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Abstract

An analytical method was developed for the speciation of elemental selenium (Se(0)) in selenized yeasts by anion-exchange HPLC-ICP-MS after its chemical transformation into $SeSO_3^{2-}$ by reaction with sodium sulfite. The presence of Se(0) in the yeasts was further confirmed by single particle ICP-MS. Indeed, Se nano-particles, if present, are expected to be, at least partly, Se(0). X-Ray Photoelectron Spectroscopy, a well-recognized technique for chemical elements speciation in the solid-state was also used with this objective. Both methods were able to confirm the presence of Se(0) in the selenized yeasts but failed to provide reliable quantitative results. Analytical performances of the HPLC-ICP-MS method were then evaluated for Se(0) determination. Quantification limits of 1 mg/kg were reached. The recovery levels from an added quantity were comprised between 93% and 101%. Within-run and between-run precision were both below 8%. The procedure developed was finally applied to quantify Se(0) content in a series of seven yeast batches from different suppliers. Se(0) was found to be present in all the studied yeasts and represented on average 10-15% of the total Se.

Keywords

Elemental selenium (Se(0)), HPLC-ICP-MS, spICP-MS, XPS, speciation

1. Introduction

The addition of selenium (Se) to diet has now become a well-established practice, especially for animal nutrition, due its anti-oxidant properties [1]. Selenized yeasts, able to accumulate up to 3000 mg/kg of selenium, are a very attractive supplementary source of selenium due to their high content of selenomethionine (SeMet) which is more bioavailable and less toxic than selenite [2]. However, they remain complex and variable mixtures in which nearly up to 20 different selenized compounds have been identified [3].The currently available methods, based on HPLC-ICP-MS and HPLC-ESI-MS/MS detection techniques, enable the determination of SeMet [4], selenocysteine [5], a number of water-soluble metabolites [6] and the residual selenite or selenate that should be present at a concentration of below 2% of total Se [7]. But the Se mass balance of all these identified species rarely exceeds 90%, which suggests the presence of other Se chemical forms [8]. It is nonetheless important to assess the comprehensive mass balance of Se in these yeasts, as it is a prerequisite to better understand its metabolism and its significance in nutrition and toxicology [9]. One hypothesis that can be made is that this unidentified selenized fraction might be, at least partly, elemental selenium (Se(0)). Indeed, selenized yeasts are prepared by exposition of high levels of selenite (typically tens of mg/L) to yeasts but selenite can undergo chemical or biotic reduction to elemental selenium by microorganisms, with a low tendency to re-oxidize [10]. However, the analytical methods developed to characterize selenized yeasts first focused on SeMet determination, expected to be the most available form of Se, then on metabolites identification and quantification to complete the mass balance but has not focused on the elemental Se determination yet.

Quantitative determination of Se(0) remains challenging as it is sparingly soluble in water and therefore difficult to extract with the common protocols used for speciation analysis [11]. It has nevertheless been shown that Se(0) can be quantitatively solubilized from sediments in the form of selenosulfate ($SeSO_3^{2-}$) after reaction with an excess of sodium sulfite at neutral pH [12]. Some authors have included this sulfite-based extraction as the last step in a sequential extraction scheme applied to garlic [13] or plants [14]. In this way, all the other selenocompounds were expected to be extracted upstream and total Se determined in the sulfite extract was assumed to correspond to Se(0). This approach was therefore not based on the on-line specific detection of the selenosulfate formed and therefore may have led to an overestimation of Se(0) if some other Se species have been simultaneously mobilized and thus erroneously identified as Se(0). Recently, Aborode *et al.* [11] proposed an anion-exchange HPLC-ICP-MS method for the specific selenosulfate determination and applied it to

quantitatively determine Se(0) levels in plants. But, to our knowledge, this approach has never been applied to selenized yeasts.

The objective of this work was therefore to develop a method to quantify Se(0) in commercial selenized yeasts by HPLC-ICP-MS after its transformation into $SeSO_3^{2-}$.

2. Materials and methods

2.1. Instrumentation

The ICP-MS used were an Agilent (Tokyo, Japan) 7500cx (for HPLC-ICP-MS measurements) and 7900 (in the single particle mode for SeNPs determination). They were fitted with Meinhard and Micromist nebulizers, respectively. ICP-MS 7900 was used in association with the single nanoparticle application module included in the ICP-MS Mass Hunter software (G5714A).

