Article

Oligosaccharide profiles of Asian commercial honeys

Mérine Dumté and Merilyn Manley-Harris

Department of Chemistry, University of Waikato, Private Bag 3105, Hamilton 3240, New Zealand (e-mail: *manleyha@waikato.ac.nz*)

Keywords: Asian commercial honey; oligosaccharides; moisture and monosaccharide content

As part of the development of a method to detect honey in imported materials, a database of the oligosaccharide composition of a range of Asian commercial honeys has been prepared. Low maltose contents were detected compared with literature values for honey from Europe, North Africa and South America; with this exception oligosaccharide contents were similar to those in the literature. Moisture contents were slightly high compared with literature values for Europe and North America but comparable with literature values for Asia. Moisture, monosaccharide and sucrose contents were generally within the limits applied by the Codex. Four honeys were apparently adulterated.

Introduction

In New Zealand an Import Health Standard (IHS) for processed bee products exists pursuant to Section 22 of the Biosecurity Act 1993. This IHS specifies the requirements to be met for the effective management of risks associated with the importation of specified processed bee products.[1, 2] Foods, confectionary, dietary supplements and medical preparations containing greater than 2% of honey require an import permit. To assist in the enforcement of this IHS by the Ministry of Agriculture and Forestry (MAF) it has been necessary to develop a method for the detection of honey at low levels in such materials. The method described here is based upon the detection and quantification of the relatively uncommon oligosaccharides that derive from reversion in honey during maturation. As part of the validation of the method we have undertaken a survey of oligosaccharides in commercial honeys of Asian origin.

Although an extensive literature describes the oligosaccharide profiles of honeys from Europe, North and South America, and North Africa, few articles describe samples of Asian origin. Studies about adulteration analysed three commercial Chinese acacia honeys but did not actually tabulate the results, as the honeys were intended only to test a validation method.[3, 4] Another study described 81 samples from three different honeybee species of Nepalese origin but only tabulated the results for sucrose, turanose and maltose, grouping the other disaccharides together without a statistical analysis,[5] while a study of honeys from the Phillipines measured only fructose, glucose and sucrose.[6]

Materials and Methods

Materials

IRC-50 resin standard grade was purchased from BDH Chemicals Ltd. $NaBH_4$ was obtained from Alfa Aesar - A Johnson Matthey Company. Tri Sil HTP reagent was obtained from Thermo Fisher Scientific Inc. Methanol was of HPLC grade and supplied by either Scharlau or Ajax Finechem Pty Ltd. Pyridine (99+%, A.C.S. reagent) was purchased from Sigma-Aldrich Co. and dried over molecular sieve. Water was obtained from a Crystal Pure Ultra Pure Water System. Glacial acetic acid (analytical reagent) was purchased from Ajax Finechem Pty Ltd.

Fructose, sucrose, turanose, maltose, nigerose, -trehalose, palatinose, melibiose, gentiobiose, isomaltose, melezitose, raffinose, maltotriose, panose, isomaltotriose, xylitol and kojibiose were obtained from Sigma-Aldrich Co.; glucose was purchased from BDH AnalaR; cellobiose was purchased from BDH Biochemical; maltulose was from CMS Chemicals Ltd.; 1-kestose was isolated from oligofructose kindly supplied by Salkat New Zealand, using HPLC and confirmed by NMR spectroscopy.

Honey samples, which had been intercepted at the New Zealand border, were supplied by MAF Biosecurity (New Zealand) and were stored at 4 °C until required. The country of origin was recorded by MAF personnel and as indicated by labelling. Samples were warmed to 40 °C and stirred to remove crystallisation before analysis.

Determination of moisture content

Moisture content of the samples was determined using a Misco Palm Abbe PA203 Digital refractometer. Measurements for each sample were taken every 10 seconds until three consecutive stable values were obtained.

Preparation of standards

Three individual solutions of xylitol were prepared as internal standards. Each of the triplicate measurement of samples and standards used a separate internal standard.

