

AUTHENTICITY OF HONEY AND OTHER BEE PRODUCTS

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ABSTRACT

Honey authenticity is defined by the Codex Alimentarius standard, the EU Honey Directive and several national legislations. The Codex and EU standards were recently revised. The authenticity of honey and other bee products have two aspects. Authenticity in respect of production i.e. to prevent adulteration by addition of other food ingredients by correct beekeeping practice and processing. The other aspect concerns authenticity in respect of geographical and botanical origin. As honeys from certain geographical regions or botanical sources attain higher prices than others, mislabelling is of economic interest.

Today, the authenticity of the botanical origin of bee products is determined by sensory analysis, pollen analysis and several physico-chemical methods while for testing of the geographical authenticity melissopalynological methods are employed. These analyses, however are time-consuming and require specialized know-how and expertise.

Objective evaluation techniques based on analytical chemistry and statistics should be developed for reliable authenticity testing in order to ensure the quality of the bee products as valuable natural products. The objective of this presentation is to discuss the different authenticity issues, the current methods used and to evaluate new methods for successful authenticity testing.

HONEY

AUTHENTICITY OF PRODUCTION

The major concern of honey quality is to ensure that honey is authentic in respect to the legislative requirements. According to the definition of the Codex Alimentarius (Codex Alimentarius Commission 2001) and other international honey standards (2001/110/EC, EU Council 2002) honey shall not have added any food ingredient than honey to it nor shall any particular constituent be removed from it. Honey shall not have any objectionable matter, flavour, aroma or taint from foreign matter during its processing and storage. The honey shall not have begun to ferment or effervesce. No pollen or constituent particular to honey may be removed except where this is unavoidable in the removal of foreign inorganic or organic matter. Honey shall not be heated or processed to such an extent that its essential composition is changed and/or its quality impaired.

Fermentation

Harvesting honey with a too high water content leads to spoilage by fermentation, resulting in a product with an off-taste, high levels of yeast, glycerol, butanediol (Russmann 1998) and ethanol (Papoff et al. 1996).

Heat defects

The use of excessive heat for liquefaction or pasteurisation of honey has adverse effects on honey quality, i.e. loss of volatile compounds, accumulation of HMF and reduction of invertase and diastase activities. Quantification of HMF content and enzyme activities are useful tools in detecting heat induced defects in honey but can not be used for the determination of botanical or geographical origin.

Honey filtration

Honey should not be strained with a mesh size smaller than 0.2 mm in order to prevent pollen removal. On the other hand, the shortly revised Codex Alimentarius Honey Standard (Codex Alimentarius Commission 2001) and EU Directive relating to honey (EU Council 2002) allow a removal of pollen if it is unavoidable for the removal of foreign matter. Such honey should be labelled as "filtered". As microscopical pollen analysis is still the most important tool for the determination of botanical and geographical origin of honey the removal of pollen by filtration will make authenticity testing much more difficult, if not impossible.

Adulteration by sweeteners

As a natural product of a relatively high price, honey has been a target for adulteration for a long time. Addition of sweeteners, feeding the bees during the nectar flow or extracting combs containing bee feed may adulteration of honey. The following sweeteners have been detected in adulterated honeys: sugar syrups and molasses inverted by acids or enzymes from corn, sugar cane, sugar beet and syrups of natural origin such as maple. Recently, there has been a major adulteration problem, predominantly concerning honey from the Far East (White 2000).

Organic honey, raw or unheated honey

Recently a European regulation for the production of organic honey has been established (EU 1999). As with all organically produced food, the control of organic honey implies only the beekeeping procedures and not the honey quality. As a consequence, no testing scheme can reveal if a honey is organically produced or not. However, the presence of veterinary drug residues will definitely demonstrate that organic production methods have not been used and will thus expose the mislabelling of a sample as organic. The label 'natural' honey is misleading, since honey is natural by definition.

