

Honey Authenticity: a Review

Stefan Bogdanov¹ and Peter Martin²

¹Swiss Bee Research Centre, Dairy Research Station, Liebefeld

²Q. P. Services, Hayes, Great Britain

Introduction

Honey is one of the very few natural foods which is offered today. The composition and the main quality criteria for honey are summarised in the Swiss Food Manual (1) and also in different monographs (2, 3).

The Codex Alimentarius Standard and the EU Council Directive

The honey standards of the Codex Alimentarius (CA) (4) and of the European Community (5) have been revised recently. The changes in the standards and the analytical methods used for their determination, following the advice of the International Honey Commission, were recently reviewed (6).

The standards of the Codex Alimentarius and the EU are very similar. The Codex Alimentarius honey standard is more detailed, containing references to quality factors such as heavy metals, pesticides and adulteration.

The definition of honey in both standards is the same:

“Honey is the natural sweet substance, produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature”.

According to the CA standard the essential composition and quality factors are:

“3.1 Honey sold as such shall not have added to it any food ingredient, including food additives, nor shall any other additions be made other than honey. Honey shall not have any objectionable matter, flavour, aroma, or taint absorbed 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.

3.2. Honey shall not be heated or processed to such an extent that its essential composition is changed and/or its quality impaired”

In the EU council directive, Annex II the same essential composition and quality factors are mentioned, but the text is formulated differently. Thus according to both standards honey should be authentic.

The new honey compositional criteria of both standards are summarised in table 1. The meaning of these standards has been discussed recently (6). Compared to the previous standards there are some changes:

- The new maximum limit for the water content is fixed at 20 %, while the old standards (and also that of the Swiss Food Directive) were at 21 %.
- There is a change in the HMF and Diastase limits: the limits of maximum 40 mg/kg HMF and minimum 8 diastase units are valid after processing and blending, while the old CA standards had limits of 80 mg/kg and 3 diastase units.
- Under some circumstances both standards will allow the fine filtration of honey, removing all the pollen, as long as the honey is described as “filtered”.

In the present standards the humidity limit is lowered to 20 %, which is the same as in the previous Swiss Food Directive. Indeed in the present directive it had to be harmonised with the old European limit of 21 %.

In both standards it is remarked that the HMF and the diastase limits are valid after processing and blending of honey. This can be interpreted to mean that these two quality factors are not valid during the whole shelf life of honey. Indeed, it has been a concern of the honey trade for a long time, mostly in non – European countries, that these two quality factors are often not fulfilled in retail honey during the shelf life of the honey, especially during storage at higher temperatures.

The removal of pollen will make the determination of botanical and geographical origin of honey much more difficult, if not impossible unless the law in each member state permits inspectors to enter packing plants to take raw material samples and insists that packers keep full records proving traceability.

The compositional criteria for honey of both standards are very similar. However there are also some differences:

- The Codex Alimentarius standard refers only to retail honey and there is no special mention of baker's and industrial honey. This is planned to be added in a future addendum to the standard.
- In the EU Directive the quality criteria hydroxymethylfurfural (HMF) content, diastase activity and honey acidity are intended for application by commercial partners and governments, while according to the CA standard they are only for voluntary application between trade partners.
- According to the EU Directive honey is referred to as a product of *Apis mellifera*, the European honey bee, while the Codex Alimentarius honey is defined as a product of all honey bees.

The honey definition of the Codex Alimentarius is more correct, as different honeys are offered on the world market, with a predominance of course of *Apis mellifera* honey. Indeed, a major part of Asian honey is produced by the Asian bee, *Apis cerana*. The honey, produced by that bee has a composition similar to that of the *Apis mellifera* honey, but has a higher water content and no special compositional criteria have been established.

The methods used for the determination of the quality factors are described only in CA standards, while the EU Directive refers to the Codex methods without naming them explicitly, as is to be expected under the principle of subsidiarity.

The Swiss Food Legislation will have to adapt to the new honey Codex Alimentarius standard and the EU honey directive. From a methodological point of view no adaptations are necessary for the Swiss Food Manual, as the same methods, which are used for the new quality factors according to the Codex Alimentarius, are already part of the present Swiss Food Manual. On the other hand, the Swiss Food Directive has to be harmonised with the new CA standard and the EU directive.

Authenticity issues

A major concern of food control is to ensure that honey is authentic in respect of the legislative requirements.

The authenticity of honey has two different aspects:

- Authenticity in respect of honey production
- Authenticity in respect of descriptions: geographical and botanical origin, 'natural', 'organic', 'raw' and 'unheated' honey

In determining honey authenticity both aspects should be considered.

There are several reviews on different honey authenticity issues (7-10). The objective of this review is to examine all authenticity issues and the methods used to prove authenticity. In order to make the review more readable and compact, an attempt is made to concentrate on the most important

issues, without giving too much detail. Researchers looking for greater details on methods and issues are advised to consult the cited references.

Authenticity of production

Processing by the beekeepers and the industry

Retail honey has always been subjected to some form of processing during honey production. Today, most if not all commercial honey is produced by centrifugation. An appellation such as "harvested in the cold" is a mislabelling, since honey is harvested naturally at temperatures between 25-32^o C, which is similar to the temperature in the beehive (35^o C). Filtering of honey is an important issue. In the regulations of many European beekeeping associations, and also of the Swiss ones, the use of honey filters is prescribed. Beekeepers should harvest honey by using filters with a mesh size not smaller than 0.2 mm in order to prevent pollen removal. On the other hand, some packers, mostly in North America, will use smaller filters in order to filter out undesirable contaminants. According to the international honey legislation, such honey should be labelled as "filtered" (see Introduction).

However, the use of excessive heat for pasteurisation and liquefaction might have adverse effects on honey quality, e.g. loss of volatile compounds and reduction of enzyme activity. Pasteurisation to kill osmophilic yeast is carried out for 7.5 minutes at 63^o or for 1 minute at 69^o C, with rapid heating and cooling involved (11). Pasteurised honey should be labelled according to the Swiss food legislation, but appellations "pasteurised honey" are seldom seen, if ever, on retail honey pots.

The great majority of Swiss honey is harvested and marketed by the beekeepers. This honey is fresher and has taken less heat load than the non-Swiss honey, which has undergone subsequent heat treatments for liquefaction and filling purposes. There is a significant difference between the HMF and the invertase and diastase activities of these two honey classes (12).

Honey crystallisation can be influenced on an industrial scale by the Dyce procedure, to produce the so called creamed honey (13). A certain amount of a fine crystallised honey is mixed to liquid honey and the crystals are allowed to grow at 14^o C. This procedure stabilises the honey consistency. This procedure will not change honey authenticity, as no foreign matter has been added or taken away from honey.

Addition of sweeteners

As a natural product with a relatively high price, honey has been for a long time a target for adulteration. Correct beekeeping practice ensures that sweeteners used to feed bees should not adulterate honey. This implies improper feeding of sugar during the honey flow or addition of sugars to honey. The following sweeteners have been used: acid inverted sugar syrups, corn syrups, syrups of natural origin such as maple, cane sugar, beet sugar, molasses, etc. In recent years, there has been a major adulteration problem in the world, concerning mainly Chinese honey (14,15). Presently these sweeteners are mainly bee feeding syrups, produced by the hydrolysis of maize, cane and beet sugar.