$NaBH_4$ (5 mg per mg of standard) was weighed into a glass vial (7 mL) and the required amount of sugar standard added. Deionised water (1 mL) was added. The vial was heated (50 °C, 4 h) then cooled and freshly washed IRC-50 resin added to remove excess $NaBH_4$ until no more gas was evolved. The standards were filtered and evaporated under reduced pressure until nearly all the solvent had evaporated. The remaining liquid was transferred quantitatively into a glass vial (7 mL) and evaporated under a stream of dry nitrogen (40 °C) followed by co-evaporation six times with acidified methanol (2 mL) to remove residual borate. Xylitol internal standard (100 µL) was added and the solution evaporated under a stream of dry nitrogen (40 °C) until no liquid was visible, then dried overnight in a vacuum oven (40 °C).

Reduced sugars were per-*O*-trimethylsilylated by adding dry pyridine (900 µL) and sonicating (5 min). Tri Sil HTP (100 µL) was added and the vials heated (10 min, 75 °C).

The vials were left to cool and subsequently centrifuged (3 min, 3000 rpm). The supernatant (0.5 mL) was transferred to a clean GC vial, diluted appropriately using dry pyridine (~1 mL) and subsequently analysed by GC-FID. Non-reducing sugars were per-*O*-trimethylsilylated without the prior reduction step.

Response factors were determined by analysing each available standard in triplicate with varying amounts of compound and a consistent amount of internal standard. The response factor was taken from the gradient of a linear fit to the graph of the ratio peak area standard: peak area xylitol versus the ratio weight standard: weight xylitol. Sugars for which standards were not available were quantified using the mean response factor for di-or trisaccharides as appropriate.

Preparation of samples

Samples were prepared in triplicate. Honey (approximately 15 mg) and $NaBH_4$ (60-70 mg) were weighed into a glass vial (7 mL) and deionised water (1 mL) was added. The vial was heated (50 °C, 4 h) then cooled and freshly washed IRC-50 resin added to the vial to remove excess $NaBH_4$ until no more gas evolved. The samples were filtered, then evaporated under reduced pressure until nearly all the solvent was removed. The remaining liquid was transferred quantitatively into a glass vial (7 mL), evaporated under a stream of dry nitrogen (40 °C) and co-evaporated six times with acidified methanol (2 mL) to remove residual borate. Xylitol internal standard (100 µL) was added and the solvent evaporated to near dryness. The vials were then dried overnight in a vacuum oven (40 °C). Reduced samples were per-*O*-trimethylsilylated by adding Tri Sil HTP (1.5 mL), sonicating for 10 mins and heating (10 min, 75 °C). The vials were left to cool and subsequently centrifuged (3 min, 3000 rpm). The supernatant (0.5 mL) was transferred to a clean GC vial and subsequently analysed by GC-FID.

Gas Chromatography with flame ionisation detection (GC-FID)

GC-FID was carried out using a gas chromatograph (Model 6890N Series, Agilent Technologies) equipped with an autosampler (Model G2614A Series Autosampler, Agilent Technologies) and injector unit (Model 7683 Series Injector, Agilent Technologies). Analyses were carried out with an on-column injector and using a 30 m × 0.32 mm × 0.25 µm Zebron ZB-5 capillary column (phase: 5%-phenyl-95%-dimethylpolysiloxane) and FID detection. Carrier gas was hydrogen at 2.6 mL/min. Two microliter samples were injected into the column, with the injector temperature tracking the oven temperature. Detector temperature was maintained at 325 °C. The oven temperature program was 150 °C (5 min) + 3 °C/min to 300 °C + 1 °C/min to 325 °C (10 min).

Results and discussion

The moisture contents of the honey samples are given in Table 1.

Compared with the average moisture content of USA floral honeys of 17.2% (range: 12.2 – 22.9%),[7] or the average moisture content of honeys from the Madrid province of Spain of 16.13% (range:13.00-18.30%),[8] the moisture contents of Asian honeys (average: 18.3% , range: 16.5-20.4%) are slightly higher. The Codex Alimentarius Commission for honey prescribes a limit of 20% moisture, except for *Calluna* honey at not more than 23%.[9] Eight of the samples supplied had moisture contents higher than the 20% limit and these are listed separately in Table 2.

Table 1. Moisture contents of Asian honey samples.

