Atomic spectrometry update. Environmental analysis. *Journal of Analytical Atomic Spectrometry, 18*(2), 170–202.

Infante, H. G., Hearn, R., & Catterick, T. (2005). Current mass spectrometry strategies for selenium speciation in dietary sources of high-selenium. *Analytical and Bioanalytical Chemistry, 382*(4), 957–967.

Lindemann, T., & Hintelmann, H. (2002a). Identification of selenium-containing glutathione S-conjugates in a yeast extract by two-dimensional liquid chromatography with inductively coupled plasma MS and nanoelectrospray MS/MS detection. *Analytical Chemistry, 74*(18), 4602–4610.

Lindemann, T., & Hintelmann, H. (2002b). Selenium speciation by HPLC with tandem mass spectrometric detection. *Fresenius' Journal of Analytical Chemistry, 372*(3), 486–490.

Lobiński, R., & Szpunar, J. (1999). Biochemical speciation analysis by hyphenated techniques. *Analytica Chimica Acta, 400*(1–3), 321–332.

Montealegre, C., Marina, M. L., & García-Ruiz, C. (2010). Separation of proteins from olive oil by CE: An approximation to the differentiation of monovarietal olive oils. *Electrophoresis, 31*(13), 2218–2225.

Montes-Bayón, M., Molet, M. J. D., González, E. B., & Sanz-Medel, A. (2006). Evaluation of different sample extraction strategies for selenium determination in selenium-enriched plants (*Allium sativum* and *Brassica juncea*) and Se speciation by HPLC-ICP-MS. *Talanta, 68*(4), 1287–1293.

Pérez-Serradilla, J. A., Japón-Luján, R., & Luque de Castro, M. D. (2007). Simultaneous microwave-assisted solid-liquid extraction of polar and nonpolar compounds from alperujo. *Analytica Chimica Acta, 602*(1), 82–88.

Pérez-Serradilla, J. A., Priego-Capote, F., & Luque De Castro, M. D. (2007). Simultaneous ultrasound-assisted emulsification-extraction of polar and nonpolar compounds from solid plant samples. *Analytical Chemistry, 79*(17), 6767–6774.

Szpunar, J. (2000). Trace element speciation analysis of biomaterials by highperformance liquid chromatography with inductively coupled plasma mass spectrometric detection. *Trends in Analytical Chemistry, 19*, 127–137.

Thomson, C. D. (2004). Assessment of requirements for selenium and adequacy of selenium status: A review. *European Journal of Clinical Nutrition, 58*(3), 391–402.

Tuck, K. L., & Hayball, P. J. (2002). Major phenolic compounds in olive oil: Metabolism and health effects. *Journal of Nutritional Biochemistry, 13*(11), 636–644.

Wake, B. D., Bowie, A. R., Butler, E. C. V., & Haddad, P. R. (2004). Modern preconcentration methods for the determination of selenium species in environmental water samples. *TrAC Trends in Analytical Chemistry, 23*(7), 491–500.