Harvesting of non-ripe honey, addition and removal of water

Normally, the water content of honey harvested in countries with a moderate climate is below 18 %. However, in some countries the harvested honey has more than 20 % water, due to climatic or harvesting conditions. Only honeys having less than 17.1 % water are regarded as safe (16). The Codex definition prescribes that the bees dehydrate and store the honey and leave it in the honeycomb to ripen and mature. This implies that the combs will be capped. In normal beekeeping practice there may be a few cells around the edge of a comb that remain uncapped but essentially the maturing process must have been allowed to occur. There are two instances which give rise to a water content high enough to require remedial action. In the United States some honeys may contain more than the 18.6% water limit prescribed by United States Department of Agriculture standards, even though they are derived from capped combs. The small excess of water may be removed by centrifuge or vacuum evaporator. In China and probably some other countries a practice occurs which results in a product which is difficult to equate with the Codex definition of

honey. The nectar is harvested before the bees have had time to 'deposit, dehydrate, store and leave in the honey comb to ripen and mature'. The water content may easily be over 25%. This 'green' honey will easily ferment, often before it has had time to reach the factory for vacuum evaporation. It therefore ferments, resulting in a product with an off-taste, high levels of dead yeast, glycerol and butanediol (17) and also ethanol (18). There is a loss of aroma compounds in the drying process. Indeed, there are modern drying technologies that would add back to honey the aroma compounds, lost after the drying procedure.

Addition of water is probably not a realistic adulteration practice because of the fermentation risk, unless the water content was very low and was adjusted back to some agreed level such as 18.6% in the United States. Some packers would argue that it is legitimate to replace the small amounts of water lost during processing. The water content of honey can naturally be as low as 13.6% and as high as 23% depending on the source of the honey, climatic conditions and other factors. Fermentation does not usually become a problem in honeys with a water content less than 18%. The crucial criterion is that the honey be produced in a way compliant with the Codex definition.

Authenticity of origin and misdescriptions

Misdescription of botanical source

Bees forage different plants, so that honey is always a mixture of different sources. When higher prices are paid for certain types of honey, beekeepers and honey packers will often designate the botanical source of the honey. The International Honey Commission is presently working on the establishment of quality criteria for the most important European unifloral honeys.

World-wide light honeys like orange blossom or acacia honey achieve higher prices than honey blends or other unifloral honeys. On the other hand, in different countries, other unifloral honeys will achieve higher prices, depending on customer preference. Blossom honeys (with some exceptions) should have an electrical conductivity of less than 0.8 mS/cm (table 1).

Currently, the honey floral type is judged on bases such as sensory analysis, pollen and chemical analysis. As the pollen content is subject to considerable variation, judgement of the honey is based on a combination of several quality criteria.

In some central European countries like Germany, Switzerland and Austria honeydew honeys achieve generally higher prices than blossom honeys. Although honeydew honeys contain microscopically visible "honeydew elements" like algae and fungi, there are no quantitative microscopic quality criteria. Honey is labelled as "forest" or "honeydew" or "fir" on the basis of sensory judgement and electrical conductivity measurements. For honeydew honeys the norm of a minimum of 0.8 milli Siemens/cm (mS/cm) has been adopted in the world standards (table 1). Fir honeys have generally conductivity values greater than 1 mS/cm (19,20). However, there are no internationally accepted quality criteria for the different types of honeydew honeys and such are fixed in individual countries.

Misdescription of the geographical and the topological source

Generally, in Western Europe honey imported from China or Latin America has a lower price than the locally produced honey. Differences persist also between countries in Europe and also between geographical regions. Thus there is a financial interest in mislabelling honeys. Pollen analysis and a number of chemical methods have been used for the characterisation of the geographical origin of honey.

In the routine control of honey the geographical origin is often checked by pollen analysis as it requires only inexpensive instrumentation. In many countries pollen analysis of the locally produced honeys is regularly carried out and the pollen specialists there have a precise knowledge of the pollen spectrum of the honeys of their country. In food control pollen analysis is very efficient for the differentiation of honeys produced in distinctly different geographical and climatic areas. If the geographical differences are less pronounced, the determination of the pollen spectrum will generally not yield a confident authenticity proof. Also it has to be borne in mind that

melissopalynological methods will not meet the quality standards for a modern validated and quality assured analytical method. The methods are based on experience and are thus subjective and have not been tested by modern proficiency test trials. The use of computerised methodology for pollen analysis is very promising.

It may be possible to characterise honeys from different topographical regions within definite, relatively small geographical areas by the measurement of common chemical parameters and subsequent evaluation with modern statistical methods (see method section).

Misdescription of the entomological source.

The present EU Directive definition defines honey as derived from *Apis mellifera*, while according to the Codex standard honey is the product of all honeybees. *Apis mellifera*, originally indigenous to Africa and Europe, has been introduced into major exporting countries such as China, where honey is also produced from *Apis cerana*. This has created two problems which need resolution. It is necessary to characterise the honey from species other than *Apis mellifera* so that the honey from these species can be accepted in international trade either as indistinguishable from *Apis mellifera* honey (*Apis cerana*, the Asian bee) or with compositional limits of its own (*Apis dorsata*, stingless bees). Indeed, it is planned that the honeys of bees others than *Apis mellifera* also be characterised and a Codex standard for these honey types be established.

Organic, raw or unheated honey

Recently a European (21) and a Swiss (22) regulation for the production of organic honey have been established. 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 regime can decide if a honey is organic 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. An appellation of 'natural' honey is a mislabelling, since honey is natural by definition.

Fresh honey has a very low hydroxymethylfurfural (HMF) level and will still contain its natural level of enzymes. Appellations "fresh" "raw" or virgin honey indicate that honey is fresh and unheated. In the EU an appellation "virgin honey" has been proposed (23). Such honeys should have a maximum HMF content of 25 mg/kg. There are also suggestions 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 (1,24). Different European beekeeping associations have accepted the latter quality criteria for their specially labelled honeys.

Methods for testing the authenticity of honey due to effects during honey production

The methods for testing the different authenticity issues are summarised in table 2.

Addition of sugars

Depending on their origin, sugars added are divided into two types: C3 and C4. Sucrose and the natural sugars of honey belong to the C3 type while cane sugar and sugars produced from the hydrolysis of maize starch are of the C4 type. Many different methods have been proposed for detecting honey adulteration with sugar. However, most of these methods have not been found useful in practice (9). Here only the methods with proven or promising efficacy will be discussed.

Adulteration by cane and maize syrups

Isotope tests

Maize and sugarcane metabolise by the Hatch-Slack or C4 metabolic pathway. As a result, sugar syrups derived from them exhibit a $^{13}\text{C}/^{12}\text{C}$ ratio, expressed as a δ value, different from that of honey the sugar of which is derived via a C3 pathway. The δ value of C4 syrups is close to -10% while the average value for honey is -25.4% . The original method for measuring the $^{13}\text{C}/^{12}\text{C}$ ratio (25,26) has been improved with the introduction of a protein internal standard (27-29). The method currently used, with or without an internal standard, allows the detection of 7-10 % adulteration with cane sugar or maize syrups. Besides the measurement of the $^{13}\text{C}/^{12}\text{C}$ ratio, deuterium NMR (30,31) can be determined to yield a greater certainty in the interpretation of the $^{13}\text{C}/^{12}\text{C}$ measurement. There are a number of other papers reporting the isotope ratios of honeys of different botanical and geographical origin (30-35).

