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Nonchromatographic Speciation of Selenium in Edible Oils Using Dispersive Liquid–Liquid Microextraction and Electrothermal Atomic Absorption Spectrometry

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ABSTRACT: A methodology for the nonchromatographic separation of the main selenium species present in edible oils is presented. Dispersive liquid–liquid microextraction is used to extract inorganic selenium (*i*Se), seleno-L-cystine ($SeCys_2$), seleno-L-methionine (SeMet), and selenocystamine (SeCM) into a slightly acidic aqueous medium. The selenium total (*t*Se) content is measured in the extracts by electrothermal atomic absorption spectrometry. By repeating the microextraction stage using an ionic liquid instead of water, the sum of $SeCys_2$, SeMet, and SeCM is obtained and *i*Se is calculated by difference. The detection limit is 0.03 ng of Se per gram of oil. The fractionation of the edible oils by solid phase extraction followed by dispersive liquid–liquid extraction and atomic absorption measurement also permits speciation of *i*Se to be carried out. Data for *t*Se and iSe levels of 15 samples of different origin are given.

KEYWORDS: *selenium, edible oils, electrothermal atomic absorption spectrometry, dispersive liquid–liquid microextraction, speciation*

INTRODUCTION

Selenium is an essential nutrient at low concentrations although it is toxic for animals and humans at high doses. The levels and bioavailability of selenium in foods have aroused increased interest due to its antioxidant and anticancer properties.[1] While a knowledge of the total content of the element is relevant, the chemical form in which it is present is also important due to the differences in the properties of the different species.[2−4] Selenium can be found as an inorganic compound (selenite and selenate) or as a variety of organic species, selenoaminoacids, selenopeptides, and selenoproteins being the most significant. All these forms can be found in foods although in low concentrations. The selenium intake recommended by the World Health Organization is 40 and 30 $\mu g\ day^{-1}$ for men and women, respectively, levels that are not reached in the diet of many countries.[1]

Despite the relevance of this nutrient and the abundant studies dealing with its presence in foods there is still a lack of data for many foods, which is probably due to the low concentrations involved and the subsequent laborious sample preparation techniques and expensive instrumentation needed. The difficulty in obtaining reliable data is further increased if speciation is required because, in addition to the need to avoid species transformation during sample manipulation, the total amount of the element is distributed into the variety of compounds. Most of the reported procedures for selenium speciation are based on liquid chromatography (LC) coupled to inductively coupled plasma mass spectrometry (ICP-MS)[5,6] or atomic fluorescence spectrometry (AFS)[7] for detection, although some procedures based on gas chromatography have been recommended for the most volatile selenium compounds.[8−10] Other analytical techniques such as electrothermal atomic absorption spectrometry (ETAAS) have also been used for the same purpose, but their application is often limited to the determination of the total amount of the element or to the inorganic forms.[11−15]

Selenium determination in oily matrices is particularly difficult, and there are few reports dealing specifically with the subject. Leaving aside a recent report that uses neutron activation analysis for its determination in the seed, oil, and oil cake of a hyperaccumulator plant,[16] the most significant works are those of Dugo et al.[17,18] and Giacomo et al.,[19] who used an extraction procedure in acidic medium followed by cathodic stripping potentiometry,

To the best of our knowledge, there is no previous reference to selenium speciation in edible oils, probably because of the above-mentioned difficulties. This manuscript reports procedures for the determination of the most common selenium compounds in this type of sample. A distinctive feature of the present manuscript is that, in addition to providing data on the total selenium content in edible oils, dispersive liquid–liquid microextraction (DLLME), a methodology for sample preparation introduced several years ago, is used with all its corresponding benefits including the use of minimal amounts of organic solvents.[20,21] When DLLME is carried out in the common way, the analytes are extracted from an aqueous phase into a low volume of organic solvent in the presence of a small amount of a third solvent so that a dispersed phase is obtained. In contrast, in one of the approaches here applied, the analytes are extracted from an organic phase (the oil sample) into an aqueous one. In this way, a preconcentration effect is achieved and the reliability of the analytical data is improved. In addition, ETAAS, an analytical technique which is commonly available in laboratories worldwide, is used for the final measurements.

