



HAL
open science

Identification and determination of selenohomolanthionine – The major selenium compound in *Torula* yeast

Katarzyna Bierla, Noriyuki Suzuki, Yasumitsu Ogra, Joanna Szpunar,
Ryszard Lobinski

► To cite this version:

Katarzyna Bierla, Noriyuki Suzuki, Yasumitsu Ogra, Joanna Szpunar, Ryszard Lobinski. Identification and determination of selenohomolanthionine – The major selenium compound in *Torula* yeast. *Food Chemistry*, 2017, 237, pp.1196-1201. 10.1016/j.foodchem.2017.06.042 . hal-03133557

HAL Id: hal-03133557

<https://hal.science/hal-03133557>

Submitted on 17 Feb 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Identification and determination of selenohomolanthionine

2 - the major selenium compound in *Torula* yeast

3 Katarzyna Bierła¹, Noriyuki Suzuki², Yasumitsu Ogra², Joanna Szpunar¹, Ryszard Łobiński^{1,3}

4 1 CNRS/UPPA, Institut for Analytical Sciences and Physical Chemistry for the Environment and Materials
5 (IPREM), UMR 5254, Hélioparc, F-64053 Pau, FRANCE

6 2 Department of Toxicology and Environmental Health, Graduate School of Pharmaceutical Sciences, Chiba
7 University, 1-8-1 Inohana, Chuo, Chiba 260-8675, Japan, Japan

8 3 Department of Chemistry, Warsaw University of Technology, Noakowskiego 3, 00-664 Warsaw, Poland

10 Abstract

11 *Torula* yeast (*Candida utilis*) was found to metabolize selenium in a totally different way to Brewer's
12 yeast (*S. cerevisiae*) leading to the biosynthesis of selenohomolanthionine (SeHLan), a major
13 selenium compound accounting for 60-80% of the total selenium. The identity of SeHLan was
14 confirmed by retention time matching in hydrophilic ion interaction chromatography (HILIC) with
15 inductively coupled plasma mass spectrometric detection (ICP MS) using a custom synthesized
16 standard molecule and by HILIC – Orbitrap MS and MS-MS fragmentation. Selenohomolanthionine
17 escapes the current assays for the organic character of Se-rich yeast based on the protein-bound
18 selenomethionine determination. A HILIC – ICP MS method was developed for the quantitative
19 determination of selenohomolanthionine in yeast supplements with a detection limit of 146 ng/g.

21 Introduction

22 The bioavailability of organic forms of selenium from dietary sources is known to be higher than that
23 of inorganic species (Fairweather-Tait, Collings, & Hurst, 2010). The most popular form of
24 supplemented organic selenium is yeast, usually *Saccharomyces cerevisiae*, grown in the presence of
25 selenite and/or selenate which is able to accumulate up to 3000 mg Se kg⁻¹ (Schrauzer, 2006). The
26 total selenomethionine (SeMet) concentration superior to 60% has been considered as a measure of
27 the “organic” character of Se-rich yeast, of the efficiency of the biotechnological enrichment process
28 and of the product quality (Bierla, Szpunar, Yiannikouris, & Lobinski, 2012).

29 The analysis for SeMet is typically based on the enzymatic proteolysis followed by HPLC with
30 selenium specific detection by ICP MS (Bierla, Szpunar, Yiannikouris, & Lobinski, 2012). The
31 availability of the SELM-1 reference material has largely contributed to the improvement of quality
32 assurance of the SeMet determination in Se-rich yeast (Mester, Willie, Yang, Sturgeon, Caruso,
33 Fernández, et al., 2006). However, there is increasing evidence that some yeast samples may contain
34 significant amounts of selenocysteine. The latter is often referred to as the 21st essential amino acid
35 because it is genetically encoded in selenoproteins (Böck, Forchhammer, Heider, Leinfelder, Sawers,
36 Veprek, et al., 1991). Selenocysteine is usually not possible to be quantified by the selenomethionine
37 assay which may mislead consumers about the organic (or not) character of Se-rich yeast. Also, most
38 of the work to date has been done on *S. cerevisiae* yeast. However, other yeast strains are being

39 investigated as tools for conversion of inorganic selenium into its organic forms. These forms are not
40 systematically identified because of the unavailability of suitable analytical methodologies.

41 One of such yeast varieties, recently used for the conversion of inorganic selenium is Torula (*Candida*
42 *utilis*) yeast. Torula yeast is widely used as a “natural” flavouring in processed foods and pet foods as
43 a replacement for the flavour enhancer monosodium glutamate (Buerth, Tielker, & Ernst, 2016). The
44 ability of selenium to be bound to the biomass of *S. cerevisiae* ATCC MYA-2200 and *Candida utilis*
45 ATCC 9950 was compared, it was observed that yeasts of the genus *Candida* were more efficient in
46 binding selenium (Kieliszek, Błazejak, & Płaczek, 2016). Effects of selenium on morphological
47 changes in *Candida utilis* yeast cells were studied (Kieliszek, Błazejak, Bzducha-Wróbel, & Kurcz,
48 2016). Our preliminary tests on Se-rich Torula indicated low (<10%) concentration of
49 selenomethionine. In the same time, the absence of inorganic selenium indicates “organic” form(s)
50 of selenium in Se-enriched Torula yeast.

51 The goal of this work was a systematic study of speciation of selenium in Se-enriched Torula yeast by
52 cataloging selenium species according to their molecular weight and hydrophobic properties,
53 identifying the detected selenium compounds, and developing an analytical method for the
54 quantitative determination of the most abundant compound(s).