Determination of sugars

This area has been recently extensively reviewed (36). Maize syrups like high fructose corn syrup (HFCS) often contain trace amounts of higher oligosaccharides, which are naturally not present in honey. The measurement of the maltose/isomaltose ratio (37,38) proved some, if not complete efficacy for the determination of adulteration with HFCS syrups. However this method has not been validated for reliable determination of sugar syrup adulteration. The reason is that the composition of these syrups is constantly changing, thus making the detection of adulteration difficult, independent of the type of syrup. HFCS sugars have been determined by isolation of the specific oligosaccharides by preparative HPLC, followed by quantitation by analytical HPLC (39). This method is very labour intensive and was not used for practical honey control. Separation of sugars by ion chromatography (40), GC (41,42) or micellar electro-kinetic chromatography (43) have been used for the detection of HFCS sugars. Carbohydrate adulteration testing has also been carried out with ^{13}C -NMR spectroscopy (44). The determination of sugars has to be updated to be able to detect the actual oligosaccharide composition of the HFCS syrups.

Routine microscope and physico-chemical methods

The determination of routine parameters allows a cheap and quick screening of doubtful honeys, which then can be tested with more sophisticated and expensive techniques.

The detection of specific microscopically visible constituents of cane sugar can be used as a cheap screening method for the detection of this type of sugar adulteration (45). This test is cheap and simple and can be used as a screening test before carrying out the more expensive isotope test.

In sugar adulterated honeys some chemical parameters such as enzyme activities, HMF content, ash content, electrical conductivity and proline content are lowered. These changes might indicate possible adulteration, if the normal variation of these parameters in different honeys is taken into account when interpreting the test for adulteration. Indeed, proline was suggested as a quality criterion for honey with respect to sugar adulteration. It was proposed that natural honeys should have a proline content of more than 180 mg/kg (46). A lower proline content could mean that the honey has been adulterated with sugar. However, this value can be higher for certain honeys, as the proline content depends on the honey type. Also, it should be borne in mind that some of these parameters like HMF and enzyme activity will change on heating and storage. Routine analysis of the sugar spectrum by HPLC or GC can also give some information on possible adulteration. Honeys adulterated by feeding of sucrose to the bees have an increased concentration of sucrose and erlose (47). However, it should be borne in mind that sucrose decreases rapidly with time, and also the erlose concentration will change slowly upon storage (47). On the other hand, certain honeys such as citrus, acacia, rhododendron, honeydew and others have a higher natural content of this sugar (48).

Other methods

A number of other methods have been described for the proof of adulteration of honeys by sugars. However, most of them have not proved useful (9). An interesting method for the detection of adulteration by beet sugar by means of testing for sugar bisulfite derivatives was developed in Russia (49) but upto the present time there is no experience with this method in other countries. Recently FTIR spectroscopy was used for testing honey adulteration by inverted beet sugar (50).

This method is based on the honey dilution effect of added sugars and might not be the universal method needed to prove adulteration of all different types of honeys. Also, it has been used for model studies and its utility remains to be tested with different honeys.

Harvesting of honey with high humidity, addition of water

Harvesting of honey with high humidity, or subsequent addition of water to honey can result in honey fermentation and spoilage (see authenticity issues). Honey spoilage can be tested by a microscopical yeast count (51), by measuring glycerol (52), butanediol or ethanol (53). The last two methods based on enzymatic determinations are quick and inexpensive. A limit of 300 mg/kg glycerol has been proposed for blossom honey (17). Honeydew honeys have higher glycerol content (17) and a limit for these honeys has not yet been proposed. A limit of 150 mg/kg ethanol has been suggested for Spanish (53) and also for Italian (54) blossom honey.

Methods for testing authenticity in respect to descriptions

The different methods are summarised in table 3.

Botanical origin

Pollen analysis

Pollen analysis (melissopalynology) with light microscopy was the first method used to determine the botanical origin of honey. It has the advantage that it needs only inexpensive instrumentation. The drawback is that it needs highly specialised personnel and cannot for the time being be aided by computers for more efficient assessment of data. The International Commission of Bee Botany set the methodology (55) which is in the process of revision in the work programme of the European Honey Commission. Pollen analysis can be qualitative and quantitative. Generally, for the determination of the botanical and geographical origin of honey qualitative pollen analysis is carried out. Here the percentage representation of the different pollen types is determined. Pollen analysis is based on the individual knowledge and appreciation of each pollen analyst and is thus subjective to a certain extent. Recently pollen analysis ring trials for the determination of the precision were carried out (56). The precision for the determination of certain pollen types was comparable to the precision determined for the measurement of chemical parameters. The criteria for the percentages of the dominant pollens of different unifloral honeys were set 30 years ago (55). However, 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. These figures can also vary depending on the relative content of the accompanying pollen. Indeed, practical experience has shown that the present melissopalynological quality criteria for unifloral honey figures are not valid for all honey. Thus, presently, pollen analysis is used in combination with the sensory and chemical analysis of the unifloral honeys.

Determination of routine physico-chemical parameters

The determination of quality parameters by modern analytical methods (57) is now routinely used for the determination of the botanical origin of honey. Of all quality parameters measurements of electrical conductivity and of the fructose and glucose content, providing the fructose glucose ratio, are most useful. The determination of electrical conductivity is the fastest method for routine honey control. Values for blossom and honeydew honeys have been recently accepted in the Codex honey standard and in the EU Directive for honey (5,6) as a criterion of the differentiation between blossom and honeydew honeys. Also, electrical conductivity values of the most important unifloral honeys of the world have been compiled recently (7). However, by using linear discriminant statistical analysis of different honey quality parameters (sugars, electrical conductivity, optical rotation, nitrogen content) a good separation between unifloral honeys (58-60) can be achieved.

Also, honeydew and blossom honeys can be distinguished by the same approach (12,61). However, it should be noted that these methods have been used for research purposes and their utility for routine purposes is doubtful.

Determination of other parameters

Although chemometric analysis will discriminate between different unifloral honeys, it will not differentiate between polyfloral and unifloral honeys by discriminant analysis using routine quality parameters, because of the great variation of parameters in polyfloral honeys. For this purpose the use of specific unifloral markers is necessary.

Determination of carbohydrates

Determination of the whole honey spectrum, especially of a great number of minor oligosaccharides by capillary gas chromatography (62,63) and HPLC (39, 64-66) has some discriminant capacity for the differentiation of unifloral honeys. With normal chromatographic methods, capable of determining the main oligosaccharides, it is not possible to differentiate the unifloral honeys, although there are some differences between unifloral honeys. Even using very thorough separation of the sugars the oligosaccharide patterns of different unifloral honeys seem very similar (67). As the minor sugars are the product of honey enzymes of bee origin, minor oligosaccharides are most probably not specific markers of unifloral honeys. The differences encountered in the different unifloral honeys are probably due to different invertase activities, as this enzyme causes most sugar transformations, also the building of most honey oligosaccharides. Moreover, the activity of this enzyme is very heat and storage dependent; this will inevitably cause a great variation of the concentration of honey oligosaccharides.

