ELSEVIER

Food Chemistry 77 (2002) 263–265

Food Chemistry

www.elsevier.com/locate/foodchem

# A simple method for the analysis of trehalose using HPTLC

T.V. Ranganathan, Pushpa R. Kulkarni*

*Food and Fermentation Technology Division, Mumbai University of Chemical Technology, Mumbai 400 019, India*

Received 10 April 2001; received in revised form 29 August 2001; accepted 29 August 2001

**Abstract**

To overcome the presence of overlapping peaks of other sugars, in the thin-layer chromatographic analysis of trehalose content in sugar-rich products, high performance thin-layer chromatographic (HPTLC) separation was successfully standardised, using silica gel impregnated with phosphotungstic acid of pH 2.5, a solvent system of *n*-butanol: pyridine: water—8:4:3, and 6.5 mM N-(1-naphthyl)-ethylenediamine dihydrochloride in methanol, containing 3% $H_2SO_4$ as the spraying agent. Commodities reported to be rich in trehalose were analysed by this method. 

*Keywords:* Trehalose; HPTLC; N-(1-naphthyl)-ethylenediamire dihydrochloride; Baker's yeast

## 1. Introduction

In the analysis of foods for trehalose content, the commonly used anthrone method (Brin, 1966; Trevelyan and Harrison, 1952) cannot be used when a mixture of sugars is present. On the other hand, the use of trehalase and the subsequent determination of the glucose formed by glucose oxidase-peroxidase reaction is cost-prohibitive (Parrou & Francois, 1997; Petit & Francois, 1994; Schulze, Larsen, & Villadsen, 1995). HPLC analysis requires good expertise and special columns (Engelhardt, & Ohs, 1987; Nikolov, Meagher, & Reilly, 1985; Wang, Shen, & Yang, 1987; Zang, Ma, & Wang, 1991). Application of gas–liquid chromatography (Mateo, Bosch, Pastor, & Jimenez, 1987) and GC–MS (Carlsson, Karlsson, & Sandberg, 1992) also suffers from an unsatisfactory separation of trehalose compared to other sugars.

A simple thin-layer chromatographic method, using the relatively expensive aminopropyl- bonded silica gel plates, has been reported (Doner & Biller, 1984). In the present work, based on the works of Mezzetti, Rufini, Ciuffini, and Lato (1971), a high performance thin-layer chromatographic separation of trehalose, in the presence of other commonly-occurring mono-, di- and trisaccharides, was attempted.

## 2. Materials and methods

### 2.1. Materials

Trehalose dihydrate, glucose, fructose, maltose, sucrose, raffinose, N-(1-naphthyl)-ethylenediamine dihydrochloride, all of AR grade, and solvents of HPLC grade were procured from M/s Sisco Research Laboratories Pvt. Ltd., Mumbai. Silica gel plates, $60F_{254}$ (HPTLC aluminium sheets 20×20 cm, particle size 4–6 nm), were procured from M/s E.Merck India Ltd. and M/s Camag HPTLC system consisting of Linomat and scanner with Cats 3.1 version were used for the analysis. Compressed baker's yeast (SAF yeast Co Ltd. Mumbai), *Selaginella lepidophylla*, button mushrooms (*Agaricus bisporus*) were procured from the local market. Mango powder, with known amounts of trehalose, was prepared in our laboratory by spray drying mango pulp (*langda* variety).

### 2.2. Methods

#### 2.2.1. Standardisation of impregnant and solvent system for HPTLC of trehalose

Preliminary experiments were carried out to find the best combination of impregnant and solvent system for the thin-layer chromatographic separation of trehalose in the presence of other sugars, such as glucose, fructose, maltose, sucrose and raffinose, on silica gel plates (10×10), based on the work of Mezzetti et al. (1971) using TLC. Impregnation was carried out by dipping

* Corresponding author. Fax: +91-22-414-5614.

