

## **NMR Honey Profiling™ update to version 2.0**

Since 2014 QSI offers NMR to evaluate the authenticity of honey and has developed in cooperation with Bruker and other partners the Honey-Profiling™. The first version 1.0 was released in September 2015 and contained approximately 3700 samples. The method is accredited at QSI and Bruker according to ISO/IEC 17025 and has been updated on the 22<sup>nd</sup> of October 2018 to version 2.0. With this update the NMR database has strongly increased to more than 18 000 samples, adulteration detection models as well as origin verification models have been improved. In this newsletter, we will give you a detailed overview about the changes as well as our first experiences during the past few months.

**Because of a strong customer demand for specific honey parameters within our Honey Profiling report, we extended our NMR service. Please find our new offers at the end of this newsletter.**

### **Honey-Profiling™ 2.0 changes**

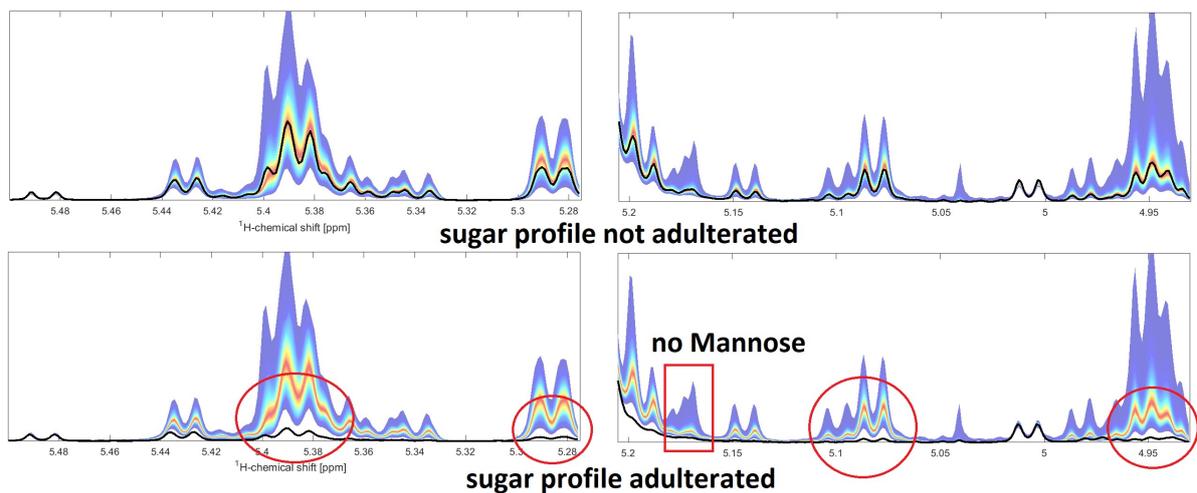
Version	1.0 (old)	2.0 (new)
Total samples	<4000	>18000
Adulteration detection models	17	60
Origin confirmation models	11	20
Variety confirmation models	3	12

### **Adulteration detection**

The database of >18 000 samples incorporates besides authentic blossom honeys and 2000 authentic honeydew honeys also 1500 industrial honey (Baker's honey) samples as well as 1900 adulterated samples (e.g. laboratory spiking experiments, pure actual syrups and market samples which were found to be adulterated according to conventional methods). For further information about the sample authentication please check our newsletter from March 2018. On the basis of these samples and in cooperation with the expert opinion of QSI, Bruker's adulteration detection algorithm was trained and it produced 60 different adulteration detection models which relate to specific markers/patterns in the NMR spectrum. Please note that this is still an ongoing process and further changes will happen with future updates. Some of these models are origin related and can only be applied if the sample is declared to be from a specific origin. The NMR profiles vary less for samples of a specific origin and therefore are more accurate if the origin of a sample in question is known, deviations can be limited and adulteration models are more efficient. Hence, we demand our customers to always declare the origin of a sample; even for blends it might be useful. We are often asked, if blending two complying (not adulterated) honeys could lead to a positive result (adulterated) in NMR afterwards and we have to vehemently deny this. However, applying the more general approach to blends (using the model including all origins of the world) in order to detect adulteration will give probably more negative results which means adulteration will not be detected. Models are focused on avoiding false positive results which is why we recommend using the Honey-Profiling™ mainly for screening the raw honey with declared origin before blending.