55

56 **EXPERIMENTAL**

57 **Samples:** Four commercial (BioProcessing, Sydney, Australia) Torula (*Candida utilis*) yeast samples,
58 referred to as samples A-D were used. Certified Reference Material SELM-1 (National Research
59 Council of Canada) was analysed in parallel with the samples as a reference of Se-enriched *S.*
60 *cerevisiae* yeast and to validate the total Se and the selenomethionine determination results.

61 **Reagents:** Analytical reagent grade chemicals and LC-MS grade solvents were purchased from
62 Sigma-Aldrich (Saint Quentin Fallavier, France) unless stated otherwise. Ultra-pure water (18 MΩ
63 cm) obtained with a MiliQ system (Millipore, Bedford, MA) was used throughout. Hydrogen peroxide
64 from Fisher Scientific (Hampton, NH) and nitric acid (INSTRANALYZED) from Baker (Central Valley,
65 PA) were used for sample digestion. Protease used was of proteomics grade. A standard solution of
66 1000 mg/l selenium was purchased from Plasma CAL standards (Teddington, UK). A standard
67 solution of SeMet was prepared by dissolving SeMet powder obtained from Sigma Aldrich, and
68 appropriately diluted with water.

69 **Instrumentation:**

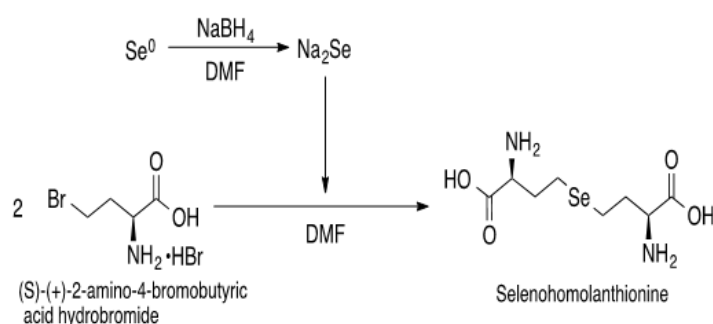
70 A Digi-Prep system from SCP Science (Quebec, Canada) was used for digestion of samples. Two types
71 of centrifuge: Jouan CR3i Model (Thermo Fisher Scientific, Waltham, MS) and a model 5415R
72 (Eppendorf, Hamburg, Germany) were used for various sample volumes. For incubation of samples
73 an ultrasonic bath Branson 2510 from Emerson (Danbury, CT), and a thermostatic shaking water
74 bath OLS 200 from Grant (Cambridge, UK) were used. pH was adjusted using a pH-213 pH meter
75 from Hanna Instruments (Woonsocket, RI). HPLC systems used were: Dionex Ultimate 3000 UHPLC
76 equipped with a UV detector from Thermo Fisher Scientific (Sunnyvale, CA) and Agilent 1200 HPLC

77 from Agilent Technologies (Tokyo, Japan). The chromatographic columns used were: size-exclusion
78 Superdex Peptide (10 x 300 mm x 13 μm) from GE Healthcare (Little Chalfont, UK), HILIC Amidogel (3
79 x 300 mm x 2.6 μm) from Phenomenex (Torrence, CA), reversed-phase Eclipse Plus C18 (4.6 x 150
80 mm x 5 μm) from Agilent (Tokyo, Japan) and anion exchange PRP x100 (4.1 x 250 mm x 5 μm) from
81 Hamilton Robotics (Reno, NV). The ICP mass spectrometer used was an Agilent 7700x (Agilent,
82 Tokyo, Japan). ESI Orbitrap LTQ Velos from ThermoFisher (Waltham, MS) was used for molecular MS
83 and MS/MS analysis. The ion source was operated in the positive or negative ion mode at 3 kV and
84 heated at 80 $^{\circ}\text{C}$. Capillary temperature was set to 300 $^{\circ}\text{C}$.

85 Procedures:

86 Synthesis of selenohomolanthionine

87 The synthesis was carried out according to the procedure of Ogra *et al.* (Ogra, Kitaguchi, Ishiwata,
88 Suzuki, Iwashita, & Suzuki, 2007) summarized in the equation below.



89

90 Briefly, elemental Se powder (80 mg) was suspended in 1 ml of DMF under nitrogen atmosphere,
91 and then sodium tetrahydroborate (300 mg) in DMF (3 ml) was added. The mixture was stirred for
92 60 min at room temperature. (S)-(+)-2-amino-4-bromobutanoic acid hydrobromide (574 mg)
93 dissolved in DMF (3.5 ml) was gradually added to the reaction mixture which was then set off at
94 room temperature for 72 h. The reaction was stopped by the addition of 1 M HCl (0.5 ml) and the
95 solvent was evaporated *in vacuo*. The residue was dissolved in water and then purified on an ODS
96 column (Wakogel[®] 100C18, Wako Pure Chemical Industries, Japan).

97 The chemical purity of selenohomolanthionine was assessed by HILIC-ICP MS. The chromatogram
98 (**Fig. 1a**), obtained in the conditions optimized for a maximum resolution, shows a major peak (>97%
99 Se) preceded by a small one (<3% Se). The HILIC-ESI MS chromatogram (**Fig. 1b**) obtained in the
100 same conditions allowed to attribute to the major peak the molecular mass of 285 corresponding to
101 the protonated form of SeHLan (**Fig. 1c**). The small peak (m/z 299, Fig. 1d) was identified as (S)-(+)-2-
102 amino-4-bromopentanoic acid, a reaction artefact formed in the reaction of the impurity present in
103 the commercially available (S)-(+)-2-amino-4-bromobutanoic acid (cf. inset in **Fig. 1**). The SeHLan
104 concentration in the solution to be used as the calibration standard was quantified by ICP MS on the
105 basis of the total Se concentration taking into account the chromatographic purity of the HILIC peak
106 and the column recovery.

