FOODSCREENER: Honey-Profiling

Results of an Interlaboratory Proficiency Test

Reproducibility of Honey-Profiling

Innovation with Integrity

Introduction

NMR spectroscopy is an analytical method which helps to study the behavior of specific nuclei of a molecule in an external magnetic field. The data which are generated during the experiments contain various information about the different molecules within a solution. For instance, it contains information about the structure, like bindings and the presence of isomers, but also information of the quantity of a particular molecule. The NMR signal, its shape, frequency (position in the spectra) and couplings are characteristic for a particular compound and can therefore be used to identify it. The intensity of the NMR signal of a specific nucleus in a molecule is directly proportional to the concentration of the molecule within the solution and can thus be used to quantify the compound.

Advantages of 1H-NMR:

- Primary method for quantification, there is no need to calibrate every single compound.
- The method is fast and highly reproducible.
- Retrospective analyses are possible.
- The NMR spectra are unique for a certain sample and can therefore be used as a fingerprint. It is therefore predestined to check consistency and monitor changes.

Need for the „Analysis of Honey on the EU market“

The honey production in the European Union (EU) shows that the demand for honey is higher than its own production. The self-sufficiency rate for honey is around 60 %. Therefore, the EU needs to import honey from third countries, which makes it the second largest importer of honey worldwide. About 80 % of the honey traded in the EU are blends. These are mixtures of honey from EU countries and countries outside EU [1].

A recently published report from the Joint Research Center of the European Commission (JRC) shows that it is important to analyze honey in the EU [2]. There is a high amount of honey in the EU market which is not compliant with the EU Honey Directive 2001/110/EC.

In 2021, the European Commission organized an EU coordinated action called "From the Hives" [1] to assess the prevalence of non-compliant honey in the market:

- In total 320 samples were collected and analyzed.
- 46 % of all samples were suspicious of being not compliant with the EU Honey Directive 2001/110/EC [3].
- Different actions were undertaken and are still ongoing to reveal illicit practices.
- One outcome of this study was that "improved, harmonized and generally accepted analytical methods are still needed…[…] to detect honey adulterated with sugar syrups" [2].

The Honey Profiling™ Method, based on 1H-NMR was introduced by Bruker in 2015. It includes various quantifications, an adulteration analysis for the detection of foreign sugars and gives information about the conformity of the variety and geographical origin.

Reproducible Results

In general, reproducibility is the ability to get equivalent results with a high level of reliability, when using the same scientific method under the same experimental conditions. In terms of an analytical analysis, reproducibility is needed to compare results within different laboratories.

Figure 1 shows 49 1H NMR Honey spectra recorded on a 400 MHz Bruker NMR spectrometer. All 49 spectra were prepared and measured on different days. The sample preparation and measurement were performed using the Bruker Honey Profiling™ SOP and measurement workflow.

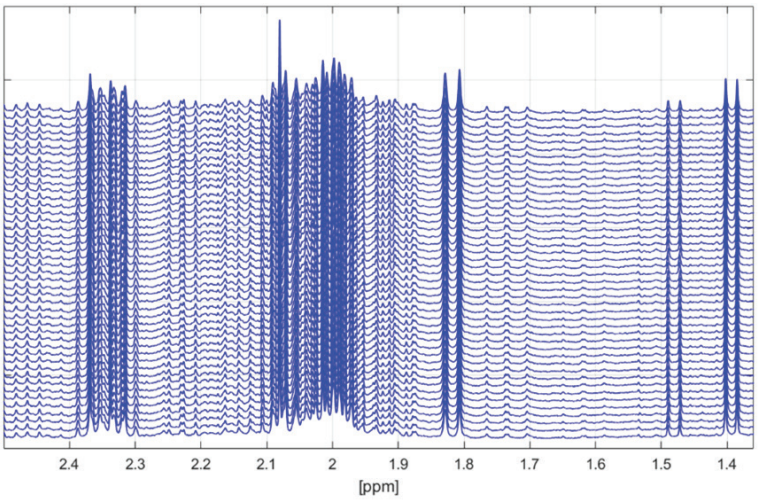

Figure 1 1H NMR spectra of 49 quality control samples of honey, prepared and measured on different days

Goal of Reproducibility

The same sample prepared and measured on different but equivalent machines (same field-strength, same hardware) generates the same fingerprint and therefore equivalent quantification and statistical results.

This is a fundamental requirement to have a reliable analysis across different laboratories.

How to achieve reproducible results?

There are three important pillars (s. Figure 2) that need to be considered to achieve reproducible results. These are "standardized quality checks", "standardized sample operating procedure (SOP)" and whenever it is possible a "standardized and automated workflow".

The standardized quality checks ensure that the system is operational, and the results are reliable. For the Honey Profiling™ Method all the quality checks are performed using defined reference samples.

The standardized quality checks are:

- **Temperature check.** The temperature adjustment is controlled and checked if it is within certain predefined limits.
- **Spectra Quality Check.** Different parameters like halfwidth of a particular signal, water suppression, signal/noise and resolution are checked and must be within specified limits.
- **Quantification Reference Check.** This sample is used as an external reference, it needs to be within certain limits to produce valid results.

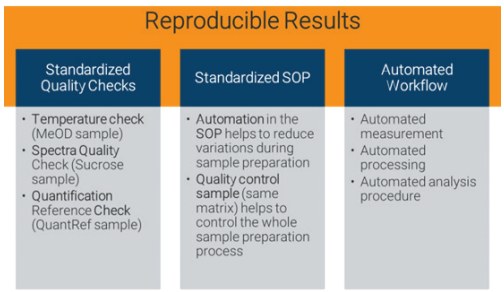

Figure 2 The three important pillars that should be considered to achieve reproducible results.