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Appropriate selection of the experimental conditions allows inorganic selenium to be differentiated from the most common organic selenium species. DLLME combined with ETAAS also permits the amounts of selenite and selenate present in edible oils to be ascertained without the need for a chromatographic stage. Complete speciation of all the selenium compounds is not possible but a reliable and relatively easy-to-perform assessment of the most common species present in edible oils can be achieved.

MATERIALS AND METHODS

Instrumentation. The atomic absorption measurements were carried out with a model 800 spectrometer (PerkinElmer, Waltham, MA) equipped with a transversely heated graphite atomizer, Zeeman effect background correction device, and autosampler (model AS-800). Pyrolytic graphite platforms inserted into pyrolytically coated tubes were obtained from the same manufacturer (reference B050-4033). Argon flowing at 250 mL min^{-1} was the inert gas. An electrodeless discharge lamp (PerkinElmer) operated at 300 mA was used as the radiation source. The instrumental parameters are summarized in Table 1.

The hydride generation atomic fluorescence spectrometric (AFS) measurements were carried out with a Millenium Excalibur spectrometer (PS Analytical, Orpington, UK) operating in the continuous flow mode. A boosted hollow cathode lamp was used as the radiation source with 200 mA as the primary current and 25 mA as the boost current. The measurements were obtained at 196.0 nm. A 3.5 mol L^{-1} hydrochloric acid pumped at 8 mL min^{-1} was used as the carrier, while a 1.2% (w/v) sodium tetrahydroborate solution stabilized with sodium hydroxide (0.075 mol L^{-1}) pumped at 3 mL min^{-1} acted as the reducing agent. Argon was used as the carrier.

Table 1. Instrumental Parameters and Experimental Conditions for Se Determination in the DLLME Extracts

parameter	value		
lamp current, mA	300		
wavelength, nm	196.0		
spectral bandwidth, nm	2.0		
atomizer type	platform		
injected simple volume, μL	30		
chemical modifier	40 μg Pd		
calibration graph, μg L^{-1}	5−150[a]; 7−120[b]		
acceptor phase, μL	300 (77% isopropyl alcohol + 23% of 3% HNO_3 v/v solution)[a]; 75 ($[C_{12}min][Tf_2N]$)[b]		
donor phase, g	10		
enrichment factor	141[a]; 129[b]		
limit of detection in oil, ng g^{-1}	0.03[a]; 0.04[b]		
RSD, %	<4.4[a]; <5.1[b]		
furnace heating program			
step	temperature, °C	ramp, °C s^{-1}	hold, s
1: dry	130; 90[c]	5	20
2: dry	450	20	30
3: ash	800; 700[d]	10	30
4: atomization[e,f]	2100	0	4
5: clean	2600	0	3
sequence for selenium determination			
A:	pipet 20 μL of the modifier and run step 1 and 2		
B:	Inject the sample and run the entire program		

[a]DLLME procedure using aqueous phase. [b]DLLME procedure using ionic liquid. [c]For oil:hexane mixtures. [d]For ionic liquid. [e]Flow of argon stopped. [f]Reading step.

The digestion of the samples was carried out with a Multiwave 3000 microwave digestion system (Anton Paar, Austria). A 50 W ultrasound bath (ATU, Valencia, Spain) was used for the ultrasonic treatment. A Büchi R-210 rotavapor (Flawil, Switzerland) was also used.