Determination of aroma compounds and phenolics

Aroma compounds and flavonoids have been used as specific markers for unifloral honeys. Extraction of honey aroma by organic solvents for the quantitative analysis of honey and subsequent determination of the honey aroma spectrum have shown differences between unifloral honeys (8,9). However the extraction of volatiles is not suitable for routine testing of the botanical origin of honey because it is too time consuming. Dynamic head-space analysis of honey aroma can be useful for routine authenticity testing of the botanical origin of honey and it has been used with promising results (68-70). From these results it seems probable that less volatile components are more typical for the different botanical sources of honey. Another new technique is the use of SPME (Solid Phase Micro Extraction) for the analysis of aroma compounds. It has the advantage over dynamic head space that it has an increased sensitivity and that less volatile aroma compounds can be analysed. This technique has been successfully used for the determination of honey volatiles of unifloral honeys (71, 72).

Phenolics are another class of compounds which have been used for the proof of botanical origin. Recently, the use of these compounds for the determination of the botanical origin of honey has been extensively reviewed (73). It seems that there are typical markers for some unifloral honeys, e.g. methyl anthranilate and hesperitin for citrus honey, kaempferol for rosemary honey, which can be used successfully for authenticity testing. On the other hand, many unifloral honeys do not have specific markers. Also, the fact that many phenolics originate from propolis, which also varies independently of the honey nectar origin, will make the search for new markers difficult.

Determination of other minor honey constituents

Unifloral honeys differ to a certain extent in their content of amino acids (74-76) and trace elements (77-79). Determination of a number of chemical parameters including water activity, free amino acid composition, reducing sugars, total sugars and pH and subsequent evaluation by chemometric methods allowed the differentiation between different Italian unifloral honeys (80).

Methods for testing the authenticity of geographical origin

Pollen analysis

Pollen analysis is also used to determine the geographical origin of honey. The possibilities of pollen analysis for the determination of the geographical origin of honey have been reviewed recently (81). Indeed, the differences of the pollen spectrum between honeys from quite different geographical and climatic zones are easy to detect. However, if the geographical zones are closer, differences are more difficult to distinguish. In such cases more sophisticated melissopalynological methods should be used. In recent years pollen analysis has been successfully used for the determination of honeys originating from close geographical zones by the use of special software for pollen analysis (82) and statistical discriminant analysis (83-84) and cluster analysis (85). No ring trials concerning the determination of accompanying pollens and on the assessment of geographical origin using these pollens, have been carried out up to the present.

Determination of routine parameters

The determination of routine quality parameters like hydroxymethylfurfural (HMF) content and honey enzyme activity (invertase, diastase) reflects honey freshness. These parameters will differ when they are determined in locally sold and imported honey (12). Indeed, in Western Europe the locally produced honeys are mostly unheated and will reach the consumer during the honey production year while the imported honey has been heated and stored for longer periods before reaching the market.

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 was achieved (86-90). However, the practical importance of the above mentioned chemometric classifications is questionable as the botanical origin of honey was mostly not considered. Also, it should be borne in mind that some parameters such as HMF, diastase and the content of individual sugars are storage- and heat-dependent.

Determination of other parameters

The determination of trace elements is widely used in food authenticity studies, also in relation to the geographical origin (91). First studies with a radioactivation method in French and Hungarian honeys were carried out in 1974 (92). By a combination of several elements it was possible to differentiate between acacia honeys from the two countries. Analysis of trace elements by atomic spectrometry in Spain showed that these honeys could be classified by the content of trace elements (93, 94).

Analysis of amino acids is another promising method. In different studies it was shown that differences in the amino acid spectra could distinguish between honeys from different geographical origins (95-97). However in these studies, as in many others, the differences in the botanical origin of the honeys studied were not considered.

Flavonoids, which are known to be markers of the botanical origin of honey, can also serve also as markers of the geographical origin of honey (73).

In recent work it was shown that pyrolysis GC is a promising technique for the differentiation of honey geographical origin (98). The drawback is that sophisticated instrumentation is used and thus for the present time the method is unsuitable for routine honey control.

The analysis of contaminants such as heavy metals and insecticides can also give information on the geographical origin of honey. Indeed, honey has been proposed as a biological indicator for contamination with heavy metals (99,100) and pesticides (101, 102) of certain geographical areas. However, the measurement of contamination parameters has not been especially used as a geographical indicator for honey.

Proof of other authenticity issues

By definition retail honey should not be overheated. Changes of honey quality resulting in overheating are not permitted and such honey has to be used as baker's or industrial honey. In the EU honey legislation there are limits for the HMF content and for the diastase activity (table 1.).

Overheated honey is detected by measuring HMF, diastatase and invertase activity (1) (57). HMF is the better criterion, as honey enzyme activities vary considerably depending on the honey type. Some honeys, such as citrus, acacia etc., have naturally low enzyme levels and this must be taken into consideration when interpreting the results.

Conclusions

Although there are powerful methods to prove honey adulteration, they have to be further improved in order to keep the image of honey as an authentic natural food.

Concerning the botanical origin of honey quality, criteria for the determination of unifloral honeys, based on a wide variety of commercially available unifloral honeys, should be developed. The International Honey Commission is working on a data bank for quality criteria of the most important European unifloral honeys. The use of SPME for the determination of honey volatiles and honey aroma should be developed as a tool for the determination of the botanical origin of honey.

Research on the determination of the geographical origin of honey is only at the beginning. There are promising methods which have to be tested with a much greater number of honeys. Here the chemometric evaluation of routine analytical data seems to be a promising method, which has to be further improved. Studies with stable isotope ratios other than carbon, such as the D/H ratio and the oxygen isotopes ^{16}O and ^{18}O may prove useful. However, it seems likely that some of the differences between honeys of different geographical origin are probably due to differences in the botanical origin. Model studies should be carried with the same unifloral honey coming from different geographical origins.

The present review can serve for the stimulation of further work on honey authenticity issues in the canton food laboratories. Projects on cheese and wine authenticity have been initiated in the framework of the Swiss Food Manual of the Federal Health Office. A project on honey authenticity has been submitted in the same framework.

Summary

Honey is the only natural sweetener offered world-wide today, which has the status of being healthy for young and old. The authenticity of honey is defined by the Codex Alimentarius standard, the EU Honey Directive and the Swiss Food Directive. The Codex and the EU standards were recently revised. The changes in these standards, as well as their consequences for the Swiss Food Directive are discussed. The authenticity of honey has two different aspects: Authenticity in respect of honey production and authenticity in respect of descriptions such as geographical and botanical origin, 'natural', 'organic', 'raw' and 'harvested in the cold'. The objective of this review is to examine the different authenticity issues and the methods used to prove the authenticity of honey, in order to enable a successful authenticity testing.

Zusammenfassung

Honig ist das einzige natürliche Süßungsmittel, welches weltweit das Image eines gesundheitsfördernden Lebensmittels für jung und alt hat. Die Honigauthentizität ist definiert im Codex Alimentarius Standard, der EU Honigverordnung und der schweizerischen Lebensmittelverordnung. Die Änderungen dieser kürzlich revidierten Codex und EU Honigverordnungen werden diskutiert, sowie die Konsequenzen für die Schweizerische Lebensmittelverordnung. Die Honigauthentizität hat zwei verschiedene Aspekte: Authentizität der Honigproduktion und Authentizität der Honigumschreibung, betreffend die botanischen und der geographischen Herkunft, sowie die Bezeichnungen „natürlich“, „roh“, „biologisch“, „kaltgeschleudert“. Das Ziel dieser Übersichtsarbeit ist, die verschiedenen Aspekte der Honigauthentizität und die Methoden, die für ihre Prüfung angewendet werden zu diskutieren, um eine erfolversprechende Prüfung der Honigauthentizität zu ermöglichen.