0308-8146/02/$ - see front matter 
PII: S0308-8146(01)00324-7

Table 1
Effect of impregnants on the resolution of trehalose in the presence of other sugars[a]

| Impregnant | $R_f$ of sugars | | | | | |
|---|---|---|---|---|---|---|
| | Raf | Tre | Mal | Suc | Glu | Fru |
| No impregnant | 0.26 | 0.36 | 0.41 | 0.45 | 0.46 | 0.48 |
| 0.2M sodium acetate | 0.06 | 0.13 | 0.14 | 0.17 | 0.22 | 0.24 |
| 0.2M monosodium phosphate | 0.03 | 0.06 | 0.09 | 0.14 | 0.14 | 0.22 |
| Saturated molybdic acid in water | 0.26 | 0.28 | 0.30 | 0.40 | 0.48 | 0.46 |
| Phosphotungstic acid—pH 2.5 | 0.3 | 0.41 | 0.46 | 0.53 | 0.55 | 0.59 |

[a] Solvent system used—*n*-butanol:pyridine:water—8:4:3 (single development). Visualising reagent—6.5 mM N-(1-naphthyl)-ethylenediamine dihydrochloride in methanol containing 3% $H_2SO_4$. raf, raffinose; tre, trehalose; mal, maltose; suc, sucrose; glu, glucose; fru, fructose.

Table 2
Trehalose content of the commodities analysed

| Commodity | Trehalose content (% dry weight)[a] |
|---|---|
| Button mushrooms | 0.697±0.001 |
| *S. lepidophylla* | 18.3±0.028 |
| Spray-dried mango powder | 4.18±0.125 |
| Compressed baker's yeast | 12.5±0.5 |

[a] Values are mean±S.D. of three individual determinations.

the silica-gel plates in a solution of the appropriate impregnants for 10 s, the plates were air-dried overnight and activated by keeping at 105 °C for 1 h in an air-oven. The sugars were then spotted quantitatively, using a Linomat system; the plates were run once in two different solvent systems in Camag TLC chambers. The solvent systems used included n-propanol: pyridine: water—5:3:2 and *n*-butanol: pyridine: water—8:4:3. Visualisation of the spots was done by spraying with N-(1-naphthyl)-ethylenediamine dihydrochloride in methanol containing 3% $H_2SO_4$ (Bounias, 1980).

### 2.3. Extraction of sugars from the commodities

Extraction of sugars was carried out by a slight modification of the method of AOAC (1990). The sample was subjected to repeated extraction with 80% alcohol by shaking in an orbital shaker for 10 min at room temperature (28±2 °C), until the extracts were colourless, and made to a known volume with 80% ethanol. A suitable dilution of the above with methanol was done before spotting. This step was incorporated to effect an exclusive extraction of only the lower oligosaccharides. The methanolic extracts were then spotted, along with the standard sugars.

#### 2.3.1. Standardisation of the method of quantification of trehalose using HPTLC

Quantification of trehalose was done from a standard curve of optical density at 546nm against concentration of each sugar (concentration of 300 ng to 1 μg/ml).

## 3. Results and discussion

Among the various solvent systems studied for an effective separation of the sugars, it was found that only two solvent systems, namely *n*-propanol: pyridine: water—5:3:2 and *n*-butanol: pyridine: water—8:4:3 could impart a better separation (unpublished data). Of these two solvent systems, *n*-butanol: pyridine: water—8:4:3-gave a comparatively satisfactory separation with all the impregnants studied. Table 1 shows the effect of various impregnant systems on the $R_f$ values of commonly present sugars, using the solvent system. The basis of impregnation in silica-gel plates was developed by Mezzetti et al. (1971), when they observed that addition of boric acid to a solvent system, led to the preferential movement of certain sugars over the others, not observed until then. This led to the trying of various impregnants, such as 0.2 N sodium acetate, 0.2M monosodium phosphate, saturated molybdic acid in water and phosphotungstic acids of varying pHs.

In the case of plates with no impregnants, it was observed that, when a solvent system of *n*-butanol: pyridine: water—8:4:3 was used, all the spots were found spread and appeared diffuse during visualisation. In the case of 0.2M sodium acetate, the $R_f$ of the respective sugars decreased with a spreading of the spots, and an unsatisfactory resolution of trehalose and maltose was observed. With 0.2M mono sodium phosphate, the observed effects were similar to that of 0.2 M sodium acetate. In case of saturated molybdic acid in water as impregnant, glucose, fructose and sucrose were found to have very close $R_f$ values. The spots were, however, characteristically sharp with less diffusion and no spreading. Finally, when phospotungstic acid of pH 2.5 was used for impregnation, an effective resolution of all sugars studied could be achieved. The spots were also sharp with less diffusion. As the desired requisites of a proper separation (good resolution and better appearance of the spot) were achieved, this combination was selected for further analysis.

Also with N-(1-naphthyl)-ethylenediamine dihydrochloride in methanol containing 3% $H_2SO_4$, as the spraying agent, linearity was observed between the concentrations of trehalose spotted and the absorption, using a Hg lamp as the illuminant, in the concentration ranges of 300 ng to 1 μg/ml ($y = 2494.9x$, $r^2 = 0.9943$).

