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Short communication

Use of gas chromatography–mass spectrometry for identification of a new disaccharide in honey

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Abstract

Gas chromatography–mass spectrometry has been used to separate and identify disaccharides of edible honey. According to the characteristic fragmentation behaviour of disaccharide TMS-oximes, fructofuranosyl-(2-1)-fructose (inulobiose) has been structurally characterized. Identification was carried out on the basis of retention time on two columns of different polarity and mass spectrometric analysis. Inulobiose was found in honeys of different origins for the first time, varying within 0.93 and 6.14 mg/g of honey. Its occurrence in honeys is discussed.

Keywords: Inulobiose; Honey; GC–MS; Disaccharides

1. Introduction

Carbohydrates in honey constitute a very complex mixture consisting of monosaccharides (glucose and fructose), disaccharides and oligosaccharides up to degree of polymerization (DP) 14 [1].

Sugars in honey are produced by enzymatic hydrolysis of sucrose (and possibly also by acid reversion) followed by partial transglycosylation. Most of the saccharides are formed by glucose and fructose units, with glycosidic linkages α and β. In the seventies, Siddiqui [2] isolated and identified 10 disaccharides. However, thanks to the use of chromatographic techniques (GC and LC) the presence of more saccharides has been determined [3,4]. A GC procedure developed by Mateo el al. [5] enabled the determination of a higher number of sugars (eight of them were disaccharides); this method was later optimized by Gomez Barez et al. [6] and 11 disaccharides were determined.

Moreover, the coupling of GC with MS affords some useful data for identification, especially when using TMS-oximes as derivatives [7], although the lack of standards (for example, fructosides) introduces an additional problem. At present, 15 disaccharides have been quantified in honey by GC–MS [8], covering all possible α- and β-glucosyl-glucoses and α-glucosyl-fructoses. Nevertheless, some peaks have been reported as unknown sugars: even with the high resolution methods available, these complex isomeric mixtures are very difficult to analyze [9].

In this work, honey disaccharides have been determined by GC–MS as their TMS-oximes. From their mass fragmentation an unidentified peak has been characterized as a disaccharide constituted by two fructose rings; the comparison of its elution behaviour on two different columns with that of inulobiose present in a commercial mixture of oligofructose afforded its identification.

2. Material and methods

2.1. Materials

All reagents were analytical grade. Thirty five honey samples covering a wide range of origins were directly purchased to beekeepers or to specialised markets. A commercial mixture of oligofructose (Beneo^{TM} P95 previously known as Raftilose-P95) was a gift from Orafti (Spain).

2.2. Derivatives

GC carbohydrate analysis was carried out according to [8], using a two-step derivatization procedure (oximation and

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trimethylsilylation). In brief, 0.5 mL of a solution prepared by dissolving 1 g of sample in 25 mL of 80% ethanol/water was mixed with 0.5 mL of a 70% ethanolic solution of phenyl-β-D-glucoside (1 mg/mL) employed as internal standard. After evaporation of the ethanolic solution under vacuum, derivatives were formed by addition of hydroxylamine chloride in pyridine. The oximes obtained in this step were silylated with hexamethyldisilazane and trifluoroacetic acid. After reaction, samples were centrifuged, and 1 μL of supernatants was taken for injection. It is noteworthy to remember that reducing sugars give two peaks after derivatization, corresponding to both *E* and *Z* isomers of the oxime, while non-reducing sugars only give one peak, corresponding to the per-TMS ethers. Besides, it has been reported that aldoses give a higher proportion of the *E*-isomer than that of the *Z*-isomer, whereas for ketoses the proportion of *E*- and *Z*-isomers is close to 1 [10].

2.3. GC analysis

GC analyses were carried out in a gas chromatograph equipped with a flame ionisation detector (FID) (HP 5890, Palo Alto, CA, USA), using nitrogen as carrier gas and working in split mode (1:40).

A 25 m × 0.25 mm i.d. × 0.25 μm fused silica column coated with SPB-1 (crosslinked methyl silicone) was used for most analysis, and a 30 m × 0.25 mm i.d. × 0.1 μm phenyl-methylsilicone column Rtx-65 TG (Crossbond, 35% dimethyl–65% diphenyl polysiloxane from Restek, Bellefonte, PA, USA) was used as medium polarity alternative. Analyses on SPB-1 were carried out at 200 °C for 20 min, then programmed to 270 °C at a heating rate of 15 °C/min, then programmed to 290 °C at 1 °C/min and finally programmed to 300 °C at 15 °C/min and held for 40 min. Injector and detector temperature was 300 °C. Analyses on Rtx-65 TG were carried out at 170 °C for 10 min, then programmed to 215 °C at a heating rate of 15 °C/min, then programmed to 240 °C at 1 °C/min and finally programmed to 320 °C at 5 °C/min and held for 20 min. Injector and detector temperatures were 300 °C and 320 °C, respectively.