Country of origin (number of samples)	Average moisture content (range) (%)
China (n = 6)	17.8 (17.2 – 19.1)
India (n = 7)	19.4 (17.8 – 20.3)
Indonesia (n = 1)	18.4
Japan (n = 2)	17.4 (16.5 – 18.2)
Malaysia (n = 2)	16.8 (16.7 – 16.8)
Russia (n = 1)	18.1
South Korea (n = 1)	18.4
Vietnam (n = 2)	19.9 (19.3 – 20.4)
Average (n = 22)	18.3 (16.5 – 20.4)

Table 2. Moisture contents of some honeys that exceeded 20% as recommended in the Codex Alimentarius.

Sample origin	Moisture content (%)
China	22.9
India	21.2
Vietnam 1	22.4
Vietnam 2	27.4
Vietnam 3	28.7
S. Korea	20.9
Sri Lanka 1	21.0
Sri Lanka 2	20.5

A comparison of moisture content of honeys by honey bee in Nepal gave 21.51 ± 2.38, 20.12 ± 2.66 and 17.14 ± 2.56 for *Apis dorsata*, *A. cerana* and *A. mellifera* respectively;[5] a similar comparison in the Phillipines gave 23.1 ± 2.3, 22.0 ± 3.7 and 19.5 ± 1.6, respectively.[6] The latter study gave possible causes for the higher range of values as bee species, handling practices and environmental humidity, although the former study narrowed the possibilities to the bee species, by collecting on the same day and from the same district. It is likely that some of the honeys in the present study may have originated from the indigenous Asian honeybees *A. dorsata* and *A. cerana* and so, with the exception of two samples from Vietnam, the moisture content difference is appropriate. Nevertheless, honeys for which the moisture content exceeded 20% have been separated in case the moisture content is due to some type of adulteration.

Because of the problems of chromatographic resolution of the very large number of di- and tri-saccharides present in honey and the difficulty and expense of obtaining

standards, many studies quantify only a representative sample of sugars. Gas chromatography of per-*O*-trimethylsilylated alditols gives good resolution but suffers from two drawbacks. Firstly, reduction of sugars with a fructose reducing end results in an epimeric pair of sugar alditols, thus complicating the chromatography. Secondly, because of symmetry considerations, reduction gives rise to three pairs of identical species which it is not possible to resolve. These are nigerose and the first peak of turanose, the second peak of turanose and the first peak of maltulose, and the second peak of maltulose and maltose. These pairs of peaks are therefore grouped in subsequent tables. Fructose and glucose cannot be distinguished by this method and so are combined and listed as monosaccharides.

The mean weight % of the sugars found in the Asian honey samples by country are given in Table 3, together with the global means and ranges.

Table 4 lists the sugar contents for honeys whose moisture contents exceeded 20%.

Several of these honeys also fell below the range specified by the Codex Alimentarius for monosaccharides of not less than 60g/100g;[9] two honeys whose moisture contents were within the specified range but whose monosaccharide sugar content fell below 60% are listed separately in Table 5.

Comparison of the data in Table 3 with the literature for honey samples from Europe, principally Spain,[8, 10, 11, 12, 13] North Africa,[14, 15] North America,[16] and South America[17] shows that the ranges for cellobiose, laminaribiose, gentiobiose and palatinose are displaced slightly higher than the literature and isomaltose and raffinose are slightly lower. A proper comparison is not possible as not all authors list all sugars and there is also considerable variation in the literature. 1-kestose, erlose and melezitose are lower than some of the literature; the latter two sugars are associated with honeydew honey,[18] and the lower values may indicate a lower contribution of this type of honey. The greatest difference is in the value for maltose: assuming that maltose is the sole contributor to the maltose + maltulose(2) peak, maltose has a mean value of 1.49 and a range of 1.04-2.03. This is considerably lower than observed in the literature with the exception of honeys from North America determined by HPLC.[16] The reasons for this are unclear, since methodologies in the literature vary; significantly lower levels of maltose were found in honeys from *A. dorsata* and *A. cerana* than in *A. mellifera*.[5] It is also possible that it relates to the current ready availability of corn syrups for feeding in Europe and the

Table 3. Mono- and oligosaccharides in Asian honey samples.

