

# Analysis Report Honey-Profiling<sup>™</sup>

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Information/Declaration provided by customer:						
Customer:	Woolworhs					
Customer Sample ID:	Off_shelf					
Type of Sample:	Honey					
Type of Honey:	Blossom					
Botanical Variety:	other					
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:	19-Nov-2021 14:15:49
Reporting Date:	02-Dec-2021 09:02:54, 11 pages, Version 3.0.2

### **Results Summary**

Type of Analysis	Result	Status
Origin, Type and Variety		
Botanical Variety	Not Applied	$\bigcirc$
Country of Origin	Not Applied	$\bigcirc$
Targeted Detection of Adulterations	No	
Composition and Comparison to Reference Group	Typical	
Compliance for EU Market for		
HMF	Compliant	
Sucrose	Compliant	
Glucose + Fructose	Compliant	

The data analysis is performed at Bruker BioSpin GmbH (Rheinstetten, Germany) according to testing method AA-72-03-11 (Honey-Profiling 3.0.2), released on 27-Aug-2021 (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.

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# **Origin, Type and Variety**

# **Botanical Variety**

No statistical model available yet for consistency verification of this botanical variety.

# **Country of Origin**

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



# **Detection of Sugar Syrups**

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

Following tests have been applied in order to detect sugar syrups:

ID	Description	Value	Threshold	Result	Deviation [%]
2	3.0004 / (1.90-2.06)	0.0180	< 0.2874	passed	
3	4.1923 / (4.54-4.56)	1.250	<8.144	passed	
4	5.3324 / (5.27-5.30)	0.0899	<0.1690	passed	
6	4.0056 / (5.27-5.30)	1.3398	3398 >0.6847 passed		
7	4.1908 / (5.37-5.41)	0.3225	>0.1484	passed	
8	4.1947 / (4.93-4.97)	0.483	<2.285	passed	
9	4.1502	445.8	>258.4	passed	
11	4.6809	41.00	>22.80	passed	
12	4.2442 / (4.54-4.56)	0.2213	>0.1409	passed	
13	3.9381 / (5.37-5.41)	0.05239	>0.01576	passed	
14	3.2873 / (1.90-2.06)	2.572	<4.064	passed	
15	3.9651 / (4.07-4.12)	0.02074	>0.01561	passed	
17	3.3135 / (5.27-5.30)	0.0382	<0.1767	passed	
19	4.2523 / (1.90-2.06)	0.1975	<0.6987	passed	
20	5.4862 / (4.60-4.67)	0.001929	< 0.002523	passed	
132	3.6649 / (4.54-4.56)	21.51	. >14.22 passed		
133	2.0377 / (5.20-5.25)	0.001230	< 0.005371	passed	
134	4.5345 / (4.54-4.56)	0.06109	>0.03596	passed	
135	5.3023 / (1.90-2.06)	0.0473	< 0.2346	passed	
137	3.5526 / (4.54-4.56)	33.94	<39.10	passed	
138	3.6679 / (3.75-3.80)	0.06783	>0.04689	passed	
140	3.2797 / (3.16-4.14)	0.0001590	>0.0001021	passed	
141	2.3670 / (1.90-2.06)	0.0509	< 0.1905	passed	
142	4.2005 / (5.27-5.30)	0.688	<1.449	passed	
143	3.2857 / (4.60-4.67)	0.01023	<0.01417 passed		
144	4.9511 / (4.93-4.97)	0.1419	<0.1822 passed		
146	4.1767 / (4.54-4.56)	0.3953	<0.7910 passed		
147	1.9385 / (5.27-5.30)	0.00873	< 0.03333	passed	
148	4.5864 / (4.07-4.12)	0.0008941	>0.0004487	passed	
150	4.4306 / (3.16-4.14)	0.00004234	>0.00002145	passed	
151	3.2168 / (4.60-4.67)	0.2548	<0.2727	passed	
152	4.0181 / (3.65-3.73)	0.03923	>0.02355	passed	
153	5.0703 / (4.07-4.12)	0.0005237	>0.0003079	passed	
154	4.3163 / (5.27-5.30)	0.0363	<0.1034	passed	
163	3.8570 / (5.37-5.41)	0.06752	>0.01924	passed	
164	5.0745	38.22	>23.15	passed	
165	3.7625 / (3.75-3.80)	0.04713	>0.03137	passed	
166	3.9471 / (5.37-5.41)	0.032908	>0.007739	passed	
167	3.5464 / (3.75-3.80)	0.010919	>0.009349	passed	
168	4.1908 / (1.90-2.06)	3.1404	>0.4642	passed	
169	3.1682 / (3.20-3.22)	0.0015254	>0.0008052	passed	



