Food Chemistry 120 (2010) 637–642

Contents lists available at ScienceDirect

Food Chemistry

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

Analytical Methods

Gas chromatographic–mass spectrometric characterisation of tri- and tetrasaccharides in honey

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ARTICLE INFO

Article history:
Received 15 July 2009
Received in revised form 7 September 2009
Accepted 19 October 2009

Keywords:
Trisaccharides
Tetrasaccharides
Honey
Gas chromatography–mass spectrometry (GC–MS)
Trimethylsilyloximes

ABSTRACT

A GC–MS method has been used to characterize tri- and tetrasaccharides in honey after their derivatization into trimethylsilyloxime derivatives. Based on retention data and mass spectra, a total of 25 trisaccharides were characterized; 12 being unequivocally identified using standards and two of them detected for the first time in honey. Erlose and panose were the major trisaccharides in the 12 honeys under analysis, their concentrations ranging 30–1214 mg 100 g^{-1} of honey and 17–863 mg 100 g^{-1} of honey, respectively. The GC–MS method also allowed the analysis of tetrasaccharides. Besides nystose, another nine tetrasaccharides were characterized; six of them were sucrose derivatives. Tetrasaccharides were present in concentrations lower than 230 mg 100 g^{-1} of honey.

1. Introduction

Honey mainly consists of monosaccharides (glucose and fructose) which constitute around 85% of total carbohydrates. Disaccharides, trisaccharides and tetrasaccharides are also present in decreasing amounts (Doner, 1977). These oligosaccharides are mainly composed of glucose and fructose units with the glycosidic bond in different positions and configurations. Several *in vitro* studies have suggested the influence of these oligosaccharides on the growth of probiotics (*Bifidobacteria* and *Lactobacilli*) (Kajiwara, Gandhi, & Ustunol, 2002; Sanz et al., 2005). However, *in vivo* studies should be done to determine their prebiotic activity, the characterisation of these oligosaccharides being a prior requirement.

Although several works have been focussed on the study of mono- and disaccharides, the knowledge about tri- and tetrasaccharide fraction is still scarce. Siddiqui (1970) isolated and characterized up to 11 trisaccharides from honey, and a critical review by Doner (1977) evidenced the presence of eight trisaccharides (isopanose, theanderose, 3-α-isomaltosyl-glucose, erlose, melezitose, maltotriose, panose and isomaltotriose); all of them with the exception of 3-α-isomaltosyl-glucose being further detected by chromatographic methods (Low & Sporns, 1988; Sanz, Sanz, & Martínez-Castro, 2004; Weston & Brocklebank, 1999). Other trisaccharides such as raffinose (Cotte, Casabianca, Chardon, Lheritier, & Grenier-Loustalot, 2004; Horváth & Molnár-Perl, 1997; Mateo & Bosch-Reig, 1997), kestoses (Cotte et al., 2004; De la Fuente, Sanz, Martínez-Castro, Sanz, & Ruiz-Matute, 2007; Rittig, 2001) and isomelezitose (Rittig, 2001) have also been identified by GC or HPLC. The number of reported tetrasaccharides is also limited: isomaltotetraose (Doner, 1977; Siddiqui, 1970); maltotetraose, α-4′-glucosyl-erlose and α-6′-glucosyl-erlose (Astwood, Lee, & Manley-Harris, 1998); stachyose, nystose and fructosyl-isomelezitose (Rittig, 2001). At present, chromatographic data about centose (Siddiqui, 1970), isopanose, 3-α-isomaltosyl-glucose and the previously mentioned tetrasaccharides are lacking.