As mentioned above, the adulteration detection models evaluate different parts of the NMR spectrum, e.g. different spectral regions and ratios. Furthermore, as in the previous version, Bruker

also quantifies 36 different parameters (e.g. sugars like glucose or amino acids like proline). For some of these quantified parameters there are also limits included in the adulteration detection, e.g. for mannose in combination with specific markers. If these limits are exceeded a positive result will be given. Nevertheless, at QSI this automatic adulteration detection was and will always be accompanied by an expert's interpretation to make sure that specific botanical sources or borderline cases are handled and verified accordingly. We want to point out that a positive or negative finding of mannose is in both cases not solely conclusive to judge adulteration and that the whole profile needs to be reviewed by an expert (see Figure 1).



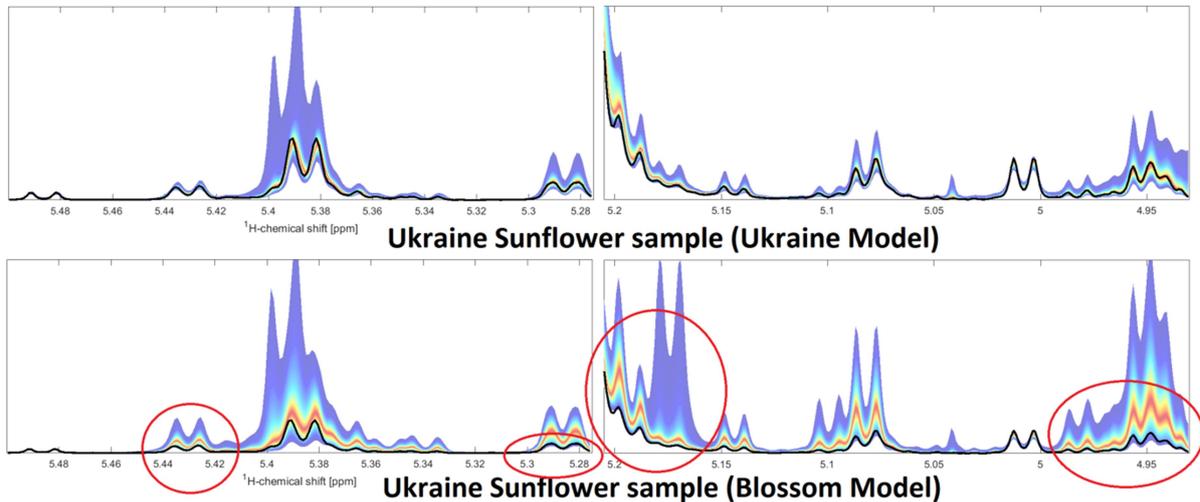
**Figure 1** Comparison of sugar profile of a not adulterated vs an adulterated honey sample from China. Red square marks the region where Mannose can be found. Note that not all adulterated samples contain Mannose. Otherwise the sugar profile in the adulterated sample is clearly diluted (red circles) compared to the database (colored background).

During the past several months since we have been testing the newest version, we have found that overall the correct prediction rate (including false and true positive rates) for adulteration has strongly increased with the Honey-Profiling™ 2.0 compared to the first Release. However, we have also had several smaller issues, i.e. newer syrups with lower amounts of Mannose or adulteration of honeys from specific botanical origins are not detected by the automatic models and still need an expert's evaluation. In our opinion this new update is a great improvement in terms of harmonization of different labs, however, in some borderline cases there will still be differences depending on a laboratories experience with the technique. We are continuously cooperating with Bruker in order to improve the current models and thus help to harmonize the interpretation.

