Identification and determination of selenohomolanthionine

- the major selenium compound in *Torula* yeast

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Abstract

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

Introduction

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

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

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

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

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

EXPERIMENTAL

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

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

Instrumentation:

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

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

Procedures:

Synthesis of selenohomolanthionine

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

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

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

Determination of the total Se content

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

Water extraction and size-exclusion HPLC fractionation of selenium

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

Selenomethionine determination

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

Selenohomolanthionine determination

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

RESULTS AND DISCUSSION

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

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

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

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

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

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

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

the three principal fractions are almost always present (Casal, Far, Bierla, Ouerdane, & Szpunar, 2010). In contrast to this, the chromatogram of *Torula* yeast shows one peak in the elution volume range corresponding to low-molecular weight (200-600 Da) metabolites. The recovery of the selenium from the size-exclusion chromatographic column in both cases was quantitative ($98\pm2\%$ and $97\pm3\%$), respectively.

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

Identification of selenohomolanthionine

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

Quantification of selenohomolanthionine

A method for the quantitative determination of SeHLan by HILIC – ICP MS was then developed. The slopes of the calibrations curves: external and by standard additions agreed within $\pm 5\%$. The recovery of the SeHLan from the column was $96\pm3\%$. Table 1 shows the recoveries of the different concentration of SeHLan measured for two Se isotopes ^{78}Se and ^{80}Se in the peak height and peak area mode. The peak height mode turned out to be more slightly more precise, especially for the most intense ^{80}Se isotope with measurement errors below 2.5%.

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

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

Selenium speciation and mass balance

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

CONCLUSIONS

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

REFERENCES

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

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

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

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

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

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

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

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

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

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

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

CAPTIONS TO FIGURES:

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

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

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

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

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

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

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

Fig. 1

Fig. 2

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Fig. 3

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Fig. 4