Résumé

Le miel est le seul édulcorant naturel mondialement reconnu pour ses propriétés bienfaitrices sur la santé aussi bien pour les jeunes que pour les personnes âgées. L'authenticité du miel est définie au sein des normes du Codex Alimentarius, dans la directive de l'UE relative au miel ainsi que dans l'ordonnance sur les denrées alimentaires. Les modifications issues de la révision récente des normes du Codex Alimentarius et de la directive de l'UE ainsi que les conséquences pour l'ordonnance sur les denrées alimentaires font l'objet de discussions. L'authenticité du miel comporte deux différents aspects: premièrement, l'authenticité de la production de miel et, deuxièmement, l'authenticité de la dénomination du miel par rapport à l'origine botanique et géographique ainsi qu'aux désignations "naturel", "brut", "biologique" et "extrait à froid". L'objectif de cette étude permettant d'avoir une vue d'ensemble consiste, sur la base des connaissances actuelles, à examiner les différents aspects de l'authenticité du miel et des méthodes utilisées pour sa détermination afin d'obtenir une détermination fiable.

Key words

Honey, Authenticity, Production, Origin, Misdescription

After: Bogdanov S., Martin P., (2002) Honey authenticity, *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene* 93, 232-254.

Table 1

Honey standard of the Codex Alimentarius Standard and EU Honey Directive: important new quality criteria.

Composition criteria	Value
Sugar content	
<i>Fructose and glucose content (sum of both)</i>	
- blossom honey	not less than 60 g/100 g
- honeydew honey, blends of honeydew and blossom honey	not less than 45 g/100 g
<i>Sucrose</i>	
- in general	not more than 5 g/100 g
- false acacia (<i>Robinia pseudoacacia</i>), alfaalfa (<i>Medicago sativa</i>), Banksia (<i>Banksia menziesii</i>), French honeysuckle (<i>Hedysarum</i>), red gum (<i>Eucalyptus camadulensis</i>), leatherwood (<i>Eucryphis lucida</i> , <i>Eucryphia milliganii</i>), Citrus spp.	not more than 10 g/100 g
- Lavender (<i>Lavandula</i> spp.), borage (<i>Borago officinalis</i>)	not more than 15 g/100 g
Moisture content	
- in general	not more than 20 %
- heather (<i>Calluna</i>), EU, CA; baker's, EU	not more than 22 %
- baker's honey from heather (<i>Calluna</i>), EU	not more than 25 %
Electrical conductivity	
- honey not listed below, and blends of these honeys	not more than 0.8 mS/cm
- honeydew honey and chestnut honey and blends of these except of those listed below	not less than 0.8 mS/cm
- exceptions: strawberry tree (<i>Arbutus unedo</i>), bell heather (<i>Erica</i>), eucalyptus, lime (<i>Tilia</i> spp.), heather (<i>Calluna</i>), manuka or jelly bush (<i>Leptospermum</i>), tea tree (<i>Melaleuca</i> spp.)	
Free acid	
- in general	not more than 50 meq/kg
- baker's honey (only EU Directive)	not more than 80 meq/kg
Diastase activity* (Schade units)	
- In general; except baker's honey (EU)	not less than 8
- Honey with low natural enzyme content (e.g. citrus honey) and an HMF content of not more than 15 mg/kg	not less than 3
HMF*	
- In general; except baker's honey (EU Directive)	40 mg/kg
- Honey of declared origin from regions with tropical regions with tropical climates and blends of these honeys	80 mg/kg

* - determined after processing and blending

Table 2.

Methods for testing of authenticity issues due to improper production, processing and storage

Method, principle	Status, remarks
<i>Detection of sugar cane adulteration</i> Detection of specific microscopically visible constituents of cane sugar determination of $^{13}\text{C}/^{12}\text{C}$ ratio	screening definite procedure
<i>Detection of adulteration by maize syrups</i> determination of $^{13}\text{C}/^{12}\text{C}$ ratio determination of specific sugars	definite procedure methods not conclusive
<i>Detection of sugar adulteration by feeding sucrose</i> measurement of sucrose, erlose content measurement of proline content determination of bisulfite sugar derivatives	screening screening used only in Russia, to be tested
<i>Detection of beet sugar adulteration</i> FTIR spectroscopy	research results, to be tested for routine use
<i>Detection of improper water removal and of fermentation</i> off taste, yeast count, glycerol and ethanol	limits are not established internationally
<i>Detection of overheating and storage defects</i> measurement of HMF, diastase and invertase activity	limits according to CA, EU-Directive or Swiss Food Manual

For references and discussion of methods see text.

Table 3.
Methods for testing of authenticity of botanical origin

Method, principle	Status, remarks
<i>Pollen analysis</i> classical and statistical assessment of data	used for routine control, to be used together with chemical parameters
<i>Sensory analysis</i>	specialised personnel necessary
<i>Determination of routine parameters</i> e.g. electrical conductivity, fructose and glucose content (fructose/glucose ratio)	used for routine control together with pollen and sensory analysis
<i>Chemometric evaluation of routine parameters</i>	used predominantly in research
<i>Determination of aroma compounds</i> volatiles: dynamic head space volatiles: SPME flavonoids: HPLC	all methods used predominantly in research, routine testing not carried out.
<i>Determination of other minor components</i> amino acids trace elements	methods used in research, routine testing not carried out.

For references and discussion of methods see text.

Table 4
Methods for testing of authenticity of geographical origin

Method, principle	Status, Remarks
<i>Classical pollen analysis</i>	used for routine control, not tested with ring trials
<i>Chemometric evaluation of routine chemical parameters</i> e.g. pH, acidity, electrical conductivity, fructose, glucose etc.	used only in research investigations
<i>Determination of other minor components</i> amino acids trace elements flavonoids	all methods used only in research investigations

For references and discussion of methods see text.