Chromatographic separations were carried out using a Model 1200 HPLC pump (Agilent, Wilmington, DE, USA) as the delivery system. The outlet of the column was directly connected to the nebulizer of the ICP-MS by means of PEEK tubing. Injections were performed using a Rheodyne valve with a 100 µL sample loop.

For selenosulfate standard preparation and for samples extraction, an OLS200 (Grant Instruments, Cambridge, United Kingdom) water bath was used. A model Mikro 120 centrifuge (Hettich, Marne la Vallée, France) was used to centrifuge the solutions. An ultrasonic bath model Elmasonic P 300H (Elma Ultrasonic, Singen, Germany) and a vortex classic model (VELP Scientifica, Usmate, Italy) were used for NPs dispersion in the extracts.

XPS analysis were carried out on a Kratos Axis Ultra spectrometer (Manchester, United Kingdom), using a monochromatic aluminum source (AlKα=1486.6eV).

2.2. Reagents, standards and solutions

Analytical reagent grade chemicals were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Water (18 MΩ.cm) obtained using a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout, unless otherwise specified. For Se(0) quantification, a 1000 µg Se/mL $SeSO_3^{2-}$ standard solution was prepared (see section 2.4.1) from a Se(0) powder purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Multi-elemental stock standard solutions for trace analysis containing 100 mg/L of Se (CCS-4) and 100 mg/L of Au (CCS-2) were used for calibration during spICP-MS measurements (Analab, Bisheim, France). Suspensions of Au nanoparticles with nominal diameters of 30 nm, 60 nm

and 100 nm (50 mg/L) (Nanocomposix, San Diego, CA, USA) were used for calibration, transport efficiency determination and quality control during spICP-MS measurements.

2.3. Samples

A set of 7 selenized yeast samples were collected from 7 different suppliers. The theoretical total Se content of these samples ranged from 2000 to 3000 mg/kg. This range of values was further confirmed by ICP-AES analysis after HNO_3/H_2O_2 mineralization.

2.4 Analytical procedures

The procedures flow chart used is summarized Fig. 1.

2.4.1. Selenosulfate standard solution preparation from Se(0) powder

A selenosulfate stock solution (1000 mg/L) was prepared by adding 5 mg of Se(0) powder to 5 mL of a 1 M sodium sulfite solution prepared in a 10 mM ammonium citrate buffer (pH 7) in a closed vessel. The solution was placed in a water bath maintained at 90°C for 1 h while being vigorously shaken. After cooling, this solution was centrifuged (14000 rpm, 10 min) and the supernatant was diluted with the sodium sulfite solution to prepare the calibration standards. All the working solutions were prepared daily. Total Se was also analyzed in the stock solution by ICP-MS to evaluate the efficiency of the Se(0) solubilization.

2.4.2. Elemental Se extraction from selenized yeasts

200 mg of yeast was extracted by 10 mL volumes of a 1 M sodium sulfite solution prepared in 10 mM ammonium citrate (pH 7) in a closed vessel. The mixture was warmed to 90°C in a water bath during 1 h while being vigorously shaken. The solution was then left to cool and centrifuge (14000 rpm, 10 min). The supernatant was collected, further diluted in the sodium sulfite solution if required and analyzed by anion-exchange HPLC-ICP-MS.

2.4.3. Chromatographic and ICP-MS conditions

The chromatographic conditions used were developed elsewhere [11]. Briefly, an aliquot (100 µL) of the sodium sulfite extract was injected into the PRP-X100 column (250 mm x 4.1 mm, 5 µm) (Hamilton, Bonaduz, Switzerland). The mobile phase was 10 mM ammonium citrate buffer (pH 7). Elution was performed by an isocratic program for 20 min at

a flow rate of 1 mL/min. The collision/reaction cell of the ICP-MS was pressurized with H_2. The ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se and ^{82}Se isotopes were monitored.