107 Determination of the total Se content

108 2.5mL of HNO₃ was added to 0.2 g of the sample in a DigiPrep tube and left overnight before adding
109 1mL H₂O₂. Then, the sample was digested in DigiPrep (0-30 min heated up to 65 °C and then kept at
110 65 °C for 4 h). After digestion, the mixture was diluted with water to 50 ml. The diluted sample was
111 analyzed by ICP-MS; isotopes: ⁷⁶Se, ⁷⁷Se, ⁷⁸Se, ⁸⁰Se and ⁸²Se were monitored. The observed isotopic
112 pattern matched the theoretical one which indicated the absence of interference. A 5 points
113 calibration curve was constructed. The content of Se in each sample was calculated as the mean of
114 results obtained for the five selenium isotopes. The ICP MS was optimized using a tuning solution
115 (containing 1 ppb of Y, Tl, Li in 2% nitric acid).

116 **Water extraction and size-exclusion HPLC fractionation of selenium**

117 5 mL of water was added to 0.2 g of sample; the mixture was placed in ultrasonic bath for one hour
118 and then centrifuged. The supernatant was eluted from a size exclusion HPLC Superdex peptide
119 column (exclusion limit of 20 kDa and the optimal separation range of 100-7000 Da) with 100 mM
120 ammonium acetate buffer (pH 7.5) at a flow rate of 0.7ml/min.

121 **Selenomethionine determination**

122 0.2 g of the sample was incubated with 5 mL of a protease solution (20 mg protease in 30 mM TRIS
123 buffer, pH 7.5). Three consecutive incubations (17 h at 37°C) with fresh portions of the enzyme
124 solution were carried out. After each incubation, the sample was centrifuged (10 min at 2500 rpm)
125 and the supernatant was transferred to a separate vial to which 5 µL of β-mercaptoethanol was
126 added. The 3 supernatants were pooled together and analysed by anion-exchange chromatography
127 (Hamilton PRP-X100 column) coupled to ICP MS. Separation was performed using gradient elution
128 with buffers A (20 mM acetic acid/10 mM triethylamine) and B (200 mM acetic acid/100 mM
129 triethylamine) according to the following program: 0-5 min: 0% B, 5-30 min: 0-100% B, 30-40 min:
130 100% B, 40-41: 100-0% B, 41-45 min: 0% B. The quantification was carried out using standard
131 additions of SeMet at three levels. The samples were analysed in duplicate. Analytical blanks and
132 SELM-1 standard reference material (National Research Council Canada) were analysed in parallel.

133

134 **Selenohomolanthionine determination**

135 0.2 g of the sample was leached with 5 mL water in an ultrasonic bath for 1 h and filtered through a
136 10 kDa cut-off filter. The solution was analysed by HILIC chromatography with dual ICP MS and ESI
137 Orbitrap MS/MS detection using a flow rate 50 µl/min and gradient elution with acetonitrile (A) and
138 10 mM ammonium formate, pH 5.5 (B). The gradient was: 0-5 min: 90% A, 5-45min 90-50% A, 45-50
139 min 50% A, 50-52min 50-35% A. 52-55 min 35% A, 55-60 35-90% A, 60-70 min 90% A. The sample
140 volume injected was 5 µl. The quantification was carried out using standard additions of
141 selenohomolanthionine at three levels by HILIC – ICP MS. The samples were analysed in duplicate.
142 Analytical blanks were analysed in parallel.

143

144

145 RESULTS AND DISCUSSION

146 ***Comparison of the selenium speciation in the S. Cerevisiae and Candida utilis yeast strains***

147 Preliminary investigations aimed at the comparison of the *Candida utilis* (Torula yeast) with *S.*
148 *Cerevisiae* (Brewer's yeast), the latter strain having served for the preparation of the SELM-1
149 reference material. This comparison was carried out in terms of 4 parameters, typically assessed in
150 order to characterize a selenium-rich yeast sample: total selenium concentration, total
151 selenomethionine concentration, percentage of the water soluble selenium, and the metabolic
152 fingerprint (Bierla, Szpunar, Yiannikouris, & Lobinski, 2012). The determined concentrations are
153 shown in **Table 1**.

154 *Total selenium concentration.* Four batches of Torula yeast were analyzed and showed total
155 selenium concentrations in the 3600-4000 ppm range. This is by 30% more than the most
156 concentrated in selenium brewer's yeast available (Bierla, Szpunar, Yiannikouris, & Lobinski, 2012;
157 Schrauzer, 2006). This observation corroborates earlier findings obtained by the comparison of the
158 selenium incorporation in *S. cerevisiae* ATCC MYA-2200 and *C. utilis* ATCC 9950 which showed that
159 yeasts of the genus *Candida* were more efficient in binding this element (Kieliszek, Błazejak, &
160 Płaczek, 2016).

161 *Selenomethionine concentration.* The contribution of selenomethionine determined in Torula yeast
162 is remarkably low (7.3-11.6%) in comparison with brewer's yeast (ca. 60%). This suggests that
163 whereas Se in *S. Cerevisiae* is incorporated into proteins, it is not in Torula yeast.

164 *Water soluble selenium.* This hypothesis is further corroborated by the important difference
165 between the two Se-rich yeast varieties in terms of the percentage of water-soluble selenium (non-
166 protein bound water-soluble fraction). Whereas it is typically fairly low in *S. cerevisiae* yeast (ca.
167 15%), it accounts for the large majority (in one batch almost the totality) of the selenium present.
168 The speciation in the latter (inorganic or organic) was therefore attempted.