Fresh honey has a very low hydroxymethylfurfural (HMF) level and shows a natural level of enzyme activity. The designation "fresh", "raw" or "virgin" honey has been proposed to indicate that the honey has not been heat-treated (EU 2002) honeys should have a maximum HMF content of 25 mg/kg. There are also proposals that the HMF level of raw, unheated honeys should be below 15 mg/kg, while the invertase activity should be higher than 10 Hadorn units (Duisberg and Hadorn 1966).

METHODS FOR TESTING OF AUTHENTICITY OF HONEY PRODUCTION

Microscopic, physico-chemical and organoleptical methods such as pollen analysis, determination of moisture, HMF-content, invertase- and diastase activity, sugar composition, electrical conductivity and proline content are used for routine quality control of honey. They are suitable as screening tools to detect impaired honeys, which can be

further submitted to analysis with more sophisticated techniques like stable carbon isotope ratio analysis. At present the determination of each parameter requires another analytical method and is thus very time consuming. In the future the routine parameters for honey quality control may be measured within minutes by infrared spectroscopy (Lichtenberg-Kraag et al. 2002).

Honey fermentation

Harvesting of honey with high moisture content, or subsequent addition of water can result in honey fermentation and spoilage. Honey spoilage can be tested by a microscopic yeast count (Beckh and Lüllmann 1999) or by determining the glycerol (Huidobro et al. 1993), butanediol or ethanol (Huidobro et al. 1994) contents.

Adulteration by addition of sugars

Adulteration by addition of cane- and corn sugar can be screened microscopically (Kerkvliet and Meijer 2000) and verified by measurement of $^{13}\text{C}/^{12}\text{C}$ isotopic ratio (Brookes et al. 1991, Rossmann et al. 1992, White 1992, White and Doner L. W. 1978, White et al. 1998). The addition of high fructose corn syrup may be detected by oligosaccharides naturally not present in honey. Recently infrared spectroscopic methods have been described for the detection of adulteration by beet and cane sugar (Sivakesava and Irudayaraj 2001a, 2001b, 2001c).

METHODS FOR TESTING THE AUTHENTICITY OF ORIGIN AND LABELLING

Misdescription of botanical source

The botanical source may be labelled if the honey originates wholly or mainly from a particular source and has the organoleptic, physico-chemical and microscopic characteristics of that origin. As bees forage on different plants, absolutely pure unifloral honeys are extremely rare. The different unifloral honeys show very distinct sensory properties.

Of the physico-chemical parameters measured electrical conductivity and the fructose and glucose content, providing the fructose glucose ratio, are most useful. By chemometrical evaluation of physico-chemical parameters (sugars, electrical conductivity, optical rotation, nitrogen content etc.) a good separation between some unifloral honeys (Vinci et al. 1997, Bogdanov 1997, Piro et al. 2002) can be achieved. However, it should be noted that these methods may not allow a discrimination between unifloral and polyfloral honeys.

Currently, a thorough examination of botanical origin includes organoleptic, physico-chemical and pollen analysis. In case of doubt the decision is very often made based on the organoleptic evaluation of an expert. This is especially true for honeydew honeys. In addition, for many unifloral honeys no internationally recognized quality criteria exist. However, the International Honey Commission is presently working on the establishment of quality criteria for the most important European unifloral honeys.

Pollen analysis is still the most important method for the determination of the botanical origin of honey. It needs highly experienced and specialised personnel and cannot for the time being be aided by computers for more efficient assessment of data. The pollen content of nectar varies to a very large extent. For example, according to this standard

citrus honey should have at least 10 % of citrus pollen, rape honey is expected to contain more than 45 % rape pollen, while a chestnut honey must contain more than 90 % of chestnut pollen. Practical experience has shown that the determination of botanical origin can not solely be based on pollen analysis.

Volatile compounds have been proposed as specific markers for unifloral honeys. Quantitative analysis of honey volatiles extracted with organic solvents have shown differences between unifloral honeys (Guyot et al. 1998, Guyot et al. 1999, Guyot-Declerck et al. 2002). However, solvent extraction of volatiles is not suitable for routine analysis of the botanical origin of honey because it is time consuming. The use of dynamic head-space techniques (Bouseta et al. 1992, Radovic et al. 2001b) or solid-phase microextraction (SPME) (Guidotti and Vitali 1998, Perez et al. 2002) of honey volatiles could prove useful for routine authenticity testing of the botanical origin of honey. Many volatile compounds are present in most of the unifloral honeys in similar concentrations and are therefore not useful for separation between different botanical origins. Marker compounds of unifloral honeys can probably be detected among the less volatile components.

