

Analysis Report Honey-Profiling™

Sample ID: Killer Bees 2023-2023-08-01

Information/Declaration provided by customer:

Customer: **515**
 Customer Sample ID: Killer Bees 2023
 Type of Sample: Honey
 Type of Honey: Unknown
 Botanical Variety: undefined
 Geographical Origin: undefined

Disclaimer: this information will affect the applicability and validity of analyses and results.

Note: it is important to fill in these information in a correct and precise manner (e.g. variety in case of monofloral honey, and country of origin). The tests applied (and therefore the results received) are different from one type of honey to the other. Bruker does not take responsibility for wrong or incomplete information given by the customer.

Measuring Date: 01-Aug-2023 21:10:39
 Reporting Date: 02-Aug-2023 06:19:45, 13 pages, Version 3.1.2

Results Summary




















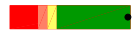















Type of Analysis	Result	Status
Origin, Type and Variety		
Type of Honey	Honeydew	○
Botanical Variety	Not Applied	○
Country of Origin	Not Applied	○
Test of Markers of Foreign Sugars	Conditional Pass	●
Composition and Comparison to Reference Group	Requires Interpretation	●
Compliance for EU Market for		
HMF	Compliant	●
Sucrose	Compliant	●
Glucose + Fructose	Compliant	●





The data analysis is performed at Bruker BioSpin GmbH (Ettlingen, Germany) according to testing method AA-72-03-17 (Honey-Profiling 3.1.2), released on 09-May-2023 (DIN EN ISO/IEC 17025:2018 Accreditation Certificate D-PL-19229-01-00). All results solely refer to the tested sample as provided by the customer.

Test of Markers of Foreign Sugars

(Analysis-ID: HO-TAD-01/0833)

Following tests have been applied in order to detect foreign sugars:

ID	Description	Value	Graphics	Result
2	3.8113 / (1.95-2.02)	31.30		pass
3	3.9238 / (3.16-4.14)	0.0005903		pass
4	3.2890 / (1.95-2.02)	3.727		pass
5	4.0363 / (3.75-3.80)	0.00793		pass
6	5.3324 / (5.27-5.30)	0.1160		pass
7	3.0123 / (1.95-2.02)	0.02725		pass
8	3.5929	1146		pass
9	5.0745 / (4.07-4.12)	0.005664		pass
10	3.2753	249.1		pass
21	3.1792 / (5.20-5.25)	0.01096		pass
22	2.1912 / (5.20-5.25)	0.000362		pass
23	5.0929 / (1.95-2.02)	0.2520		pass
24	4.0566 / (3.20-3.22)	0.04799		pass
149	2.7405 / (1.95-2.02)	0.0455		pass
150	4.5705	63.9		pass
151	4.4264 / (5.20-5.25)	0.002103		pass
152	4.9500 / (4.93-4.97)	0.0837		pass
153	4.9658 / (4.93-4.97)	0.04186		conditional pass
155	4.1511	459.9		pass
156	3.3701 / (3.20-3.22)	0.09731		pass
157	4.1781 / (4.07-4.12)	0.0010267		pass
158	3.9283 / (5.27-5.30)	0.0964		pass
159	3.9246 / (5.20-5.25)	0.02053		pass
162	3.1784 / (5.20-5.25)	0.006577		pass
163	3.5929 / (3.16-4.14)	0.0004830		pass
166	3.6627 / (4.07-4.12)	0.04751		pass
167	3.2977 / (4.07-4.12)	0.004342		pass
168	3.3753 / (4.60-4.67)	0.1627		pass
169	3.7579 / (3.65-3.73)	0.11331		pass
170	3.8885 / (3.75-3.80)	0.05496		pass
171	4.1651 / (1.95-2.02)	2.948		pass
172	3.1637	47.375		pass
192	4.0570	1153		pass
1000	min. fructose/g glucose	1.39		pass
1001	max. fructose/g glucose	1.39		pass



ID	Description	Value	Graphics	Result
1002	turanose [g/100g]	1.64		pass
1003	sucrose [g/100g]	1.8		pass
1004	total sugar [g/100g]	61.2		pass
1005	proline [mg/kg]	236		pass
1006	DHA(D), mannose(M) [mg/kg]	D=1.7, M=120		pass

Result: There is no indication for the presence of foreign sugars. However, for one or more markers the determined values are close to the threshold.

