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Determination of carbohydrates in honey

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ABSTRACT

Purpose: To develop an HPAE-PAD method for the determination of carbohydrates in honey samples to evaluate their quality and to assess the possibility of adulteration.

Methods: Separation of individual honey sugars was achieved on the recently introduced Thermo Scientific™ Dionex™ CarboPac™ PA210-Fast-4µm column. Carbohydrate detection was by pulsed amperometric detection (PAD) with a gold working electrode and, therefore, no sample derivatization was required.

Results: An HPAE-PAD method was successfully developed and validated for the sugar analysis of 12 commercial honey samples using a Dionex CarboPac PA210-Fast-4µm column. This column allows the separation of 15 sugars in honey with minimal sample preparation and an overall cycle time of 45 min.

INTRODUCTION

Honey is a complex mixture of sugars produced in nature by honeybees. The sugar composition of honey varies and is mainly dependent on its floral source. Fructose and glucose are the major components (~85–95%) of the honey's carbohydrates. The remaining carbohydrates are a mixture of di-, tri-, and several larger oligosaccharides. The sugars present at low concentrations are useful for the determination of floral origin.

We developed a high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) method to measure and quantify the mono-, di-, and trisaccharides in honey. Separation of honey sugars was achieved on the recently introduced Dionex CarboPac PA210-4µm column, which provides fast, high-resolution separations for most mono- through tetrasaccharides using a KOH or NaOH mobile phase. Prior HPAE-PAD methods for honey carbohydrate analysis required sodium acetate-containing mobile phases. Carbohydrate detection was by PAD; and therefore, no sample derivatization was required. This method resolved 15 honey sugars in 45 min, injection to injection. PAD is sensitive thus allowed the determination of low concentration carbohydrates in honey, while at the same time detecting the high concentrations of the major components, glucose and fructose. These properties allowed us to show differences in a collection of honey samples and show that HPAE-PAD profiling could detect a 10–20% addition of industrial sugar syrups (adulteration) to honey.

For more details please refer to Thermo Scientific Application Note 1158 in the Thermo Scientific AppsLab Library of Analytical Applications.[1]

MATERIALS AND METHODS

Table 1. List of honey samples used in this study.

Honey	Floral source	Honey	Floral source	Honey	Floral source
HS1	Clover	HS5	Clover	HS9	Clover
HS2	Clover, sunflower and alfalfa	HS6	Mixed	HS10 *	Wildflower
HS3 *	Wildflower	HS7	Manuka tree	HS11 *	Blackberry blossoms
HS4	Manuka tree	HS8	Mixed	HS12 *	Mixed

* - local beekeeper honey

Preparation of honey samples: Dissolve 0.1 g of honey sample in 100 mL of DI water to achieve a 1:1000 fold dilution. Store the honey sample at -20 °C. Filter through a 0.2 µm filter before analysis.

Preparation of adulterated honey samples: Six commercial honey samples and five commercial sugar syrup samples are used. Dilute the honey samples and sugar samples (1:3000 fold) by dissolving 0.1 g sample in 300 mL DI water. Then mix the sugar syrup sample and honey sample in a 20:80 ratio. For honey sample HS6 we mixed the sugar syrup sample and honey sample in a 10:90 ratio. Filter through a 0.2 µm filter before analysis.

Table 2. Method conditions.

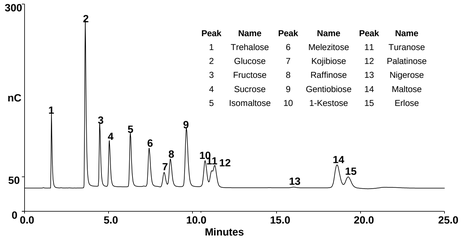
System	Dionex ICS-5000⁺ HPIC System
Column	Dionex CarboPac PA210-4µm + guard
Eluent source	Dionex EGC 500 KOH
Eluent	0–25 min: 30 mM KOH 25–30 min: 100 mM KOH (Wash step) 30–45 min: 30 mM KOH (equilibration step)
Temp.	30 ºC
Flow rate	0.8 mL/min
Inj. Vol.	10 µL
Detection	PAD (Au) Disposable Waveform A (TN21)

