

Analysis Report Honey-Profiling™

Sample ID: 2463-2022-10-13

Information/Declaration provided by customer:

Customer: **515**
 Customer Sample ID: Killer Bees Honey Wildflower
 Type of Sample: Honey
 Type of Honey: Blossom
 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: 14-Oct-2022 15:33:40
 Reporting Date: 15-Oct-2022 00:42:54, 11 pages, Version 3.0.5

Results Summary

Type of Analysis	Result	Status
Origin, Type and Variety		
Botanical Variety	Not Applied	○
Country of Origin	Not Applied	○
Markers of Foreign Sugars	No	●
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-72-03-14 (Honey-Profiling 3.0.5), released on 11-Apr-2022 (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.

Origin, Type and Variety

Botanical Variety

Verification of consistency is not possible as the declared botanical variety of the sample is unknown or Polyfloral.

Country of Origin

Verification of consistency is not possible as the declared country of origin is unknown or not available.

Markers of Foreign Sugars

(Analysis-ID: HO-TAD-01/0189)

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

ID	Description	Value	Threshold	Result	Deviation [%]
2	3.0004 / (1.90-2.06)	0.0122	<0.2874	passed	
3	4.1923 / (4.54-4.56)	0.901	<8.144	passed	
4	5.3324 / (5.27-5.30)	0.0933	<0.1690	passed	
6	4.0056 / (5.27-5.30)	1.0774	>0.6847	passed	
7	4.1908 / (5.37-5.41)	0.2931	>0.1484	passed	
8	4.1947 / (4.93-4.97)	0.092	<2.285	passed	
9	4.1502	487.1	>258.4	passed	
11	4.6809	40.17	>22.80	passed	
12	4.2442 / (4.54-4.56)	0.2724	>0.1409	passed	
13	3.9381 / (5.37-5.41)	0.05439	>0.01576	passed	
14	3.2873 / (1.90-2.06)	1.319	<4.064	passed	
15	3.9651 / (4.07-4.12)	0.02446	>0.01561	passed	
17	3.3135 / (5.27-5.30)	0.0246	<0.1767	passed	
19	4.2523 / (1.90-2.06)	0.1402	<0.6987	passed	
20	5.4862 / (4.60-4.67)	0.002239	<0.002523	passed	
132	3.6649 / (4.54-4.56)	20.72	>14.22	passed	
133	2.0377 / (5.20-5.25)	0.002213	<0.005371	passed	
134	4.5345 / (4.54-4.56)	0.06739	>0.03596	passed	
135	5.3023 / (1.90-2.06)	0.0518	<0.2346	passed	
137	3.5526 / (4.54-4.56)	35.22	<39.10	passed	
138	3.6679 / (3.75-3.80)	0.06615	>0.04689	passed	
140	3.2797 / (3.16-4.14)	0.0001449	>0.0001021	passed	
141	2.3670 / (1.90-2.06)	0.0304	<0.1905	passed	
142	4.2005 / (5.27-5.30)	0.492	<1.449	passed	
143	3.2857 / (4.60-4.67)	0.01087	<0.01417	passed	
144	4.9511 / (4.93-4.97)	0.1486	<0.1822	passed	
146	4.1767 / (4.54-4.56)	0.3884	<0.7910	passed	
147	1.9385 / (5.27-5.30)	0.01787	<0.03333	passed	
148	4.5864 / (4.07-4.12)	0.0008018	>0.0004487	passed	
150	4.4306 / (3.16-4.14)	0.00004758	>0.00002145	passed	
151	3.2168 / (4.60-4.67)	0.2562	<0.2727	passed	
152	4.0181 / (3.65-3.73)	0.04230	>0.02355	passed	
153	5.0703 / (4.07-4.12)	0.0011989	>0.0003079	passed	
154	4.3163 / (5.27-5.30)	0.0305	<0.1034	passed	
163	3.8570 / (5.37-5.41)	0.10205	>0.01924	passed	
164	5.0745	90.17	>23.15	passed	
165	3.7625 / (3.75-3.80)	0.05046	>0.03137	passed	
166	3.9471 / (5.37-5.41)	0.057300	>0.007739	passed	
167	3.5464 / (3.75-3.80)	0.014773	>0.009349	passed	
168	4.1908 / (1.90-2.06)	2.0217	>0.4642	passed	
169	3.1682 / (3.20-3.22)	0.0021845	>0.0008052	passed	