ID	Description	Value	Threshold	Result	Deviation [%]
170	4.2990 / (4.93-4.97)	1.110	<2.868	passed	
171	3.8440 / (3.16-4.14)	0.005621	>0.004518	passed	
174	3.2349 / (4.60-4.67)	0.5659	<0.5947	passed	
175	5.2188 / (4.60-4.67)	0.5730	<0.6248	passed	
176	4.1677 / (5.20-5.25)	0.010724	>0.006368	passed	
177	3.5464 / (5.27-5.30)	1.5761	>0.6349	passed	
196	3.5914 / (4.54-4.56)	1.951	<3.663	passed	
197	5.2549	40.25	>19.48	passed	
1000	fructose/glucose	1.12	0.85-1.95	passed	
1001	total sugar $[g/100g]$	76.5	>45	passed	
1002	turanose [g/100g]	0.90	>0.35	passed	
1003	sucrose [g/100g]	0.9	<15	passed	
1004	DHA(D), mannose(M) [mg/kg]	D=86.3, M=20	D<5, M<200	passed	
1005	proline [mg/kg]	296	>160	passed	

**Result:** There are no indications for adulteration.

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< td=""><td>g/100g</td><td>0.01</td><td>&lt;0.01 0.03</td><td></td></loq<>	g/100g	0.01	<0.01 0.03	
dihydroxyacetone (DHA)	86	mg/kg	5	<5 431	$\bigcirc$

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 syrups or industrial
  processing practices which are not suitable for honey. An expert interpretation is suggested
  when mannose is present in the honey.</li>
- 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 syrups or industrial processing 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 *	76.5	g/100g	20.0	61.9 83.0	
fructose / glucose *	1.12	-	-	0.93	
fructose	40.4	g/100g	10.0	33.9 46.8	
glucose	36.1	g/100g	10.0	25.1 40.8	
sucrose	0.9	g/100g	0.5	<0.5 4.4	
turanose	0.9	g/100g	0.2	0.4 2.8	
maltose	2.5	g/100g	0.5	<0.5	
melezitose	<loq< td=""><td>g/100g</td><td>1.0</td><td>&lt;1.0</td><td></td></loq<>	g/100g	1.0	<1.0	
maltotriose	<loq< td=""><td>g/100g</td><td>1.0</td><td><math>{&lt;}1.0~{ m g}/100{ m g}</math> in reference dataset</td><td></td></loq<>	g/100g	1.0	${<}1.0~{ m g}/100{ m g}$ in reference dataset	
gentiobiose	<loq< td=""><td>g/100g</td><td>0.3</td><td>&lt;0.3 0.5</td><td></td></loq<>	g/100g	0.3	<0.3 0.5	
raffinose	0.1	g/100g	0.1	0.1 0.6	

## Acids:

Compound	Value	Unit	LOQ	Reference Range	Flag
citric acid	481	mg/kg	50	<50 489	
malic acid	215	mg/kg	100	<100 494	
quinic acid	<loq< td=""><td>mg/kg</td><td>300</td><td>&lt;300 mg/kg in reference dataset</td><td></td></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< td=""><td>mg/kg</td><td>150</td><td>&lt;150 210</td><td></td></loq<>	mg/kg	150	<150 210	
glutamine	<loq< td=""><td>mg/kg</td><td>200</td><td>&lt;200</td><td></td></loq<>	mg/kg	200	<200	
leucine	<loq< td=""><td>mg/kg</td><td>40</td><td>&lt;40 139</td><td></td></loq<>	mg/kg	40	<40 139	
proline	296	mg/kg	150	185 1056	
valine	<loq< td=""><td>mg/kg</td><td>10</td><td>&lt;10 51</td><td></td></loq<>	mg/kg	10	<10 51	
tyrosine	<loq< td=""><td>mg/kg</td><td>50</td><td>&lt;50</td><td></td></loq<>	mg/kg	50	<50	
phenylalanine	118	mg/kg	100	<100 1413	