Gas chromatography–mass spectrometry (GC–MS) is a useful technique to characterize complex mixtures of carbohydrates up to degree of polymerisation (DP) 4; the derivatization of the sample being a prior requirement. Trimethylsilyloximes (TMSO) are adequate, since reducing sugars give rise to only two different derivatives corresponding to the *syn* (*E*) and *anti* (*Z*) isomers (Molnár-Perl & Horváth, 1997). The study of the different *m/z* fragments produced by electronic impact (EI) ionisation in MS analysis provides valuable information about the chemical structure of a molecule. However, difficulties are found when the compounds to be analysed are complex mixtures of carbohydrates with the same molecular weight and which only differ in the position of their glycosidic linkages and the configuration of their hydroxyl groups. Therefore, the relative intensity of MS fragments together with the differences in GC retention time of each isomer are the only available tools to identify these compounds. A GC–MS method

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doi:10.1016/j.foodchem.2009.10.050

developed in our laboratory (De la Fuente, Sanz, Martínez-Castro, & Sanz, 2006; Sanz et al., 2004) has allowed obtaining new structural data for disaccharides present in different honeys (De la Fuente et al., 2007) and for unusual both commercial and non-commercial trisaccharide standards (Brokl, Soria, Martínez-Castro, Sanz, & Ruiz-Matute, 2009).

In this work, experimental GC and MS data of honey trisaccharides have been obtained and used for their detailed characterisation. Moreover, this method has been extended to the analysis of honey tetrasaccharides, allowing the characterisation of a high number of compounds.

2. Materials and methods

2.1. Standards

Cellotriose (β-Glc-(1 → 4)-β-Glc-(1 → 4)-Glc), erlose (α-Glc-(1 → 4)-α-Glc-(1 → 2)-β-Fru), isomaltotriose (α-Glc-(1 → 6)-α-Glc-(1 → 6)-Glc), 1-kestose (β-Fru-(2 → 1)-β-Fru-(2 → 1)-α-Glc), laminaritriose (β-Glc-(1 → 3)-β-Glc-(1 → 3)-Glc), maltotriose (α-Glc-(1 → 4)-α-Glc-(1 → 4)-Glc), maltotetraose (α-Glc-(1 → 4)-α-Glc-(1 → 4)-α-Glc-(1 → 4)-Glc), nigerotriose (α-Glc-(1 → 3)-α-Glc-(1 → 3)-Glc), nystose (β-Fru-(2 → 1)-β-Fru-(2 → 1)-β-Fru-(2 → 1)-α-Glc), panose (α-Glc-(1 → 6)-α-Glc-(1 → 4)-Glc), raffinose (α-Gal-(1 → 6)-α-Glc-(1 → 2)-β-Fru) and stachyose (α-Gal-(1 → 6)-α-Gal-(1 → 6)-α-Glc-(1 → 2)-β-Fru) were acquired from Sigma Chemical Co. (St. Louis, USA). Melezitose (α-Glc-(1 → 3)-β-Fru-(2 → 1)-α-Glc) was from Fluka (Madrid, Spain). Gentianose (β-Glc-(1 → 6)-α-Glc-(1 → 2)-β-Fru), α-3′-glucosyl-isomaltose (α-Glc-(1 → 3)-α-Glc-(1 → 6)-Glc), kojitriose (α-Glc-(1 → 2)-α-Glc-(1 → 2)-Glc), planteose (α-Gal-(1 → 6)-β-Fru-(2 → 1)-α-Glc) and theanderose (α-Glc-(1 → 6)-α-Glc-(1 → 2)-β-Fru) were kindly gift by Dr. G.R. Côté (USDA, Peoria, USA).

Neo-kestose (β-Fru-(2 → 1)-α-Glc-(6 → 2)-β-Fru) was obtained from an onion (*Allium ascalonicum*) extract in hot water for 2 h after filtration. 6-Kestose (β-Fru-(2 → 6)-β-Fru-(2 → 1)-α-Glc) was prepared according to Manley-Harris and Richards (1991) by pyrolysis of amorphous sucrose with citric acid as catalyst.