Reagents and Samples. Pure water (18 MΩ.cm) obtained with a Millipore purification system (Millipore, Bedford, MA) was used exclusively. The glassware was thoroughly acid-washed with a 10% (v/v) nitric acid solution and rinsed with water prior to use. Stock solutions of 1000 μg mL^{-1} were prepared in water by dissolving sodium selenite and sodium selenate (Sigma, St. Louis, MO). Seleno-L-cystine ($SeCys_2$), seleno-L-methionine (SeMet), and selenocystamine dihydrochloride (SeCM) were also obtained of Sigma and dissolved in a 0.5% (v/v) hydrochloric acid solution or isopropyl alcohol. The ionic liquids, 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ($[C_6min][Tf_2N]$), 1-methyl-3-octylimidazolium bis(trifluoromethylsulfonyl)imide ($[C_8min][Tf_2N]$), and 1-dodecyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide ($[C_{12}min][Tf_2N]$), were obtained from IOLITEC (Heilbronn, Germany). Other chemicals used, including the chemical modifier for ETAAS measurements (a 2000 μg mL^{-1} palladium nitrate solution) and the ammonium pyrrolidine dithiocarbamate (APDC, 0.1 M solution prepared in methanol), were obtained from Fluka (Buchs SG, Switzerland).

For the separation of polar and nonpolar compounds, HLB solid-phase cartridges (6 mL, 200 mg) were obtained from Supelco (Bellefonte, PA).

Commercial edible oils marketed as olive, sunflower, avocado, and macadamia, as well as cans of fish (tuna, mackerel, sardine, and anchovy) prepared in oil were acquired in a local supermarket. Five samples commercialized by Sigma, namely F8020 (fish oil from menhaden), 74380 (fish liver oil from *Gradus Morrhua*), 85067 (sesame oil from *Sesamum indicum*), P1244 (peanut oil), and C8267 (corn oil), were also used. Nutritional supplements marketed in the form of pills as fish oil (salmon and cod) were acquired in a specialized market.

Determination of the Total Selenium Content by AFS. To mineralize the samples, 0.25 g were taken and digested in the microwave oven with a solution containing concentrated hydrogen peroxide (3 mL) and nitric (5 mL) and hydrochloric acid (1 mL). Once the heating program had finished and the solution cooled, 10 mL of 6 M hydrochloric acid were incorporated and the mixture heated at 80 °C for 50 min. The solution was finally made up to 25 mL with water. Aliquots were submitted to AFS measurements using the conditions summarized in Table 1. Solutions containing 0.1−20 μg L^{-1} selenium were used for calibration. The detection limit (criterion based on three times the standard deviation of the blank) was found to be 5 ng g^{-1} selenium in the original oil.

Determination of the Total Selenium Content by ETAAS. The samples were analyzed directly without a mineralization stage. A 3 g portion of the sample was diluted with hexane up to 10 mL, and aliquots of this solution were used for the measurement. The heating program is given in Table 1. Palladium nitrate (2000 μg L^{-1}) was used as the chemical modifier. A 20 μL aliquot of the modifier solution was first injected into the electrothermal atomizer, and stages 1 and 2 of the heating program were run. Next, 30 μL of the sample solution in hexane were injected, and the entire heating program was run to obtain the analytical signal. Calibration was carried out by means of the standard additions method spiking each sample with three amounts of SeMet dissolved in ethanol. The detection limit was found to be 4 ng g^{-1} selenium in the original oil.

Fractionation of Selenium Compounds. *Speciation of Inorganic Selenium.* The HLB cartridges were first preconditioned with hexane and then loaded with a mixture of 4 mL of hexane and 3 g of the sample. Next, 4 mL of hexane were passed through the cartridge and the nonpolar (NP) fraction collected. The organic solvent was evaporated in a rotavapor and the residue dissolved in 300 μL of hexane. Aliquots were introduced into the electrothermal atomizer,

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Table 2. Content of Selenium in the Samples Analyzed by the Different Proposed Procedures