169 *Fractionation of the water-soluble Se fraction according to the molecular mass.* This fraction is
170 supposed to contain intermediate products (metabolites) characteristic of the pathway of its
171 incorporation into proteins. Also, the distribution of the Se compounds according to the molecular
172 size is known to be characteristic of yeast strain and of fermentation parameters and can be a useful
173 fingerprint of the origin of the preparations available on the market and of the reproducibility of the
174 production process (Casal, Far, Bierla, Ouerdane, & Szpunar, 2010).

175 **Fig. 2** compares the metabolite distribution in the two types of yeast strains investigated by
176 size-exclusion HPLC. The chromatogram of the water extract of *S. cerevisiae* shows 5 peaks: three
177 corresponding to selenium incorporated in proteins (Encinar, Ouerdane, Buchmann, Tortajada,
178 Lobinski, & Szpunar, 2003; Encinar, Śliwka-Kaszyńska, Połatajko, Vacchina, & Szpunar, 2003); three
179 corresponding to the metabolome fraction (ca. 50 identified metabolites) (Arnaudguilhem, Bierla,
180 Ouerdane, Preud'homme, Yiannikouris, & Lobinski, 2012; Far, Preud'homme, & Lobinski, 2010) and
181 one corresponding to Se-adenosylhomoselenocysteine and its derivatives (Bierla, Szpunar,
182 Yiannikouris, & Lobinski, 2012; Casal, Far, Bierla, Ouerdane, & Szpunar, 2010). (Arnaudguilhem,
183 Bierla, Ouerdane, Preud'homme, Yiannikouris, & Lobinski, 2012; Far, Preud'homme, & Lobinski,
184 2010). Some variations of the selenometabolite pattern in Se-enriched *S. cerevisiae* are possible but

185 the three principal fractions are almost always present (Casal, Far, Bierla, Ouerdane, & Szpunar,
186 2010). In contrast to this, the chromatogram of *Torula* yeast shows one peak in the elution volume
187 range corresponding to low-molecular weight (200-600 Da) metabolites. The recovery of the
188 selenium from the size-exclusion chromatographic column in both cases was quantitative ($98\pm 2\%$
189 and $97\pm 3\%$), respectively.

190 *Fractionation of the water-soluble selenometabolite fraction in Torula yeast according to*
191 *hydrophobicity.* The chromatographic purity of the SEC – ICP MS peak in *Torula* yeast was
192 investigated by other chromatographic mechanisms. The reversed-phase (RP) HPLC–ICP MS
193 chromatogram (not shown) of the water extract indicated that >80% of Se eluted in the void of the
194 column followed by a number of minor peaks. The attempts to retain this selenocompound in RP
195 HPLC had been unsuccessful and therefore the separation mechanism was changed to HILIC. The
196 chromatogram obtained in optimized gradient conditions by HILIC – ICP MS is shown in **Fig. 3a**. The
197 retention time of the major species, accounting for the majority of selenium (> 50 %), was
198 reproducible within 0.06 min (n=3).

199

200 **Identification of selenohomolanthionine**

201 An extract of *Torula* yeast spiked with the selenohomolanthionine standard showed a proportional
202 increase of the major peak without an increase in width (Fig. 3b) which suggests that the major
203 compound is selenohomolanthionine. Electrospray MS confirms (Fig. 4) this identification in terms of
204 molecular mass, match of the molecule isotopic pattern with the theoretical one, and MS/MS
205 fragmentation. Selenohomolanthionine was earlier identified as a minor compound in selenized *S.*
206 *cerevisiae* yeast (Arnaudguilhem, Bierla, Ouerdane, Preud'homme, Yiannikouris, & Lobinski, 2012;
207 Dernovics, Far, & Lobinski, 2009; Far, Preud'homme, & Lobinski, 2010; Ogra, Kitaguchi, Ishiwata,
208 Suzuki, Iwashita, & Suzuki, 2007) and in Japanese pungent radish (ca. 5 % of total Se) (Ogra,
209 Kitaguchi, Ishiwata, Suzuki, Iwashita, & Suzuki, 2007). The biosynthetic pathway of SeHLan in plants
210 was presented by Ogra (Ogra, 2015). Its tissue distribution differs from that of selenomethionine
211 (Ogra, 2015); it can be metabolized in the same way as SeMet but, in contrast to SeMet, does not
212 accumulate in pancreas (Anan, Mikami, Tsuji, & Ogra, 2011; Ogra, 2015).

213

214 **Quantification of selenohomolanthionine**

215 A method for the quantitative determination of SeHLan by HILIC – ICP MS was then developed. The
216 slopes of the calibrations curves: external and by standard additions agreed within $\pm 5\%$. The
217 recovery of the SeHLan from the column was $96\pm 3\%$. Table 1 shows the recoveries of the different
218 concentration of SeHLan measured for two Se isotopes ^{78}Se and ^{80}Se in the peak height and peak
219 area mode. The peak height mode turned out to be more slightly more precise, especially for the
220 most intense ^{80}Se isotope with measurement errors below 2.5%.

221 The instrumental detection limits calculated as three times the standard deviation of the noise at the
222 retention time of SeHLan were 3 ng/ml in the injected solution which corresponded to 146 ng/g of
223 selenohomolanthionine in the yeast sample. The quantification limits (10 times of the SD of the
224 noise) were 10 ng/ml and 485 ng/g, respectively.

225 The selenohomolanthionine concentrations in the four analysed samples were: Sample A: 2344 ± 56
226 $\mu\text{g/g}$ (58.3%); B: $2715 \pm 397 \mu\text{g/g}$ (73.6%); C: $2888 \pm 45 \mu\text{g/g}$ (78.7%), and D: $3306 \pm 350 \mu\text{g/g}$
227 (84.5%).