2.2. Samples

Six Spanish honey samples were directly obtained from beekeepers: two rosemary (*Rosmarinus officinalis*), one heather (*Erica* sp.), one orange blossom (*Citrus* sp.), one multiflower and one honeydew honey. Six commercially available honeys labelled as borage (*Borago officinalis*), clover (*Trifolium* sp.), manuka (*Leptospermum scoparium*), rewarewa (*Knightia excelsa*), kamahi (*Weinmannia racemosa*) and tawari (*Ixerba brexioides*) were acquired in a local market in New Zealand.

2.3. Derivatization of carbohydrates

About 1 ml of carbohydrate standards (1 mg ml^{-1} in methanol:water 30:70 v/v) was evaporated under vacuum and treated with 350 μl of 2.5% hydroxylamine chloride in pyridine at 75 °C for 30 min. The silylation reaction was carried out with 350 μl of hexamethyldisilazane (HMDS) and 35 μl of trifluoroacetic acid (TFA) at 45 °C for 30 min (Ruiz-Matute, Sanz, & Martínez-Castro, 2007). Derivatised samples were centrifuged and 1 μl of supernatant was injected onto the injection port of the gas chromatograph.

For honey samples, 0.5 g were diluted in 25 ml of a methanol:water solution (30:70 v/v) and 1 ml was evaporated under vacuum. Derivatization conditions were identical to those described above.

2.4. GC–MS analysis

GC–MS analysis 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). Analyses were carried out in splitless mode (valve close 0.5 min) on a polycarborane–siloxane HT-5 column (SGE Europe Ltd., UK) at 200 °C for 15 min, then programmed to 270 °C at a heating rate of 15 °C min^{-1}, then programmed to 290 °C at 1 °C min^{-1} and finally programmed to 350 °C at 15 °C min^{-1} and held for 30 min. Injector temperature was 300 °C and the transfer line was thermostatised at 280 °C. Helium at ~1 ml min^{-1} was used as carrier gas. Acquisition was done using a HP ChemStation software (Hewlett–Packard, Palo Alto, CA, USA).

Linear retention indices (I^T) were calculated from the retention times of TMSO of carbohydrates and suitable *n*-alkanes. Honey tri- and tetrasaccharides were identified by comparison of their experimental spectra and I^T with those of standard compounds run in the laboratory under identical experimental conditions (Brokl et al., 2009). Data published in the literature were used for further confirmation. Identifications were considered to be positive when both MS and I^T data were consistent with those obtained from standard compounds or with those in the literature. Identities were considered to be tentative when only experimental MS and GC data were available and no further comparison was possible due to the lack of standards or literature data.

2.5. GC–FID analysis

GC analyses were carried out in a gas chromatograph equipped with a flame ionisation detector (FID) (HP 6890, Palo Alto, CA, USA), using nitrogen at ~1 ml min^{-1} as carrier gas. Operating conditions other than detector temperature (300 °C) were identical to those previously described for GC–MS analysis. Chromatographic peaks were recorded using a HP ChemStation software (Hewlett–Packard, Palo Alto, CA, USA).

Quantitative analyses were carried out using solutions of trisaccharide and tetrasaccharide standards over the expected concentration range in honey samples. Phenyl-β-D-glucoside (0.1 mg ml^{-1}) was used as internal standard. All the analyses were carried out in duplicate.

3. Results and discussion

3.1. Qualitative analysis

Fig. 1 shows a close-up view of the tri- and tetrasaccharide elution range in the GC–MS profile of one of the honey samples under analysis. As previously mentioned, identities were considered to be positive when both MS and I^T data were consistent with those of standards or with literature data. Both the characteristic MS and GC data (Table 1) and the possible formation pathway in honey were considered to tentatively characterize other tri- and tetrasaccharides present in honey. It is worth noting that all the honey samples analysed showed a qualitatively similar tri- and tetrasaccharide profile irrespective of their botanical or geographical origin.