Notes:

- The column *ID* is the marker's identification number.
- The column *Description* indicates either the NMR spectral region(s) (in ppm) concerned by the marker, or the molecule when it has been identified.
- The column *Value* is the result obtained for the marker.
- For columns *Graphics* and *Result* please refer to detailed description in section "General Remarks" at the end of this report.

DHA and Mannose

Compound	Value	Unit	LOQ	Reference Range	Flag
mannose	<LOQ	g/100g	0.02	<0.02  0.06	●
dihydroxyacetone (DHA)	<LOQ	mg/kg	5	<5  16	●

Note: the reference range is based on *1993 Honeydew* samples in the Honey-Profiling Database.

Guideline:

- Mannose is a mono saccharide not found in honeys with a pH value lower than 5, but that is regularly found in industrial sugars. In rare cases, however, the presence of mannose cannot be excluded for certain geographic origins and/or botanical varieties. A concentration of mannose exceeding 0.02 g/100g in honey with a pH < 5 could indicate the presence of foreign sugars or industrial processing practices which are not suitable for honey. An expert interpretation is suggested when mannose is present in the honey.
- Dihydroxyacetone and/or methylglyoxal are only known to be naturally present in *Leptospermum* genus honeys from Australia and New Zealand. A concentration exceeding 5 mg/kg in other types of honey is not typical and could indicate the presence of foreign sugars or industrial processing practices which are not suitable for honey. An expert interpretation is suggested in such cases.
- DHA and mannose are often observed simultaneously in various types of sugar syrups.

Deviations in the sugar profile, fermentation parameters and comparison to reference group could also indicate adulterations. Please check the section "Composition and Comparison to reference group" in addition.




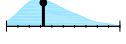
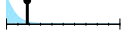

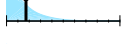




Composition and Comparison to Reference Group

Quantitative Analysis of Compounds

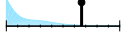


(Analysis-ID: HO-Q/3.1.0)

Parameters labelled with * are calculated parameters. The reference range is based on *1993 Honeydew* samples in the Honey-Profiling Database.

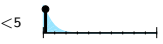

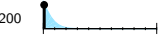
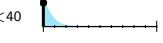
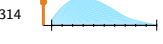
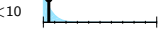


Sugars:

Compound	Value	Unit	LOQ	Reference Range	Flag
glucose + fructose *	61.2	g/100g	20.0	47.9  77.6	●
fructose / glucose *	1.39	-	-	1.02  1.75	●
fructose	35.6	g/100g	10.0	27.5  43.6	●
glucose	25.6	g/100g	10.0	19.9  37.4	●
sucrose	1.8	g/100g	0.5	<0.5  7.7	●
turanose	1.64	g/100g	0.2	0.6  2.8	●
maltose	2.5	g/100g	0.5	<0.5  12.1	●
melezitose	<LOQ	g/100g	1.0	<1.0  13.1	●
maltotriose	<LOQ	g/100g	1.0	<1.0  3.1	●
gentiobiose	<LOQ	g/100g	0.3	<0.3  0.6	●
raffinose	1.8	g/100g	0.1	0.1  2.7	●

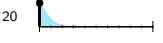
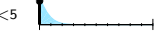
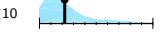
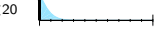

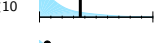
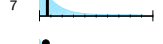


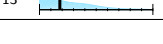
Acids:

Compound	Value	Unit	LOQ	Reference Range	Flag
citric acid	631	mg/kg	50	<50  926	●
malic acid	273	mg/kg	100	<100  1396	●
quinic acid	<LOQ	mg/kg	300	<300  4281	●




Amino Acids:

Compound	Value	Unit	LOQ	Reference Range	Flag
alanine	21	mg/kg	5	<5  829	●
aspartic acid	177	mg/kg	150	<150  320	●
glutamine	208	mg/kg	200	<200  1271	●
leucine	<LOQ	mg/kg	40	<40  612	●
proline	236	mg/kg	200	314  1302	●
valine	16	mg/kg	10	<10  140	●
tyrosine	<LOQ	mg/kg	50	<50  616	●
phenylalanine	<LOQ	mg/kg	100	<100  1127	●

Indicators for Fermentation and Processing:

Compound	Value	Unit	LOQ	Reference Range	Flag
2,3-butanediol	<LOQ	mg/kg	20	<20  529	●
5-hydroxymethylfurfural (HMF)	<LOQ	mg/kg	5	<5  159	●
acetic acid	66	mg/kg	10	<10  262	●
acetoin	<LOQ	mg/kg	20	<20  112	●
ethanol	<LOQ	mg/kg	5	<5  2125	●
lactic acid	254	mg/kg	10	<10  687	●
formic acid	88	mg/kg	5	7  1173	●
fumaric acid	12	mg/kg	5	<5  123	●
pyruvic acid	15	mg/kg	10	11  39	●
succinic acid	119	mg/kg	5	13  602	●

Markers:

Compound	Value	Unit	LOQ	Reference Range	Flag
3-phenyllactic acid	<LOQ	mg/kg	300	<300  818	●
methylglyoxal (MGO)	<LOQ	mg/kg	30	<30 mg/kg in reference dataset	●
kynurenic acid	<LOQ	mg/kg	60	<60  593	●
shikimic acid	647	mg/kg	80	<80  1837	●

Guideline:

- Values of fructose/glucose ratio exceeding 1.95 or below 0.85 are not typical for honey and could indicate the presence of sugar syrups.
- Low concentrations of turanose (less than 0.35 g/100g) is a marker for the presence of foreign sugars (see section "Markers of Foreign Sugars").
- Atypical concentrations of sugars in comparison to reference range can be related to foreign sugars/adulteration or to the botanical varieties present in the honey. Expert interpretation is suggested.
- The presence of gentiobiose is common for Linden Tree honey.
- The presence of quinic acid is common for honeydew.
- HMF is a sugar degradation product formed under influence of heat. According to EU-Directive (2001/110/EC), HMF can be found naturally in honey in concentrations up to 80 mg/kg in regions with tropical climate. A higher concentration of HMF is due to processing of honey which can sometime correlate with adulterations. A concentration of HMF exceeding 200 mg/kg should be regarded as suspicious.
- Concentration of ethanol exceeding 400 mg/kg indicates fermentation of the honey, which could be related to unripe honey.
- Acetoin can be elevated in Eucalyptus honey.
- The presence of kynurenic acid is common for Chestnut honey.
- The presence of shikimic acid is common for honeydew.

Codex Alimentarius and EU-Directive 2001/110/EC:

Following parameters are required according to Codex Alimentarius and EU-Directive 2001/110/EC. The concentrations are obtained by direct quantification. Parameters labelled with * are calculated parameters.

Compound	Value	Unit	LOQ	Official Reference		
				min	max	Flag
glucose + fructose *	61.2	g/100g	20.0	45	-	●
sucrose	1.8	g/100g	0.5	-	15	●
5-hydroxymethylfurfural (HMF)	<LOQ	mg/kg	5	-	80	●

Following flags are used according to Codex Alimentarius and EU-Directive 2001/110/EC:

Compound	Flag	Concentration	Declaration	Interpretation
glucose + fructose	●	< 45 g/100g	All	Not compliant
		< 60 g/100g	Blossom	Not compliant for blossom honey
	●	≥ 60 g/100g	All	Compliant
	●	≥ 45 g/100g	Honeydew	Compliant for honeydew honey
sucrose	●	≥ 45 g/100g, < 60 g/100g	Unknown	Compliant for honeydew honey and blends of honeydew honey with blossom honey. Not compliant for blossom honey.
	●	> 15 g/100g	All	Not compliant
		10-15 g/100g	Acacia, Eucalyptus	Not compliant for false acacia (<i>Robinia pseudoacacia</i>), and red gum (<i>Eucalyptus camadulensis</i>)
	●	≤ 5 g/100g ≤ 10 g/100g	All Acacia, Eucalyptus	Compliant Compliant for false acacia (<i>Robinia pseudoacacia</i>), and red gum (<i>Eucalyptus camadulensis</i>)
HMF	●	≤ 15 g/100g 5-10 g/100g	Lavender All, except Acacia, Eucalyptus, Lavender	Compliant for <i>Lavandula spp.</i> If ≤ 15g/100g: compliant for lavender (<i>Lavandula spp.</i>) and borage (<i>Borago officinalis</i>). If ≤ 10g/100g: compliant for false acacia (<i>Robinia pseudoacacia</i>), alfalfa (<i>Medicago sativa</i>), Menzies Banksia (<i>Banksia menziesii</i>), French honeysuckle (<i>Hedysarum</i>), red gum (<i>Eucalyptus camadulensis</i>), leatherwood (<i>Eucryphia lucida</i> , <i>Eucryphia milliganii</i>) and <i>Citrus spp.</i>
	●	> 80 mg/kg	All, except Industrial honey	Not compliant, except for baker's honey
HMF	●	≤ 40 mg/kg	All	Compliant
	●	> 80 mg/kg	Industrial honey	Compliant for baker's honey
	●	40-80 mg/kg	All	Not compliant, except for baker's honey and honeys of declared origin from regions with tropical climate and blends of these honeys