2.4.4. Analysis of the Se nanoparticulate fraction by single particle ICP-MS

The sample preparation used for SeNPs characterization was described in detail elsewhere [8]. Briefly, a sequential extraction procedure based on water, Driselase and protease was applied before resuspension of the pellet in a 4% SDS (w/v) solution. This suspension was sonicated for 1 h and centrifuged (4500 g, 10 min). The supernatant was analyzed by single particle ICP-MS after dilution (1/1000) and NPs dispersion (30 s, vortex) according to the method developed by Jiménez-Lamana *et al.* [8]. Settling time during data acquisition was eliminated, the dwell time and the total acquisition time were 100 µs and 60 s, respectively. The sample flow rate was calculated daily by measuring the mass of water taken up by the peristaltic pump for five minutes. This operation was repeated three times and the average value was used for calculations. Transport efficiency (TE) was determined daily from particle size calibration using a 30 nm Au NPs solution diluted to obtain a particle concentration of 50 ng/L, and Au dissolved calibration obtained from standard solutions of 1 µg/L. TE obtained was 3.6 %. Au NPs suspensions (60 nm and 100 nm at 250 and 1000 ng/L, respectively) and Au dissolved solutions (5 and 10 µg/L) were also employed for quality control during spICP-MS analysis. TE was applied to SeNPs analysis taking into account a density of 3.79 g/cm^3.

2.4.5. Analysis of the selenized yeasts by XPS

The analyzed area of the samples was 300 µm x 700 µm. Peaks were recorded with constant pass energy of 20 eV. Charge neutralization was used for all the acquisitions. The pressure in the analysis chamber was around 10^{-8} Torr. The binding energy scale was calibrated using the C 1s peak at 285.0 eV from the hydrocarbon contamination invariably present. The curves fit for core peaks were obtained using a minimum number of components in order to match with the experimental curves. The samples, in the form of powder, were pasted on sample holders using conductive tape. For selenium, the recorded peaks were Se3d and Auger spectra Se_{LMM}, the latter corresponding to relaxation phenomena of excited ionized state of the atoms. This Auger peak was used to differentiate the selenium chemical environment.

3. Results and discussion

The analytical strategy applied to identify the presence and the concentration of Se(0) in yeast samples is summarized in Fig. 1. The HPLC-ICP-MS method was developed to identify and quantify Se(0) in selenized yeasts. The two other analytical techniques, spICP-MS and XPS, were used as alternative approaches to confirm the presence of elemental selenium already characterized by HPLC-ICP-MS. Both methods were therefore applied without further development.

3.1. Determination of Se(0) in selenized yeasts by HPLC-ICP-MS

3.1.1. Efficiency of transformation of Se(0) into selenosulfate

Elemental Se is difficult to solubilize in water and, as a consequence, to extract with aqueous buffers [11]. It can however be converted into a soluble compound, selenosulfate, after reaction with an excess of sodium sulfite at high temperature while being vigorously shaken [12]. Different authors have shown that this reaction was stoichiometric and quantitative [12, 15] and obtained a complete dissolution whereas Aborode *et al.* [11] only reached an incomplete conversion of 83%. Here, to evaluate the efficiency of the conversion, a series (n = 10) of $SeSO_3^{2-}$ standards were prepared by reacting Se(0) and sulfite to reach a final concentration of 1000 mg/L. The resulting supernatants were measured for their total Se content to evaluate the efficiency of this reaction. The recoveries were evaluated at 104 ± 6% showing a complete dissolution of Se(0) in the conditions used. Indeed, if part of the Se(0) had not reacted with sulfite, it would have remained in the solid fraction and would not have been measured in the supernatant. The supernatants were then further analyzed by HPLC-ICP-MS. A typical chromatogram obtained is shown in Fig. 2a. As expected with the protocol used, only one peak corresponding to $SeSO_3^{2-}$ is observed which confirms that Se(0) was converted into a single species after reaction with sulfite. Therefore, as no $SeSO_3^{2-}$ standard is commercially available, the solution prepared in this way can be used for further calibration.

3.1.2. Effect of sulfite-based extraction on other selenocompounds

Yeasts are complex mixtures which may contain up to 20 different selenized compounds including SeMet, a number of water-soluble metabolites, and residual Se(IV) and Se(VI). It is thus important to ensure that these compounds will not interfere with selenosulfate detection. However, only a few of these compounds are commercially available and could be subjected to the sulfite-based extraction procedure. SeMet, the main compound in selenized yeasts, and Se(IV) and Se(VI), even if both are only present at trace level in these samples, were investigated. Each of these three Se compounds were incubated with the sulfite

solution to check if they could also react with it to cause potential chromatographic interference with the $SeSO_3^{2-}$ detection. No shift in retention times and no appearance of peaks in the chromatogram were observed between these standards prepared in water or in the sulfite solution, showing that they were not reacting with sulfite. These retention times, marked with a dotted line in Fig. 2a, were also well separated from the peak of $SeSO_3^{2-}$. In consequence, SeMet, Se(IV) and Se(VI) present in selenized yeasts do not interfere with Se(0) detection. Due to the unavailability of standards for selenized metabolites, the same kind of experiment could not be conducted with them. It should however be pointed out that they account for minor compounds, the main one being SeMet.