A standardized SOP is key to any reproducible result. Automation of the SOP helps to reduce variations during the sample preparation. To control the sample preparation process, a "quality control sample" is needed. This sample is preferably from the same matrix as the samples tested and should be prepared together with the samples. It is used to uncover variations that could occur during sample preparation. Monitoring this sample helps to identify preparation practices that are not being performed correctly and therefore could lead to error-prone results.

A standardized and automated workflow, such as automated sample measurement with defined experiments, automated processing without interaction of any operator, and an automated analysis procedure help to minimize errors due to personal preferences.

Interlaboratory Proficiency Test

To check the reproducibility and consistency of NMR results an interlaboratory proficiency test was performed. Three different laboratories participated in this interlaboratory proficiency test.

In total 45 different honey samples were analyzed by all the laboratories. The samples were selected to cover a wide range of honey varieties available on the market.

This includes:
- Different honey types: honeydew, blossom, and mixture of both
- Different honey varieties
- Different country of origin (also blends of different origins)
- Authentic, non-authentic and suspicious honey samples

All the honeys were prepared and measured in each of the three laboratories using the same SOP and the same automated workflow of measurement, processing and analysis procedure (Bruker Honey Profiling™).

The adulteration analysis using the Bruker Honey Profiling™ version 3.1 (i.e. „Test of Markers of Foreign Sugars") was used to compare the different analysis results.

Test of Markers of Foreign Sugars

This adulteration test aims at detecting foreign sugars. It relies on the analysis of the concentrations of certain known molecules and on absolute intensities or intensities ratios of different marker signals.

To identify and handle borderline cases, the "Non-Binary Statement with Guard Band" according to ILAC G8:09/2019 [4] was implemented in the Bruker Honey Profiling™ version 3.1 (s. Figure 3).

The reported results consider a borderline region, related to the analytical measurement uncertainty.

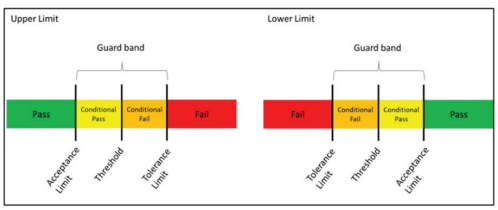

Figure 3 Graphical display of the results of the Test of Markers of Foreign Sugars in the Bruker Honey Profiling™ version 3.1 report.

Results of the Interlaboratory Proficiency Test

Each of the 45 honey samples were tested with the Test of Markers of Foreign Sugars. Table 1 shows the overall results of this adulteration test for each honey tested in each laboratory. The first column contains the Sample ID, the second to forth column ("Lab1", "Lab2", "Lab3") the respective test result and the last column ("Consistency") shows whether the overall result of the laboratories agree with each other or not.

Sample	Lab 1	Lab 2	Lab3	Consistency
1	pass	pass	pass	ok
2	pass	pass	pass	ok
3	pass	pass	pass	ok
4	fail	fail	fail	ok
5	pass	pass	pass	ok
6	fail	fail	fail	ok
7	fail	fail	fail	ok
8	pass	pass	pass	ok
9	fail	fail	fail	ok
10	pass	pass	pass	ok
11	pass	pass	pass	ok
12	pass	pass	conditional pass	ok
13	pass	pass	conditional pass	ok
14	pass	pass	conditional pass	ok
15	fail	fail	fail	ok
16	pass	pass	pass	ok
17	pass	pass	pass	ok
18	fail	fail	fail	ok
19	pass	pass	conditional pass	ok
20	pass	pass	pass	ok
21	pass	pass	pass	ok
22	fail	fail	fail	ok
23	fail	fail	fail	ok
24	pass	pass	pass	ok
25	pass	pass	pass	ok
26	pass	pass	pass	ok
27	pass	pass	pass	ok
28	pass	pass	pass	ok
29	fail	fail	fail	ok
30	fail	fail	fail	ok
31	pass	pass	pass	ok
32	pass	pass	pass	ok
33	pass	pass	conditional pass	ok
34	pass	pass	conditional pass	ok
35	pass	pass	pass	ok
36	fail	fail	fail	ok
37	pass	pass	pass	ok
38	pass	pass	pass	ok
39	fail	conditional fail	fail	ok
40	conditional pass	conditional pass	conditional pass	ok
41	fail	fail	fail	ok
42	pass	pass	pass	ok
43	pass	pass	pass	ok
44	pass	pass	pass	ok
45	fail	fail	fail	ok

Table 1 Results of Test of Markers of Foreign Sugars

In total, 24 honey samples showed no indication of adulteration, while 13 honey samples showed clear indication of adulteration in all three laboratories. Eight samples were identified to be borderline for one of the laboratories or in all the laboratories. Thereof, seven samples were conditional pass and one sample was conditional fail.

For an easier assessment of the overall results (Table 1, last column "Consistency") the results for "conditional pass" were grouped together with the results for "pass" and the results for "conditional fail" were grouped together with the results for "fail". The overall results of this adulteration analysis are consistent for all 45 honey samples, in all three laboratories.

The results of this interlaboratory proficiency test show that NMR spectra-based results are highly reproducible within different laboratories, when using the same standardized SOP and the same standardized automated workflow.

References:

1. EU Coordinated Action "From the hives" Sampling, investigations and results (2023), Publications Office of the European Union, p. 1-22.
2. JRC technical report EU Coordinated action to deter certain fraudulent practices in the honey sector, Analytical testing results of imported honey (2023), Publication Office of the European Union , EUR 31461 EN, p. 1-16.
3. Council- Directive 2001/110/EC relating to honey (2001), Official Journal of the European Communities, L10 47-52.
4. ILAC G8:09/2019 - Guidelines on Decision Rules and Statements of Conformity (2019), ILAC Guidance Documents (G-Series).

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