RESULTS

Separation

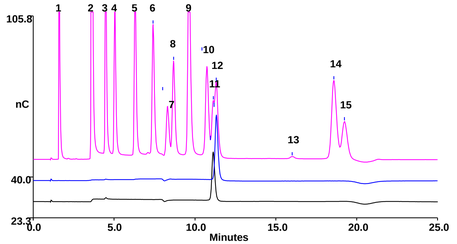
Honey sugars were separated using a Dionex CarboPac PA210-Fast-4µm column (150 × 4 mm) in series with a Dionex CarboPac PA210 guard column (50 × 4 mm). The column selectivity allow carbohydrates to be separated with only a hydroxide eluent generated using an eluent generator. A solution of honey sugar standards was prepared and an aliquot (10 µL) of the solution was injected onto the column and eluted at 0.8 mL/min with 30 mM hydroxide. Figure 1 displays the chromatogram of the honey sugars standard showing the separation of 15 sugar standards in a single run. Of the 15 sugars, two are monosaccharides (glucose and fructose), nine are disaccharides (trehalose, sucrose, kojibiose, gentiobiose, turanose, palatinose, nigerose, isomaltose and maltose), and four are trisaccharides (melezitose, raffinose, 1-kestose, and erlose).

Figure 1. Chromatogram of 15 honey sugar standards mix.



All 15 honey sugars were separated within 25 min with good resolution, except turanose/palatinose, which are poorly resolved under these conditions. It is noteworthy that standards containing turanose (Figure 2) showed a slight increase of the baseline (~ 0.5 nC) around 4 min. This is due to partial on-column hydrolysis of turanose to fructose and glucose, resulting in the baseline rise at the retention times where they elute. This effect was observed earlier and with additional experiments it was demonstrated that the potential impact on the determination of turanose, glucose, and fructose was negligible.[2]

Figure 2. Chromatogram of 5 mg/L turanose, 5 mg/L palatinose , and 15 honey sugar mix standards.

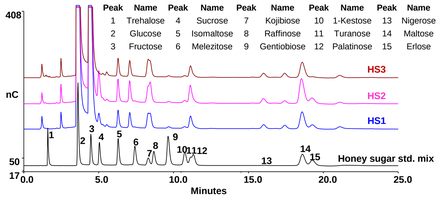


SAMPLE ANALYSIS

Honey sugar analysis

For this study, we purchased 12 commercial honey samples (Table 1) and analyzed them using HPAE-PAD. Figure 3 shows the representative chromatograms of 3 honey samples. For all 12 investigated honey samples, fructose and glucose (Peak 2 and Peak 3), were found to be the major constituents.

Figure 3. Chromatogram of honey samples (HS1- HS3) along with 15 sugar standard mix.



Quantification was performed with 13 sugar standards; two monosaccharides, seven disaccharides , and four trisaccharides. The calibration curves for these 13 sugars were plotted and concentrations were determined for all 12 honey samples (Table 3).

Table 3. Amount (g/100g) of monosaccharides, disaccharides and trisaccharides in honey samples (HS1-HS12).