	selenium content (±SD (n = 3)), ng g^{-1}							
					SPE fractions			
							HNO_3 3% v/v	
oil sample	direct	HG-AFS	Aq-DLLME	IL-DLLME	hexane	ethanol	Se(IV)	Se(VI)
olive[a]	<5	<4	0.55 ± 0.05	<0.04	<0.1	0.7 ± 0.3	0.27 ± 0.04	0.32 ± 0.05
sunflower[a]	<5	<4	1.59 ± 0.06	<0.04	<0.1	<0.1	0.90 ± 0.03	0.49 ± 0.05
macadamia[a]	<5	<4	0.60 ± 0.04	0.08	<0.1	0.8 ± 0.2	0.42 ± 0.05	0.24 ± 0.04
avocado[a]	<5	<4	0.38 ± 0.05	<0.04	<0.1	<0.1	0.18 ± 0.06	0.21 ± 0.04
maize[b]	9.4 ± 0.5	9.1 ± 0.6	0.84 ± 0.04	<0.04	<0.1	0.5 ± 0.2	0.53 ± 0.04	0.35 ± 0.06
peanut[b]	5.9 ± 0.5	6.1 ± 0.7	0.71 ± 0.07	<0.04	<0.1	0.5 ± 0.2	0.49 ± 0.05	0.28 ± 0.06
sesame[b]	8.2 ± 0.5	8.4 ± 0.6	0.64 ± 0.06	<0.04	<0.1	<0.1	0.33 ± 0.06	0.24 ± 0.06
fish[b]	48.2 ± 0.6	48.8 ± 0.8	1.49 ± 0.08	0.22 ± 0.04	22.8 ± 0.5	27.8 ± 0.5	0.41 ± 0.05	0.82 ± 0.05
fish liver[b]	65.9 ± 0.7	63.1 ± 0.9	1.76 ± 0.09	0.19 ± 0.03	23.3 ± 0.6	40.0 ± 0.6	0.71 ± 0.07	0.98 ± 0.05
cod liver[c]	24.7 ± 0.6	26.0 ± 0.7	1.01 ± 0.08	0.10 ± 0.04	10.0 ± 0.5	13.0 ± 0.5	0.51 ± 0.06	0.44 ± 0.07
salmon liver[c]	17.6 ± 0.5	16.1 ± 0.8	1.39 ± 0.07	0.13 ± 0.05	6.7 ± 0.4	12.8 ± 0.5	0.63 ± 0.07	0.71 ± 0.07
canned tuna[d]	28.2 ± 0.7	29.2 ± 0.8	2.26 ± 0.09	0.28 ± 0.04	10.0 ± 0.4	18.1 ± 0.5	0.43 ± 0.08	1.05 ± 0.06
canned mackerel[d]	25.9 ± 0.7	26.1 ± 0.8	2.62 ± 0.09	0.47 ± 0.04	6.2 ± 0.4	17.5 ± 0.6	0.84 ± 0.05	1.53 ± 0.08
canned sardine[d]	30.6 ± 0.8	31.3 ± 0.9	1.83 ± 0.08	0.57 ± 0.05	10.3 ± 0.5	18.1 ± 0.4	1.06 ± 0.05	0.73 ± 0.06
canned anchovy[d]	50.6 ± 0.8	52.0 ± 0.9	1.87 ± 0.08	0.33 ± 0.04	20.5 ± 0.5	26.0 ± 0.5	1.49 ± 0.08	0.34 ± 0.06

[a]Commercial edible oil. [b]Laboratory product (Sigma or Fluka). [c]Nutritional supplement marketed as pearls. [d]Olive oil from canned fish.

and the optimized furnace program (Table 1) was run. The polar (P) fraction was recovered by eluting with 4 mL of ethanol. The liquid collected was evaporated in the rotavapor, the residue was dissolved in 300 μL of ethanol, and aliquots were injected into the electrothermal atomizer. Finally, selenite and selenate were recovered from the cartridge by eluting with 4 mL of a 3% v/v nitric acid solution.

To speciate inorganic Se (IV) and Se(VI), the DLLME procedure was applied. To this purpose, the liquid of this third fraction was made up to 10 mL and 200 μL of 0.1 M APDC solution and 50 μL of carbon tetrachloride were incorporated. Dispersion occurred almost instantaneously. The organic phase was separated by centrifugation (4000 rpm for 5 min), and 30 μL of this phase were injected in the electrothermal atomizer. The application of the heating program (Table 1) allowed the selenite level to be calculated. For the determination of Se(VI), the aqueous phase recovered after centrifugation was passed through a polyvinylidene fluoride filter to remove the remains of organic solvent, concentrated hydrochloric acid was incorporated up to 4 M, and the liquid was heated at 80 °C for 50 min. After cooling, 0.5 mL of concentrated ammonium hydroxide solution were added and the DLLME procedure followed by ETAAS measurement was repeated.