3.1.3. Tracking for endogenous selenosulfate in selenized yeasts

Speciation analysis is generally based on the analysis of the undisturbed analyte which is not the case here as Se(0) is transformed into $SeSO_3^{2-}$. It was therefore important to check that endogenous $SeSO_3^{2-}$ was absent from the yeast samples to avoid any interference with Se(0) determination. A typical yeast sample was therefore extracted in the same conditions as the Se(0) determination but without addition of sulfite. The HPLC-ICP-MS chromatogram obtained for this extract is shown in Fig. 2b. No peak was observed in the range of retention times where $SeSO_3^{2-}$ elutes, confirming the absence of this compound in yeast. Therefore, $SeSO_3^{2-}$ detected after sulfite extraction fully corresponds to elemental selenium.

3.1.4. Optimization of sulfite-based extraction for selenized yeasts

As it was demonstrated that $SeSO_3^{2-}$ was not present in selenized yeasts, and as sulfite has been shown to be efficient to fully transformed Se(0) into $SeSO_3^{2-}$ without affecting the other selenocompounds potentially present, the sulfite-based extraction was applied to selenized yeasts. Fig. 2c shows a typical chromatogram. No significant peak corresponding to SeMet, expected to be the main compound in selenized yeasts, was detected. This is explained because SeMet in yeasts is incorporated into proteins, thus its extraction requires the use of proteolytic enzymes which was not the case here. Some minor peaks were also detected at the beginning of the chromatogram probably corresponding to selenometabolites. The main peak of the chromatogram was found at a retention time matching the one of $SeSO_3^{2-}$ which proves the presence of elemental selenium in the sample.

HPLC-ICP-MS allows the determination of Se(0) in selenized yeasts but this approach is based on its chemical transformation into $SeSO_3^{2-}$ and not on its direct determination. Therefore, two alternative analytical approaches, expected to detect Se(0) directly, were used

to confirm its presence. The first one was spICP-MS and the second one, XPS, was a solid-state speciation technique.

3.2. Determination of nanoparticulate fraction of Se by single particle ICP-MS

Jimenez-Lamana *et al.* have already shown the presence of biogenic selenium nanoparticles (SeNPs) in selenized yeasts by single particle ICP-MS [8]. Even if not clearly stated in this paper, one can assume that these nanoparticles are, at least partly, composed of Se(0). This assumption can be made from the sample preparation protocol used which aimed to sequentially eliminate the water-soluble Se, then the polysaccharides bound Se and finally the proteins bound Se, that is to say all the selenium not expected to be Se(0). And then, the residue, expected to contain mainly Se(0), was dispersed to be analyzed by spICP-MS. Moreover, Khoei *et al.* have shown that during the reduction of selenite into elemental selenium by proteobacteria, nanoparticles of selenium were formed [16]. Finally, Alvarez-Fernandez Garcia *et al.* have shown the presence of Se nanoparticles that disappeared after reaction with sulfite to become selenosulfate [17]. The transformation of elemental selenium nanoparticles into selenosulfate by sulfite has already been shown in animal tissue [18]. All these studies suggest that there is a link between elemental selenium and selenium nanoparticles.

Therefore, in order to confirm the presence of Se(0) in selenized yeasts, a typical sample was analyzed by the protocol developed by Jimenez-Lamana *et al.* [8] to evaluate the presence of SeNPs. The size particle distribution obtained is presented in Fig. 3. Even if the high dissolved amount of Se in the suspension prevented the detection of NPs with sizes lower than 60 nm, particles with sizes between 80 and 150 nm were observed. The average, median and most frequent diameters measured were 108 ± 1, 100 ± 3 and 87 ± 2 nm, respectively. These results are in agreement with those reported by Jimenez-Lamana *et al.* [8], namely an average median diameter of 108 ± 4 nm.

In conclusion, the analysis of biogenic SeNPs tends to confirm the presence of Se(0) in the selenized yeasts since, as previously discussed, there is a link between biogenic SeNPs and Se(0). However, the high level of dissolved Se prevented the analysis of particles with sizes lower than 60 nm. Therefore, the evaluation of the concentration level of particulate Se in order to compare it to Se(0) concentration measured by HPLC-ICP-MS would be irrelevant since the concentration level of particulate Se would be grossly underestimated.