Samples	HS1	HS2	HS3	HS4	HS5	HS6	HS7	HS8	HS9	HS10	HS11	HS12
	Monosaccharides (g/100g)											
Glucose	39.5	36.8	37.9	34.3	35.7	35.5	26.8	37	35.6	34.9	34.7	35.7
Fructose	41.9	41.1	39.9	38	37.9	37.8	36.9	38.1	38.1	39.3	38.3	36
F+G	81.4	77.9	77.8	72.3	73.6	73.2	63.7	75.1	73.7	74.2	73	71.8
F/G ratio	1.06	1.12	1.05	1.11	1.06	1.07	1.37	1.03	1.07	1.13	1.1	1.01
	Disaccharides (g/100g)											
Trehalose	0.016	0.011	0.027	0.017	0.013	0.031	0	0.899	0.014	0.317	0.09	0.025
Sucrose	0.668	1.82	0.032	0.351	1.71	0.118	0.489	0.025	1.33	0.852	0.219	0.609
Isomaltose	0.623	0.817	0.639	0.98	0.68	0.575	1.52	0.639	0.541	0.874	1.16	0.282
Gentiobiose	0.04	0.032	0.033	0.097	0.057	0.093	0.125	0.021	0.035	0.052	0.037	0.06
Turanose	1.48	2.45	1.46	1.97	1.79	1.37	2.12	1.5	1.49	1.38	1.67	0.878
Palatinose	0.186	0.26	0.12	0.268	0.26	0.186	0.433	0.191	0.17	0.224	0.21	0.075
Maltose	1.99	3.01	1.77	1.1	1.77	1.79	1.99	1	2.24	2.29	1.37	1.67
	Trisaccharides (g/100g)											
Melezitose	0.014	0.07	0.005	0.119	0.011	0.003	0.142	0.015	0.009	0.173	0.081	0
Raffinose	0	0	0	0.476	0	0	0.41	0	0	0.155	0	0
1-Kestose	0.125	0.138	0.091	0.143	0.135	0.203	0.23	0.082	0.128	0.098	0.101	0.147
Erlose	0.852	1.172	0.266	0.642	0.867	0.585	2.261	0.186	0.795	0.205	0.382	0.284

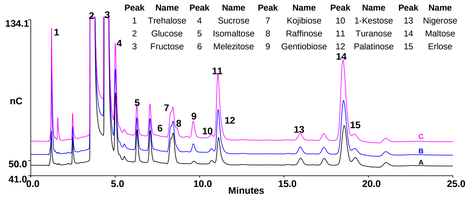
- In almost all types of honey, fructose is the carbohydrate in greatest proportion, and the ratio of fructose to glucose (F/G) is greater than 1.
- The sum of fructose and glucose for the honey samples used in this study all exceeded the limit required by the Codex; i.e., 60g/100 g.[3]
- The disaccharide and trisaccharide profiles of honey depend upon the sugars and the enzymes present in the bee and nectar.
- Among disaccharides, maltose was the main component in the majority of the honey samples followed by turanose, sucrose, and isomaltose. The sucrose contents of the honey samples ranged from 0.03 to 1.82 g/100 g. For two of the honey samples in our study; HS3 (a local bee keeper honey) and HS8, the sucrose content was very low or not detected.
- The values obtained for sucrose contents of the honey samples in this study were all within the limits of international standards (<5 g/100 g sucrose)[3].
- For trisaccharides, four sugars were analyzed and quantitated i.e., melezitose, raffinose, 1-kestose, and erlose. Out of the 12 honey samples, HS4, HS7, and HS10 had higher percentages of trisaccharides.

METHOD ACCURACY

Sample Recovery

Method accuracy was evaluated by measuring recoveries of 10 sugar standards spiked into honey samples. For spiking experiments, four honey samples were used (HS7–HS10) and spiked with a 10-sugar standard mix at two concentration levels. Figure 4 shows the representative chromatograms of unspiked and spiked honey sample HS7.

Figure 4. Chromatogram of A) unspiked HS7 and B) spiked HS7 at spike level 1, and C) spiked HS7 at spike level 2.



The recovery percentages were calculated using the formula shown below:

$$Recovery\ \% = (C_{spiked\ sample} - C_{unspiked\ sample}) / (C_{analyte\ added}) \times 100$$

Table 4. Spike recovery results for honey sample HS7, HS8, HS9, and HS10.

Peak Name	HS7		HS8		HS9		HS10	
	at spike level 1	at spike level 2	at spike level 1	at spike level 2	at spike level 1	at spike level 2	at spike level 1	at spike level 2
Trehalose	94.3	95.2	95.9	96.4	82.3	101	105	100
Glucose	81.8	79.5	86.9	84.1	84.7	84.1	81.1	78.5
Fructose	81	80.9	88.9	89.3	82.9	82.9	84.5	78.2
Sucrose	113	94.2	112	98.3	107	106	79.1	84.4
Raffinose	95.2	98.5	104	110	89.6	98.4	103	103
Gentiobiose	102	96.3	106	103	107	105	106	101
Turanose	93.9	94.6	98.1	101	96.9	96	96.5	95.2
Palatinose	103	78.9	86.2	104	89.8	92.2	96.5	83.9
Maltose	85.5	102	79.8	88.7	102	96.7	101	94.3

Table 4 lists the percentage recovery results for honey samples HS7 through HS10. For all of the four honey samples spiked, recoveries were in the range of 78.2–113%.