We are also often asked about the minimum detection level of a possible adulteration. A quantification of adulteration is still also with Release 2.0 like with other methods, e.g. LC-IRMS, not possible without knowing the specific syrup. Some syrups can be detected at 5% addition, while others might be undetected even at higher concentration. Other methods like foreign enzyme activity or oligosaccharide analysis can be more sensitive or detect in some cases other syrups than NMR. More sensitive tests also might detect rests of feeding more easily. Results of different adulteration tests can still differ from NMR results. With the NMR profile we just get more information about the general quality and origin than with the other individual methods. We recommend using different methods in combination, whereas NMR can give a first general tendency to preselect for further testing.

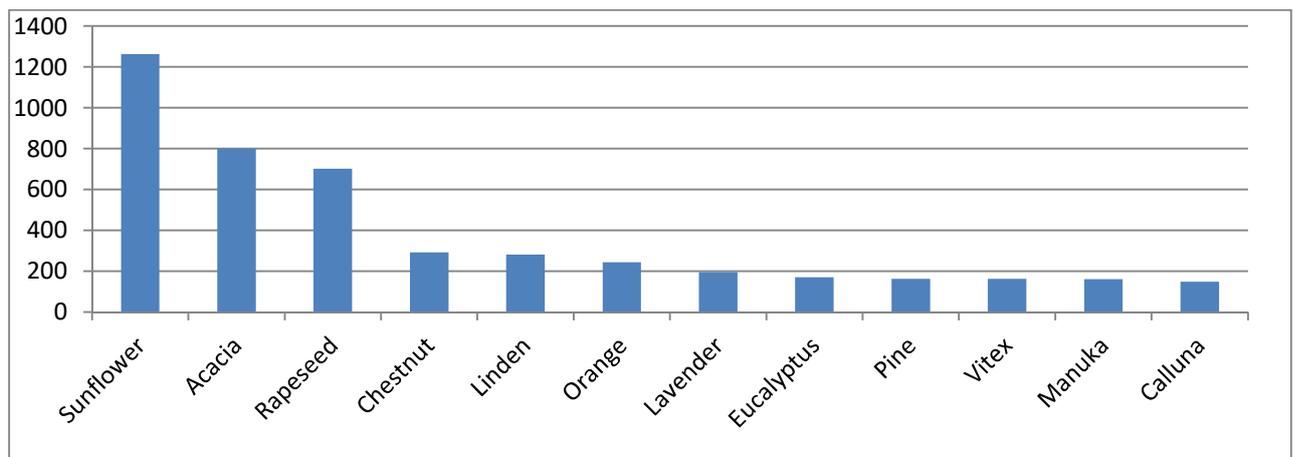
## Origin / Variety verification

Declaring the origin of a sample can have a positive influence on the ability to detect adulterations with NMR, since the complete profile for a certain origin, e.g. Ukraine, can be much sharper than of e.g. all blossom honeys (see colored backgrounds in Figure 2). The example below also shows why not declaring an origin can in some rare cases lead to a false positive.