α-Glucosidase is an enzyme which hydrolyses the sucrose of nectar into fructose and glucose (Doner, 1977; Siddiqui, 1970), but it also has transglycosylating action, since the main disaccharides in honey (maltulose, nigerose, turanose, maltose, kojibiose, trehalulose and isomaltose) are α-glucosyl derivatives of these monosaccharides. Following this reasoning, trisaccharides in honey are probably α-glucosyl derivatives of the main disaccharides; sucrose derivatives being the main components. Tetrasaccharide

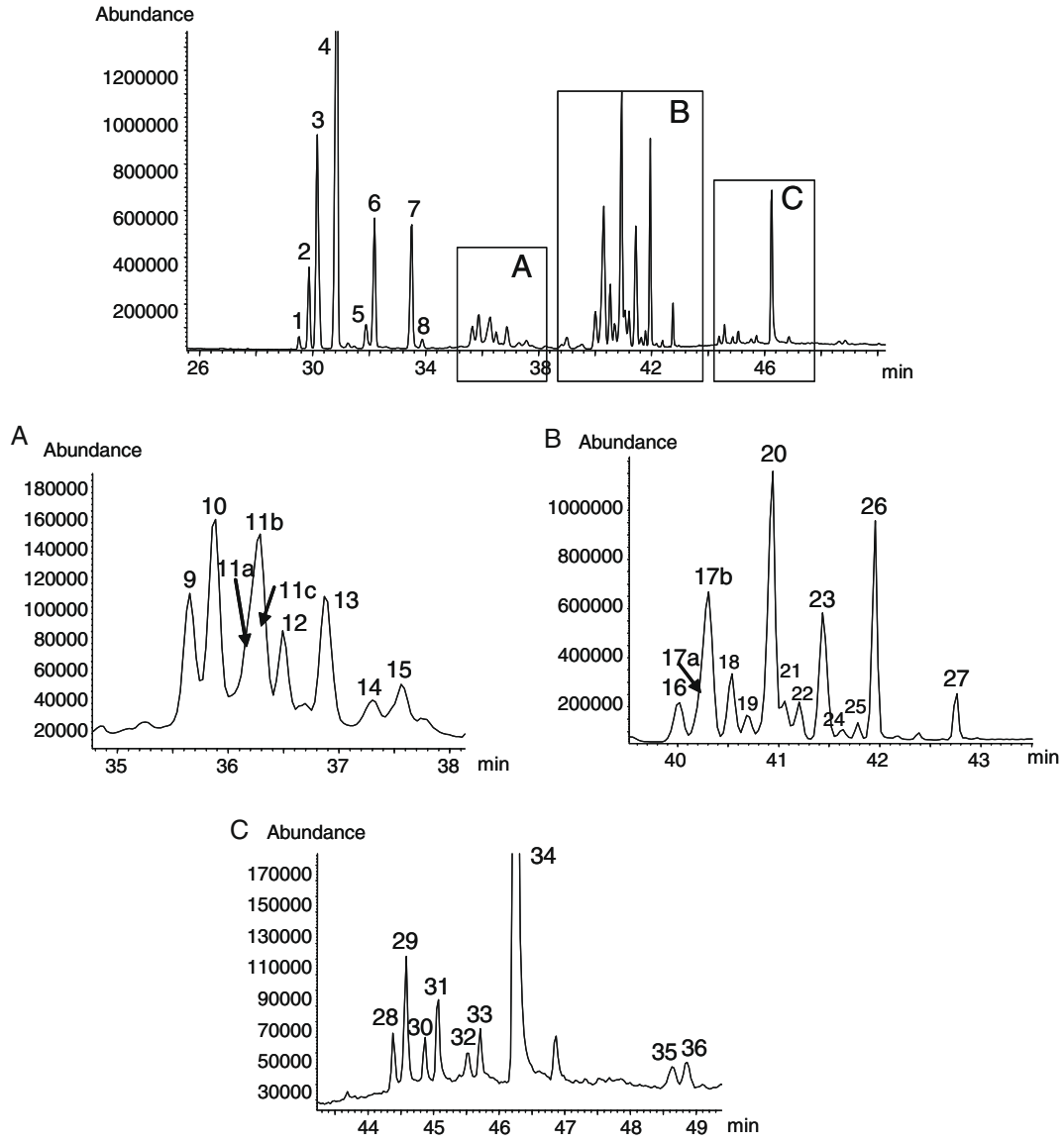

Fig. 1. GC–MS profile of TMSO derivatives of tri- and tetrasaccharides of honeydew honey. For peak identification, see Tables 1 and 2.