HONEY ADULTERATION

For adulteration experiments, we used five different sugar syrup samples (Table 5). Six honey samples (HS1-6) were adulterated with all five sugar syrups at 20% and 10%. Table 6 lists the adulteration parameters for honey sample HS1, adulterated with 20% of three sugar syrups.

Table 5. Sugar profile of five sugar syrups used in this study.

Designation	Type	Glucose (ppm)	Fructose (ppm)	Sucrose (ppm)	Maltose (ppm)
SS1	Corn syrup	51.5	<1	28.3	35.1
SS2	Corn syrup	62.1	<5	6.10	42.8
SS3	Corn syrup	65.8	<5	6.53	46.3
SS4	Beet syrup	76.6	74.2	42.3	0.00
SS5	Maple syrup	<5	<5	59.7	0.00

Table 6. Adulteration parameters for honey sample HS1.

Adulteration Parameters	HS1			
	100% Honey	+ 20% SS1	+ 20% SS2	+ 20% SS3
Glucose(G)	132	121	125	126
Fructose(F)	140	115	120	120
F/G ratio	1.06	0.95	0.96	0.95
Sucrose (S)	2.22	10.9	2.68	2.7
Turanose(T)	4.95	3.45	3.81	3.33
S/T ratio	0.45	3.16	0.71	0.81
Maltose(M),	6.64	9.62	11.37	11.27
S/M ratio	0.33	1.13	0.24	0.24

Table 7. Adulteration parameters for HS6 adulterated with 10% SS1 through SS5.

Adulteration Parameters	HS6 (Wild Mountain Honey)					
	100% Honey	+ 10% SS1	+ 10% SS2	+ 10% SS3	+ 10% SS4	+ 10% SS5
Glucose(G), mg/L	121	115	116	117	119	107
Fructose(F), mg/L	127	115	115	116	126	116
F/G ratio	1.04	1.00	1.00	0.99	1.06	1.09
Sucrose(S), mg/L	0.37	5.08	0.68	0.90	9.23	11.85
Turanose(T), mg/L	4.99	4.43	4.50	4.40	4.44	4.41
S/T ratio	0.07	1.15	0.15	0.20	2.08	2.68
Maltose(M), mg/L	5.96	8.47	8.82	9.11	5.48	5.48
S/M ratio	0.06	0.60	0.08	0.10	1.69	2.16

- The fructose/glucose ratio decreased and is less than 1 for these samples, indicating adulteration.
- Upon addition of sugar syrups the amount of sucrose increased in all honey samples (HS1 through HS6).
- Similarly, maltose content increased, while turanose decreased upon addition of sugar syrup.
- For the 10% level, the amount of fructose and glucose decreased on addition of all sugar syrups except SS4. The F/G ratio decreased on addition of SS1, SS2, and SS3, but increased slightly with SS4 and SS5. This is due to the fact that SS4 has a higher amount of fructose than glucose. In SS5, both glucose and fructose are present in small amounts, thus the F/G ratio is due primarily to unadulterated honey. The amount of sucrose increased upon addition of sugar syrup samples and a significant increase was seen with SS4 and SS5.
- The S/T ratio increased, but the increase is almost 10 times higher upon addition of SS4 and SS5, in comparison to the corn syrups (SS1, SS2, and SS3).

CONCLUSIONS

- Developed a 45 min HPAE-PAD method for honey sugar analysis that requires only a hydroxide eluent, which can be prepared automatically, with no sample derivatization required.
- The method was validated with 12 commercial honey samples.
- The method showed good precision and accuracy with a recovery range of 78.2–113%.
- The method can authenticate honey and is able to detect adulteration with 10% of a commercial sugar syrup.

REFERENCES

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3. Codex standard 12, revised Codex Standard for Honey. Standards and Standard Methods. Codex Alimentarius Committee on Sugars. 2001, 11, 1–7.

TRADEMARKS/LICENSING

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