Determination of Sucrose in Honey with Derivatization/Solid-Phase Microextraction and Gas-Chromatography/Mass Spectrometry

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Received 15 August 2014; revised 5 March 2015

A new method for the determination of sucrose in honey with derivatization solid-phase microextraction and gas chromatography/mass spectrometry (D-SPME–GC/MS) was developed. The method incorporates a sample derivatization with acetic anhydride using N-methylimidazole as the catalyst and the subsequent enrichment of the analyte in a Polyacrylate-SPME fiber. Results show that 100 μL N-methylimidazole and 800 μL acetic anhydride were sufficient to complete the acetylation for sucrose in 100 μL aqueous sample at room temperature. For SPME, an enrichment time of 30 min was sufficient. SPME was performed by immersing the fiber into the solution with additional vibration. Then, the analyte was desorbed for 5 min at 280°C in the GC/MS injection port with splitless mode. The present method exhibits good linearity at a concentration range of 0.3–8% of sucrose in honey with excellent regression (R = 0.9993). The method has been successfully applied to the control of sucrose adulteration in honey.

Introduction

Honey is a supersaturated natural solution of sugars, in which the monosaccharide fructose and glucose are the most abundant (typically ~80–85% of the solids in the honey). Other disaccharides (i.e., sucrose, trehalose, isomaltose, etc.) and higher sugars (trisaccharides and oligosaccharides) are also present, although in quite small quantities (1).

Sucrose (saccharose) is present in honey at a concentration of ~1% of dry weight. However, this level can be increased if the beekeeper has over-fed the bees with sugar during spring (2, 3). According to British and German honey regulations the sucrose content should generally not exceed 5%.

The sucrose content in honey can be determined by spectroscopic, electrophoretic and chromatographic methods: Fourier transform infrared spectroscopy (FTIR) (4), thin layer chromatography (1, 5), capillary electrophoresis (6), high performance liquid chromatography (HPLC) (7), gas chromatography (8–12) and gas chromatography–mass spectrometry (GC–MS) (13, 14).

FTIR has been proven to provide reliable results for fructose, glucose, sucrose, turanose, maltose, trehalose, isomaltose, erlose, as well as for a number of physical properties of honey using multivariate partial least squares (PLS) calibration (4). However, it is necessary for PLS calibration to hold numerous calibration and verification samples all with known concentrations of its constituents. For example, Lichtenberg-Kraag et al. (4) used 1600 FTIR spectra of honey samples for PLS calibration, which had been characterized by HPLC and other methods previously. Although HPLC does not necessarily require derivatization of sugars, they are not detectable by common UV/VIS and require refraction (RI) or light scattering detectors. On the other hand, conventional GC–MS of sugars necessitates time-consuming derivatization, bearing a high risk of error and requiring additional quality control measures. It was the aim of this study to develop a GC–MS method which overcomes these drawbacks and to make sugar analysis accessible to laboratories where the requirements for FTIR or HPLC are not available.

In order to minimize the sample preparation time for GC–MS, we propose a new method with derivatization/solid-phase microextraction (D-SPME), which was firstly described in 1997 by Pawliszyn and coworkers (15), and was later used for detection of aldehydes, amines, phenols, steroids, thiols, organic acids and amino acids. To our knowledge, this is the first study to demonstrate the D-SPME method also for the determination of carbohydrates.

Experimental

Chemicals and reagents

Sucrose, fructose, glucose, acetic anhydride and N-methylimidazole were purchased from Sigma-Aldrich (Steinheim, Germany). Mixtures of standard working solutions were prepared by dilution with water purified by a water purification system from Seral Reinstwassersysteme GmbH (Germany).