formation could also follow a similar behaviour. However, some exceptions could be expected for certain saccharides not cleaved by this enzyme.

About the retention behaviour, elution order of trisaccharides showed some parallelism with that of disaccharides previously studied by Sanz, Díez-Barrio, Sanz, and Martínez-Castro (2003). According to this, sucrose derivatives eluted first and those of isomaltose the latter, the remaining eluting at intermediate positions.

Peaks 1 and 2 in Fig. 1 were identified as raffinose and 6-kestose by comparison with data from the corresponding standards and from the literature (Kamerling, Vliegenthart, Vink, & de Ridder, 1972; Sanz et al., 2004). Peak 3 was an unresolved mixture of 1-kestose and neo-kestose, as shown by the different *m/z* ratio 437/451 obtained in the fronting and tailing part of the peak (>1 and <1, respectively) (Kamerling et al., 1972).

Peaks 4, 6 and 7 were identified as erlose, melezitose and theanderose, respectively. Peak 5, which displayed a spectrum similar to that of melezitose, was assigned as planteose by comparing its I^T (3278) and mass spectrum with those of a standard (Brokl et al., 2009). This is the first report about the presence of planteose in honey.

Peak 8 was characterized by the unusually high abundance of the group of *m/z* ions 191, 204 and 217 (see Table 1), which have been found in aldosyl aldoses with (1 → 1) linkages (Brokl et al., 2009; Sanz, Sanz, & Martínez-Castro, 2002). This peak could therefore correspond to a trisaccharide derived from α,β-trehalose substituted with glucose in 3 or 4 position; the low retention of trehaloses also supports this assignation. A glucotriose derived from trehalose with 1 → 4 substitution has been reported in *Bemisia* honeydew (Hendrix & Wei, 1994).

Mass spectra of peaks 9 and 10 (Fig. 1A) were compatible with glucotrioses; their retention data point at derivatives from maltose or nigerose. Three overlapping compounds could be detected in the broad peak 11: the front part (peak 11a) was assigned to maltotriose *E*; maltotriose *Z* was peak 14. The middle part (peak 11b) showed a relatively high 319 *m/z* ion. As this ion has been observed in glucosylglucoses with (1 → 2) linkage, a glucosyl-kojibiose structure was tentatively assigned to this peak. The tail part (peak 11c) was very similar to peaks 12 and 13. These peaks could corre-

Table 1
Linear retention indices (I^T) and relative abundance for characteristic *m/z* ratios of trisaccharides (peaks 8–25) and tetrasaccharides (peaks 28–36) characterized in honey by GC–MS.