# Indicators for Fermentation and Processing:

Compound	Value	Unit	LOQ	Reference Range	Flag
2,3-butanediol	<loq< td=""><td>mg/kg</td><td>20</td><td>&lt;20 139</td><td></td></loq<>	mg/kg	20	<20 139	
5-hydroxymethylfurfural (HMF)	7	mg/kg	5	<5 63	
acetic acid	29	mg/kg	10	<10 85	
acetoin	<loq< td=""><td>mg/kg</td><td>20</td><td>&lt;20 66</td><td></td></loq<>	mg/kg	20	<20 66	
ethanol	7	mg/kg	5	<5 1325	
lactic acid	25	mg/kg	10	<10 324	
formic acid	73	mg/kg	5	<5 321	
fumaric acid	<loq< td=""><td>mg/kg</td><td>5</td><td>&lt;5 12</td><td></td></loq<>	mg/kg	5	<5 12	
pyruvic acid	<loq< td=""><td>mg/kg</td><td>10</td><td>&lt;10 36</td><td></td></loq<>	mg/kg	10	<10 36	
succinic acid	83	mg/kg	5	<5 192	



#### Markers:

Compound	Value	Unit	LOQ	Reference Range	Flag
3-phenyllactic acid	<loq< td=""><td>mg/kg</td><td>300</td><td>&lt;300 694</td><td></td></loq<>	mg/kg	300	<300 694	
methylglyoxal (MGO)	<loq< td=""><td>mg/kg</td><td>30</td><td>&lt;30 220</td><td></td></loq<>	mg/kg	30	<30 220	
kynurenic acid	<loq< td=""><td>mg/kg</td><td>60</td><td>&lt;60 137</td><td></td></loq<>	mg/kg	60	<60 137	
shikimic acid	<loq< td=""><td>mg/kg</td><td>80</td><td>&lt;80 205</td><td></td></loq<>	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 adulteration (see section "Markers for adulteration").
- Atypical concentrations of sugars in comparison to reference range can be related to 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

Applied Model: Blossom Result: Typical for Blossom.

#### Multivariate Verification

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

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

# Applied Model: Blossom Result: Typical for Blossom.



log-Mahalanobis Parameter



# **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.

				Official Reference		
Compound	Value	Unit	LOQ	min	max	Flag
glucose + fructose *	76.5	g/100g	20.0	60	-	
sucrose	0.9	g/100g	0.5	-	15	
5-hydroxymethylfurfural (HMF)	7	mg/kg	5	-	80	

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

Compound	Flag	Concentration	Declaration	Interpretation
glucose +		< 45 g/100g	All	Not compliant
fructose		< 60 g/100g	Blossom	Not compliant for blossom honey
		$\geq$ 60 g/100g	All	Compliant
		$\geq$ 45 g/100g	Honeydew	Compliant for honeydew honey
	0	$\geq$ 45 g/100g, < 60 g/100g	Unknown	Compliant for honeydew honey and blends of honeydew honey with blossom honey. Not compliant for blossom honey.
sucrose		> 15 g/100g	All	Not compliant
		10-15 g/100g	Acacia, Eucalyp- tus	Not compliant for false acacia ( <i>Robinia pseudoacacia</i> ), and red gum ( <i>Eucalyptus camadulensis</i> )
		$\leq$ 5 g/100g	All	Compliant
		$\leq$ 10 g/100g	Acacia, Eucalyp- tus	Compliant for false acacia ( <i>Robinia pseu- doacacia</i> ), and red gum ( <i>Eucalyptus ca- madulensis</i> )
		$\leq$ 15 g/100g	Lavender	Compliant for Lavandula spp.
	•	5-10 g/100g	All, except Aca- cia, Eucalyptus, Lavender	If $\leq 15g/100g$ : compliant for lavender (Lavandula spp.) and borage (Borago of- ficinalis). If $\leq 10g/100g$ : compliant for false acacia (Robinia pseudoacacia), al- falfa (Medicago sativa), Menzies Banksia (Banksia menziesii), French honeysuckle (Hedysarum), red gum (Eucalyptus ca- madulensis), leatherwood (Eucryphia lu- cida, Eucryphia milliganii) and Citrus spp.
HMF		> 80 mg/kg	All, except Indus- trial honey	Not compliant, except for baker's honey
		$\leq$ 40 mg/kg	All	Compliant
		> 80 mg/kg	Industrial honey	Compliant for baker's honey
	0	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 Adulteration**

The detection of adulteration 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 an adulteration. Indeed, the false positive rate of each marker is less than 0.5%. Bee feeding can sometimes also result in an adulteration of the honey, even though it may not be intentionally.

#### **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.

#### 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.

#### 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.

#### 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