228

229 **Selenium speciation and mass balance**

230 In addition to selenomethionine, the HILIC-ICP MS chromatograms obtained for the investigated
231 samples (cf. Fig 3a) show the presence of a number of minor selenocompounds of relative
232 abundances between 2 and 7 % depending on the sample. The molecular masses ($M+H^+$) measured
233 for these compounds by ESI Orbitrap MS were : 286.01910, 308.98390, 490.03806 and 433.07332.
234 Except for the latter compounds identified as Se-adenosyl-L-homocysteine ($C_{14}H_{21}O_5N_6Se^+$), the other
235 compounds could not be identified by the molecular mass measurement only and their identification
236 was beyond the scope of this study.

237

238 **CONCLUSIONS**

239 *Torula (Candida utilis)* yeast metabolizes selenium in a totally different way to *S. cerevisiae* leading
240 to the biosynthesis of selenohomolanthionine, a major selenium compound accounting for 60-80%
241 of total selenium. The high percentage of biosynthesized selenohomolanthionine together with the
242 capacity of *Torula* strain to incorporate up to $4000 \mu\text{g/g}$ of selenium make it one of the richest
243 sources of organic selenium available. In contrast to selenomethionine (the major Se-compound in *S.*
244 *cerevisiae* Se-rich yeast) which is protein-bound, selenohomolanthionine is water soluble, and
245 potentially more easily bioavailable. Interestingly, selenohomolanthionine escapes classic assays for
246 the organic character of Se-rich yeast. Therefore, these assays which are based on the
247 selenomethionine determination should be revised or complemented in order to account for other
248 forms of "organic" selenium in yeast.

249

250 **REFERENCES**

- 251 Anan, Y., Mikami, T., Tsuji, Y., & Ogra, Y. (2011). Distribution and metabolism of
252 selenohomolanthionine labeled with a stable isotope. *Analytical and Bioanalytical Chemistry*, *399*(5),
253 1765-1772.
- 254 Arnaudguilhem, C., Bierla, K., Ouerdane, L., Preud'homme, H., Yiannikouris, A., & Lobinski, R. (2012).
255 Selenium metabolomics in yeast using complementary reversed-phase/hydrophilic ion interaction
256 (HILIC) liquid chromatography-electrospray hybrid quadrupole trap/Orbitrap mass spectrometry.
257 *Analytica Chimica Acta*, *757*, 26-38.
- 258 Bierla, K., Szpunar, J., Yiannikouris, A., & Lobinski, R. (2012). Comprehensive speciation of selenium
259 in selenium-rich yeast. *TrAC - Trends in Analytical Chemistry*, *41*, 122-132.
- 260 Böck, A., Forchhammer, K., Heider, J., Leinfelder, W., Sawers, G., Veprek, B., & Zinoni, F. (1991).
261 Selenocysteine: the 21st amino acid. *Molecular Microbiology*, *5*(3), 515-520.
- 262 Buerth, C., Tielker, D., & Ernst, J. F. (2016). *Candida utilis* and *Cyberlindnera (Pichia) jadinii*: yeast
263 relatives with expanding applications. *Applied Microbiology and Biotechnology*, *100*(16), 6981-6990.
- 264 Casal, S. G., Far, J., Bierla, K., Ouerdane, L., & Szpunar, J. (2010). Study of the Se-containing
265 metabolomes in Se-rich yeast by size-exclusion - Cation-exchange HPLC with the parallel ICP MS and
266 electrospray orbital ion trap detection. *Metallomics*, *2*(8), 535-548.
- 267 Dernovics, M., Far, J., & Lobinski, R. (2009). Identification of anionic selenium species in Se-rich yeast
268 by electrospray QTOF MS/MS and hybrid linear ion trap/orbitrap MSn. *Metallomics*, *1*(4), 317-329.

269 Encinar, J. R., Ouerdane, L., Buchmann, W., Tortajada, J., Lobinski, R., & Szpunar, J. (2003).
 270 Identification of water-soluble selenium-containing proteins in selenized yeast by size-exclusion-
 271 reversed-phase HPLC/ICPMS followed by MALDI-TOF and electrospray Q-TOF mass spectrometry.
 272 *Analytical Chemistry*, 75(15), 3765-3774.

273 Encinar, J. R., Śliwka-Kaszyńska, M., Połatajko, A., Vacchina, V., & Szpunar, J. (2003). Methodological
 274 advances for selenium speciation analysis in yeast. *Analytica Chimica Acta*, 500(1-2), 171-183.

275 Fairweather-Tait, S. J., Collings, R., & Hurst, R. (2010). Selenium bioavailability: Current knowledge
 276 and future research requirements. *American Journal of Clinical Nutrition*, 91(5).

277 Far, J., Preud'homme, H., & Lobinski, R. (2010). Detection and identification of hydrophilic selenium
 278 compounds in selenium-rich yeast by size exclusion-microbore normal-phase HPLC with the on-line
 279 ICP-MS and electrospray Q-TOF-MS detection. *Analytica Chimica Acta*, 657(2), 175-190.

280 Kieliszek, M., Błażejczak, S., Bzducha-Wróbel, A., & Kurcz, A. (2016). Effects of Selenium on
 281 Morphological Changes in *Candida utilis* ATCC 9950 Yeast Cells. *Biological Trace Element Research*,
 282 169(2), 387-393.

283 Kieliszek, M., Błażejczak, S., & Płaczek, M. (2016). Spectrophotometric evaluation of selenium binding
 284 by *Saccharomyces cerevisiae* ATCC MYA-2200 and *Candida utilis* ATCC 9950 yeast. *Journal of Trace*
 285 *Elements in Medicine and Biology*, 35, 90-96.