References

- 1 *Anonymous*: Kapitel 23 A, Honig. Schweizerisches Lebensmittelbuch (1995).
- 2 *Crane, E.*: A book of honey. Oxford University Press, Oxford, New York, Toronto, Melbourne 1975.
- 3 *Lipp, J., Zander, E. und Koch, A.*: Der Honig. Eugen Ulmer, Stuttgart 1994.
- 4 *Codex Alimentarius*: Draft revised standard for honey (at step 10 of the Codex procedure). Alinorm 01/25 19-26 (2001).
- 5 *EU Council*: Council Directive 2001/110/EC of 20 December 2001 relating to honey. Official Journal of the European Communities **L10**, 47-52 (2002).
- 6 *Bogdanov, S., Lüllmann, C., Martin, P., von der Ohe, W., Russmann, H., Vorwohl, G., Persano Oddo, L., Sabatini, A. G., Marcazzan, G. L., Piro, R., Flamini, C., Morlot, M., Lheretier, J., Borneck, R., Marioleas, P., Tsigouri, A., Kerkvliet, J., Ortiz, A., Ivanov, T., D'Arcy, B., Mossel, B. and Vit, P.*: Honey quality, methods of analysis and international regulatory standards: review of the work of the International Honey Commission. Mitt. Lebensm. Hyg. **90**, 108-125 (1999).
- 7 *Martin, P., Sharman, M. and Scotter C.N.G.*: Honey - product definition and manufacturing processes. In: Food authenticity - issues and methodologies, 169-182. Eurofins Scientific, 1998.
- 8 *Molan, P.*: Authenticity of honey, food authentication. In: Ashhurst, P.R. and Dennis, M.(eds), 259-303, Blackie, London, 1996.
- 9 *Singhal R., Kulkarni, P. und Rege, D.* In: Honey, quality criteria. Handbook of indices of food quality and authenticity, 358-385, Woodhead publishing ltd, Cambridge, England, 1997.
- 10 *Anklam, E.*: A review of the analytical methods to determine the geographical and botanical origin of honey. Food Chem. **63**, 549-562 (1998).
- 11 *Townsend, G. F.*: Processing and storing liquid honey. In: Crane E.: Book of honey, 269-292, Oxford University Press, Oxford, New York, Toronto, Melbourne 1975.
- 12 *Bogdanov, S., Rieder, K. und Rüegg, M.* Neue Qualitätskriterien bei Honiguntersuchungen. Apidologie **18**, 267-278 (1987).
- 13 *Dyce, E.*: Producing finely granulated or creamed honey. In: Crane E.: A book of honey. 293-306, Oxford University Press, Oxford, New York, Toronto, Melbourne 1975.
- 14 *SPMF*: Der Skandal der Honigverfälschung. Ein weltweiter Betrug - Hauptangeklagter: China. Imkerei-Technik Magazin 14-24 (1998).
- 15 *White, J. W.*: Isotope ratio testing of honey: Demystifying the internal standard test. Am. Bee J. **140**, 318-321 (2000).
- 16 *Stephen, W. A.*: The relationship of moisture content and yeast count in honey fermentation. Sci. Agricult. **26**, 258-264 (1946).
- 17 *Russmann, H.*: Hefen und Glycerin in Blütenhonigen - Nachweis einer Gärung oder einer abgestoppten Gärung. Lebensmittelchemie **52**, 116-117 (1998).
- 18 *Papoff, C. M., Floris, I. e Farris G. A.*: Contenuto di etanolo e fermentazione da lieviti nel miele. Apicolt. Moderno **87**, 123-126 (1996).
- 19 *Talpay, B.*: Spezifikationen für Trachthonige. Dtsch. Lebensm. Rundsch. **81**, 148-152 (1985).
- 20 *Accorti, M., Persano Oddo L., Piazza M. G. e Sabatini A. G.*: Schede di caratterizzazione delle principali qualità di miele Italiano. Apicoltura **2**, 1-35 (1986).
- 21 *EU*: Council Regulation No 1804 On organic farming, Chapter Beekeeping and Beekeeping Products. Off. J. Eur. Comm. of 19 July 1999, L 222, C. Bruxelles, Belgium. (1999).

- 22 *Anonymous*: Verordnung des EVD über die biologische Landwirtschaft, vom 22. September 1997 (Stand am 12. Februar 2002), 2. Abschnitt: Bestimmungen an die Bienenhaltung und Imkereierzeugnisse. Schweiz. Eidgenössisches Volkswirtschaftliches Departement 2002.
- 23 *EU*: Application for registration of a certificate of specific character, Council Regulation No.2082/92. Off. J. Eur. Comm. **C 63**, 23-24 (2002).
- 24 *Duisberg, H. und Hadorn, H.*: Welche Anforderungen sind an Handelshonige zu stellen? Mitt. Lebensm. Hyg. **57**, 386-407 (1966).
- 25 *Brookes, S. T., Barrie, A. and Davies, J. E.*: A rapid ¹³C/¹²C test for determination of corn syrups in honey. J. Ass. Off. Anal. Chem. **74**, 627-629 (1991).
- 26 *White, J. W. and Doner, L. W.* Mass spectrometric detection of high-fructose corn sirup in honey by use of ¹³C/¹²C ratio: Collaborative study. J. Ass. Off. Anal. Chem. **61**, 746-750 (1978).
- 27 *Rossmann, A., Lüllmann, C. und Schmidt, H. L.*: Massenspektrometrische Kohlenstoff- und Wasserstoff-Isotopen-Verhältnismessung zur Authentizitätsprüfung bei Honigen. Z. Lebensm. Unters. Forsch. **195**, 307-311 (1992).
- 28 *White, J. W.*: Internal standard stable carbon isotope ratio method for determination of C-4 plant sugars in honey: Collaborative study, and evaluation of improved protein preparation procedure. J. Ass. Off. Anal. Chem. **75**, 543-548 (1992).
- 29 *White, J. W., Winters, K., Martin, P. and Rossmann A.*: Stable carbon isotope ratio analysis of honey: Validation of internal standard procedure for worldwide application. J. Ass. Off. Anal. Chem. **81**, 610-619 (1998).
- 30 *Lindner, P., Bermann, E. and Gamarnik, B.*: Characterization of citrus honey by deuterium NMR. J. Agr. Food Chem. **44**, 139-140 (1996).
- 31 *Giraudon, S., Danzart, M. and Merle, M. H.*: Deuterium nuclear magnetic resonance spectroscopy and stable carbon isotope ratio analysis/mass spectrometry of certain monofloral honeys. J. Ass. Off. Anal. Chem. **83**, 1401-1409 (2000).
- 32 *Floris, I., Vacca, V., Franco, M. A., Del Caro, A., Marras, P. M. e Reniero, F.*: Fenoli totali e rapporto isotopico ¹³C/¹²C di mieli uniflorali della Sardegna. Apicoltura **9**, 119-133 (1994).
- 33 *Serra Bonvehi, J. and Coll, F. V.*: Determination of stable carbon isotope ratio delta c-13 by mass spectrometry in spanish honeys. Food Sci. Techn. Intern. **1**, 25-28 (1995).
- 34 *Cienfuegos, E., Casar, I. and Morales, P.*: Carbon isotopic composition of Mexican honey. J. Apic. Res. **36**, 169-179 (1997).
- 35 *Muro, N., Torregrosa, M., Blanch, A. and Ruperez, M.*: Studies of honey authenticity using mass spectroscopy of carbon isotope ratio and its relation to other physicochemical parameters, Alim. Equipos Tecnol. **18**, 75-78 (1999).
- 36 *Prodoliet, J. and Hischenhuber, C.*: Food authentication by carbohydrate chromatography. Z. Lebensm. -Unters. -Forsch A **207**, 1-12 (1998).
- 37 *Doner, L. W., Kushnir, I. and White, J. W.*: Assuring the quality of honey. Is it honey or syrup? Anal. Chem. **51**, 224-232 (1979).
- 38 *Allegretti, M., Ambrosoli, G. e Cantoni, C.*: Individuazione dell'isoglucosio nel miele. Industrie alimentari 566-573 (1987).
- 39 *Lipp, J., Ziegler, H. and Conrady, E.*: Detection of high fructose- and other syrups in honey using high-pressure liquid chromatography. Nachweis von Honigfructose- und anderen Sirupen in Honig mit HPLC. Z. Lebensm. Unters. Forsch. **187**, 334-338 (1988).
- 40 *Swallow, K. W. and Low, N. H.*: Determination of honey authenticity by anion-exchange liquid chromatography. J. Ass. Off. Anal. Chem. **77**, 695-702 (1994).