ID	Description	Value	Threshold	Result	Deviation [%]
170	4.2990 / (4.93-4.97)	0.401	<2.868	passed	
171	3.8440 / (3.16-4.14)	0.005592	>0.004518	passed	
174	3.2349 / (4.60-4.67)	0.5589	<0.5947	passed	
175	5.2188 / (4.60-4.67)	0.5779	<0.6248	passed	
176	4.1677 / (5.20-5.25)	0.010831	>0.006368	passed	
177	3.5464 / (5.27-5.30)	1.0112	>0.6349	passed	
196	3.5914 / (4.54-4.56)	2.355	<3.663	passed	
197	5.2549	34.69	>19.48	passed	
1000	fructose/glucose	1.47	0.85-1.95	passed	
1001	total sugar [g/100g]	66.0	>45	passed	
1002	turanose [g/100g]	1.79	>0.35	passed	
1003	sucrose [g/100g]	0.4	<15	passed	
1004	DHA(D), mannose(M) [mg/kg]	D=0.0, M=37	D<5, M<200	passed	
1005	proline [mg/kg]	236	>160	passed	

Result: There are no indications for the presence of foreign sugars.

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.
- The column *Threshold* corresponds to the maximum or minimum value of the marker in authentic honey. See section "Remarks" for more information.
- The column *Deviation* corresponds to the deviation from the threshold (in %) for a given marker.

DHA and Mannose

Compound	Value	Unit	LOQ	Reference Range	Flag
mannose	<LOQ	g/100g	0.01	<0.01  0.03	●
dihydroxyacetone (DHA)	<LOQ	mg/kg	5	<5  431	●

Note: the reference range is based on *18077 Blossom* 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.

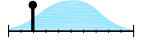

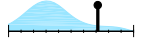



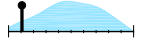

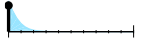

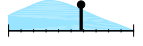

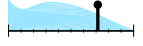






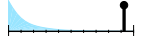

Composition and Comparison to Reference Group

Quantitative Analysis of Compounds






(Analysis-ID: HO-Q/3.0.0)

Parameters labelled with * are calculated parameters. The reference range is based on *18077 Blossom* samples in the Honey-Profiling Database.

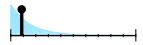
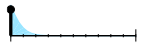
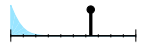

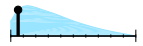



Sugars:

Compound	Value	Unit	LOQ	Reference Range	Flag
glucose + fructose *	66.0	g/100g	20.0	61.9  83.0	
fructose / glucose *	1.47	-	-	0.93  1.68	
fructose	39.2	g/100g	10.0	33.9  46.8	
glucose	26.8	g/100g	10.0	25.1  40.8	
sucrose	<LOQ	g/100g	0.5	<0.5  4.4	
turanose	1.8	g/100g	0.2	0.4  2.8	
maltose	2.2	g/100g	0.5	<0.5  2.9	
melezitose	<LOQ	g/100g	1.0	<1.0  1.5	
maltotriose	<LOQ	g/100g	1.0	<1.0 g/100g in reference dataset	
gentiobiose	<LOQ	g/100g	0.3	<0.3  0.5	
raffinose	0.5	g/100g	0.1	0.1  0.6	

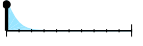







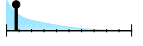
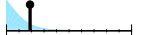
Acids:

Compound	Value	Unit	LOQ	Reference Range	Flag
citric acid	274	mg/kg	50	<50  489	
malic acid	193	mg/kg	100	<100  494	
quinic acid	<LOQ	mg/kg	300	<300 mg/kg in reference dataset	

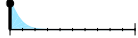



Amino Acids:

Compound	Value	Unit	LOQ	Reference Range	Flag
alanine	12	mg/kg	5	<5  82	●
aspartic acid	<LOQ	mg/kg	150	<150  210	●
glutamine	250	mg/kg	200	<200  278	●
leucine	<LOQ	mg/kg	40	<40  139	●
proline	236	mg/kg	150	185  1056	●
valine	<LOQ	mg/kg	10	<10  51	●
tyrosine	<LOQ	mg/kg	50	<50  721	●
phenylalanine	<LOQ	mg/kg	100	<100  1413	●

Indicators for Fermentation and Processing:

Compound	Value	Unit	LOQ	Reference Range	Flag
2,3-butanediol	<LOQ	mg/kg	20	<20  139	●
5-hydroxymethylfurfural (HMF)	<LOQ	mg/kg	5	<5  63	●
acetic acid	17	mg/kg	10	<10  85	●
acetoin	<LOQ	mg/kg	20	<20  66	●
ethanol	<LOQ	mg/kg	5	<5  1325	●
lactic acid	33	mg/kg	10	<10  324	●
formic acid	47	mg/kg	5	<5  321	●
fumaric acid	6	mg/kg	5	<5  12	●
pyruvic acid	12	mg/kg	10	<10  36	●
succinic acid	40	mg/kg	5	<5  192	●

Markers:

Compound	Value	Unit	LOQ	Reference Range	Flag
3-phenyllactic acid	<LOQ	mg/kg	300	<300  694	●
methylglyoxal (MGO)	<LOQ	mg/kg	30	<30  220	●
kynurenic acid	<LOQ	mg/kg	60	<60  137	●
shikimic acid	199	mg/kg	80	<80  205	●

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.

Statistical Comparison with the Reference Group

The models are based on 18076 samples of group *Blossom* in the Honey-Profiling Database.

Univariate Verification

(Analysis-ID: HS3-NTV-1001-18153)

Applied Model: Blossom

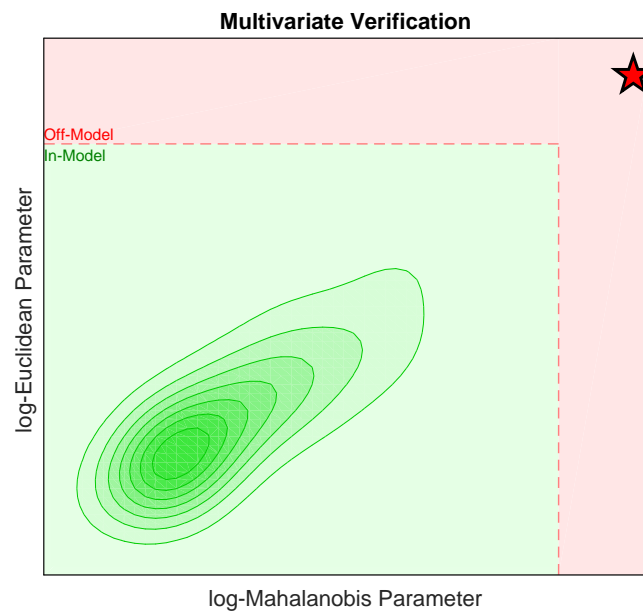
Result: Typical for Blossom.

Multivariate Verification

(Analysis-ID: HS3-NTV-1001-18153)

Applied Model: Blossom

Result: Not typical for Blossom.



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 *	66.0	g/100g	20.0	60	-	●
sucrose	<LOQ	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. In case one or several markers exceed the maximum threshold or are below the minimum threshold for purity, there is a very strong indication for the presence of foreign sugars. 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 "Binary Statement for Simple Acceptance Rule" according to ILAC-G8:09/2019.

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>