Food Control 78 (2017) 183–186

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Short communication

Determination and occurrence of 5-hydroxymethyl-2-furaldehyde in white and brown sugar by high performance liquid chromatography

Michaela Polovková, Peter Šimko*

Institute of Food Science and Nutrition, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovak Republic

ARTICLE INFO

Article history:
Received 20 January 2017
Received in revised form
24 February 2017
Accepted 25 February 2017
Available online 28 March 2017

Keywords:
5-Hydroxymethyl-2-furaldehyde
HPLC
White sugar
Brown sugar
Food safety

Chemical compound studied in this article:
5-Hydroxymethyl-2-furaldehyde (PubChem CID: 237332)

ABSTRACT

5-hydroxymethyl-2-furaldehyde (HMF) is not only indicator of food freshness and quality, but also contaminant forming during Maillard reaction, or by dehydratation of saccharides, respectively. While data about presence of HMF in white and brown sugar are scarce, 13 kinds of white sugar and 25 kinds of brown sugar were analysed. Sugar was dissolved in deionised water, clarified with Carrez solutions, filtered and content of HMF was determined using high performance liquid chromatography with diode array detector (HPLC-DAD) with detection at 284 nm when separation run on Poroshel 120 EC C18 at 32 °C. Elution was performed under isocratic conditions using 95:5 water/acetonitrile mobile phase at a flow rate of 0.9 mL/min and analysis run 5 min. Method was validated in *in house* regime and its parameters such as limit of detection – (LOD = 0.05 mg/kg), limit of quantification (LOQ = 0.15 mg/kg), specificity, repeatability and recovery enabled its application for sugar analysis. While white sugar was free of HMF, all kinds of brown sugar exhibited presence of HMF, when content in 15 kinds varied between 0.17 and 6.45 mg/kg, content in other 10 kinds was under LOQ. On the base of obtained results was postulated that brown sugar contains HMF either due to absence of refining processes, or it is re-contaminated by treacle adding to white sugar during production of brown sugar.

1. Introduction

Sugar is being produced almost exclusively from sugar cane or sugar beet. As a member of saccharide group, it belongs to disacharides, it means that it is composed of two reducing monosaccharide – hexoses such as glucose and fructose. Sometimes, sugar is also named as sucrose, or saccharose, respectively. By The *International Union of Pure and Applied Chemistry (IUPAC)* nomenclature, it is (2*R*,3*R*,4*S*,5*S*,6*R*)-2-[(2*S*,3*S*,4*S*,5*R*)-3,4-dihydroxy-2,5-bis(hydroxymethyl)oxolan-2-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol and a more frequent "chemical" name is *O*-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside. In its pure state it is normally available as white crystals (Manley, 2011). Whether extracted from cane or sugar-beet, sugar manufacture uses only very simple purification and extraction processes, without using any additives or synthetic products (Linden & Lorient, 1999).

As mentioned above, sugar can be extracted either from sugar beet or sugar cane, respectively. Normally, these two sources are equivalent even though some trace impurities are different. However, there is one area where the two sources are not equivalent and that is regarding brown sugars. Cane sugar that has not been completely purified has a pleasant taste and can be used either as an ingredient for food production, or direct consumption. Beet sugar, however, is not acceptable unless it is completely white due to unpleasant "beety" sub flavour of non-refined sugar. In some products, brown sugar or even molasses are used to add colour and flavour to them. Alternatively, in some products a less than completely white product, brown sugar is used simply to save money (Edwards, 2000). Molasses is the product left when no more sugar can be obtained from thick syrup. Beet sugar molasses is unpleasant in taste and flavour and therefore it is not normally used for human food. However, cane sugar molasses does have some direct food use, normally in the form of treacle, what is clarified molasses. Treacle is normally stored at 50 °C to maintain its liquidity (Edwards, 2007). In final, beet sugar refiners do production of brown sugar by addition of cane sugar molasses to refined – white beet sugar (Edwards, 2000).

HMF is spontaneously formed compound by the Maillard reaction (the nonenzymatic browning) or during dehydration of

* Corresponding author.
E-mail address: qsimko@stuba.sk (P. Šimko).

http://dx.doi.org/10.1016/j.foodcont.2017.02.059
0956-7135/

reducing saccharides (fructose and glucose) under acidic conditions and, in general, its concentration tends to rise as a result of heating processes or long-term storage, respectively. Therefore, HMF is found in fruit, vegetable, cereal food products containing reducing monosaccharides, infant formulas and sugar (Demirhan et al., 2015; Zhang, Wei, Liu, Lin, & Yuan, 2014), and in other heat-processed food such as juices (Lee, Sakai, Manaf, Rodhi, & Saad, 2014) and treacle (Edris, Murkovic, & Siegmund, 2007), sugar cane molasses (Ruiz-Matute, Soria, Sanz, & Martínez-Castro, 2010), or honey (Castoldi, Milani, Rossini, Pezza, & Pezza, 2016).

Apart from criterion quality, HMF is also frequently discussed as the compound with adverse effects to living organisms. As found, HMF can be metabolically transformed to 5-sulfooxymethylfurfural which may play role as an ultimate electrophilic metabolite in toxification of the parent compound *in vivo* (Lee, Shlyankevich, Jeong, Douglas, & Surh, 1995; Surh, Lieč, Miller, & Tannenbaum, 1994). Toxicity and risk assessment aspects were lately summarised and comprehensively discussed by Capuano and Fogliano (2011).