	China (n = 6)	India (n = 7)	Japan (n = 2)	Malaysia (n = 2)	Vietnam (n = 2)	Indonesia (n = 1)	Russia (n = 1)	Thailand (n=1)	South Korea (n = 1)	Mean	Std Dev	Range
Sugar	%w/w											
Monosaccharides	68.84	68.84	72.13	66.92	69.53	68.3	85.05	59.91	77.79	70.81	7.10	59.91–85.05
Sucrose	0.73	0.73	0.15	0.06	0.11	0.27	0.12	0.04	0.02	0.25	0.28	0.01–0.73
Trehalose	0.1	0.1	0.08	0.1	0.09	ND	0.05	0.12	0.02	0.08	0.03	0–0.12
Cellobiose	0.38	0.38	0.3	0.47	0.39	0.49	0.4	0.66	0.42	0.43	0.10	0.3–0.66
Laminaribiose	0.35	0.35	0.25	0.6	0.43	0.17	0.25	0.23	0.12	0.31	0.15	0.12–0.6
Nigerose + Turanose1	1.16	1.16	0.8	0.52	0.66	0.83	1.92	2.17	1.55	1.20	0.57	0.52–2.17
Turanose2 + Maltulose1	0.66	0.66	0.44	0.28	0.36	0.14	1.26	1.32	0.95	0.67	0.42	0.14–1.32
Maltulose2 + Maltose	1.39	1.39	1.45	1.31	1.38	1.04	1.66	2.03	1.76	1.49	0.29	1.04–2.03
Kojibiose	0.32	0.32	0.15	0.21	0.18	0.09	0.49	0.69	0.56	0.33	0.20	0.09–0.69
Melibiose	0.4	0.4	0.13	0.33	0.23	0.2	0.79	1.13	1	0.51	0.37	0.13–1.13
Gentiobiose	0.21	0.21	0.05	0.28	0.17	0.13	0.11	0.37	0.4	0.21	0.12	0.05–0.4
Palatinose	0.56	0.56	0.24	0.51	0.38	0.25	1.15	1.65	1.58	0.76	0.55	0.24–1.65
Isomaltose	0.37	0.37	0.56	0.77	0.67	ND	ND	0.09	ND	0.47	0.25	0–0.77
Raffinose	0.03	0.03	0.01	ND	0.01	ND	0.01	ND	ND	0.02	0.01	0–0.03
Kestose	0.12	0.12	0.08	0.02	0.05	0.01	0.18	0.11	0.15	0.09	0.06	0.01–0.18
Erlose	0.32	0.32	0.17	0.02	0.10	0.01	0.73	0.25	0.16	0.23	0.22	0.01–0.73
Melezitose	0.03	0.03	0.01	0.02	0.02	ND	0.07	0.05	0.05	0.03	0.02	0.01–0.07
Maltotriose	0.09	0.09	0.05	0.12	0.09	0.04	0.17	0.17	0.1	0.10	0.05	0.04–0.17
Panose	0.08	0.08	0.13	0.16	0.15	0.18	0.18	0.24	0.2	0.16	0.05	0.08–0.24
Isomaltotriose	0.01	0.01	0.01	0.03	0.02	0.04	0.04	0.03	0.02	0.02	0.01	0.01–0.04

Table 4. Mono- and oligosaccharides in Asian honey samples whose moisture content exceeded 20%.

	China	India	Vietnam 1	Vietnam 2	Vietnam 3	S. Korea[1,2]	Sri Lanka 1[2]	Sri Lanka 2[2]
Sugar								
Monosaccharides	57.43	57.43	57.79	63.29	56.16	52.82	77.30	85.75
Sucrose	0.96	0.96	0.22	0.10	0.03	1.19	0.06	0.06
Trehalose	0.03	0.03	0.08	0.03	0.02	0.06	ND	0.14
Cellobiose	0.15	0.15	0.32	0.16	0.26	0.35	0.47	0.71
Laminaribiose	0.21	0.21	0.13	0.29	0.05	0.26	0.18	0.78
Nigerose + Turanose1	0.50	0.50	0.59	0.60	1.13	0.78	2.63	2.00
Turanose2 + Maltulose1	0.22	0.22	0.08	0.34	0.57	0.49	1.67	1.41
Maltulose2 + Maltose	1.20	1.20	0.53	1.50	1.04	2.33	2.20	2.79
Kojibiose	0.14	0.14	0.05	0.28	0.28	0.28	0.82	0.56
Melibiose	0.18	0.18	0.08	0.20	0.24	0.47	1.03	0.71
Gentiobiose	0.07	0.07	0.07	0.08	0.08	0.21	0.11	0.34
Palatinose	0.28	0.28	0.11	0.44	0.01	0.46	1.70	0.79
Isomaltose	ND	ND	ND	0.32	ND	ND	ND	ND
Raffinose	0.01	ND	0.01	0.00	0.01	0.02	ND	ND
Kestose	0.14	ND	0.01	0.09	0.04	0.33	0.09	ND
Erlose	0.49	ND	0.02	0.05	ND	2.37	0.26	0.17
Melezitose	0.03	ND	ND	ND	0.00	0.11	0.09	0.03
Maltotriose	0.12	ND	0.02	0.07	0.03	0.29	0.18	0.24
Panose	0.04	ND	ND	0.14	0.05	0.16	0.21	0.15
Isomaltotriose	0.00	ND	ND	0.01	ND	ND	0.04	ND

