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Short communication

Simple assay of trehalose in industrial yeast

Małgorzata Kus-Liśkiewicz [a,*], Anna Górka [a], Mykhailo Gonchar [a,b]

[a] Biotechnology Centre for Applied and Fundamental Sciences, Department of Biotechnology, Department of Animal Physiology and Breeding, University of Rzeszow, Sokołowska St. 26, 36-100 Kolbuszowa, Poland
[b] Institute of Cell Biology, NAS of Ukraine, Drahomanov Street 14/16, Lviv 79005, Ukraine

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ABSTRACT

Trehalose is an essential chemical marker to control a quality of the industrial yeast strains and to assess a tolerance of the yeasts products to different physical stresses. A high-performance liquid chromatography analysis with charged aerosol detection (HPLC–CAD) was developed for trehalose determination in industrial yeasts. The method offers a linearity in the range of 5.0–15 mM with linear regression coefficient $R^2 = 0.9995$, a good reproducibility and relatively short analysis time (7 min). Trehalose can be detected at concentrations as low as 0.07 mM, and limit of precise quantification is 0.2 mM. The coefficient of variation (CV%) is 0.3%. The developed method is more sensitive compared with conventional chromatography procedure with UV absorbance detection. It was shown that the proposed method can be used in baker's industry to control a quality of the yeast products and to assess biotechnological significance of the yeast strains.

1. Introduction

Trehalose is a very important natural metabolite and a prospective food additive. This non-reducing sugar, consisting of two glucose residues (α-D-glucopyranosyl-1-1α-D-glucopyranoside) was found in different living cells, including fungi, bacteria, insects and some plants. So far, it was thought that trehalose plays a role only as their spare material and/or as one of the energy sources (Bolat, 2008; Chuanbin, Jian, Fengwu, & Zhiguo, 1998; Kienle, Burgert, & Holzer, 1993). Now it is known that the primary role of trehalose is to protect cell membranes and proteins in response to cellular stress conditions, e.g. heat shock, ethanol, osmotic stress in dehydration processes (Managbanag & Torzilli, 2002; Murray & Liang, 1999; Neves & Francois, 1992).

Baker's yeasts and non-conventional yeast species are widely used in brewing and food traditional biotechnology, as well as for production of biofuels, pharmaceuticals and heterologous proteins in the field of modern biotechnology. For this purpose, industry needs the yeast strains with a high content of trehalose as the most suitable organisms for bioprocessing. During fermentation and biopreparation stages, as well as at storage conditions, industrial yeast cells are exposed to many stresses such as high-sucrose concentrations, freeze–thaw or air-drying. For this reason, it is important to optimize existing methods for assessing the potential of the used strains and to control the commercial yeast products (Ferreira, Paschoalin, Panek, & Trugo, 1997). Similarly, the level of trehalose is assessed in different plant species used in the food industry (El-Bashiti, Hamamci, Oktem, & Yucel, 2005). Furthermore, assessing the content of trehalose in food is also important; the data shows its influence on bone metabolism and prevention of osteoporosis (Higashiyama, 2002). Many researchers have shown that trehalose is an attractive substance for industrial application: to improve the bioethanol production (Tao et al., 2012), to increase stability of the membranes, protein and nucleic acid to environmental stressors (Chen & Lu, 2006; Di et al., 2012; Katenz et al., 2007; Vianna, Silva, Neves, & Rosa, 2008) and to improve the effectiveness of protection against mold decay of apples (Zhao, Zhang, Lin, Zhang, & Ren, 2012). Trehalose is also used for different application in medicine field, e.g. to increase the viability of microbial cells which are ingredient of oral capsule probiotics (Higashiyama, 2002; Jalali et al., 2012).

Now, there are many different techniques for analysis of trehalose content. The most common are colorimetric, enzymatic and chromatographic methods. Among the colorimetric methods, one procedure is based on the reaction of the anthrone with carbohydrates, followed by determination of the colour change (Brin, 1966). Unfortunately, due to the high reactivity of anthrone with other sugars, this method is not specific to trehalose (Ferreira et al., 1997). To avoid this, the other sugars should be separated from trehalose, although it is laborious and time-consuming. Another trehalose quantification method is based on using an enzyme which specifically hydrolyzes trehalose (acid

* Corresponding author. Tel.: +48 17 8723710; fax: +48 17 8723261.
E-mail address: mkus@univ.rzeszow.pl (M. Kus-Liśkiewicz).

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trehalase) followed by glucose oxidase–peroxidase assay of the released glucose (Neves, Terenzi, Leone, & Jorge, 1994). The enzymatic assays have a high selectivity, but because of high cost of the enzymes (trehalase, glucose oxidase, peroxidase), they are not widely used in industry (Ferreira et al., 1997). It is worth mentioning that there was proposed a novel biosensor method for trehalose detection for genetically engineered strains of *Pseudomonas fluorescens*, but the proposed device is only at the research stage (http://www.enterprise-europe-scotland.com/sct/services/enquire.asp?id=12+FR+32j3+3O7T&enquirytype=BBS, 2012). Therefore, a more selective, sensitive and cost-effective methods are required for trehalose determination.

