IHC Working Group “Authenticity of Bee Products”

Summary Report of the annual meeting, 1 October 2013
(during the 43th Apimondia Conference in Kiev, Ukraine)

Meeting Agenda

1. Welcome, existing members, new member applications (Lutz Elflein)
2. Modification of AOAC method 998.12 (C-4 sugars in honey) regarding the manuka honey issue (Lutz Elflein)
3. Manuka honey: C4 sugar false positives and determining manuka honey ID (Karyne Rogers)
4. Manuka honey authenticity - new options (Ralf Schlothauer)
5. Unifloral honeys - some remarks about manuka honey (Karl Speer)
6. Using NMR technique for honey authenticity and NMR metabonomics for geographical/botanical origin destination (Stefan Schwarzinger)
7. ISO/TC 34/WG 13 – latest draft of the Royal Jelly ISO Standard (Giancarlo Quaglia)
8. GC-MS technique used for detection of beeswax adulteration with hydrocarbons of alien origin – results of monitoring in Poland (Ewa Was)
9. The determination of phenolic profiles of Serbian unifloral honeys using ultra-high-performance liquid chromatography/high resolution accurate mass spectrometry (Dušanka Milojković-Op senica)
10. Overview of relevant new scientific publications in 2013 (Lutz Elflein)
11. Various, conclusions, next group meeting (Lutz Elflein)

1. Welcome, existing members, new member applications (Lutz Elflein)

It was decided at the last IHC Meeting 2012 in Braganca, Portugal, to reorganize the IHC working groups, because of the presence of many non-active or quietly retired members, so that there was no actual cooperation and work progress possible in the past year. Call-up for active members and scientists who want to join and actively participate in the authenticity working group fortunately attracted a great interest, so that and many new members could be acquired and welcomed in Kiev. Also, five new members were admitted to the working group in the Kiev meeting:

- Professor Karl Speer, Food Chemistry Department, TU Dresden, Germany
- Dr. Karyne Rogers, Senior Scientist, National Isotope Center, GNS Science, New Zealand
- Dr. Stefan Schwarzinger, Alnumed/University Bayreuth, Germany
- Dr. Ralf Schlothauer, Comvita, New Zealand
- Dr. Jinzhong Xu, Jiangsu Sinography Testing Co. Ltd., China

The authenticity group is now consisting of 23 members, mainly from European countries as well as Turkey, New Zealand and China.
2. Modification of AOAC method 998.12 (C-4 sugars in honey) regarding the manuka honey issue (Lutz Elflein)

In 2012 and 2013, manuka honey authenticity was one of the most discussed topics regarding honey authenticity. A large percentage of New Zealand manuka honey was reported to fail C4 sugar testing although they were claimed to be pure (Rogers et al., Eliminating false positive C4 sugar tests on New Zealand manuka honey, Rapid communications in mass spectrometry 24 (2010), 2370-2374; Rogers et al., Modification of AOAC Official Method 998.12 to Add Filtration and/or Centrifugation: Interlaboratory Comparison Exercise, Journal of AOA C International 96/3 (2013), 607-614).

Based on these findings, the AOAC revised its method for the determination of C4 sugars in honey (AOAC 998.12) by amending the result interpretation with an exemption for unusual varieties. While previously every honey which fails to comply with the limit value of ≤ 7% C4 sugars (referring to a natural variation of d13C protein and d13C honey values of max. ± 1 ‰ due to the fact that bees can forage on nectar and pollen from different plants), the new result interpretation now allows for “an exception of a few unusual varieties which may slightly exceed the range, but will have d13C for honey which are in the normal range (more negative than –24.0 ‰)”.

Not surprising, this new interpretation opened the door for numerous speculations about the reliability of the AOAC method and which honeys might be considered as unusual varieties (not only manuka) and also led to a severe confusion within the international honey industry, particularly in the import-export business, due to the vague wording. Basically, every result > 7% C4 sugars could now be questioned in trade contracts or legally, so that a great uncertainty arouse within international trade which requires clear rules and definite acceptance criteria. In the meanwhile discussions within AOAC and among stakeholders is ongoing to define the new wording for exemptions more precisely by explicitly mentioning honey varieties which have been scientifically proven to require different purity criteria. Additionally, the general cut-off value of -24 ‰ is also scientifically unjustified, as the d13C honey value depends on the botanical origin of the honey (natural range: -22.5 to -28 ‰). Thus, an isotopic cut-off value can only be defined along with the honey type for which it is valid. Otherwise, especially for honey varieties having d13C honey values more negative than -24 ‰, the new wording of the interpretation would mean a weakening of the current purity criteria by accepting possible C4 sugar contaminations larger than 7 % (up to 20%).