Peak no.[a]	Assignation	I^T	*m/z*													
			191	204	205	217	271	305	307	319	361	422	437	448	451	538
8	Glc-(1 → 1)-Glc-(1 → *x*)-Glc (*x* = 3 or 4)	3331	73	100	27	89	14	5	0	7	85	0	0	0	2	0
9	Glc–Glc–Glc	3394	30	100	31	49	16	5	3	11	96	0	0	0	3	0
10	Glc–Glc–Glc	3403	21	100	31	47	12	4	2	5	82	0	0	0	3	2
11b	Glc-(1 → *x*)-Glc-(1 → 2)-Glc	3417	13	100	33	14	7	2	2	13	34	0	0	0	0	3
11c	Fru-(2 → *x*)-Glc-(1 → 3)-Fru	3418	24	100	36	67	10	5	20	13	54	0	2	0	2	0
12	Fru-(2 → *x*)-Glc-(1 → 3)-Fru	3424	21	100	51	60	10	5	20	7	46	0	5	6	0	3
13	Fru-(2 → *x*)-Glc-(1 → 3)-Fru	3438	21	100	36	63	8	5	30	8	42	0	1	0	0	1
15	Glc–Glc–Glc	3463	47	100	22	63	12	6	0	6	80	0	0	0	0	0
17a	α-Glc-(1 → *x*)-α-Glc-(1 → 1)-Fru, 1	3560	22	100	32	55	6	4	22	1	55	0	0	1	1	0
17b	α-Glc-(1 → 6)-α-Glc-(1 → 2)-Glc *E*	3561	19	100	40	54	6	6	3	52	49	0	0	1	2	3
18	α-Glc-(1 → *x*)-α-Glc-(1 → 1)-Fru, 2	3570	17	100	36	53	5	4	52	10	30	0	0	0	1	2
19	Reducing ring substituted in C6 derived from isomaltose	3575	21	100	37	54	6	4	14	5	58	0	0	4	1	7
21	Glc-(1 → *x*)-Glc-(1 → 6)-Glc (*x* = 3 or 4)	3588	17	100	26	52	7	4	2	6	65	2	0	0	3	6
22	Glc–Glc–Glc	3593	25	100	24	58	8	6	2	10	99	0	0	0	2	0
25	α-Glc-(1 → 6)-α-Glc-(1 → 2)-Glc *Z*	3631	19	100	46	47	7	5	12	50	35	0	0	0	0	4
29	Sucrose derivative	3872	11	12	6	100	19	3	1	6	56	0	4	0	12	0
30	Sucrose derivative	3901	14	51	14	100	20	4	0	2	63	0	6	0	16	0
31	Sucrose derivative	3921	16	71	18	100	19	5	0	6	80	0	14	0	18	0
32	Sucrose derivative	3968	22	94	24	61	24	3	0	7	100	0	3	0	7	0
33	Tetrasaccharide	3985	21	100	25	53	16	8	0	8	97	0	0	0	0	0
34	Sucrose derivative	4041	16	100	23	57	16	4	1	5	100	0	9	0	13	0
35	Tetrasaccharide	4279	23	100	37	47	20	2	0	5	70	0	0	0	0	0
36	Sucrose derivative	4303	25	100	40	44	17	6	15	7	46	0	0	0	0	0

[a] Peak number refers to the elution order of tri- and tetrasaccharides in Fig. 1.

spond to reducing ketoses substituted in C1 or C3, as shown by the relative high intensity of ion at *m/z* 307 and an unusual small fragment at *m/z* 334, which we have only found in turanose; these trisaccharides could be turanose derivatives.

The spectrum of peak 15, characterized by a moderately high *m/z* ion at 191, was compatible with that of a glucotriose. Its I^T was similar to that of cellotriose *Z* (I^T = 3460); however, the probability of this carbohydrate being present in honey is very low, taking into account the low abundance of cellobiose in honey and the low probability of formation of β linkages by honey enzymes; the corresponding trisaccharides should be then present at trace levels.

In Fig. 1B, peaks 16 and 24 were identified as glucotrioses based on their mass spectra. Their high retention times indicate that these carbohydrates could share structural features with panose or isomaltose. The comparison of experimental data with those of standards compounds run in the laboratory allowed the identifications of peaks 16 and 24 as the *E* and *Z*-isomers of α-3′-glucosyl-isomaltose, respectively; this compound is here identified by GC–MS for the first time in honey.