3.3. Se speciation by XPS analysis

The second alternative method investigated was XPS which is a solid-state speciation technique. It enables to highlight the chemical environment of selenium atoms present at the surface of the sample and to quantify them. It can therefore provide speciation data and has already been used to perform selenium speciation [19]. Its main advantage is that it allows to avoid the sample preparation step. However, it is commonly accepted that qualitative results can be reached for concentrations higher than 0.05% whereas getting reliable quantitative results requires concentrations higher than 1% [20].

The spectra obtained for yeast B sample are shown in Fig. 4. First, the Se(0) standard spectrum was compared to the one obtained for SeMet (the main compound expected in the yeasts). For Se(0) (Fig. 4a and 4d), the Se3d and Se3p peaks are well defined, confirming the presence of one single environment for the selenium. The Se3d spectrum resembled a doublet due to the spin orbit splitting effect associated with a d core level. These two peaks, $Se3d_{5/2}$ and $Se3d_{3/2}$, correspond to a single environment of selenium atoms. The binding energies of $Se3d_{5/2}$ and $Se3p_{3/2}$, respectively located at 55.4 eV and 161.7 eV, correspond to the position expected for Se(0) [21]. The SeMet signals (Fig. 4b and 4e) are less well-defined than the Se(0) equivalents, which is characteristic of a more disturbed chemical environment. This phenomenon could be explained by the fact that the carbon bound to selenium into SeMet is not electronegative enough to shift the corresponding peak toward high binding energy, as it is usually observed when considering a chemical shift based on an initial state effect. Therefore, both signals are very close and the discrimination in terms of binding energy of Se(0) from SeMet by XPS is ambiguous. Another source of information is given by Auger features (Fig. 4g and 4h) which are induced by the phenomenon of relaxation. Indeed, the Auger peaks present different shapes and positions : at 180 eV for Se(0) and at 182 eV for SeMet.

The yeast B sample was then analyzed in the same conditions (Fig. 4c, 4f and 4i). The Se3d and Se3p peaks characterizing the selenium atoms appeared at the same reference positions, 55.4eV and 161.7eV respectively (Fig. 4c and 4d). But, as expected, attributions were not obvious probably because the Se concentration was not high enough for XPS analysis. Moreover, the differentiation between the Se-Se (Se(0)) and the C-Se-C (SeMet) bonds is ambiguous. Another source of difficulty to attribute the signal is caused by the presence of sulfur in the yeast sample which interferes with the Se3p signal (Fig. 4f). But focusing on the deconvolution of the Auger spectrum of the yeast B sample (Fig. 4i), it seems that both species, Se(0) and SeMet, are present in the yeast. The SeMet shape tends to cover 75% of the total Auger spectrum of the yeast, the remaining 25% part of the signal

corresponding to Se(0). However, even if experiments were performed using the yeast with the highest Se(0) concentration (determined by HPLC-ICP-MS, see Table 1), this amount of selenium appears critical compared to the quantification limits of XPS. As a consequence, the Se distribution in the yeast sample determined by XPS was probably affected by a large uncertainty. Again, this alternative technique to HPLC-ICP-MS tends to confirm the presence of Se(0) in the yeast sample but fails to give reliable quantitative results.

Both alternative methods allow to assess Se(0) without its chemical transformation which is the main drawback of the HPLC-ICP-MS approach. However, both fail to provide reliable quantitative results. In the case of XPS, this is mainly due to a lack of sensitivity. For spICP-MS, assuming that SeNPs were present as Se(0), the high Se dissolved concentration in the suspension was responsible for an underestimation of the amount of SeNPs. Therefore, HPLC-ICP-MS appears to be the most promising technique to quantitatively evaluate Se(0) in selenized yeasts. The analytical figures of merit were therefore evaluated.