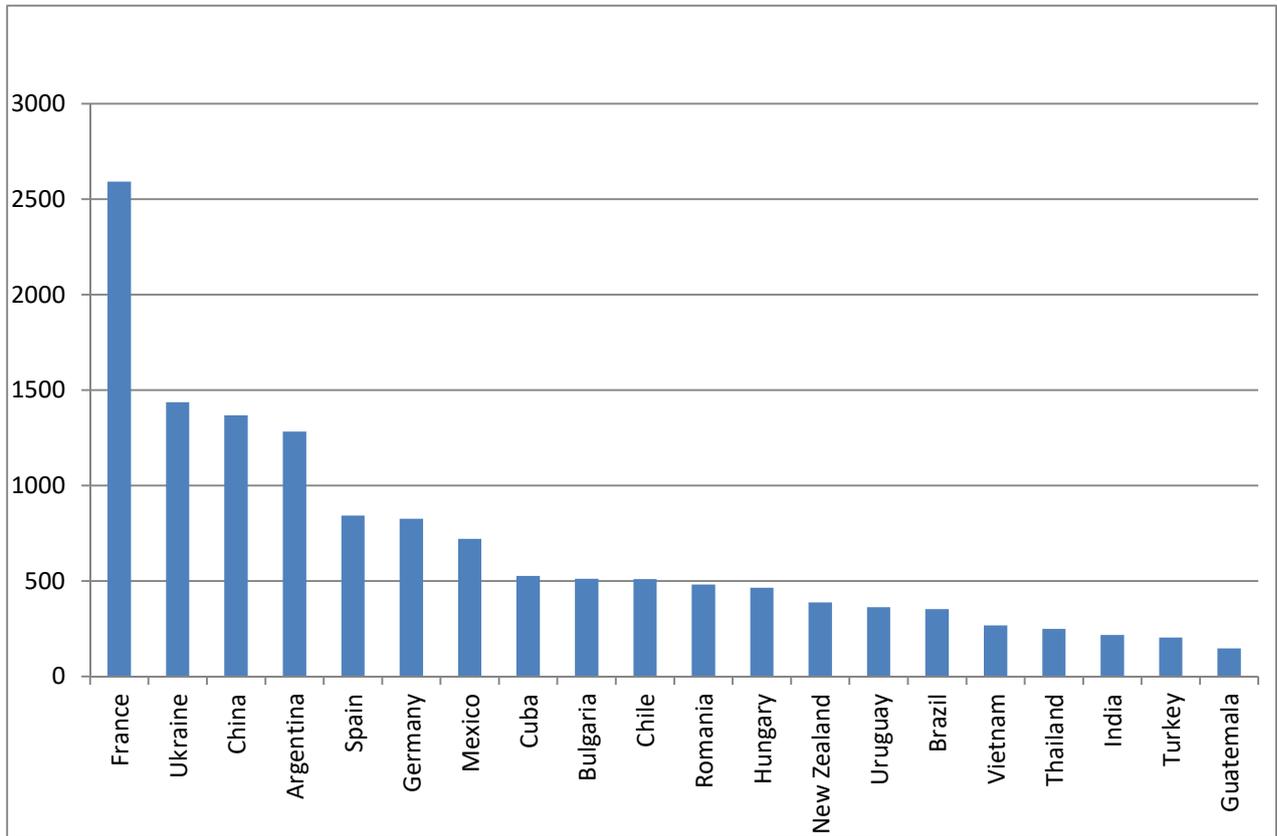


**Figure 2** The same Sunflower sample from Ukraine was analyzed once with the Ukraine model (top pictures) and once with the more general Blossom model (bottom pictures). Even though the sugars are identical, compared to the general Blossom model they are seemingly diluted which in some rare cases might be reason for a false positive evaluation, if the customer does not declare the honey accordingly. Overall the country / variety models are much sharper (pictures above are scaled differently).

In the first version of the Honey-Profiling 3 models for botanical varieties (Manuka, Linden and Acacia) and 11 country models for geographical origin were available. In the new version of the Honey-Profiling™ this list could be extended (see Figures 2 and 3). With these models the origin of a sample can be verified and specific adulteration models can be applied for certain origins which enables a much greater accuracy of the adulteration detection.



**Figure 3** Varieties available for origin verification sorted by sample count.



**Figure 4 Countries of origin available for origin verification sorted by sample count.**

While testing the new and improved origin verification models, we have found that they can predict and correctly verify the origin in the vast majority of cases. However, there are still some rare cases for which declared origins are not correctly identified by the statistical evaluation. This can only be improved, if further samples are added to the database and the statistical models get even better. Hence, we will continue to grow the database, especially with a focus on rarer origins and models that only have a small number of samples; i.e. models with samples <500.

The models are generated by differentiating samples from a specific origin against all other samples (see Figure 4). These are then applied to actual samples in a second step (see Figure 5).