For determination of HMF, HPLC coupled with DAD operating in UV spectra is frequently used as a reference method for its analysis in various food matrix such as bakery, fruit and vegetable products (Zhang et al., 2014), honey (Truzzi et al., 2012; Zappalá, Fallico, Arena, & Verzera, 2005; Risner, Kiser, & Dube, 2006), coffee, soft drinks (Xu, Liu,Yu, Yu, & Zhao, 2015), vinegars and cereal based baby foods (Bignardi, Cavazza, & Corradini, 2014), or even royal jelly (Ciulu et al., 2013).

Up to present time, there is no comprehensive information about determination of HMF and any survey about its content in sugar in literature. Therefore, the aim of this work was elaboration and *in house* validation of HPLC-UV method for determination of HMF in white and brown sugar and find real situation regarding its content in both kind of sugar available on Slovakian market.

2. Materials and methods

2.1. Samples

All available kinds of crystal sugar (mesh size 0.8–1 mm) were bought in local markets in territory of Bratislava, capital city of Slovak Republic during the spring of 2016. Samples were packed in 1 kg, or 5 g paper package, respectively. Samples were stored in dry and dark room at ambient temperature until analysed.

2.2. Chemicals

HMF (as analytical standard) was purchased from Sigma-Aldrich, Darmstad, Germany. The standard was used to prepare the working standard solutions (4.0–60.0 mg/L) dissolving in deionised water. Acetonitrile (HPLC grade) was purchased from Mikrochem Ltd., Pezinok, Slovak Republic, *potassium ferrocyanide* $K_4[Fe(CH)_6]$ and *zinc sulphate* $ZnSO_4$, both of analytical grade from Lachema Brno, Czech Republic. Membrane disc filters (0.2 μm) were obtained from Hermes Lab Systems Ltd., Bratislava, Slovak Republic.

2.3. Sample preparation

4 g of sugar were dissolved in 8 mL of deionised water using a magnetic stirrer. Then, solution was transferred into 10 mL volumetric flask and 0.3 mL of Carrez I (15% aqueous solution of $K_4[Fe(CH)_6]$) and 0.3 mL Carrez II (20% aqueous solution of $ZnSO_4$) solutions were added. After thorough stirring, the volume of sample solution was adjusted to 10 mL with deionised water, filtered using a disc filter, placed in a vial and analysed by HPLC.

2.4. HPLC analysis

HPLC analysis were performed employing Agilent 1260 Infinity HPLC system (Agilent Technologies, Palo Alto, CA, USA), equipped with autosampler and a diode array detector set at 284 nm. Samples aliquots of 100 μL were injected onto pre-separation column Agilent UHPLC Guard 3PK SB-C18 (4.6 × 2.1 mm, 1.8 μm) coupled with separation column Agilent Poroshel 120 EC C18 (4.6 × 50 mm, 2.7 μm) maintained at 32 °C. Elution was performed under isocratic conditions using 95:5 (v/v) water/acetonitrile mixture as the mobile phase at a flow rate of 0.9 mL/min. At these conditions, time of analysis was set to 5 min, while HMF eluted in 2.5 min of analysis. Confirmation of HMF identity was made by comparison of retention time and comparison of scanned UV spectrum of peaks and standard solution of HMF. All analysis were done in triplicate.

3. Results and discussion

3.1. Sample treatment

From the analytical point of view, sugar is relative simple matrix and therefore it does not need time consuming and complicated pre-treatment. However, some protein based impurities, especially in brown sugar could be present and therefore Carrez solutions were preventively applied. Mobile phase composition was adopted from Ramírez-Jiménez, Guerra-Hernández, and García-Villanova (2003).

3.2. In-house validation of the method

European Commission Regulation No. 836/2011 EC (2011) defines performance criteria for methods of analysis for contaminants in foods. So, in accordance with the regulation, parameters such as specificity, repeatability, reproducibility, recovery, limit of detection (LOD), limit of quantification (LOQ) are required. Instead of collaborative trial validation, *in-house* validation may also be used when it fulfils set out performance criteria (Suranová, Semanová, Skláršová, & Šimko, 2015).

3.2.1. LOD, LOQ, specificity and linearity

LOD and LOQ values were calculated from the calibration curve as 3 *s*/slope and 10 *s*/slope, respectively, where *s* is the standard deviation of the signal obtained from five independent measurements (International Conference on Harmonisation, 2005). So, while LOD was equal to 0.05 mg/kg, LOQ was equal to 0.15 mg/kg. Specificity was tested for eventual co-eluting impurities and spectral interferences during HPLC analysis by comparison of scanned UV spectrum of the HMF standard and HMF in samples, identified by external standard addition procedure. As follows from Fig. 1, both spectra exhibit very high similarity, what confirms sufficient quality of separation of HMF from other interferences in analysed sugar. Linearity of detector response to HMF amounts was followed by analysis of standard solutions of HMF in the range of 4.0–60.0 mg/L, loaded on the column. As confirmed value of regression coefficient ($R^2 = 0.99995$), there was a sufficient linear accordance between amount of HMF and intensity of absorbance measured during analysis.

3.2.2. Repeatability and recovery

Repeatability and recovery are other criteria of the European Commission regulation (2011). Repeatability, expressed by $HORRAT_r$ value, has to be less than value 2 (Horwitz & Albert, 2006). $HORRAT_r$ value calculated for two concentration levels of 0.5 and 5 mg/L were 0.27 and 0.22 what indicates good repeatability. According to the regulation, recovery sets should be in the range of