GC–MS analysis

GC–MS analysis was performed using a gas chromatograph VARIAN 450-GC coupled with a VARIAN 300MS TQ mass spectrometer. The GC was equipped with a VARIAN FactorFour capillary column (30 m × 0.25 μm film thickness × 0.25 mm i.d.). The injection was carried out in splitless mode. High purity helium gas (99.999%) was used as a carrier gas with a flow rate of 1.0 mL min⁻¹. The oven temperature programming was as follows: the initial oven temperature was held at 50°C for 1 min, then increased to 300°C at a rate of 30°C min⁻¹ and held for 4 min. The ion source and interface temperature were set to 200°C. All the samples were analyzed in selected ion monitoring mode (sucrose: RT = 11.81 min, m/z = 331 quantification ion, m/z = 169, 221 confirmation ions).

Preparation of honey samples

Honey was obtained from a local supplier and was harvested in summer 2014 in the Spreewald region in Germany. It was prepared by mixing 1 g honey with 20 mL pure water.

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Preparation of the sucrose standards
The sucrose standards were prepared in an aqueous solution of fructose and glucose. Initially, 20 g fructose and 17.5 g glucose were dissolved in 1 L pure water. Then, 10–400 mg sucrose was added to 100 mL portions of this solution. This corresponds to 0.2–8% of sucrose in honey.

SPME fibers
Commercially available polydimethylsiloxane (PDMS)-, polyacrylate (PA)- and divinylbenzene-polydimethylsiloxane (DVB-PDMS-SPME) fibers were purchased from Supelco (Bellefonte, PA). Prior to use, the fibers were conditioned in the hot injector of the gas chromatograph in helium atmosphere according to the manufacturer's directions.

Table I
Specifications of the Method According to the German Standard DIN 32645 (17)

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression equation</td>
<td>$y = 161667x + 8105$</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9993</td>
</tr>
<tr>
<td>Method standard deviation</td>
<td>0.084</td>
</tr>
<tr>
<td>LOD (%)</td>
<td>0.3</td>
</tr>
<tr>
<td>Recovery (2% standard)</td>
<td>0.91</td>
</tr>
<tr>
<td>Linear dynamic range (LDR) (%)</td>
<td>0.3–8</td>
</tr>
</tbody>
</table>

Figure 1. Effects of the derivatization conditions on peak area of sucrose octaacetate. (A) Effect of the volume of acetic anhydride, (B) effect of the volume of N-methylimidazole and (C) effect of reaction time.

Figure 2. Peak areas obtained by performing analyses in immersion modes ($n = 3$) with three different fibers. PDMS, polydimethylsiloxane; PA, polyacrylate; DVB-PDMS, divinylbenzene-polydimethylsiloxane.

Figure 3. Effect of the enrichment time on peak area of sucrose octaacetate.
Optimized derivatization SPME procedure
One hundred microliters of the aqueous honey sample or standard solution were added into 20 mL vials. Then 100 µL of N-methylimidazole and 800 µL of acetic anhydride were added. To ensure complete derivatization, the mixture was thoroughly mixed by hand and allowed to rest for another 10 min. Then 10 mL of pure water were added, and the vials were sealed with PTFE septa. SPME was performed by immersing the PA-fiber into the solution for 30 min with additional vibration of the fiber with a Varian 8200 SPME agitation device. Desorption time and temperature were 5 min and 280°C, respectively.

Results
Optimization of the derivatization procedures
In order to obtain the best derivatization yields, the volume of acetic anhydride, the amount of N-methylimidazole and the derivatization time were optimized.

Effect of acetic anhydride volume
Different volumes of acetic anhydride were added to 100 µL standard solution and 100 µL N-methylimidazole, the reaction was performed at room temperature for 10 min. As shown in Figure 1A, the peak area markedly increased at the volume of acetic anhydride ranging from 200 to 800 µL. When the volume was beyond 800 µL, the yields did not improve further. Thus, 800 µL of acetic anhydride were considered as the optimum amount.