As it has been already shown that the pollen contained in manuka honey, previously claimed to be responsible for a shift of the d13C protein values towards more negative figures and thus causing an increase in the apparent C4 sugar content, is not the root cause for test failures / false positives (Frew et al., Modified sugar adulteration test applied to New Zealand honey, Food Chem. 141/4 (2013), 4127-31), the research will have to go on in order to clarify potential false positive results of pure manuka honey and identify specific purity acceptance criteria for this honey type as required.

3. Manuka honey: C4 sugar false positives and determining manuka honey ID (Karyne Rogers)

Dr. Rogers held two presentations at the meeting. The first presentation dealt with the manuka honey false positives in C4 sugar testing. She has conducted new investigations which gave indication that active manuka honey containing high levels of dihydroxyacetone (DHA) and methylglyoxal (MGO) fails the C4 sugar test, because some yet unknown reaction mechanism between these compounds and amino acids/proteins in the honey seems to cause a shift of the d13C protein value towards more negative values. A publication about these findings will follow soon.

The second presentation gave an overview about the initiative of the NZ Ministry of Primary Industries in order to fine criteria for manuka honey ID. The current proposal includes the manuka pollen count and the MGO level as parameters, either alone or in combination. However there are
some significant drawbacks (difficult differentiation of manuka/Kanuka pollen by microscopy; possible artificial manipulation of DHA/MGO content and variation of their concentrations with storage time), so that additional parameters are likely to be required to accurately define the key characteristics and composition of pure manuka honey.

4. Manuka honey authenticity - new options (Ralf Schlothauer)

Dr. Schlothauer described the manuka honey history and the discovery of its unique properties (antimicrobial, anti-inflammatory, immunostimulatory) which eventually led to the development of value-added and high price products. Especially, the non-peroxide-activity (NPA) determines the price of active manuka honey. There is a direct correlation between the UMF activity and MGO concentration, but the correlation factor varies between different studies. Both UMF and MGO can change over time during storage. After an initial increase, MGO and UMF will finally decrease after 40 months. This should be considered in respect to shelf life and labeling. Dr. Schlothauer also discussed the possibilities for defining Manuka honey ID, including pollen count (manuka, kanuka) and various chemical marker compounds. The studies showed that Manuka honeys from different regions of New Zealand contain different amounts of pollen, DHA and MGO, which do not necessarily correlate with each other. It can be presumed that honeys with high pollen count but low levels of DHA and MGO are likely derived from kanuka. However, there are also honeys with low pollen count exhibiting high DHA/MGO levels. Furthermore, the occurrence of chemical markers, 4-methoxyphenyllactic acid (kanuka) and 2-methoxybenzoic acid (manuka) is variable. Therefore, in order to define manuka honey ID, it is very important to conduct a representative and independent sample collection of authentic manuka honeys and verify their origin by analyzing plant nectar samples in order to prove that the chemical signature is identical.

5. Unifloral honeys - some remarks about manuka honey (Karl Speer)

Prof. Speer presented his research work about honey analysis of volatile compounds, phenolic acids, flavonoid, aromatic amino acids, nor-isoprenoids and N-containing substances, including manuka honey. Besides details about the analytical methods applied, he found interestingly three groups/types of manuka honeys which can be distinguished by characteristic components:

**Group A:** 4-OH-benzoic acid, dehydrofomivoliol, benzoic acid
**Group B:** kojic acid, 2-methoxybenzoic acid
**Group C:** syringic acid, 4-methoxyphenyllactic acid, methylsyringate

The three groups of manuka honeys also differed in their MGO content:

**Group A:** av. 123 (58-178) mg/kg
**Group B:** av. 655 (507-897) mg/kg
**Group C:** av. 349 (41-1178) mg/kg

Regarding volatiles/aroma compounds, several characteristic marker compounds were identified for acacia, buckwheat, chestnut, dandelion, eucalyptus, heather, lavender, lime, manuka, orange, oregano, pine, rosemary, sage, sunflower and thyme honeys which can be used for unifloral classification. The three above mentioned groups of manuka honeys could also be differentiated by their volatile profile. Volatile markers of all manuka honeys investigated were benzofuran, dimethylbenzofuran, 2'-hydroxyacetophenone and 2'-methoxyacetophenone.