2.4. GC–MS analysis

It was carried out using a Hewlett-Packard 6890 gas chromatograph coupled to a 5973 quadrupole mass detector operating in electronic impact (EI) mode at 70 eV (both from Agilent, Palo Alto, CA, USA). Operating conditions other than carrier gas (He at 1 mL/min) were identical to those previously described for GC analysis. Acquisition was done using an HPChem Station software (Hewlett-Packard, Palo Alto, CA, USA).

3. Results and discussion

3.1. Identification

Fig. 1A shows a GC disaccharide profile of a typical honey sample, obtained using a methyl silicone column, with some unidentified peaks eluting after α,β-trehalose. The highest unknown peak (marked with U), showed a retention time relative

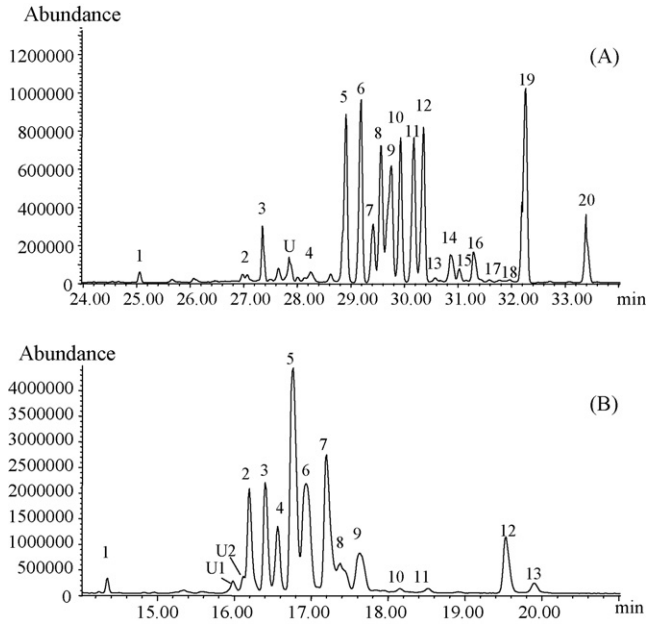

Fig. 1. Disaccharide profiles of a honey sample analyzed by GC on a: (A) methylsilicone column: (1) Sucrose, (2) α,α-trehalose, (3) α,β-trehalose, (U) unknown disaccharide, (4) cellobiose (*E*), (5) cellobiose (*Z*) + laminaribiose (*E*) + maltulose (*E*), (6) maltulose (*Z*), (7) nigerose (*E*) + unknown, (8) turanose 1, (9) laminaribiose (*Z*) + turanose 2 + maltose (*E*), (10) kojibiose (*E*), (11) maltose (*Z*) + trehalulose 1, (12) nigerose (*Z*) + trehalulose 2, (13) unknown, (14) palatinose 1 + gentiobiose (*E*), (15) kojibiose (*Z*), (16) palatinose (*Z*), (17) gentiobiose (*Z*), (18) melibiose (*E*), (19) isomaltose (*E*), (20) melibiose (*Z*) + isomaltose (*Z*); and (B): phenyl methyl silicone column (conditions in text): (1) sucrose, (U1+U2): unknown disaccharide, (2) cellobiose (*E*) + maltulose (*E*), (3) maltulose (*Z*), (4) cellobiose (*Z*) + laminaribiose (*E*), (5) turanose 1 + maltose (*E*), (6) nigerose (*E*) + turanose 2 + maltose (*Z*), (7) laminaribiose (*Z*) + kojibiose (*E*) + trehalulose 1 + unknown, (8) nigerose (*Z*) + trehalulose 2 + α,β-trehalose, (9) kojibiose (*Z*), (10) palatinose 1, (11) palatinose 2, (12) isomaltose (*E*), (13) isomaltose (*Z*).

to sucrose of 1.11. It eluted in the zone clearly corresponding to disaccharides [11].

Mass spectrum of this unknown peak is shown in Fig. 2A. The intensity of 307 fragment as high as the 361 is indicative of a free ketose linked to the non-reducing unit through the hydroxyl group in position 1 or 3. The unit with the free reducing end was probably fructose, the only ketohexose reported in honey. The relative proportions of fragments 217, 204 and 191, as well as the high ratio of fragments at 437/451 indicate that the non-reducing unit was probably also fructose. However, two peaks should appear for this compound, and only one was detected, although their width seemed to indicate that they were two overlapped compounds.

When the sample was analyzed using a phenyl silicone column two unidentified compounds with almost identical mass spectra as the compound U eluted with retention times relative to sucrose of RRT 1.11 and 1.12 (Fig. 1B). Peak U2 partially overlapped with maltulose (*E*) + cellobiose (*E*), but resolution was enough to obtain a satisfactory mass spectrum. The similar mass spectra of both peaks suggested that they corresponded to *E*- and *Z*-isomers of the same compound, namely a reducing sugar. Therefore, this compound could be probably identified as a fructosyl-fructose.