3.4. HPLC-ICP-MS analytical figures of merit

The criteria performance of the methods, including linearity, sensitivity, recovery level and within-run and between-run precision, were evaluated. Calibration graphs, acquired up to 3000 µg Se/L, were linear ($R^2 > 0.995$). In the absence of Certified Reference Material, the recovery level of Se(0) added to a yeast sample at a concentration about five times higher than the natural Se(0) concentration was evaluated. Five independent analyses were performed on three different days. The recovery level ranged between 93 and 101% which is considered as acceptable. Within-run precision ranged between 4.6 and 7.1% and the between-run precision was evaluated at 5.9%. Both were below 8% which confirms the precision of the method. These figures of merit confirm that the quantitative transformation of Se(0) into $SeSO_3^{2-}$ could be as efficient in standard conditions as in real samples. The limit of detection and the limit of quantification were calculated according to the IUPAC criteria as the concentration corresponding to three and ten times the standard deviation of the blank solution, respectively. They were evaluated at 0.3 and 1 mg/kg, respectively.

3.5. Application to evaluation of Se(0) concentration in different selenized yeast batches

Seven different selenized yeasts obtained from different suppliers were analyzed for their Se(0) content. The results are shown Table 1. The level of Se(0) in the yeasts was usually between 150 and 400 mg/kg, accounting for about 10-15% of total Se since total Se

contents were in the range of 2000 – 3000 mg/kg. In any case, one sample contained a much higher level of elemental Se. This higher amount may have been expected as this sample displayed a more reddish coloring compared to the other samples, specific to the presence of elemental selenium [16]. Considering that the mass balance of selenized amino-acids + selenometabolites + residual Se(IV) and (VI) rarely exceeds 90% [8], Se(0) appears to be one of the species that should be measured to complete the Se budget in selenized yeasts.

The level of elemental Se found here is higher than the level of residual Se(IV) and Se(VI) (below 2%) for example. This significant concentration should probably be considered when investigating the metabolism and the nutritional and toxicological impacts of selenized yeasts.

4. Conclusion

A method, based on the chemical conversion of Se(0) into $SeSO_3^{2-}$ by sulfite followed by HPLC-ICP-MS analysis, has been developed for the selective determination of elemental Se in selenized yeasts. The presence of Se(0) in these samples was confirmed by spICP-MS and XPS analysis but both failed to provide reliable quantitative results. Due to its ease of use and its analytical performances, HPLC-ICP-MS appears to be the preferential approach for Se(0) routine characterization in selenized yeasts, even if it requires the chemical transformation of the species to be measured.

Se(0) was recovered in the seven different batches of commercial yeasts investigated, representing on average 10-15% of the total Se. This level of concentration is significant and therefore the presence of Se(0) should be considered when trying to elucidate the metabolism of selenized yeasts.

Compliance with ethical standards

The authors declare that there are no known conflicts of interest associated with this publication.