A recent paper shows that pyrolysis-mass spectrometry is a promising technique for the differentiation between several unifloral honeys (Radovic et al. 2001a).

Misdescription of geographical origin

Generally, in Western Europe and also in countries like the United States and Japan, honey imported from Far East or South America has a lower price than the locally produced honey, and is therefore prone to mislabelling because of economic reasons. Pollen analysis and a number of chemical methods have been used for the characterisation of the geographical origin of honey. Pollen analysis is very efficient for the differentiation of honeys produced in distinctly different geographical and climatic areas. If the climatic differences are less pronounced, the determination of the pollen spectrum may not yield a confident authenticity proof. However, recently the computer aided interpretation of data has been successfully used for the determination of honeys originating from zones geographically close by the use of special software for pollen analysis (Battesti and Goeury 1992) and also statistical discriminant analysis and cluster analysis (Sancho et al. 1991, Floris and Satta 2002).

By chemometric combination of different parameters such as water, proline, ash content, electrical conductivity, acidity (free and lactone), pH, HMF, diastase and sugars, a good separation of honeys from different geographical regions of Spain has been shown (Barez et al. 2000, Gonzales Paramas et al. 2000, Lopez et al. 1996, Pena Crecente and Herrero Latorre 1993, Sanz et al. 1995). The botanical origin has not been considered and may have contributed to the separation of the different regions.

The determination of trace elements such as calcium, magnesium, zinc and strontium is widely used in food authenticity studies, also in relation to the geographical origin (Crews 1998). The first study was carried out by activation analysis with thermic neutrons in French and Hungarian honeys (Lasceve and Gonnet 1974). By a combination of several elements it was possible to differentiate between acacia honeys from the two countries. Canadian honeys from regions with a continental climate could be distinguished from honeys produced in a maritime climate by the total mineral content (Feller-Demalsy 1990). Analysis of amino acids might be another promising approach. In several studies it was shown the amino acid spectra varied between honeys from different geographical origins (Davies 1975, Davies and Harris 1982, Gilbert et al. 1981). For a reliable evaluation of the

usefulness of the amino acid composition of for the determination of geographical origin the botanical origin of the honeys studied should be considered.

The Swiss Bee Research Centre is currently running a project attempting to determine the botanical origin of honey by chemometrical evaluation of physico-chemical parameters in cooperation with the Swiss Federal Institute of Technology Zürich and the Swiss Federal Office of Public Health.

BEESWAX

Beeswax is the most important bee product besides honey and apart from its use in beekeeping it is predominately used in cosmetic industry. A very serious problem of beeswax used for pharmaceutical applications is the contamination of persistent acaricide residues resulting from the world wide use of chemicals used for the control of *Varroa destructor* (Wallner 1997).

The current quality criteria for pure beeswax according several pharmacopoeia i.e. acid value, ester value, saponification value, drop point, tests for paraffin and other waxes as well as for glycerol and other polyols are inadequate for it's reliable determination. An adulteration by oleochemical or paraffin hydrocarbon addition is relatively easy and common. The official methods for detection of beeswax adulteration are outdated, on the other hand adulteration can be detected very sensitively by gas chromatographic determination of wax components Unambiguous detection of beeswax adulteration can be achieved by GC (Brüschweiler et al. 1989).

Most of the beeswax on the world market is produced by *Apis mellifera ligustica* (60 %) and *Apis mellifera scutellata* (25 %) (Brand-Garnys and Sprenger 1988). Modern gas chromatographic methods show that the wax produced by different bee races has the same components, but in different proportions (Brand-Garnys and Sprenger 1988). Also high-temperature gas chromatography at about 380 °C can supply characteristic fingerprints of waxes from different honeybee species (Aichholz and Lorbeer 2000). There is a need to develop new analytical methods and to establish composition criteria for pure beeswax that can allow reliable quality control.