Peak 17 was broad and clearly formed by two overlapping compounds. The front (peak 17a) corresponded to a reducing ketose substituted in C1 or C3 as shown by the *m/z* ion at 307; based on its retention data, this peak may correspond to a trisaccharide derived from trehalulose (α-Glc-(1 → x)-α-Glc-(1 → 1)-Fru). The spectrum of peak 18 was very similar to that of peak 17a and it probably corresponds to its isomer. The tailing part of peak 17 (17b) was probably a glucosyl-kojibiose as indicated by the high abundance of *m/z* 319 (α-Glc-(1 → *x*)-α-Glc-(1 → 2)-Glc).

Spectrum of peak 19 was characterized by the proportion of *m/z* ions 307, 448 and 538; this peak could contain a reducing ring substituted in C6 derived from isomaltose. Peaks 20 and 23 corresponded to panose and peaks 26 and 27 to isomaltotriose.

Peak 21 is probably a derivative of isomaltose substituted in C3 or C4, whereas peak 22 could only be characterized as a glucotriose.

The mass spectrum of peak 25 clearly corresponds to a reducing aldose substituted at the C2 position, as shown by the ion at *m/z* 319. This mass spectrum was similar to that of peak 17b and could correspond to the *Z*-isomer of the same TMSO carbohydrate. Therefore, peak 25 was tentatively assigned as (α-Glc-(1 → *x*)-α-Glc-(1 → 2)-Glc). Considering its high retention time, the structure of this compound would be in agreement with α-Glc-(1 → 6)-α-Glc-(1 → 2)-Glc.

As commented above, several trisaccharides arising from major disaccharides have been detected in honeys (maltotriose and panose from maltose; isomaltotriose from isomaltose). Other glucotrioses may also be formed by enzymatic transglycosidation, as the above characterized. α-Glucosidase from honey may glycosylate all disaccharides present, but the most abundant will probably give rise to noticeable amounts of trisaccharides. For example, glycosylation of maltulose, turanose and trehalulose makes possible the presence of the corresponding trisaccharides in honey (e.g. peaks 11c, 12, 13, 17a and 18 in Fig. 1A). Kestoses and other fructosyl saccharides are probably formed by the transfructosylating action of fructosidases from pollen (Siddiqui, 1970) or yeasts (Park, Koo, & Oliveira, 1996). Reversion reactions at the acidic pH of honey could also be partially responsible for oligosaccharide formation (Doner, 1977).

Honey tetrasaccharides (Fig. 1C) apparently showed the same general chromatographic behaviour as trisaccharides: sucrose

Table 2
Concentration range of trisaccharides (peaks 1–27) and tetrasaccharides (peaks 28–37) in honey samples under analysis.

Peak no.[a]	Assignation	Honey sample (mg 100 g^{-1})	
		Maximum	Minimum
1	Raffinose	209.7	0.0
2	6-Kestose	148.9	11.7
3	1-Kestose + neo-kestose	845.3	23.2
4	Erlose	1214.8	30.8
5	Planteose	70.4	0.0
6	Melezitose	246.5	21.5
7	Theanderose	301.3	33.6
8	Glc-(1 → 1)-Glc-(1 → *x*)-Glc (*x* = 3 or 4)	29.9	8.4
9	Glc–Glc–Glc	134.7	15.2
10	Glc–Glc–Glc	113.5	14.7
11b + 11c	Glc-(1 → *x*)-Glc-(1 → 2)-Glc + Fru-(2 → *x*)-Glc-(1 → 3)-Fru	58.9	tr
12	Fru-(2 → *x*)-Glc-(1 → 3)-Fru	60.5	0.0
13	Fru-(2 → *x*)-Glc-(1 → 3)-Fru	120.4	0.0
11a + 14	Maltotriose	124.2	0.0
15	Glc–Glc–Glc	23.2	0.0
16 + 24	α-3′-Glucosyl-isomaltose	291.4	0.0
17a + 17b	α-Glc-(1 → *x*)-α-Glc-(1 → 1)-Fru, 1 + α-Glc-(1 → 6)-α-Glc-(1 → 2)-Glc *E*	810.8	19.8
18	α-Glc-(1 → *x*)-α-Glc-(1 → 1)-Fru, 2	407.4	6.1
19	Reducing ring substituted in C6 derived from isomaltose	127.9	0.0
20 + 23	Panose	863.2	17.4
21	Glc-(1 → *x*)-Glc-(1 → 6)-Glc (*x* = 3 or 4)	142.7	0.0
22	Glc–Glc–Glc	152.6	0.0
25	α-Glc-(1 → 6)-α-Glc-(1 → 2)-Glc *Z*	58.8	0.0
26 + 27	Isomaltotriose	534.3	0.0
28	Nystose	166.8	0.0
29	Sucrose derivative	47.5	0.0
30	Sucrose derivative	23.9	0.0
31	Sucrose derivative	16.0	0.0
32	Sucrose derivative	27.1	0.0
33	Tetrasaccharide	28.4	0.0
34	Sucrose derivative	230.0	0.0
35	Tetrasaccharide	24.2	0.0
36	Sucrose derivative	27.2	0.0