Ultrasound-Assisted DLLME with Water. A 10 g oil sample was placed in a conical centrifuge tube and heated up to 80 °C. After adding 300 μL of a 4:1 isopyl alcohol:3% v/v nitric acid solution, the tube was shaken for a few seconds and then introduced into the ultrasound bath at 80 °C for 3 min. After centrifuging at 4000 rpm for 15 min, 30 μL of the aqueous phase were injected into the electrothermal atomizer and the heating program given in Table 1 was applied. Calibration was carried out with oil samples containing selenium levels below the detection limit, which were spiked with a SeMet solution prepared in isopropyl alcohol. The calibration graph was linear in the range 0.05−1.2 μg kg^{-1} selenium in the oil.

Ultrasound-Assisted DLLME with an Ionic Liquid. To a 10 g oil sample placed in a conical tube and heated to 80 °C, 75 μL of $[C_{12}min][Tf_2N]$) were added instead of the isopropyl alcohol/nitric acid mixture. The rest of the procedure was the same as described for DLLME with water. The calibration graph was linear in the range 0.1−1.5 μg kg^{-1} selenium in the oil.

RESULTS AND DISCUSSION

Total Selenium Content. As a first stage, the total selenium content of the 15 samples studied was obtained. For this purpose, the samples were completely dissolved by digestion in a microwave oven and the concentrations of selenium were measured in the resulting solutions using hydride generation atomic fluorescence spectrometry (AFS). The results, which are summarized in Table 2, showed wide variations, depending on each particular sample. Thus, oils of vegetal origin were selenium-free (detection limit 5 ng g^{-1}) or contained low levels of the element, while oils from fish or used in canned fish showed the highest selenium concentrations. To confirm these data, all the samples were again analyzed by means of a different analytical technique. The various oils were diluted with hexane to facilitate handling and then directly introduced into the electrothermal atomizer of an atomic absorption spectrometer. The conditions for the heating program were optimized in the usual way,[22] and the concentrations of selenium again calculated. The results, which are also given in Table 2, showed no significant statistical differences (95% confidence level) from those obtained by AFS. Because the detection limits for both procedures are similar, namely 4 and 5 ng g^{-1} for the ETAAS and AFS procedures, respectively, the first procedure is recommended because the digestion stage is avoided and sample handling is minimal.

Selenium Content in Fractions of Edible Oils. The distribution of selenium compounds present in the oils was studied by fractionating the samples and measuring the signal due to the element by ETAAS. To this end, 3 g of each sample were mixed with 4 mL of hexane and passed through a polymeric reversed-phase sorbent (HLB) that had been preconditioned with this solvent. The data showed that, when using these proportions, selenium compounds were retained completely. Next, three fractions were obtained, eluting first the nonpolar (NP) fraction with pure hexane, then eluting a polar (P) fraction with ethanol and, finally, obtaining a strongly polar (SP) fraction by elution with a 3% (v/v) nitric acid solution. Recovery experiments carried out by spiking the samples with the most common selenium compounds showed that SeMet, SeCM, and $SeCys_2$ eluted in the P fraction, while selenite and selenate eluted in the SP fraction. The results for the two first fractions are given in Table 2.

Regarding the SP fraction, very low signals, often below the detection limit, were obtained. To improve the reliability of the data for this fraction and, at the same time, to achieve the speciation of selenite and selenate, a DLLME procedure was applied. The procedure is based on the fact that selenium(IV) is complexed with ammonium pyrrolidine dithiocarbamate (APDC)[23,24] and extracted into the fine droplets formed when mixing carbon tetrachloride, an auxiliary disperser solvent (such as methanol), and the sample solution. Introduction into the electrothermal atomizer of the organic phase recovered after a centrifugation step allows the analytical signal due to the element to be obtained. In this way, the preconcentration effect inherent in microextraction and the low background during atomization result in an increase in sensitivity. Because only Se(IV) is extracted, speciation is possible if a second microextraction step is carried out after the reduction of Se(VI) to Se(IV). The experimental conditions were studied for the particular case here studied, and the optimal values were found to be those detailed in the Materials and Methods section. The detection limit was 0.1 ng g^{-1} selenium referred to the original oil. The data obtained using this procedure are included in Table 2.