- 41 *Low, N. and South, W.*: Determination of honey authenticity by capillary gas chromatography. *J. Assoc. Off. Anal. Chem. Int.* **78**, 1106-1113 (1995).
- 42 *Alamanni, M. C.*: Indagine sulla presenza di isoglucosio in campioni di miele sardo. *La Rivista Soc. Ital. Sci. Aliment.* **24**, 517-522 (1995).
- 43 *Corradini, C., Canali, A., Cavazza, A., Cogliandro, R. and Nicoletti, I.*: Determination of honey authenticity and its botanical origin by miscelar electrokinetic chromatography and HPLC. *Euro Food Chem IX, Authenticity and adulteration of food, the analytical approach, Interlaken* 664-670 (1997).
- 44 *Mazzoni, V., Bradesi, P., Casanova, T. and Casanova, J.*: Direct qualitative and quantitative analysis of carbohydrate mixtures using ¹³C NMR spectroscopy: application to honey. *Magn. Res. Chem.* **35**, 81-90 (1997).
- 45 *Kerkvliet, J. D. and Meijer, H. A. J.*: Adulteration of honey: relation between microscopic analysis and deltaC-13 measurements. *Apidologie* **31**, 717-726 (2000).
- 46 *Von der Ohe, W., Dustmann, J. H. und von der Ohe, K.*: Prolin als Kriterium der Reife des Honigs. *Dtsch. Lebensm. Rundsch.* **87**, 383-386 (1991).
- 47 *Deifel, A., Gierschner, K. und Vorwohl, G.*: Saccharose in Honig: Saccharose und deren Transglycosidierungsprodukte in natürlichen und Zuckerfütterungshonigen. *Dtsch. Lebensm. Rundsch.* **81**, 356-362 (1985).
- 48 *Sabatini, A. G., Persano, O. L., Piazza, M. G., Accorti, M. and Marcazzan, G.*: Glucide spectrum in the main Italian unifloral honeys. II. Di- and trisaccharides. *Apicoltura* **6**, 63-70 (1990).
- 49 *Chepurnoi, I. P. and Dmitrenko, A. S.*: Method for testing of honey adulteration by sucrose (in Russian) USSR Patent, SU 1 281 233 A (1987).
- 50 *Sivakesava, S. and Irudayaraj, J.*: Detection of inverted beet sugar adulteration of honey by FTIR spectroscopy. *J. Sci. Food and Agric.* **81**, 683-690 (2001).
- 51 *Beckh, G. and Lüllmann, C.*: Natürliche Bestandteile des Honigs - Hefen und deren Stoffwechselprodukte. Tel 1: Hefegehalt. *Dtsch. Lebensm. Rundsch.* **95**, 457-463 (1999).
- 52 *Huidobro, J. F., Rea, M. E., Branquinho de Andrade P. C., Sancho, M. T., Muniategui, S. and Simal-Lozano, J.*: Enzymatic determination of glycerol in honey. *J. Agr. Food Chem.* **41**, 557-559 (1993).
- 53 *Huidobro, J. F., Rea, M. E., de Andrade, P. C. B., Sanchez, M. P., Sancho, M. T., Muniategui, S. and Simal-Lozano, J.*: Enzymatic determination of primary normal alcohols as apparent ethanol content in honey. *J. Agr. Food Chem.* **42**, 1975-1978 (1994).
- 54 *Floris, I., Satta, A., Carpana, E. and Sabatini, A. G.*: The ethanol content as a useful parameter for the identification of honey. *Symposio Il Ruol della Ricerca in Apicoltura, Bologna, 2002.*
- 55 *Louveaux, J., Maurizio, A. and Vorwohl, G.*: Methods of melissopalynology. *Bee World* **59**, 139-162 (1978).
- 56 *Behm, F., Ohe, K. v. d. und Henrich, W.*: Zuverlässigkeit der Pollenanalyse von Honig: Bestimmung der Pollenhäufigkeit. *Dtsch. Lebensm. Rundsch.* **92**, 183-188 (1996).
- 57 *Bogdanov, S., Martin, P. and Lüllmann, C.*: Harmonised methods of the European honey commission. *Apidologie* 1-59 (1997).
- 58 *Bogdanov, S.*: Charakterisierung von Schweizer Sortenhonigen. *Agrarforschung* **4**, 427-430 (1997).
- 59 *Vinci, G., Carunchio, F., D'Ascenzo, F., Ruggieri, R. and Tarola, A.*: Chemical composition (water, sugars, HMF, N) of different botanical origin honey samples: a statistical evaluation.

Euro Food Chem IX, Authenticity and adulteration of food, the analytical approach, Interlaken 671-676 (1997).

- 60 *Piro, R., Guidetti, G., Persano, L. and Piazza, M.*: Mathematical diagnosis of unifloral honeys. Symposium "Il Ruolo della ricerca in apicoltura" Bologna, 235-240 2002.
- 61 *Kirkwood, K. C., Mitchell, T. J. and Smith, D.*: An examination of the occurrence of honeydew in honey. *Analyst* **85**, 412-416 (1960).
- 62 *Horvath, K. and Molnar, I.*: Simultaneous quantitation of mono-, di- and trisaccharides by GC-MS of their TMS ether oxime derivatives in honey. *Chromatographia* **45**, 328-335 (1997).
- 63 *Radovic, B., W.R., Parker, I., Sharman, M., Geiss, H. and Anklam, A.*, Contribution of high temperature gas chromatographic analysis of oligosaccharides and ion chromatographic cations and anions to authenticity testing of honey, *Dtsch. Lebensm. Rundsch.* **97**, 380-384(2001).
- 64 *Goodall, I., Dennis, M. J., Parker, I. and Sharman, M.*: Contribution of high-performance liquid chromatographic analysis of carbohydrates to authenticity testing of honey. *J. Chrom. A* **706**, 353-359 (1995).
- 65 *Mateo, R. and Bosch-Reig, F.*: Sugar profiles of Spanish unifloral honeys. *Food Chem.* **60**, 33-41 (1997).
- 66 *Da Costa, L., Trugo, L., Quintero, L., Barth, O., Dutra, V. and De Maria, C.*: Determination of oligosaccharides in Brazilian honeys of different botanical origin. *Food Chem.* **70** 93-98. (2000).
- 67 *Low, N. H., Nelson, D. L. and Sporns, P.*: Carbohydrate analysis of western Canadian honeys and their nectar sources to determine the origin of honey oligosaccharides. *J Apic Res* **27**, 245-251 (1988).
- 68 *Bouseta, A., Collin, S. and Dufour, J. P.*: Characteristic aroma profiles of unifloral honeys obtained with a dynamic headspace GC-MS system. *J. Apic. Res.* **31**, 96-109 (1992).
- 69 *Barcarolo, R., Centeleghe, M., Zanatta, P. and Conte, L. S.*: GC-MS coupled with head space sampling with reverse carrier flow in sampling step applied to honey characterization. 5th International Symposium of Hyphenated Technique in Chromatography, Bruges, Belgium, 11-21 (1998).
- 70 *Radovic, B. S., Careri, M., Mangia, A., Musci, M., Gerboles, M. and Anklam, E.*: Contribution of dynamic headspace GC-MS analysis of aroma compounds to authenticity testing of honey. *Food Chemistry* **72**, 511-520 (2001).
- 71 *Guidotti, M. and Vitali, M.*: Identification of volatile organic compounds present in different honeys through SPME and GC/MS. *Industrie Alimentari* **37**, 351-353 (1998).
- 72 *Conte, L., Piasenzotto, Bogdanov, S., Rouff, K., Sanz, J. and Denemont, J.*: Determination of honey volatiles in relation to their botanical origin. Symposium "Il Ruolo della ricerca in apicoltura", Bologna, 247-252 (2002).
- 73 *Tomas-Barberan, F. A., Martos, I., Ferreres, F., Radovic, B. S. and Anklam, E.*: HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. *J. Sci. Food Agr.* **81**, 485-496 (2001).
- 74 *Bosi, G. and Battaglini, M.*: Gas chromatographic analysis of free and protein amino acids in some unifloral honeys. *J. Apicult. Res.* **17**, 152-166 (1978).
- 75 *Pirini, A., Conte, L., Francioso, O. and Lerkcer, G.*: Capillary gas chromatographic determination of free amino acids in honey as means of discrimination between botanical sources. *J. High Res. Chromat.* **15**, 165-170 (1992).
- 76 *Sanchez, M. D., Huidobro, J. F., Mato, I., Muniategui, S. and Sancho M. T.*: Correlation between proline content of honeys and botanical origin. *Dtsch. Lebensm. Rundsch.* **97**, 171-175 (2001).