General Remarks

Targeted Markers for Foreign Sugars

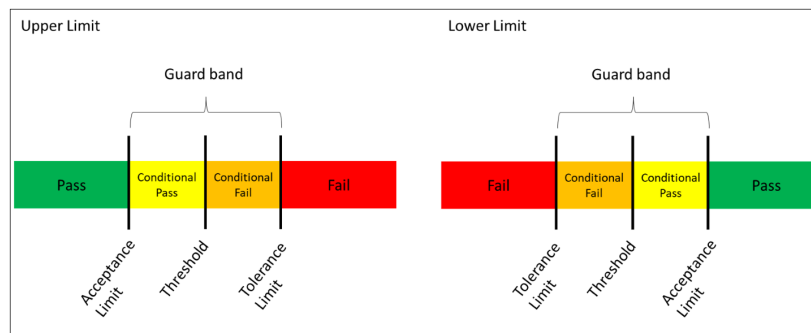
This test aims at detecting foreign sugars. It does rely on the analysis of the concentrations of certain known molecules and on absolute intensities or intensities ratios of marker peaks. The Honey-Profiling report contains several tens of markers. Some of these markers are independent on the variety and the origin, in order to be able to analyze blends, while there are also many markers specific to countries or varieties.

The values obtained for each marker are compared to the thresholds for purity.

Reported and visualized results:

- Result Pass (green):**
 Markers with an upper limit: The value of the marker is below the acceptance limit
 Markers with a lower limit: The value of the marker is above the acceptance limit
 Conclusion: There are no indications for the presence of foreign sugars.
- Result Conditional Pass (yellow):**
 The value of the marker is between the acceptance limit and the threshold
 Conclusion: There is no indication for the presence of foreign sugars. However, for one or more markers the determined values are close to the threshold.
- Result Conditional Fail (orange):**
 The value of the marker is between the threshold and the tolerance limit
 Conclusion: There is a strong indication for the presence of foreign sugars. However, the determined values are close to the threshold. It is recommended to perform additional tests in order to better evaluate the risk.
- Result Fail (red):**
 Markers with an upper limit: The value of the marker is above the tolerance limit
 Markers with a lower limit: The value of the marker is below the tolerance limit
 Conclusion: There is a strong indication for the presence of foreign sugars.

The black dot represents the current sample.



Presence of foreign sugars is due to deliberate dilution of honey with sugar syrups or wrong beekeeping practices in terms of artificial feeding of bees.

All conformity statements in this section are based on the "Non Binary Statement with Guard Band" according to ILAC G8:09/2019. Please find in the following table the values for acceptance limit (conditional pass), threshold (conditional fail) and tolerance limit (fail).

ID	Description	Conditional Pass	Conditional Fail	Fail
2	3.8113 / (1.95-2.02)	>68.95	>69.44	>69.92
3	3.9238 / (3.16-4.14)	<0.0003172	<0.0003039	<0.0002905
4	3.2890 / (1.95-2.02)	>5.792	>5.838	>5.884
5	4.0363 / (3.75-3.80)	>0.01113	>0.01131	>0.01148
6	5.3324 / (5.27-5.30)	>0.1683	>0.1703	>0.1723
7	3.0123 / (1.95-2.02)	>0.06929	>0.07134	>0.07340