Thus, the presented work can make an important contribution for defining a manuka Honey ID.
6. **Using NMR technique for honey authenticity and NMR metabonomics for geographical and botanical origin destination (Stefan Schwarzinger)**

Dr. Schwarzinger presented the analytical possibilities of NMR measurements for compositional analysis as well as NMR metabonomics and component profiling for determining the authenticity of honey. This technique has a great potential for the future scientific work regarding authenticity issues. While quite a lot of research has already been done regarding honey composition and botanical origin determination. However, the application of this technique for adulteration testing has to be still developed. Dr. Schwarzinger offered his cooperation in measuring samples and creating databases of authentic reference samples in order to set up additional decision criteria for the assessment of honey authenticity.

7. **ISO/TC 34/WG 13 – latest draft of the Royal Jelly ISO Standard (Giancarlo Quaglia)**

Dr. Quaglia presented the current status of the draft Royal Jelly ISO norming process. Two types of royal jelly (type 1: traditionally produced by feeding honey and pollen; type 2: produced by sugar feeding) have been included in the royal jelly definition and composition criteria were established to differentiate between the two types which differ significantly in the market price. Also, ranges of acceptable d13C values for both types were set. As a final agreement of the draft norm was reached in the last ISO meeting, it is now hoped that the norm can be published in the near future.

8. **GC-MS technique used for detection of beeswax adulteration with hydrocarbons of alien origin – results of monitoring in Poland (Ewa Was)**

Ms. Was presented a further refined method for determination of paraffin adulteration of beeswax. This type of adulteration not only causes economic damage but can also negatively affect bee colony development. Unlike the commonly applied quantitative gravimetric determination of the hydrocarbon fraction in beeswax, the hydrocarbons were directly quantified with GC-MS, which is much more convenient and more precise. Furthermore, minimum-maximum concentrations of individual hydrocarbons in pure beeswax were defined for the first time, which can be used for a more sophisticated and sensitive (> 3% addition) authenticity assessment of beeswax in future.

9. **The determination of phenolic profiles of Serbian unifloral honeys using ultra-high-performance liquid chromatography/high resolution accurate mass spectrometry (Dušanka Milojković-Opsenica)**

Dr. Milojković-Opsenica presented her research about phenolic profiles of unifloral Serbian honeys. This work has just been published in the Food Chemistry journal. Besides detailed methodical information (UHPLC-LTQ-Orbitrap), the data of phenolic compound profiling along with multivariate data analysis allowed for identification of 43 characteristic compounds as well as differentiation between different botanical origins. This work gives valuable information for botanical and geographical origin determination of Serbian honeys.

10. **Overview of relevant new scientific publications in 2013 (Lutz Elflein)**

There had been a number of relevant publications in the last year for which as short summary and reference was given:

11. Conclusions, various, next group meeting (Lutz Elflein)

- The Head of the working group wants to thank all participants for attending the meeting, their valuable contributions and the fruitful discussions. As the group has now been formed with new active members, new scientific work can now be gathered, evaluated and published through IHC.
- Several scientists are currently dealing with the manuka honey authenticity problem. The IHC working group authenticity should also participate in the ongoing research about this issue. The interested parties agreed on an international and interdisciplinary cooperation for further research in order to help defining a manuka honey ID and find generally accepted and scientifically sound analytical methodologies to assess manuka honey authenticity in the routine control of commercial honey batches.
- While mainly focused on adulteration detection and purity assessment of honey in the recent years, the newly formed working group “authenticity of bee products” will also deal in future with analytical tools related to the botanical and geographical origin determination which is also an important part of authenticity assessments, interlinking with the working group “Pollen Analysis”.
- Furthermore, other bee products like royal jelly and beeswax, are gaining more attention in current and future authenticity research. Pollen and Propolis could also be potential matrices for authenticity testing, but this will be much more complex than for the other matrices and potential work has to be coordinated in advance with the other two IHC working groups “Bee Pollen” and “Propolis” specialized in these two products.
- The time schedule of 2 hours for the meeting was very tight considering the broad range of contributions. Therefore, apologies and thanks for patience to the sensory working group which followed up. For the next IHC meeting it should be considered to organize separate
meeting rooms for the individual working group sessions as far as possible, so that more time (half-day to one day) is available.

- The next meeting will take place during the annual IHC meeting in Opatija, Croatia, somewhere around the end of September 2014. The exact date and meeting details will be communicated as soon as they are available.

Sgd. Lutz Elflein, Bremen 11 December 2013