286 Mester, Z., Willie, S., Yang, L., Sturgeon, R., Caruso, J. A., Fernández, M. L., Fodor, P., Goldschmidt, R.
 287 J., Goenaga-Infante, H., Lobinski, R., Maxwell, P., McSheehy, S., Polatajko, A., Sadi, B. B. M., Sanz-
 288 Medel, A., Scriver, C., Szpunar, J., Wahlen, R., & Wolf, W. (2006). Certification of a new selenized
 289 yeast reference material (SELM-1) for methionine, selenomethionine and total selenium content and
 290 its use in an intercomparison exercise for quantifying these analytes. *Analytical and Bioanalytical*
 291 *Chemistry*, 385(1), 168-180.

292 Ogra, Y. (2015). Selenohomolanthionine and its potential use in medicine and nutrition. In V. R.
 293 Preedy (Ed.), *Selenium: Chemistry Analysis, Function and Effects*. Cambridge: RSC.

294 Ogra, Y., Kitaguchi, T., Ishiwata, K., Suzuki, N., Iwashita, Y., & Suzuki, K. T. (2007). Identification of
 295 selenohomolanthionine in selenium-enriched Japanese pungent radish. *Journal of Analytical Atomic*
 296 *Spectrometry*, 22(11), 1390-1396.

297 Schrauzer, G. N. (2006). Selenium yeast: Composition, quality, analysis, and safety. *Pure and Applied*
 298 *Chemistry*, 78(1), 105-109.

299

300 **CAPTIONS TO FIGURES:**

301 Fig. 1. Verification of the purity of the selenohomolanthionine standard. a) HILIC-ICP MS; b) HILIC ESI
 302 MS; c) zoom of spectrum of selenohomolanthionine (m/z 285), d) zoom of spectrum of
 303 impurity (m/z 299) (the chemical reaction leading to this impurity is given in the inset)

304 Fig.2. Size-exclusion liquid chromatography screening for the water-soluble fraction of a) SELM-1
 305 (*Saccharomyces cerevisiae*) and b) *Torula* yeast.

306 Fig. 3. HILIC-ICP MS analysis of the water extract of *Torula* yeast. a) extract; b) extract spiked with
 307 selenohomolanthionine standard

308 Fig. 4. Identification of selenohomolanthionine by ESI MS/MS. (a) mass spectrum (the zoom of the
 309 isotopic pattern is given in inset); b) MS/MS fragmentation pathways

310

311 **Table 1.** Total selenium, selenomethionine and water-soluble metabolite fraction found in Torula
 312 and SELM-1 yeast

Sample	Total Se, μg/g	Water soluble, μg/g	%	SeMet, μg/g as Se	SeMet, %
A	4019 ± 185	3306 ± 204	82.3	466 ± 58	11.6
B	3686 ± 168	3150 ± 197	85.5	270 ± 28	7.3
C	3669 ± 219	3106 ± 272	84.7	312 ± 47	8.5
D	3910 ± 147	3797 ± 142	97.1	369 ± 41	9.4
SELM-1	2048 ± 26*	256 ± 4	13.8	1401 ± 99**	68.4

313 * certified value 2059± 64 μg/g, **certified 1369 ± 112 μg Se/g

314

315 **Table 2.** Recoveries of selenohomolanthionine (as selenium) added to a Torula-yeast sample

added	Peak height, ⁷⁸ Se		Peak height, ⁸⁰ Se		Peak area, ⁷⁸ Se		Peak area, ⁸⁰ Se	
	Found, ng/ml	Error, %	Found, ng/ml	Error, %	Found, ng/ml	Error, %	Found, ng/ml	Error, %
50	47,56	-4,86	48,76	-2,47	52,45	4,90	52,27	4,53
100	98,35	-1,66	100,3	0,30	95,00	-5,00	93,75	-6,25
250	237,9	-4,85	249,1	-0,34	242,7	-2,91	245,5	-1,78
500	507,8	1,56	509,6	1,92	510,7	2,14	510,2	2,05

