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# Honey Quality, Methods of Analysis and International Regulatory Standards: Review of the Work of the International Honey Commission

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## Introduction

Honey quality criteria are specified in a European Directive (1) and in the Codex Alimentarius standard (2), both presently under revision (3, 4). As many of the analysis methods and regulations are outdated, an International Honey Commission (IHC) was formed in 1990 to revise the methods and standards for honey. The authors of this review, as members of the IHC, have all contributed with data, which made possible the present review. The commission compiled the methods of analysis currently used in routine honey control and carried out ring trials in collaboration with the honey commission of the Swiss Food Manual (SFM). The methods were first published in the SFM (5) and then in a slightly modified form elsewhere (6). In this paper we review the present knowledge on the different quality criteria and on the methods used for their determination. The review is intended to inform food chemists and authorities on the work done by the IHC. It should also help the regulatory authorities in the decision-making process when revising the present honey standard.

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## The collaborative work of the International Honey Commission

With the exception of the methods for the determination of apparent sugars and of specific rotation, the precision parameters concerning the intra- and inter-laboratory variation of all methods have been determined in ring trials (for details see references 5 and 6). Most of the methods have been collaboratively tested by the SFM and the IHC (methods Nr. 1, 4, 5, 6, 7, 8, 9, 10, 11, 14 and 15, in table 1), using the modern robust statistics method (7-9). The rest were tested by DIN (Deutsches Institut für Normung; methods Nr. 2, 3, 13, 17 in table 1) or by a UK collaborative study (method Nr. 16 in table 1) using the ISO 5725 standard for collaborative studies (10, 11). The complete precision parameters of the methods are published elsewhere (5, 6). The ISO method has drawbacks, especially in that outlier laboratories are eliminated from the computation of the precision parameters. The robust method does not have these drawbacks, taking into account the results of all laboratories participating in the trial (8). It is gaining increasing acceptance and in the next edition of the guide for conducting collaborative studies (reference 11, currently in revision) both the ISO protocols and the robust method will be included. The interlaboratory variation, determined as the coefficient of variation  $VK_R$  of the reproducibility  $R$  is the quality parameter most often used to compare the precision of analytical methods (12).  $VK_R$  in % is calculated as:  $100 \times R/\bar{x} \times 2.8$ . Generally  $VK_R$  decreases exponentially with increasing concentration of the measured variable. In the methods for analysis of major foodstuff components, in a concentration range between 0.01 g/100 g and 10 g/100 g, the coefficient of variation will mostly lie between 0.1 and 10 % (12). A  $VK_R$  value greater than 10 % indicates an insufficient precision of a method.

The extreme  $VK_R$  values for each method are summarized in table 1. With the exception of methods 5 (acidity by equivalent point titration), 14 (sugars by GC) and 18 (specific rotation), which are part only of the European harmonized methods, the same methods are part of the SFM (5) and in the European harmonised methods for honey analysis (6).

## Scope and precision of the methods

### Moisture content

The determination of moisture by refractometry does not yield the true water content and the determined values are lower than those obtained by the Karl Fischer method (13). However, it is a very simple and reproducible method, used successfully in routine honey control. Thus, although the refractometric values are not absolutely correct, there is no need for an alternative method. The  $VK_R$  values are acceptable and vary from 0.8 to 2 % over the entire determination range.