DLLME of the Oils. The common practice of DLLME involves the extraction of an aqueous sample into an organic solvent. In the case considered here, the oil samples play the role of the organic solvent so that DLLME can be exploited in a different way, that is, for the extraction of the selenium compounds present in the oil with a small volume of an aqueous phase. A large number of experiments were carried out in which 10 g oil samples were extracted with diluted nitric acid solutions containing different proportions of acetonitrile, acetone, methanol, ethanol, or isopropyl alcohol as the disperser solvent. In all cases, aliquots of the extracts recovered by centrifugation were injected into the electrothermal atomizer and the selenium signal was obtained. Maximum signals were achieved in the presence of isopropyl alcohol, which was finally selected. The volume of the aqueous phase, which plays an important role, was also optimized. Of note is the fact that this volume affects the analytical signal obtained from the liquid recovered after centrifugation and that the volume recovered is always less than that initially used. The experimental results demonstrated that for 10 g oil samples, the most suitable volume for the aqueous phase is 300 μL of a mixture containing diluted nitric acid and isopropyl alcohol. The volume recovered by centrifugation was 67 $\pm$ 2 μL, which allowed the measurements to be carried out in duplicate.

To speed up the transfer of selenium compounds from the sample to the aqueous phase, the effect of ultrasounds was considered. Similar results were obtained when this auxiliary energy was supplied by an ultrasound probe or by a simple ultrasound bath. Because the bath facilitates sample heating, which resulted in faster extraction, this simple device was finally used for the remaining experiments.

Using the optimized conditions, all the samples were submitted to the DLLME process and selenium was determined in the aqueous extracts by ETAAS. The heating program for these measurements was practically the same as that used for the determination of the total selenium content. It should be noted that as a palladium salt acts as the chemical modifier, the atomic signal corresponds to the total amount of selenium irrespective of the particular species present. To check the effectiveness of the DLLME process for extracting different selenium compounds, two samples, one of maize oil and another of olive oil, were spiked separately with Se (IV), Se (VI), $SeCys_2$, SeMet, and SeCM and recovery experiments were carried out. The recoveries for the five compounds were in the 96−101% range. Similar results were obtained for fish liver and fish oils, the recoveries for the five compounds being in the 94−103% range.

The use of DLLME involves a preconcentration of the analytes extracted, and so very low concentrations of the five above-mentioned selenium compounds may be measured. The enrichment factor calculated as the ratio of the slopes of the calibration lines obtained with and without application of the DLLME stage was found to be 140. The detection limit was calculated to be 0.03 ng g^{-1} referred to the original oil sample. This extremely low detection limit is the consequence of both the low final volume of the solution in which the analyte is present and the well-defined atomization profile with low background signal, as shown in Figure 1.