316

317

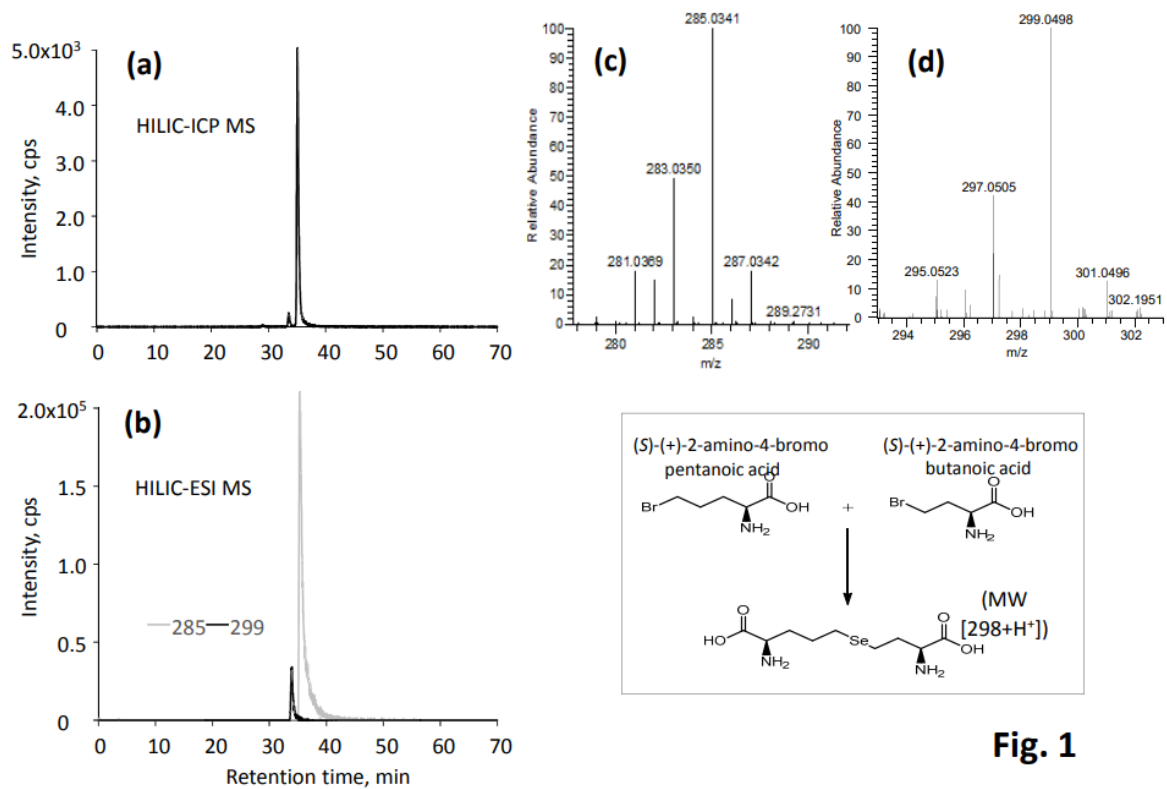


Fig. 1

318
319

320

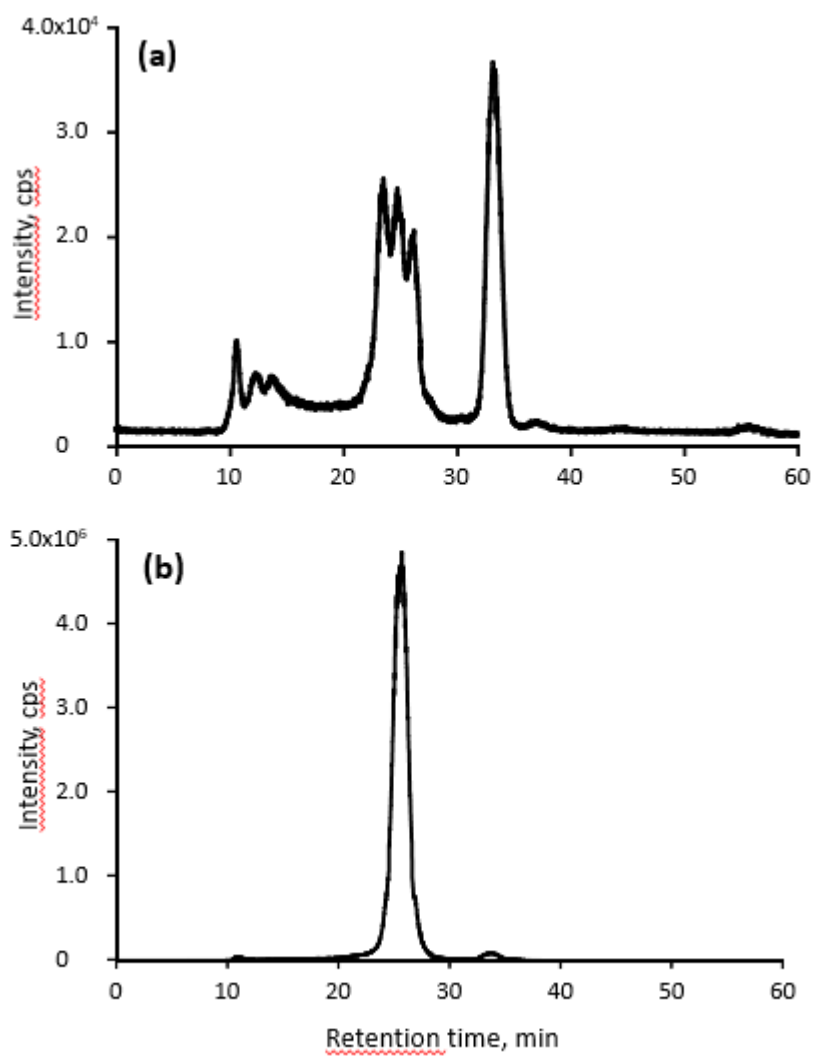


Fig. 2

321

322