Table 1

Precision of the harmonised honey analysis methods:  $VK_R$  % values

Parameter, method, unity	VALUE <sup>1</sup> MIN-MAX	$VK_R$ % <sup>2</sup> MIN-MAX
1. Moisture content, g/100 g	16.4–20.0	1.0–2.5
2. Mineral content (ash), g/100 g	0.06–0.49	4.3–13.2
3. Electrical conductivity, mS/cm <sup>-1</sup>	0.22–1.52	3.4–4.4
4. Acidity by endpoint titration to pH 8.3, meq/kg	11.2–46.2	10.3–22.0
5. Acidity by equivalence point titration, meq/kg	7.0–32.5	8.7–46.8
6. Hydroxymethylfurfural content by HPLC, mg/kg	5.2–42.3	6.1–10.9
7. Hydroxymethylfurfural content after White, mg/kg	3.8–42.1	3.7–22.0
8. Hydroxymethylfurfural content after Winkler, mg/kg	7.5–42.9	7.9–15.2
9. Diastase activity after Schade in diastase numbers	8.7–37.7	20.5–26.1
10. Diastase activity with Phadebas in diastase numbers	8.6–37.6	11.0–17.9
11. Invertase activity in invertase numbers	6.5–17.7	2.7–9.6
12. Apparent reducing sugar content, g/100 g apparent sucrose content, g/100 g	— —	nd nd
13. Sugar Content by HPLC, g/100 g fructose glucose sucrose	31.2–42.4 23.0–32.0 0–2.8	1.5–1.9 1.6–3.2 11.4
14. Sugar Content by GC, g/100 g fructose glucose sucrose	31.4–39.4 23.8–31.4 0–7.7	3.9–8.6 2.6–7.5 7.2
15. Sugar Content by HPLC, pulsed amperometric detection, g/100 g fructose glucose sucrose	36.3–38.9 26.9–28.2 1.2–2.4	6.0–7.4 7.3–7.8 6.8–12.5
16. Water Insoluble Content, g/100 g	0.01–0.03	26.5–84.4
17. Proline Content, mg/kg	171–762	2.3–3.4
18. Specific rotation	—	nd

<sup>1</sup> = range of the values, determined in the collaborative trials<sup>2</sup> =  $VK_R$  %, determined for the values in column two, calculated from the precision data of the collaborative trials (for more details see references 5 and 6).

Min = minimum value, Max = maximum value, nd = not determined

### *Mineral content (ash)*

The  $VK_R$  values varied from 4 to 11 % over the entire determination range. This method is now very often replaced by the faster and easier electrical conductivity measurement (see below).

### *Electrical conductivity*

This measurement depends on the ash and acid content of honey: the higher their content, the higher the resulting conductivity (14). The  $VK_R$  values varied from 3 to 4 % over the entire determination range and are lower than the corresponding values of the ash determination method.

### *Acidity*

Two methods have been proposed: a fixed and an equivalent point titration method. Titration of the acidity to a fixed endpoint of pH 8.3 has the major drawback that the endpoint of the titration is not well defined because of lactone hydrolysis, which leads to a persistent drift in the titration endpoint. Also, the final point of titration depends on the honey type. Thus, the equivalence point titration is the correct method for determination of honey acidity. Except in France, where the equivalence point titration is used (15), the fixed endpoint titration method is used in most other countries, as the maximum free acidity fixed in food regulations is based on this method. The two methods were tested collaboratively (6), using the same honey samples. There was a very good correlation ( $r = 0.999$ ) between the acidity values, determined with both methods. However, the equivalent point titration yielded values which were on average 35 % lower than the ones measured with the fixed endpoint titration method. For both methods the  $VK_R$  values were very high: 11 to 22 % for the endpoint titration and 9 to 47 % for the equivalent point titration. Thus the reproducibility of these methods is unsatisfactory in both cases, especially in honeys with low acidity. One should bear in mind the poor precision of the method when interpreting results close to the limit.

### *Hydroxymethylfurfural content*

Three methods can be used for the determination of hydroxymethylfurfural (HMF): a colorimetric one (with p-toluidine), a spectrophotometric one (with sodium bisulfite) and an HPLC method. Because p-toluidine may be carcinogenic, the Winkler method should not be used if one of the other methods is available. These methods were tested collaboratively with the same three honey samples to cover the determination range between 5 and 40 mg/kg (5, 6). At very low levels, of no interest for assessing honey quality, there were small differences between the methods. In the higher range all three methods yielded practically the same values. The  $VK_R$  values of the White and the HPLC method were slightly lower than the one of the Winkler method. With the exception of the measurements at the lowest concentrati-

on range with  $VK_R$  values above 10 %, the interlaboratory precision of all methods is acceptable.