The trehalose contents of various commodities reported to be rich in trehalose, as determined by the newly developed method, are shown in Table 2. The values obtained were found to be in agreement with earlier reports (Muller, Boller, & Wiemken, 1995; Yoshida, Sugahara, & Hayashi, 1986). The trehalose content of spray-dried mango powder was also found to be in agreement with the amount added to the pulp before drying. In the case of compressed baker's yeast, it was found that the content of trehalose, determined by the

modified AOAC method and HPTLC, was 12.5%. From the results, it is clear that this method can be used as a possible alternative to the reported methods.

## References


AOAC. (1990). Sugars in plants. Official methods of analysis of the Associaiton of Official Analytical Chemists. In K. Helrich (Ed.), *Fifteenth edition No, 931,02* (pp. 58–59). Virginia, USA: Pub. AOAC Inc.

Bounias, M. (1980). N-(1-Naphthyl) ethylenediamine dihydrochloride as a new reagent for nanomole quantification of sugars on thin layer plates by a mathematical calibration process. *Analytical Biochemistry*, *106*(2), 291–295.

Brin, M. (1966). Transketolase: clinical aspects, in methods in enzymology, 9. In S. P. Kolowick, & N. O. Kaplan (pp. 506–514). New York: Academic Press.

Carlsson, N. G., Karlsson, H., & Sandberg, A. S. (1992). Determination of oligosaccharides in foods, diets and intestinal contents by high temperature gas chromatography and gas chromatography/mass spectrometry. *Journal of Agriculture and Food Chemistry*, *40*(12), 2404–2412.

Doner, L. W., & Biller, L. M. (1984). High-performance thin-layer chromatographic separation of sugars: preparation of amino-propyl bonded-phase silica plates impregnated with monosodium phosphate. *Journal of Chromatography*, *287*(2), 391–398.

Engelhardt, H., & Ohs, P. (1987). Trace analysis for sugars by HPLC and post-column derivatization. *Chromatographia*, *23*(9), 657–662.

Mateo, R., Bosch, F., Pastor, A., & Jimenez, M. (1987). Capillary column gas chromatographic identification of sugars in honey as trimethylsilyl derivatives. *Journal of Chromatography*, 410(2), 319-328. c.f. *CA* 108: 36395v.

Mezzetti, T., Rufini, S., Ciuffini, G., & Lato, M. (1971). Thin-layer chromatography of oligosaccharides with tungstic or molybdic acid as impregnant. *Journal of Chromatography*, *63*, 329–342.

Muller, M., Boller, T., & Wiemken, A. (1995). Trehalose and trehalase in plants: recent developments. *Plant Science*, *112*, 1–9.

Nikolov, Z. L., Meagher, M. M., & Reilly, P. J. (1985). High performance liquid chromatography of disaccharides on amine-bonded silica columns. *Journal of Chromatography*, *319*(1), 51–57.

Parrou, J. L., & Francois, J. (1997). A simplified procedure for a rapid and reliable assay of both glycogen and trehalose in whole yeast cells. *Analytical Biochemistry*, *248*, 186–188.

Petit, T., & Francois, J. (1994). Accumulation of trehalose in *Saccharomyces cerevesiae* growing on maltose is dependent on TPS1 gene encoding the UDP glucose-linked trehalose synthase. *FEBS Letters*, *355*(3), 309–313.

Schulze, U., Larsen, M. E., & Villadsen, J. (1995). Determination of intracellular trehalose and glycogen in *S. cerevesiae*. *Analytical Biochemistry*, *228*(1), 143–149.

Trevelyan, W. E., & Harrison, J. S. (1952). Studies on yeast metabolism. 1. Fractionation and Micro determination of cell carbohydrates. *Biochemical Journal*, *50*, 298– 303.

Wang, B., Shen, Z., & Yang, R. (1987). High-performance liquid chromatographic analysis of trehalose in Xianggu mushroom. *Shiyong Jun*., *51*, 39.

Yoshida, H., Sugahara, T., & Hayashi, J. (1986). Studies on free sugars, free sugar alcohols and organic acids of wild mushrooms. *Nippon Shokuhin Kogyo Gakkaishi*, *33*(3), 426–433.

Zang, Y., Ma, Z., & Wang, Y. (1991). Determination of carbohydrates in silkworm eggs by HPLC. *Fenxi Ceshi Tongbao*, *10*(4), 60–62.