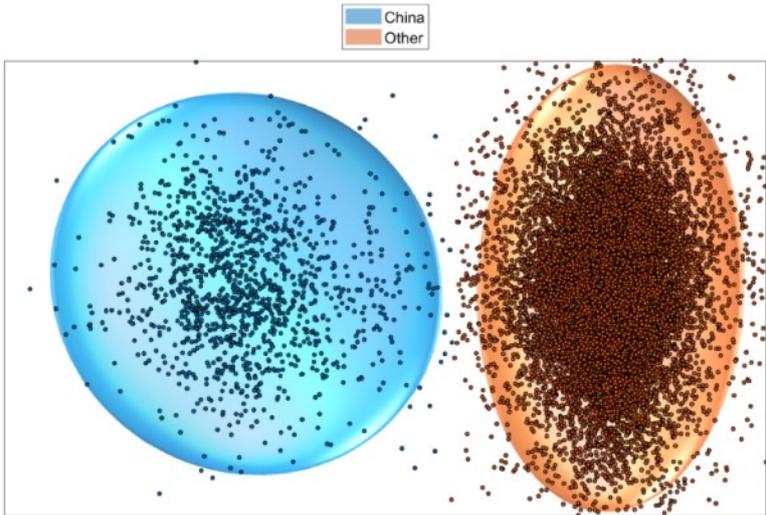


Figure 5 Build of model china (all chinese samples are statistically evaluated against all other samples).

### Analysis of declared Information

Statistical Model: Origin China

(Analysis-ID: HO-1105-01/0025)

This model is based on 15946 samples, thereof 1367 samples of reference group *China*.

**Result:** Consistent with declared country *China*. The probability of consistency is 100.0%.

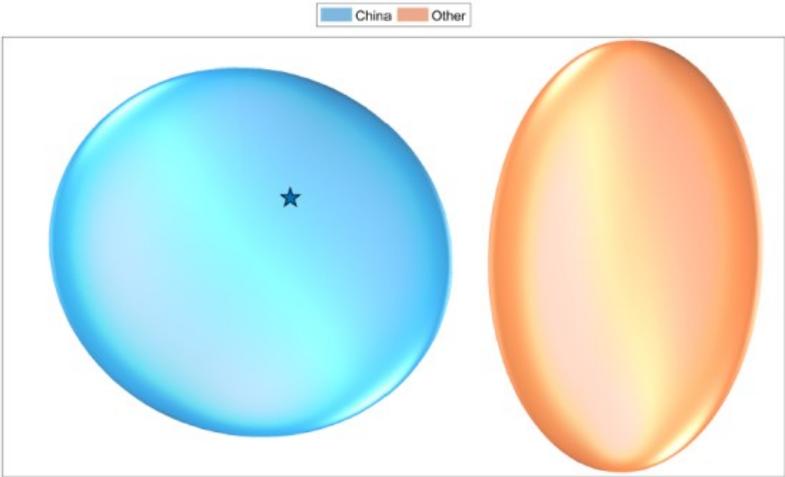


Figure 6 Analysis of a specific sample utilizing the china model. Sample is found to be within reference group.

## QSI NMR Offer

- General Honey-Profiling (Code 40500)
  - Cost = 150 €
  - Included are adulteration, authenticity (e.g. botanical and geographical origin) and quality (HMF, fermentation) analysis
- **NEW:** Full Service Honey-Profiling (Code 40505)
  - Cost = 250€
  - Included is everything from the General Honey-Profiling
  - Additional 15 quantified parameters of Bruker report (Glucose, Fructose, Sucrose, Turanose, Mannose, Maltose, Melizitose, Citric acid, Lactic acid, Acetic acid, Formic acid, Shikimic acid, HMF, Proline, Ethanol)
- **NEW:** Full service Honey-Profiling (Manuka; Code 40506)
  - Cost = 250€
  - Included is everything from the General Honey-Profiling
  - Additional 15 quantified parameters of Bruker report (Glucose, Fructose, Sucrose, Turanose, Mannose, Maltose, Lactic acid, Acetic acid, Formic acid, HMF, Prolin, Ethanol, Phenyllactic acid, MGO, DHA)

**Adulteration analyses includes QSI expert interpretation which might differ from Bruker report for borderline cases.**

Please do not hesitate to contact us if you have any further questions on this subject.

Best regards

Quality Services International GmbH