ROYAL JELLY

Authenticity of production

Royal jelly is a very perishable product that has to be deep-frozen immediately after collection. In quality control of royal jelly the water content, the amount of sugars, lipids and proteins as well as pH and free acidity is determined (Pourtallier et al. 1987, Serra Bonvehí 1992). The measurement of these parameters allows to a certain extent also the determination of adulterations with different products, which is most cases honey (Serra Bonvehí 1991). However, specific determination of the royal jelly marker, trans-10-hydroxydecanoic acid (Bloodworth et al. 1995) is generally a better and a more specific method for testing of royal jelly authenticity.

Authenticity of geographical origin

Royal jelly is predominately produced in China and other far eastern countries and is marketed world wide. The far eastern production has a highly competitive price on the world market and the determination of geographical origin of royal jelly is an important issue. At present the only method described is based on the analysis of the pollen spectrum. Pollen spectra from Chinese, Mexican, North-American, Russian, Polish, Romanian, Yugoslavian, Czech, Bulgarian and French royal jelly have been described (Ricciardelli d'Albore and Battaglini 1978).

POLLEN

Pollen are produced in various countries as a feeding supplement of bees and for human nutrition. Because of its unique appearance no adulterations with other products are known. Between the different production areas considerable differences in price prevail. In countries with higher prices there is a certain risk of selling lower priced pollen from abroad as a domestic product. Pollen analysis is at present the only way to detect misdescriptions of geographical origin. Specific software tools could prove useful in the detection of mislabelled pollen.

PROPOLIS

Authenticity of origin

The chemistry and plant origin of propolis have recently been reviewed. The chemical composition of propolis varies considerably depending on the plant species and the geographical region it is derived from (Bankova et al. 2000). In countries with moderate climate this resinous product is collected by bees predominately from the buds of poplar trees. The majority of Eurasian propolis is derived from *Populus nigra* bud extrudates. In northern areas or at high altitudes *Populus tremula* can also contribute to propolis production. Brazilian propolis is predominantly derived from *Baccharis dracunculifolia*. In tropical regions, there are probably other plant sources, but these have not yet been elucidated. At present the botanical origin of propolis can be determined by thin layer, gas chromatographic and HPLC procedures (Bankova et al. 2000) while the geographical origin of propolis can be determined by pollen analysis (Ricciardelli d'Albore 1979).

Authenticity of production

Another important quality authenticity issue is the contamination of propolis with acaricides, as this valuable product is used for many medicinal purposes. Today propolis, produced in conventional beekeeping contains high amounts of acaricides, used for the control of *Varroa destructor* (Wallner 1995). Also heavy metals may accumulate to high levels in this bee product (Leita et al. 1996).

Naturally, propolis contains up to about 30 % of beeswax (Ghisalberti 1979) The appropriate quality control of propolis has to include determination of the amount of beeswax and other insoluble matter (Bankova and Marcucci 2000). For the determination of propolis freshness the volatile content is an important indicator.

CONCLUSIONS

Although there are powerful methods to prove honey adulteration, they have to be further improved in order to ensure honey quality. Concerning the botanical origin of honey, criteria for the determination of unifloral honeys, based on a wide variety of commercially available unifloral honeys, should be fixed. The International Honey Commission is working on a data bank for quality criteria of the most important European unifloral honeys. Analytical methods for the determination of honey volatiles and honey aroma should be developed as a tool for the determination of the botanical origin of honey.

The development of new alternative methods for the determination of the geographical origin of honey is at the beginning. There are promising methods which have to be tested with a much greater number of honeys. Studies with stable isotope ratios other than carbon, such as the D/H ratio and the oxygen isotopes ^{16}O and ^{18}O , which have proved useful in geographical authenticity testing of other foods can be tested in honey.

Compared to honey the analytical methods and criteria for the authentication of other bee products are far behind. There is a need to fix appropriate criteria for quality control and to develop powerful analytical methods for authenticity testing in the near future.

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