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Figures

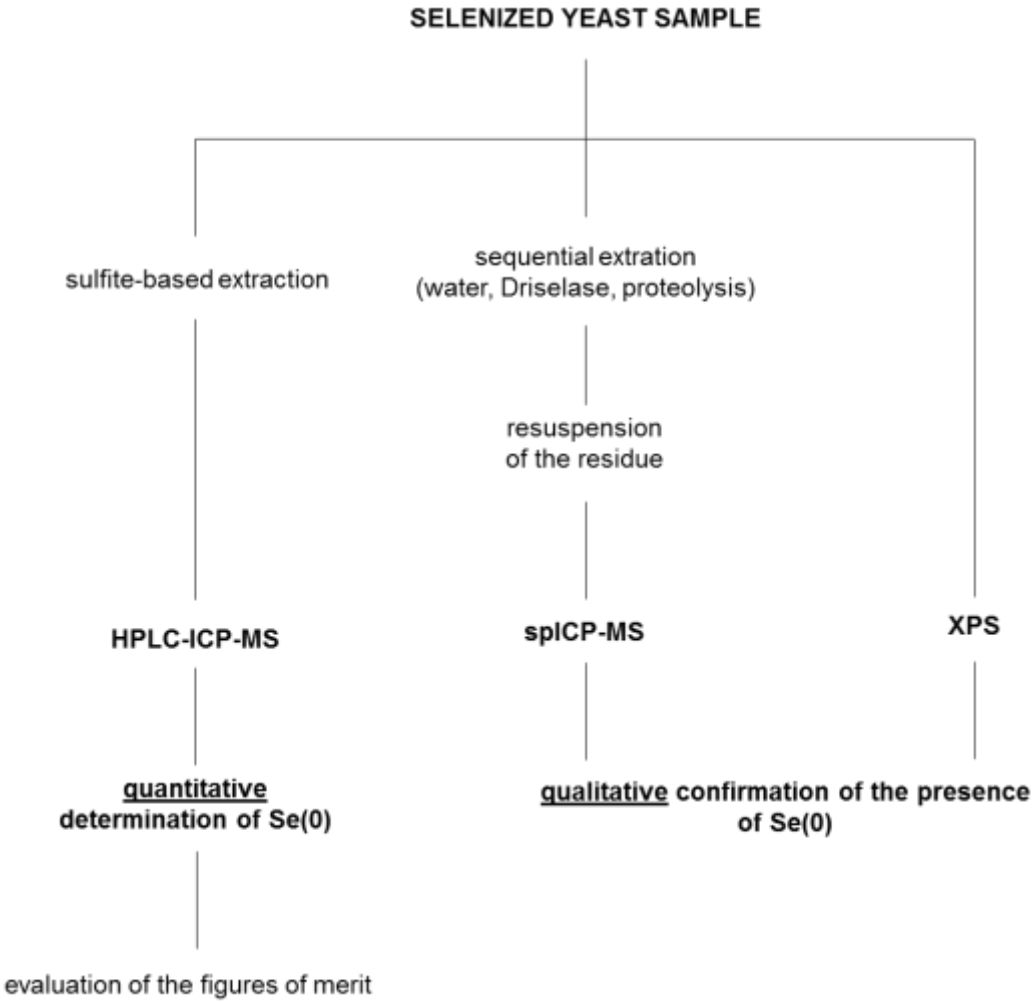

Fig1 flow chart of the analytical strategy for Se(0) characterization in selenized yeast samples

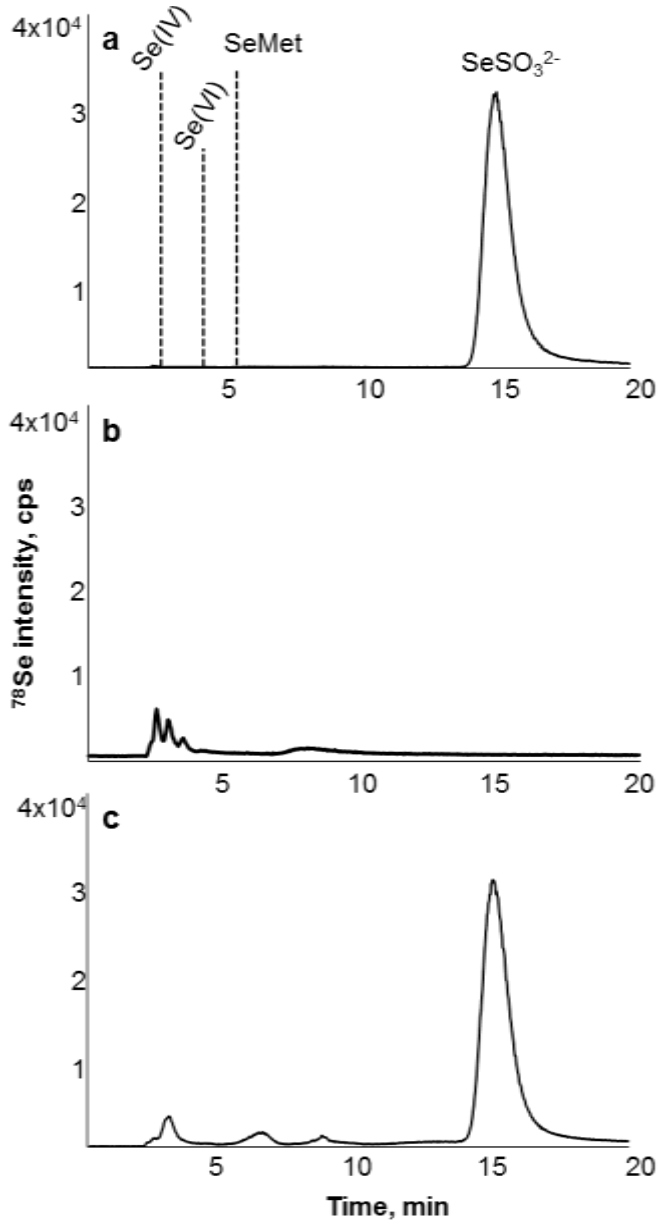

Fig2 typical HPLC-ICP-MS chromatograms. (a) SeSO32- standard. (b) extract from a selenized yeast without sulfite. (c) extract from a selenized yeast with sulfite.