Effect of N-methylimidazole volume
Different volumes of N-methylimidazole were added to 100 µL standard solution and 800 µL acetic anhydride, and the reaction was performed at room temperature for 10 min. As shown in Figure 1B, the peak area was no further increased when the volume was greater than 100 µL. Thus, 100 µL of N-methylimidazole anhydride were considered as the optimum amount which also corresponds to the finding of Wu et al. (16).

Effect of reaction time
One hundred microliters of standard solution, 100 µL N-methylimidazole and 800 µL acetic anhydride were mixed, the reaction was performed at room temperature from 5 to 15 min. As shown in Figure 1C, 10 min of reaction time was considered as sufficient.

Selection of the SPME fiber
The commercially available fiber coatings PDMS, PA and PDMS/DVB were evaluated. Best extraction efficiencies were obtained with DVB-PDMS as shown in Figure 2. To avoid possible discrimination effects due to the high concentration of fructose and glucose, the PA fiber was used for the further investigation.

Determination of the SPME enrichment time
For the investigation of the influence of the SPME enrichment time it was varied from 15 to 75 min. To circumvent method errors associated with nonequilibrium states, 30 min were considered as sufficient (Fig. 3).

Calibration and validation
Calibration and validation of the method were performed according to the German DIN 32645 (17). The specifications are given in Table I. The calibration curve is given in the Supplementary data. The positive increment of y at x = 0 results from the fiber blank. The carryover of a blank run immediately following a SPME-injection of a 5% sucrose standard amounted to 2.9% of the initial peak height of the standard.

Discussion
SPME is a rapid, portable, rugged and solvent-free extraction/concentration technique widely used in analytical chemistry for almost 20 years, predominantly for the determination of nonpolar substances. The enrichment of polar metabolites in the relatively nonpolar commercially available SPME fibers is
Derivatization of sugars into volatile acetates is one of the most widely used methods for GC analysis. Using \(N\)-methylimidazole as catalyst, the reaction is very rapid even at room temperature and the use of an aqueous sample is possible (16, 21, 22). Figure 4 shows the reaction equation for the derivatization of sucrose.

With the help of \(^1\)H-NMR we have shown that the reaction takes place completely already after 10 min without side reactions (see Supplementary data 1).

The EI-MS-spectrum of sucrose octaacetate showed no molecular peak \((m/z = 678)\). For the quantification of sucrose, therefore, we used the mass fragment \(m/z = 331\). An example of the mass spectrum of sucrose octaacetate recorded in this study is shown in Supplementary data 2.

Figure 5 shows in the upper chromatogram the sucrose peak of a honey sample from the local market. The sucrose content was 0.5%. The lower chromatogram shows the same sample to which 1% sucrose were added.

**Conclusions**

A simple and rapid D-SPME-GC/MS method was developed for the determination of sucrose in honey. Prior to GC analysis, the sample was derivatized by \(N\)-methylimidazole catalyzed acetylation. Derivatization was completed rapidly at room temperature in an aqueous sample. For SPME, with a polyacrylate fiber an enrichment time of 30 min was sufficient. The method is sensitive enough to safely detect adulteration of honey by sucrose feeding. The proposed method may be adapted for the determination of other mono- and disaccharides in honey.

**Supplementary data**

Supplementary data are available at *Journal of Chromatographic Science* (http://chromsci.oxfordjournals.org).

**Acknowledgements**

The authors thank Dr Heather Lord (Maxxam Analytics, Canada) for review of the text and the helpful hints.

**References**

1. Puscas, A., Hosu, A., Cimpoiu, C.; Application of a newly developed and validated high-performance thin-layer chromatographic method...


12. Kaskoniene, V., Venckutonis, P.R., Cekstere, V.: Carbohydrate composition and electrical conductivity of different origin honeys from Lithuania; *Food Science and Technology*, (2010); 43: 801–807.


15. Kaskoniene, V., Venckutonis, P.R., Cekstere, V.: Carbohydrate composition and electrical conductivity of different origin honeys from Lithuania; *Food Science and Technology*, (2010); 43: 801–807.