Method ring test
NMR in honey
P2528-MRT

Summary

The entire report is available to participants only.

The method ring test was designed, realised, evaluated, and authorised on behalf of PROOF-ACS GmbH by

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DAkkS
Deutsche
Akkreditierungsstelle
D-EP-22211-01-00

PROOF-ACS is a DAkkS accredited proficiency testing provider according to DIN EN ISO 17043:2010 (D-EP-22211-01-00). This method ring test is covered by the scope of accreditation.

PROOF-ACS GmbH does not have any analytical laboratory facilities of its own. Homogeneity testing and stability testing are subcontracted to laboratories, accredited according to DIN EN ISO 17025. The subcontracted laboratory may also participate in the ring tests. If so, the laboratory is treated in the same way as other participants and the same rules of confidentiality apply.

The Honey-Profiling™ method by Bruker BioSpin GmbH identifies adulterations of honey by quantification of 36 parameters in one measurement.

The so-called NMR-profiling identifies adulterations by automated fingerprint analysis and comparison against a spectra library. Typical patterns of e.g. sugars, amino acids, organic acids are used to identify the type of honey as well as the geographical and botanical origin of the honey samples. Specific parameters like HMF, ethanol and the ratios of the different sugars help to identify adulterations by heating, fermentation or addition of sugar syrups.

The aim of the method ring test is to evaluate the performance of the laboratories in quantification of the 36 parameters as mentioned above, the resulting assessments of the adulterations and origins as well to identify shortcomings of the applied analytical methods if present.

The method ring test as well as the report consists of three parts:

- Part 1: Evaluation of the analytical results

  The performance of laboratories is evaluated with respect to their ability to quantify parameters of the Honey-Profiling™ method in three different samples of honey.

- Part 2: Analytical methods and assessments

  The applied analytical methods and the assessments related to the type of honey, the botanical variety, and the geographical origin of the honey samples are discussed. The information, which was provided by the labs is summarised. The provided information is considered for interpretation of the analytical results as well as presented for further evaluation by the experts in the labs.

- Part 3: Bruker reports

  The analytical procedure in quantifying with the Honey-Profiling™ method is based on the automatised evaluation of the respective NMR-spectra. The Bruker reports of all laboratories are collected and summarised. The provided information is considered for the interpretation of the assessments in part 2 of the report and presented for further evaluation of the experts in the labs.

Three honeys, a honeydew honey from Spain, a rapeseed honey from Germany and a honey blend with honeys from different origins are chosen as matrices for the method ring test. The rapeseed honey was not adulterated. The honeydew honey and the honey blend were adulterated with a rice sirup at different percentages. Fructose was added to the honey blend for further adulteration.

Seven laboratories across four countries (France, Germany, New Zealand, and Turkey) took part in the test. One of the laboratories provided two independent results from different spectrometers. Thus, eight different results are considered for evaluation.

The laboratories were asked to report analytical results related to three honey samples. Besides the pure analytical data, the laboratories were asked to provide comprehensive data related to the applied analytical methods and assessments in a questionnaire and the Bruker reports related to the three honey samples.

The labs were asked to report results related to

- ten sugars:
  fructose, glucose, sucrose, turanose, maltose, melezitose, maltotriose, gentiobiose, raffinose, and mannose.
- twelve organic acids:
  citric acid, malic acid, quinic acid, acetic acid, lactic acid, formic acid, fumaric acid, pyruvic acid, succinic acid, 3-phenyllactic acid, kynurenic acid, and shikimic acid.
- eight amino acids:
  alanine, aspartic acid, glutamine, leucine, proline, valine, tyrosine, and phenylalanine, and
- six honey specific parameters:
  dihydroxyacetone (DHA), 2,3-butanediol, 5-hydroxymethylfurfural (HMF), acetoin, ethanol, and methylglyoxal (MGO).

Some of the parameters are reported below the respective limits of quantification of the labs. The respective honey samples naturally contain the parameters at low levels only. The results related to all parameters, which were quantified by the labs are considered for evaluation. All seven labs reported results and all eight results (one of the labs took part with two spectrometers) are considered for evaluation.

The performance of laboratories in the test is evaluated according to the comparability of the results. The evaluation of the comparability is based on the z-score model. The absolute value of the z-score should be at least ≤ 2. The comparability criterion is applied to all quantified parameters, except citric acid, and aspartic acid in honeydew honey, and raffinose in honey blend. The statistical evaluation of the results is summarised in the tables below.

The assessments on the botanical and geographical origin of the honey samples are not considered for evaluated but summarised and presented for information in part 2 of the report.

All laboratories identified the honeydew honey and the honey blend correctly as adulterated. The labs correctly identified the rapeseed honey as not adulterated.

All labs correctly confirmed the botanical origin of the honeydew honey and of the rapeseed honey correctly. The geographical origin of the honeydew honey was correctly confirmed as Spain by all labs. The geographical origin of the rapeseed honey was correctly confirmed by three out of eight labs, while five labs did not confirm the geographical origin of the rapeseed honey correctly.

Test material honeydew honey

The results related to 19 parameters are considered for evaluation. A summary of the overall performance of the laboratories is provided in the table below.