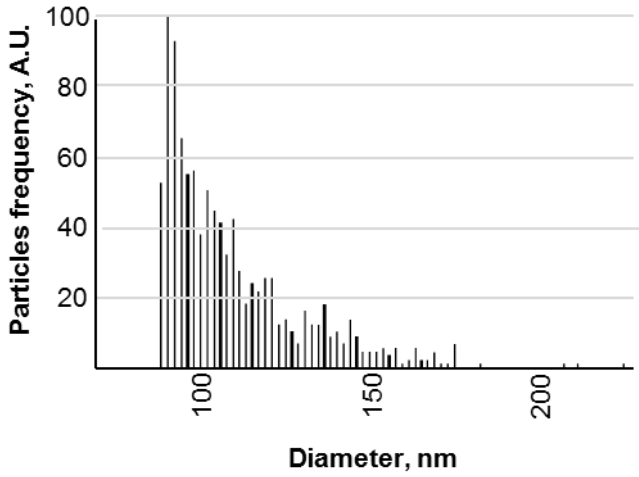

Fig3 size distribution obtained by spICP-MS from a selenized yeast.

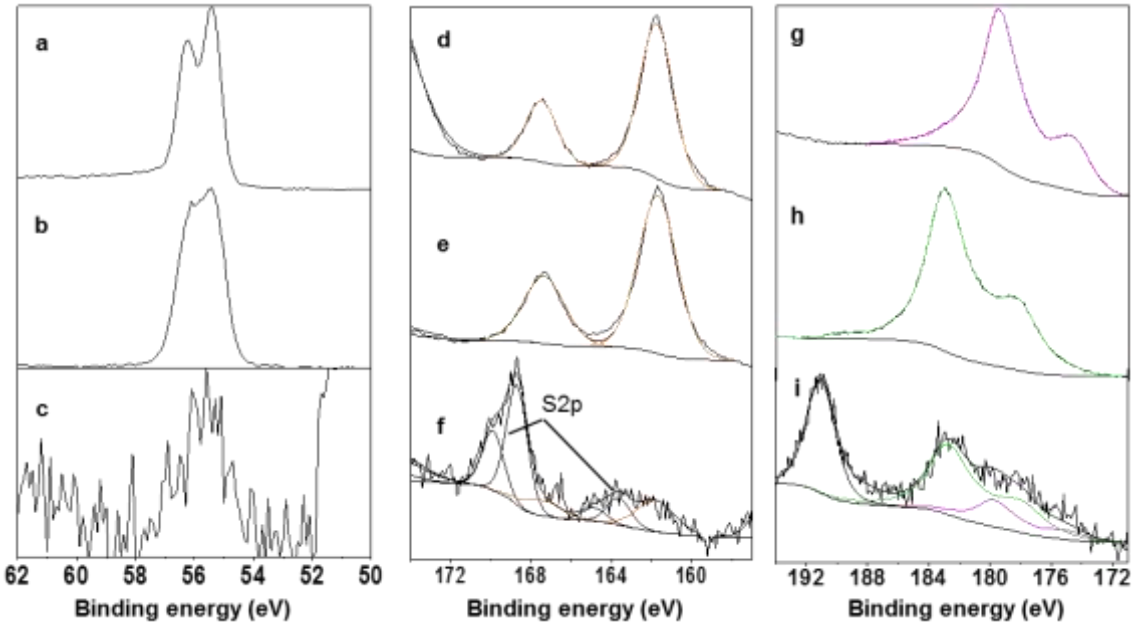

Fig4 XPS spectra. (a) Se3d for Se(0). standard, (b) Se3d for SeMet standard, (c) Se3d for yeast B. (d) Se3p for Se(0) standard, (e) Se3p for SeMet standard, (f) Se3p (□) and S2p (□) for yeast B, (g) Se(0) Auger feature for Se(0) standard, (h) SeMet Auger feature for SeMet standard, (i) Se(0) Auger feature for yeast B.

Table 1 : Se(0) concentration evaluated by HPLC-ICP-MS for the 7 different yeast batches

Samples	Se(0) concentration (mg/kg)	Se(0) rounded percentage relative to total Se (%)
Yeast A	317 ± 9	11%
Yeast B	1085 ± 81	40%
Yeast C	421 ± 27	14%
Yeast D	306 ± 12	15%
Yeast E	334 ± 5	16%
Yeast F	374 ± 9	18%
Yeast G	158 ± 10	8%