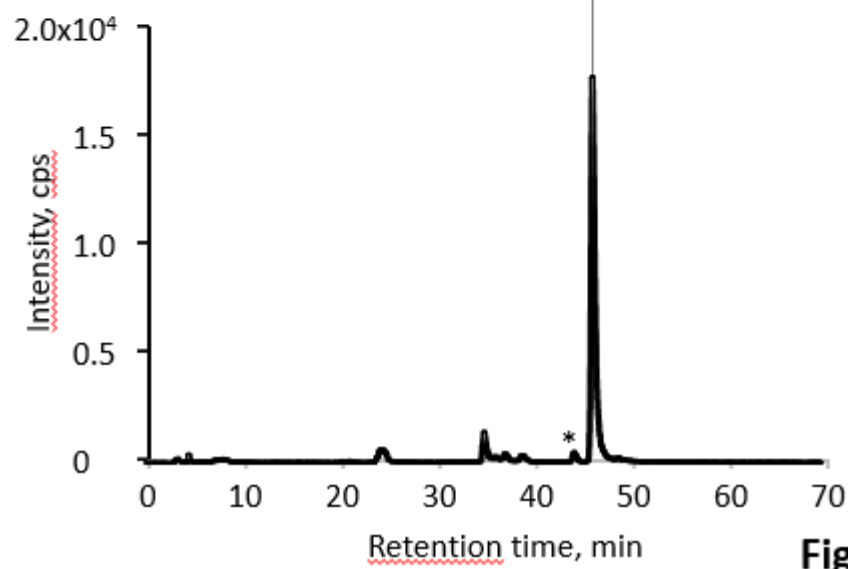
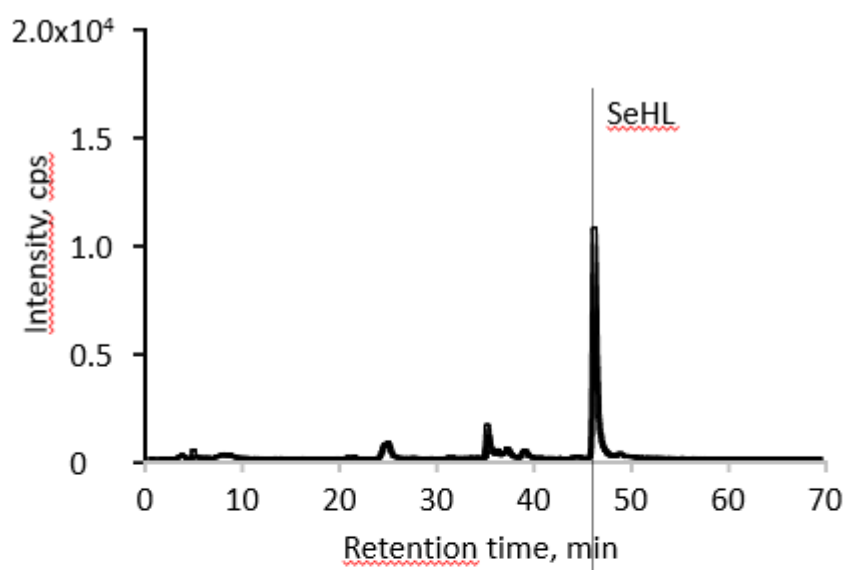


Fig. 3

323

324

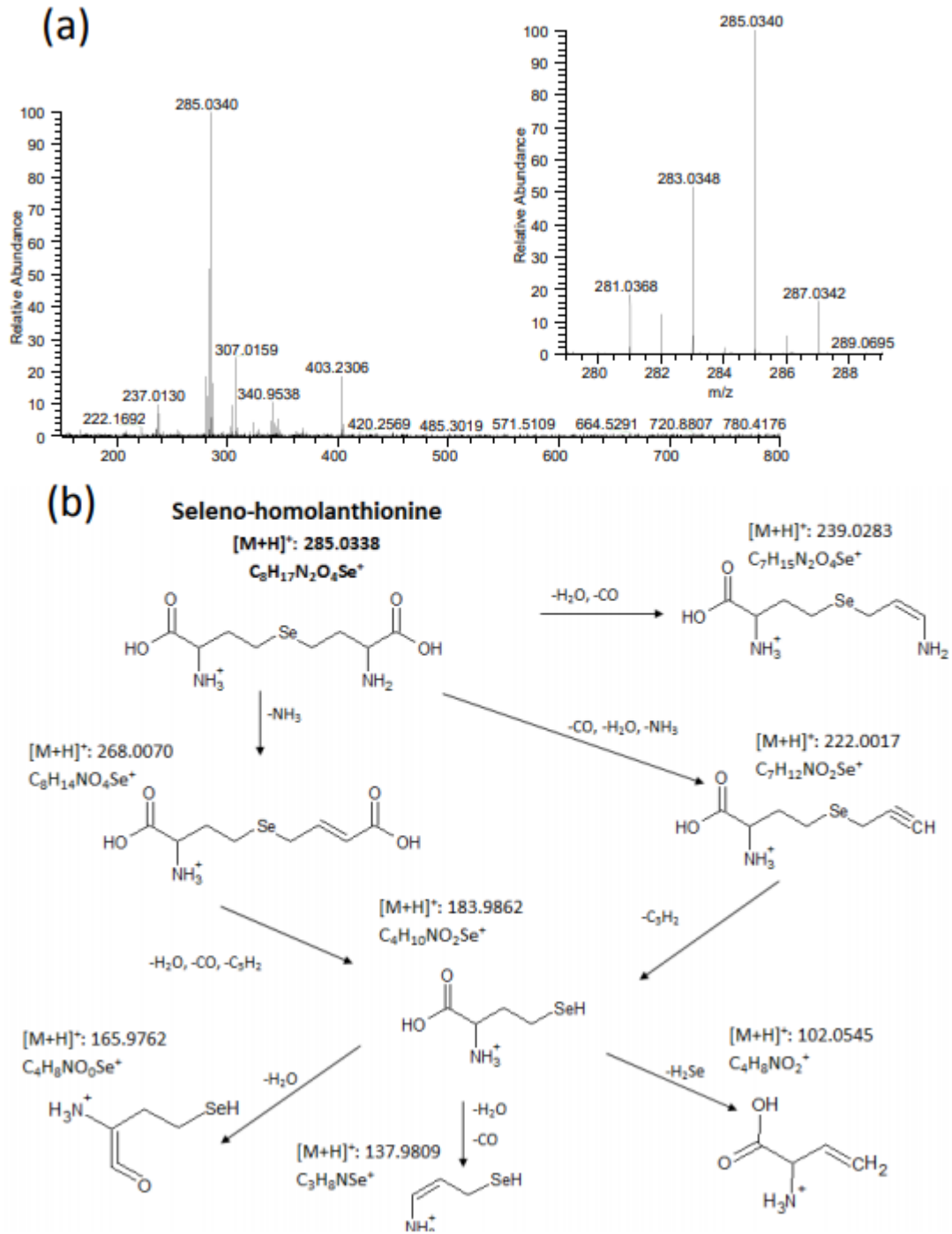


Fig. 4

325

326