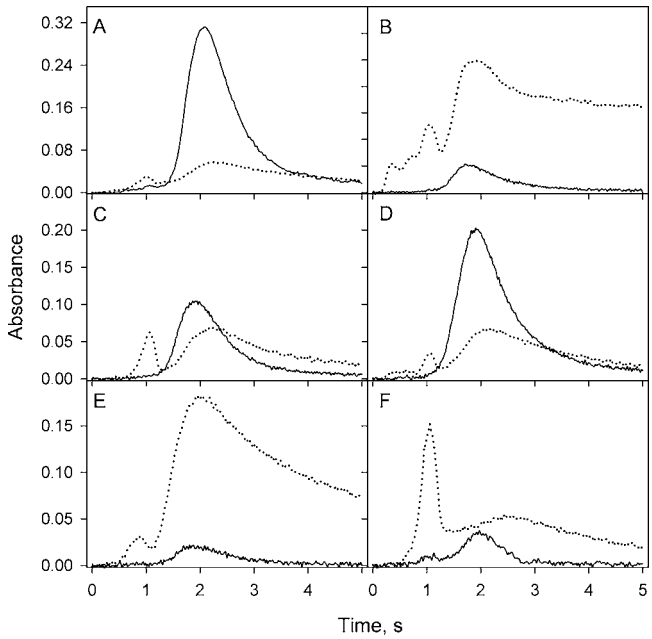

Figure 1. Analytical signals obtained of a fish liver oil sample. Graphs A−F correspond to the procedures using DLLME with an aqueous solution, IL-DLLME, NP fraction, P fraction, and the speciation of Se(IV) and Se(VI) in the SP fraction, respectively. Solid and dotted lines indicate the atomic and background signals, respectively.

The results obtained for the 15 samples studied are given in Table 2. It is important to note that these data reflect the sum of the inorganic selenium plus $SeCys_2$, SeMet, and SeCM, although the presence in this fraction of other selenium-containing species not studied in the recovery experiments cannot be excluded. In addition, these levels represent a part of the total selenium detected in the oil samples. The difference between these data and the total contents would correspond to other unknown selenium compounds, probably including selenolipids, confirming the wide diversity of forms in which the element may be present in edible oils.

Taking into account the complexity of the chemistry involved, in an attempt to further fractionate the selenium compounds, the oil samples were submitted to a different extraction stage by replacing the acidified aqueous solution and the disperser solvent by an ionic liquid. This is also a DLLME procedure, while the dispersion of a solvent (the ionic liquid) in

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the other phase (the oil sample) is achieved by means of ultrasound.[25] Three commercial ionic liquids, namely $[C_6min][Tf_2N]$, $[C_8min][Tf_2N]$, and $[C_{12}min][Tf_2N]$, were evaluated for this purpose. The experiments were carried out by spiking 10 g of the oils with inorganic selenium, $SeCys_2$, SeMet, and SeCM, followed by 75 μL of the IL and submitting the mixture heated to 80 °C to ultrasound to produce dispersion and extraction. After centrifugation, aliquots of the IL phase were injected in duplicate in the electrothermal atomizer and the analytical signal due to selenium was obtained. It was verified that the recoveries of $SeCys_2$, SeMet, and SeCM were in the 95−102% range when using $[C_{12}min][Tf_2N]$ as the IL. For the other two ILs assayed, the recoveries were low and did not exceed 70%. Inorganic selenium was not extracted into the IL. The enrichment factors were calculated for the three selenium compounds extracted and found to be in the 139−142 range. The final results obtained for this DLLME process are shown in Table 2.

The results obtained by the different approaches (summarized in Table 2) are consistent and support the reliability of the data. Thus, it is of note that the content of inorganic selenium calculated after fractionation with hexane agreed with the difference in the levels found using the DLLME procedure with water (sum of inorganic selenium plus $SeCys_2$, SeMet, and SeCM) and the DLLME procedure with IL (sum of $SeCys_2$, SeMet, and SeCM).

The procedures here reported could, at first glance, seem complicated or tedious but in fact they represent a relatively easy way to check the level of inorganic selenium in edible oils. There is no need to use a chromatographic technique coupled to ICP-MS because a simple ETAAS instrument provides the results in a short time.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

AFS, atomic fluorescence spectrometry; APDC, ammonium pyrrolidine dithiocarbamate; $[C_6min][Tf_2N]$, 1-hexyl-3-methylimidazolium bis(trifluoromethyl sulfonyl)imide; $[C_8min][Tf_2N]$, 1-methyl-3-octylimidazolium bis(trifluoromethyl sulfonyl)imide; $[C_{12}min][Tf_2N]$, 1-dodecyl-3-methylimidazolium bis(trifluoro methylsulfonyl) imide; DLLME, dispersive liquid liquid microextraction; ETAAS, electrothermal atomic absorption spectrometry; ICP-MS, inductively coupled plasma mass spectrometry; IL, ionic liquid; LC, liquid chromatography; NP, nonpolar fraction; P, polar fraction; SeCM, selenocystamine; $SeCys_2$, seleno-L-cystine; SeMet, seleno-L-methionine; SP, strongly polar fraction

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