[a] Peak number refers to the elution order of tri- and tetrasaccharides in Fig. 1.

derivatives eluted first, followed by other tetrasaccharides and they were the main compounds. However, mass spectra of tetrasaccharides are more difficult to interpret because of the lower number of available standards and the accumulation of identical fragments coming from four sugar rings in the same molecule (Table 1).

Peaks 28–34 (except for peak 33) were clearly derivatives of sucrose, as shown by the presence of *m/z* fragments 437 and 451 (Kamerling et al., 1972). Nystose was identified as peak 28 by comparison with the commercial standard. Peak 29 was characterized by a very high *m/z* ratio 217/204, which points at the presence of at least two fructose rings in the molecule. Taking into account their mass spectra and relative retention, peaks 30 and 31 could be derivatives of kestoses. Peaks 32 and 34 are probably derivatives of erlose; the most abundant tetrasaccharide described by Astwood et al. (1998) was α-4′-glucosyl-erlose whose structure is compatible with the mass spectrum of peak 34. Peaks 33, 35 and 36 showed a mass spectrum without specific features. Other tetrasaccharides previously described in honeys such as stachyose or maltotetraose (Astwood et al., 1998; Rittig, 2001) eluted at higher retention times and were not detected by this method probably due to their low concentration in the honey samples analysed here.

3.2. Quantitative results

Table 2 shows quantitative data of tri- and tetrasaccharides detected in the 12 honey samples analysed. As no noticeable differences were observed between honeys of different botanical or geographical origin, data for tri- and tetrasaccharides were grouped and shown as concentration ranges. In general, erlose was the most abundant trisaccharide followed by panose and kestoses. This agrees with previous data found in the literature (Cotte et al., 2004; De la Fuente et al., 2007; Sanz et al., 2004). Since raffinose usually coelutes with kestoses in non-polar columns, it has probably been overestimated in some reports (Mateo & Bosch-Reig, 1997). Honey tetrasaccharides appeared at a lower average concentration than trisaccharides, sucrose derivatives showing the highest concentration.

4. Conclusions

This study has allowed the conclusive identification of 12 trisaccharides in honeys; planteose and α-3′-glucosyl-isomaltose have been reported in honey for the first time. Thirteen other trisaccharides have been characterized by their retention indices and mass spectrum data; some structural features have been also deduced. The method has also allowed the analysis of tetrasaccharides. Ten tetrasaccharides were detected: nystose was unequivocally identified and six compounds were characterized as sucrose derivatives. Formation of these oligosaccharides is mainly attributed to transglycosidation by honey enzymes.

Acknowledgements

This work has been funded by projects CTQ2006-14993/BQU (financed by CICYT) and ANALISYC S-505/AGR-0312 (financed by Comunidad de Madrid). M. Brokl thanks Consejería de Educación de la Comunidad de Madrid and Fondo Social Europeo for his predoctoral contract.

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