- 77 *Ivanov, T. and Chervenakova, Y.*: Content of some macro-, oligo- and microelements in bee honey, royal jelly and pollen. *Animal Science (Bulg.)* **21**, 65-69 (1984).
- 78 *Feller-Demalsy, M. J., Vincent, B. and Beaulieu, F.*: Mineral content and geographical origin of Canadian honeys. *Apidologie* **20**, 77-91 (1989).
- 79 *Bengsch, E.*: Spurenelemente in Bienenprodukten fördern die Gesundheit. *Allg Dtsch Imkerztg* **27**, 12-14 (1993).
- 80 *Conte, L. S., Miorini, M., Giomo, A., Bertacco, G. and Zironi, R.*: Evaluation of some fixed components for unifloral honey characterisation. *J. Agr. Food Chem.* **46**, 1844-1849 (1998).
- 81 *Piana, M. L.*: La determinazione dell'origine geografica nel miele e le frodi collegate. *Apis* **5**, 8-17 (1997).
- 82 *Battesti, M. et Goeury, C.*: Efficacité de l'analyse mélitopalynologique quantitative pour la certification des origines géographique et botanique des miels: le modele des miels corses. *Revue Paleobo. Palynol.* **75**, 77-102 (1992).
- 83 *Sancho, M. T., Muniategui, S., Huidobro, J. F. and Simal-Lozano, J.*: Discriminant analysis of pollen spectra of Basque Country (northern Spain) honeys. *J. Apicult. Res.* **30**, 162-167 (1991).
- 84 *Floris, I. and Satta, A.*: Approach to the diagnostics of the botanical and geographical origin of honey. Symposium "Il ruolo della ricerca in apicoltura" Bologna. 229-234 (2002).
- 85 *Ferrazzi, P. and Medrzycki, P.*: First approach of application of cluster analysis of melissopalynological data for the determination of the geographical origin of honey. Symposium "Il ruolo della ricerca in apicoltura" Bologna, 223-228 (2002).
- 86 *Pena Crecente, R. and Herrero Latorre, C.*: Pattern recognition analysis applied to classification of honeys from two geographic origins. *J. Agr. Food Chem.* **41**, 560-564 (1993).
- 87 *Sanz, S., Perez, C., Herrera, A., Sanz, M. and Juan, T.*: Application of a statistical approach to the classification of honey by geographic origin. *J. Sci. Food Agr.* **69**, 135-140 (1995).
- 88 *Lopez, B., Latorre, M., Fernandez, M., Garcia, M., Garcia, S. and Herrero, C.*: Chemometric classification of honey according to their type based on quality control data, *Food Chemistry* **55**, 281-287 (1996).
- 89 *Gonzales Paramas, A. M., Gomez Barez, J. A., Garcia Villanova, R. J., Rivas Pala, T., Ardanuy Albajar, R. and Sanchez, J.*: Geographical discrimination of honeys by using mineral composition and common chemical quality parameters. *J. Sci. Food and Agr.* **80**, 157-165 (2000).
- 90 *Barez, J. A. G., Garcia Villanova, R. J., Garcia, S. E., Pala T. R., Paramas, A. M. G. and Sanchez J. S.*: Geographical discrimination of honeys through the employment of sugar patterns and common chemical quality parameters. *Eur. Food Res. Techn.* **210**, 437-444 (2000).
- 91 *Crews, H. M.*: Trace elements analysis for food authenticity. In: Ashhurst, P., Dennis, M. eds. *Analytical methods of food authentication* 270-291, Blackie, London 1998.
- 92 *Lasceve, G. and Gonnet, M.*: Analyse par radioactivation du contenu minéral d'un miel. Possibilité de préciser son origine géographique. *Apidologie* **5**, 201-223 (1974).
- 93 *Latorre, M. J., Pena, R., Pita, C., Botana, A., Garcia, S. and Herrero, C.*: Chemometric classification of honeys according to their type. II. Metal content data. *Food Chemistry* **66**, 263-268 (1999).
- 94 *Paramas, A. M. G., Barez J. A. G., Garcia Villanova, R. J., Pala T. R., Albajar, R. A. and Sanchez, J. S.*: Geographical discrimination of honeys by using mineral composition and common chemical quality parameters. *J. Sci. Food Agr.* **80**, 157-165 (2000).

- 95 *Davies, A. M. C.* : Amino acid analysis of honeys from eleven countries. *J. Apicult. Res.* **14**, 29-39 (1975).
- 96 *Davies, A. M. C. and Harris, R. G.*: Free amino acid analysis of honeys from England and Wales: application to the determination of the geographical origin of honeys. *J Apic Res* **21**, 168-173 (1982).
- 97 *Gilbert, J., Shepherd, M. J., Wallwork, M. A. and Harris, R. G.*: Determination of the geographical origin of honeys by multivariate analysis of gas chromatographic data on their free amino acid content. *J. Apicult. Res.* **20**, 125-135 (1981).
- 98 *Radovic, B., Goodacre, R. and Anklam, E.*: Contribution of pyrolysis-mass spectrometry (Py-MS) to authenticity testing of honey. *J. Anal. Pyrol.* **60**, 79-87 (2001).
- 99 *Tong, S. S. C., Morse, R. A., Bache, C. A. and Lisk, D. J.* Elemental analysis of honey as an indicator of pollution. *Arch. Environ. Health* **30**, 329-332 (1975).
- 100 *Jones, K. C.*: Honey as an indicator of heavy metal contamination. *Water, Air and Soil Pollution* **33**, 179-189 (1987).
- 101 *Celli, G. e Porrini, C.*: I bioindicatori nel monitoraggio dell' inquinamento ambientale. *Biologi Italiani* **3**, 29-38 (1991).
- 102 *Celli, G., Porrini, C., Radeghieri, P., Sabatini, A. G., Marcazzan, G. L., Colombo, R., Barbattini, R., Greatti, M. and D'Agaro, M. D.*: Honeybees (*Apis mellifera* L.) as bioindicators for the presence of pesticides in the agroecosystem. Field tests. *Insect Social Life* **1**, 207-212 (1996).

Corresponding author: Stefan Bogdanov, Swiss Bee Research Centre, Federal Dairy Station, Liebefeld, CH-3003 Bern. E-mail: stefan.bogdanov@fam.admin.ch