HPLC with a charged aerosol detection (CAD) is a new analytical approach that has been developed by Dixon and Peterson (Dixon & Peterson, 2002). CAD is based on evaporative light-scattering detection and is referred to be an universal detector. It is used as an alternative to UV/Vis detection in many applications, in particular for the analysis of compounds lacking strong UV chromophores such as many carbohydrates, lipids, amino acid derivates, polymers and surfactants. A few detection methods for mono-, di- and oligosaccharides using CAD have already been reported (Inagaki, Min, & Toyo'oka, 2007; Vehovec & Obreza, 2010), but in the literature there is no information about detection of trehalose using CAD. Trehalose quantification has been done with using different detectors or/and types of column (Ferreira et al., 1997; Hallsworth & Magan, 1997; Inagaki, Min, & Toyo'oka, 2007; Murray, Hayashida, & Nishimura, 1997; Wannet, Hermans, van Der Drift, & Op Den Camp, 2000), but each of them has some disadvantages, e.g. derivatization is time- and cost-consuming. It is also known that usage of the refractive index detector has a low sensitivity compared to other detectors.

It is obvious that specific treatment procedures can influence trehalose detection validity (Jin et al., 2011; Vehovec & Obreza, 2010). Trehalose can be extracted from cells and tissues with different methods (Al-Naama, Ewaze, Green, & Scott, 2009; Chuanbin et al., 1998; El-Bashiti et al., 2005; Jin et al., 2011; Murray et al., 1997; Roustan & Sablayrolles, 2002; Saharan & Sharma, 2010; Wannet et al., 2000). For example, Jin and other authors have shown that trehalose yield by the use of high-intensity pulsed electric field (PEF) was the lowest, compared to microwave and ultrasound treatment. When compared trehalose extraction methods with perchloric acid and water, there were no significant differences (Jin et al., 2011). On the other hand, traditional methods, including extraction by trichloroacetic acid are also useful. The advantage of TCA method is a high solubility of sugars and simple conditions (without heating) of extraction procedure (at 0 °C).

In the present work, for the first time, a high-performance liquid chromatography with a charged aerosol detection was used to assay trehalose in yeast biomass. This simple and reliable method is developed to optimize the technique for quantitative detection of trehalose as a biomarker of stress resistance of industrial yeast strains and can be used also to determine the trehalose content in food.

2. Materials and methods

2.1. Chemicals

Trehalose, sucrose and glucose were obtained from Sigma. Acetonitrile was purchased from J.T. Baker (Netherland). All solvents and reagents were of HPLC grade. Water was purified by a Aquinity E30 analytical systems (MembraPure, Bodenheim, Germany). Trichloroacetic acid (TCA) was obtained from Merck (Darmstadt, Germany). The stock solution of trehalose standard (100 mM) was prepared in a trichloroacetic acid and stored at −20 °C. Working solutions were diluted with HPLC grade water and were prepared in the form of 0.5; 1; 5; 10 and 15 mM concentrations.

2.2. Preparation of the yeast samples and trehalose extraction

As the tested samples, three commercial baker's yeast preparations produced by Ukrainian plant were used which differ in regard to strain type (strain I – for sample 1; strain II – for samples 2 and strain III – for samples 3) and technological form (dried – sample 1 and pressed form – samples 2 and 3). All preparations were obtained by aerobic fed-batch cultivation at 33 °C in industrial fermentors using beet molasse (as a carbon source) and NH_4OH (as a nitrogen source). For induction of trehalose biosynthesis, in the end of cultivation, incubation at 33 °C during 2 h without adding melasse was used.

Before analytical testing, yeast commercial samples were stored at +4 °C.

Trehalose was extracted from 1 g industrial baker's yeast products (samples were suspended in 10 mL of cold water and centrifuged at 3000*g*, 10 min). Procedure was repeated three times. The cells were washed with H_2O after centrifugation (1500*g*, 5 min) and cell pellets were extracted with 15 mL 0.5 M trichloroacetic acid for 30 min at 0 °C. After the incubation, cell extracts were centrifuged (3000*g*, 10 min) and the supernatant was collected. To the pellet, 5 mL H_2O was added and centrifuged. The both supernatants were combined and the extract was adjusted by H_2O to the final volume 25 mL. These supernatants were stored at −20 °C before assay.

2.3. HPLC analysis

Measurement of trehalose content was performed using a HPLC device (Dionex, UltiMate 3000 RSLC systems, Sunnyvale, CA, USA) with Corona ultra RS detector (CAD). The Shodex SZ5532 column (6.0 × 150 mm, with a particle size of 6 μm), with Zn^{2+} counter ion (a ligand-exchange type of separation) was used. The mobile phase was CH_3CN/H_2O (70:30) with the flow rate 1 mL/min. A column temperature was 50 °C, and 10 microliters samples or standards were injected at a constant flow rate. The yeast extract components were detected by Corona (Dionex system). Data processing was carried out with Chromeleon 6.8 software (Dionex). Elution was performed at ambient temperature. N_2 gas pressure of the CAD was adjusted to 35 psi. Analyses of standard solution of trehalose were carried out five times. Trehalose quantification was based on comparison of the areas for the tested samples with those of the standards (external standard method). Trehalose content (%) in the yeast samples (Table 1) was calculated using the following formula:

$$\text{Trehalose content } [\%] = \left(\frac{\text{Trehalose concentration in the extract } [\text{mg/mL}]}{\text{Quantity for extraction } [\text{mg}]/25\ [\text{mL}]}\right) \cdot 100\%$$

Table 1
Trehalose content (per dry weight) for different industrial baker's strains.

Yeast preparation	Quantity for extraction, recalculated for dry weight [mg]	Volume of the extract [mL]	Trehalose concentration in the extract [mg/mL]	Trehalose content [%]
(1) Dried	1596	25	1.21 ± 0.012	1.89 ± 0.017
(2) Pressed	1437	25	4.75 ± 0.051	8.26 ± 0.088
(3) Pressed	1395	25	3.76 ± 0.051	6.74 ± 0.091