Parameter	Unit	Assigned value	Total number of results	**Comparability:** no. of results, which correspond to |z-score| ≤ 2
Fructose	g/100 g	28.3	7	7
Glucose	g/100 g	23.4	7	6
Turanose	g/100 g	2.34	8	8
Maltose	g/100 g	6.80	7	4
Maltotriose	g/100 g	1.17	8	5
Raffinose	g/100 g	0.659	8	6
Mannose	g/100 g	0.0648	8	7
Malic acid	mg/kg	995	8	8
Alanine	mg/kg	25.4	8	8
Proline	mg/kg	416	8	8
2,3-Butanediol	mg/kg	309	8	7
Acetic acid	mg/kg	126	8	7
Ethanol	mg/kg	14.0	8	6
Lactic acid	mg/kg	216	8	8
Formic acid	mg/kg	86.9	8	8
Fumaric acid	mg/kg	20.4	8	7
Pyruvic acid	mg/kg	28.5	8	8
Succinic acid	mg/kg	213	8	8
Shikimic acid	mg/kg	195	8	8

Test material rapeseed honey

The results related to 9 parameters are considered for evaluation. A summary of the overall performance of the laboratories is provided in the table below.

Parameter	Unit	Assigned value	Total number of results	**Comparability:** no. of results, which correspond to |z-score| ≤ 2
Fructose	g/100 g	39.6	8	7
Glucose	g/100 g	39.8	8	7
Turanose	g/100 g	0.851	7	7
Alanine	mg/kg	6.79	8	8
Proline	mg/kg	321	8	8
Acetic acid	mg/kg	17.5	7	4
Lactic acid	mg/kg	12.5	8	7
Formic acid	mg/kg	23.6	8	8
Succinic acid	mg/kg	9.08	8	8

Test material honey blend

The results related to 17 parameters are considered for evaluation. A summary of the overall performance of the laboratories is provided in the table below.

Parameter	Unit	Assigned value	Total number of results	**Comparability:** no. of results, which correspond to |z-score| ≤ 2
Fructose	g/100 g	37.0	8	8
Glucose	g/100 g	33.3	8	7
Turanose	g/100 g	1.21	7	7
Maltose	g/100 g	4.24	8	7
Raffinose	g/100 g	-	8	8
Citric acid	mg/kg	187	8	8
Malic acid	mg/kg	174	8	4
Alanine	mg/kg	14.0	8	8
Proline	mg/kg	445	8	8
2,3-Butanediol	mg/kg	38.6	7	6
5-HMF	mg/kg	5.70	7	6
Acetic acid	mg/kg	29.9	8	4
Ethanol	mg/kg	15.2	8	7
Lactic acid	mg/kg	54.0	8	7
Formic acid	mg/kg	37.7	8	8
Pyruvic acid	mg/kg	15.7	8	8
Succinic acid	mg/kg	55.7	8	8

The Honey-Profiling™ method is designed to be a plug-and-play analysis. An official SOP for sample preparation was published by Bruker. On request, all reagents are provided as well. Thus, the analytical method is fully standardised. However, still slightly different approaches and concepts for sample preparation are applied by the laboratories. The measurement of some parameters, the organic acids like citric acid, malic acid and acetic acid depends on the pH value, because the shifts in the spectrum are pH sensitive. Depending on the pH value, the outcome might differ a lot especially for organic acids. Therefore, the labs should carefully adjust the pH values to the recommended pH values of Bruker. Otherwise, shifts in the spectra might result in misinterpretation of interferences, false negative results, or mistakes in quantification during evaluation by the software.

The summary of the information for the method and assessments (part 2 of the report) can support laboratories to improve the quality of the applied analytical method e.g. the choice of the most suitable conditions for sample preparation. Furthermore, the method details can

build the basis for further discussion, point out shortcomings and thus lead to a further improvement of the analytical method related to Honey-Profiling™ by Bruker.

The Bruker reports of all participants are summarised in part 3 of the report. The provided Bruker reports contain detailed information besides the one that is considered for evaluation in the report. The Bruker reports in part 3 of the report thus offer the chance to the experts in the lab to dig deeper in the details of the applied analytical methods of all participants. E.g. assessments related to the type of honey, the origin, and the outcome with respect to adulteration or not depends on the selection if analysis IDs and reference groups.

Expert knowledge is indispensable for a correct interpretation of the resulting Bruker reports and NMR spectra. The laboratories must be able to identify interferences to avoid misinterpretation and thus over- or underestimation of the values of especially the organic acids.

The method ring test revealed shortcomings in the analytical method resp. in the Bruker report. The Bruker report showed significant shortcomings in the reporting of the results in at an adequate level of significance. Results are reported in one to three significant figures. The reports should be adjusted to ensure a reasonable reporting of the analytical results.

Further shortcomings were identified for the parameters citric acid, aspartic acid and 2,3-butanediol, especially for the matrix honeydew honey. Reproducibility of the applied analytical method is low, as results as well as false negative results were reported for identical samples. The method requires adjustment by Bruker. Probably, a possibility for the laboratories to manually adjust the measurement if problems in the shifts or the matrix are identified might help to solve this issue.

If the labs are experienced and this sophisticated analytical method is correctly applied, a reliable quantification with the Honey-Profiling™ method in honey is possible.