[1] possibly a honeydew honey as it has elevated erlose content.

[2] These honeys have maltose contents that fall outside the range for other Asian honeys but not outside values quoted in the literature for Europe, N. Africa and S. America.

Americas and that the North American paper predates this practice.

Four of the samples were apparently adulterated: two from China and one (possibly) from the Phillipines, either by inappropriate feeding with sucrose or addition of sucrose syrup; this was deduced from the presence in these samples of a large sucrose peak.[4, 10] The sample from the Phillipines (which was labelled "pure honey") may possibly be a honeydew honey as it has an elevated erlose content, but the sucrose content (9.94%) is considerably higher than the 5% permitted by the Codex.[9] It should be noted, however, that a mean of 9.51% was found from colonies of *A. cerana* in the Phillipines;[6] and that in another study sucrose ranged to higher levels in the two Asian honeybees compared with *A. mellifera*.[5] One sample from Malaysia was adulterated and exhibited enlarged peaks for maltose (-6 % w/w) and maltotriose (~5%) probably due to addition of, or inappropriate feeding with starch syrups.[4, 19, 20] The presence of adulteration makes it difficult to accurately quantify minor oligosaccharides. The mean weight % of sugars in apparently adulterated samples by country is given in Table 6.

Acknowledgements

The authors would like to thank Zoran Sinovcic of MAF Biosecurity (New Zealand) for kindly supplying the honey samples.

References

1. Biosecurity New Zealand, *Import Risk Analysis: Honey Bee Products*, Ministry of Agriculture and Forestry: Wellington, New Zealand, 2004.

2. Ministry of Agriculture and Forestry, *Import Health Standard for Specified Processed Bee Products*: Wellington, New Zealand, 13 November 2006: Wellington.

3. Cotte J.F., Casabianca, H., Chardon, S., Lheritier, J., Grenier-Loustalot, M.F. *J.Chromatogr. A*, **2003**, *1021,* 145-155.

4. Cotte, J.F., Casabianca, H., Chardon, S., Lheritier, J., Grenier-Loustalot, M.F. *Anal. Bioanal. Chem.* **2004**, *380*, 698-705.

5. Joshi, S.R., Pechhacker, H., Willam, A., von der Ohe, W. *Apidologie* **2000**, *31*, 367-375.

6. Laude, V.T., Naegel, L., Horn, H. *Apidologie* **1991**, *22*, 371-380.

7. White, J.W. Jr. "Honey". In J.M. Graham, (Ed.), *The Hive and the Honey Bee* (pp. 869-925), Dadant & Sons: Hamilton, Illinois, 1992.

8. Sanz, M.L., González, M., de Lorenzo, C., Sanz, J., Martínez-Castro, I. *J.Sci.Food Agric.* **2004**, *84*, 1577-1584.

9. Codex Alimentarius Commission, *Revised Codex Standard for Honey. Codex STAN 12-1981*, FAO and WHO: Rome, 2001.

10. Ruiz-Matute, A.I., Weiss, M., Sammataro, D., Finely, J., Sanz, M.L. *J. Agric. Food Chem.* **2010**, *58*, 7317-7322; Ruiz-Matute, A.I., Brokl, M., Soria, A.C., Sanz, M.L., Martínez-Castro, I. *Food Chem.*

Table 5. Mono- and oligosaccharides in Asian honey samples whose monosaccharide content was less than the 60% prescribed by the Codex alimentarius but whose moisture content was within the specified range.