ID	Description	Conditional Pass	Conditional Fail	Fail
8	3.5929	>2186	>2227	>2269
9	5.0745 / (4.07-4.12)	<0.001662	<0.001495	<0.001329
10	3.2753	>407.8	>414.9	>422.0
21	3.1792 / (5.20-5.25)	>0.01528	>0.01555	>0.01582
22	2.1912 / (5.20-5.25)	>0.001219	>0.001232	>0.001246
23	5.0929 / (1.95-2.02)	>0.4552	>0.4604	>0.4656
24	4.0566 / (3.20-3.22)	>0.06636	>0.06846	>0.07057
149	2.7405 / (1.95-2.02)	>0.1724	>0.1759	>0.1794
150	4.5705	>159.8	>171.2	>182.7
151	4.4264 / (5.20-5.25)	>0.005618	>0.005757	>0.005896
152	4.9500 / (4.93-4.97)	>0.1059	>0.1085	>0.1111
153	4.9658 / (4.93-4.97)	>0.04147	>0.04202	>0.04257
155	4.1511	<307.6	<268.2	<228.8
156	3.3701 / (3.20-3.22)	<0.07742	<0.07561	<0.07380
157	4.1781 / (4.07-4.12)	<0.0004571	<0.0003843	<0.0003115
158	3.9283 / (5.27-5.30)	>0.4156	>0.4226	>0.4295
159	3.9246 / (5.20-5.25)	<0.01303	<0.01167	<0.01031
162	3.1784 / (5.20-5.25)	<0.002753	<0.002590	<0.002427
163	3.5929 / (3.16-4.14)	>0.0008186	>0.0008317	>0.0008449
166	3.6627 / (4.07-4.12)	<0.02052	<0.02000	<0.01949
167	3.2977 / (4.07-4.12)	<0.002394	<0.002260	<0.002126
168	3.3753 / (4.60-4.67)	>0.1892	>0.1935	>0.1977
169	3.7579 / (3.65-3.73)	<0.08739	<0.08315	<0.07891
170	3.8885 / (3.75-3.80)	<0.03880	<0.03725	<0.03570
171	4.1651 / (1.95-2.02)	>6.362	>6.421	>6.480
172	3.1637	<10.563	<8.554	<6.544
192	4.0570	>1290	>1355	>1419
1000	min. fructose/glucose	<0.90	<0.85	<0.80
1001	max. fructose/glucose	>1.90	>1.95	>2.00
1002	turanose [g/100g]	<0.38	<0.35	<0.32
1003	sucrose [g/100g]	>14	>15	>16
1004	total sugar [g/100g]	<46	<45	<44
1005	proline [mg/kg]	<171	<160	<149
1006	DHA(D), mannose(M) [mg/kg]	D>3, M>150	D>5, M>200	D>7, M>250

Classification Models

Analysis of origin, type and variety relies on a statistical classification analysis. The test applied is a classification analysis with the aim to check the consistency of the declared meta-information of the sample (geographical origin or botanical variety). The consistency with a group is expressed as posterior probability in the range from 0% to 100%. A posterior probability exceeding 50% is being regarded as consistent with the respective group. The underlying statistical models are based on a dimension reduction (Principal Component Analysis and/or Linear Discriminant Analysis used) followed by a Linear (or Quadratic) Discriminant Analysis for final classification.

Within the discrimination space figure, the ellipsoids are representing the modeling samples and the star represents the actual sample under investigation.

Verification of origin is not possible on blends from different countries.

Expert interpretation is necessary before deducing any conclusions.

All conformity statements in this section are based on the "Binary Statement for Simple Acceptance Rule" according to ILAC-G8:09/2019.

Quantitative Analysis

Quantitative values are compared with the distribution of concentration of the reference samples in the Honey-Profiling Database, for the same type of honey. Deviations to the reference range can be linked with adulterations or with specificities of the honey (e.g. untypical floral/ plant sources or production regions). For this reason, an expert interpretation is suggested in case of deviations.

All conformity statements in this section are based on the "Binary Statement for Simple Acceptance Rule" according to ILAC-G8:09/2019.

Univariate and Multivariate Verification Models

Verification models are non-targeted analyses comparing the whole NMR-Profile of a specific sample with one corresponding group of reference spectra (within the Honey-Profiling Database). All spectral data points are taken into account irrespective of whether the signals are caused by already identified molecules or not.

In the univariate analysis, the NMR spectrum is checked for any unusual low or high signal intensities, while taking into account the natural variability of a respective reference group. The chemical shifts (positions of the signals in the spectra) of the deviating signals are indicated. A guideline gives a list of possible molecules with their chemical shifts that could be responsible for the deviations.

The multivariate models take into account the relation between different signals in the NMR spectrum.

Deviations to the group of reference spectra can be linked with adulterations or specificities of the honey (e.g. untypical floral/ plant sources or production regions). For this reason, an expert interpretation is suggested in case of deviations.

All conformity statements in this section are based on the "Binary Statement for Simple Acceptance Rule" according to ILAC-G8:09/2019.

FAQ - Frequently asked Questions

For more information please visit our website and read our FAQ at

<http://www.bruker.com/en/products-and-solutions/mr/nmr-food-solutions/honey-profiling/honey-profiling-faq.html>

END OF REPORT