	China	Thailand
Sugar		
Monosaccharides	54.06	53.08
Sucrose	0.30	0.02
Trehalose	0.16	0.03
Cellobiose	0.19	0.60
Laminaribiose	0.23	0.24
Nigerose + Turanose1	0.76	2.13
Turanose2 + Maltulose1	0.64	1.21
Maltulose2 + Maltose	0.88	1.56
Kojibiose	0.14	0.62
Melibiose	0.20	1.03
Gentiobiose	0.03	0.38
Palatinose	0.23	1.57
Isomaltose	ND	ND
Raffinose	0.01	ND
Kestose	0.08	0.09
Erlose	0.94	0.06
Melezitose	0.09	0.03
Maltotriose	0.10	0.18
Panose	0.02	0.29
Isomaltotriose	0.00	0.05
Moisture content	18.9	17.6

Table 6. Mono- and oligosaccharides in Asian honey samples which were apparently adulterated

	China	China	Malaysia	Philippines[1]
Sugar	% w/w			
Monosaccharides	50.02	41.46	51.43	61.74
Sucrose	22.98	30.06	0.27	9.94
Trehalose	0.19	0.24	0.05	0.33
Cellobiose	0.24	0.27	0.53	0.13
Laminaribiose	0.09	0.26	0.17	0.37
Nigerose + Turanose1	0.21	0.31	0.72	1.59
Turanose2 + Maltulose1	0.18	0.20	0.35	1.11
Maltulose2 + Maltose	0.36	0.41	6.12	3.57
Kojibiose	0.09	0.08	0.17	0.27
Melibiose	0.18	0.17	0.30	0.26
Gentiobiose	0.15	0.16	0.29	0.10
Palatinose	0.15	0.19	0.43	0.32
Isomaltose	ND	ND	ND	ND
Raffinose	0.10	0.13	0.01	0.09
Kestose	0.08	0.11	0.01	0.69
Erlose	0.15	0.79	0.04	5.53
Melezitose	0.02	0.03	ND	0.11
Maltotriose	0.01	0.01	5.29	0.27
Panose	0.01	0.00	0.03	0.09
Isomaltotriose	ND	ND	ND	ND
Moisture content:	17.7	17.7	15.9	21.7

[1] Possibly a honeydew honey and possibly an *A. cerana* honey; see section 4.

2010, *120*, 637-642.

11. Sanz , M.L., Gonzalez, M., de Lorenzo, C., Sanz, J., Martínez-Castro, I. *Food Chem.* **2005**, *91*, 313-317.

12. Bentabol Manzanares, A., Hernández García, Z., Rodríguez Galdón, B., Rodríguez Rodríguez, E., Díaz Romero, C. *Food Chem.* **2011**, *126*, 664-672.

13. de la Fuente, E., Ruiz-Matute, A.I., Valencia-Barrera, R.M., Sanz, J., Martínez Castro, I. *Food Chem.***2011**,*129*, 1483-1489.

14. Terrab, A., Vega-Pérez, J.M., Diez, M.J., Heredia, F.J. *J.Sci.Food Agric.* **2001**, *82,* 179-185.

15. Ouchemoukh, S., Schweitzer, P., Bachir Bey, M., Djoudad-Kadji, H., Louaileche, H. *Food Chem.* **2010**, *121*, 561-568.

16. Swallow, K.W., Nicholas H., Low, N.H. *J.Agric.Food Chem.* ***1990***, *38*, 1828-1832.

17. Da Costa Leite, J.M., Trugo, L.C., Costa, L.S.M., Quinteiro, L.M.C., Barth, O.M., Dutra, V.M.L., De Maria, C.A.B. *Food Chem.***2000**, *70*, 93-98.

18. Astwood, K., Lee,B., Manley-Harris, M. *J. Agric. Food Chem.* **1998**, *46*, 4958-4962.

19. Cordella, C., Militão, J.S.L.T., Clément, M-C., Drajnudel, P., *Anal. Chim. Acta* **2005**, *531*, 239-248.

20 Megherbi, M., Herbreteau, B., Faure, R., Salvador, A. *J. Agric. Food Chem.* **2009**, *57*, 2105-2111.