### *Diastase activity*

Diastase (amylase) catalyzes the transformation of starch to maltose. There is practically no starch in honey or its precursors, but the enzyme is added by bees to honey and plays a role in bee physiology. Two different methods can be used to determine honey diastase. The traditional Schade method uses starch as a substrate and determines the diastase activity expressed in Schade units. In the Phadebas method the substrate is an artificial, blue-dyed cross-linked type of starch. There is a very good correlation ( $r = 0.987$ ) between the two measurements. Linear regression of  $y$  (diastase number, DN) against  $x$  ( $\Delta A_{620}$ ) yielded the following relation:

$$DN = 28.20 \times \Delta A_{620} + 2.64 \quad (1)$$

where 28.2 and 2.64 are respectively the slope and the intercept of the best straight line obtained by linear regression of  $\Delta A_{620}$  ( $x$  axis) on DN ( $y$  axis). For each measurement of  $\Delta A_{620}$  with the Phadebas method the DN can be calculated using the above formula. However, when measurements were conducted in honeys with lower diastatic activity, it was found that actually, the relation between the measurements with Phadebas and with starch was composed of two linear regions. The first linear region is for honeys having a DN between 0 and 6 and there is a second region for honeys having a DN greater than 6. For the latter group the above formula (1) is valid, while in honeys with DN numbers lower than 6 the diastatic activity can be calculated from the following equation:

$$DN = 35.16 \times \Delta A_{620} - 0.46 \quad (2)$$

The  $VK_R$  values for both methods are above 10 %. The precision of the Phadebas method, as expressed by the  $VK_R$  value, was almost twice as good as the Schade method. A possible explanation might be that the Phadebas method uses a defined substrate, whereas commercially available starch varies considerably in its quality.

### *Invertase activity*

Invertase (sucrase, glucosidase, transglucosidase) catalyses mainly the hydrolysis of sucrose to glucose and fructose, but also other sugar transformations. It is common usage to express the invertase activity as an invertase number (IN). The IN indicates the amount of sucrose per g hydrolysed in 1 hour by the enzymes contained in 100 g of honey under test conditions. In this method p-Nitrophenyl- $\alpha$ -D-glucopyranoside (pNPG) is used as an enzyme substrate. If the invertase activity is determined simultaneously by this method and by the original polarimetric method (16) the following relation between IN and  $\Delta A_{400}$  results:

$$IN = 21.64 \times \Delta A_{400}$$

where 21.64 is the slope of the best straight line obtained by linear regression of  $\Delta A_{400}$  (x axis) on IN (y axis).

The  $VK_R$  values for this method are considerably better than that of the diastase determination methods. This can be explained by the fact that in the invertase assay a chemically defined substrate is used.

### ***Sugar content***

The apparent reducing sugars as well as the apparent sucrose content are measured by the Fehling method. The «apparent reducing sugars» correspond roughly to the sum of the main honey sugars fructose and glucose and some minor reducing disaccharides, mainly maltose. The «apparent sucrose» is calculated as the difference in apparent reducing sugars before and after sucrose hydrolysis. These methods are very time consuming and although they have been used for almost 100 years, their precision has not been tested collaboratively. They do not satisfactorily characterise honey quality and origin (see next section) and for this reason have been replaced by specific chromatographic methods.

Specific chromatographic methods have been used for the determination of honey sugars for the last 30 years. The advantage of these methods is that they yield data of all honey sugars, which is important for the determination of different aspects of honey quality (see next section). Modern gas chromatography uses capillary columns for separation of silylated sugar derivatives. This method gives a very good separation of the many honey sugars, but is very time consuming and thus unsuitable for routine analysis. HPLC with silica-based amino columns and refractometric detection or with ion-exchange columns and pulsed amperometric detection is simpler and more suitable for routine analysis. Although all the main honey sugars can be determined with these methods, in routine control mostly fructose, glucose and sucrose are measured.

The  $VK_R$  values of all three methods are below 10 %, with the exception of measurements of low levels of sucrose. The HPLC method showed the least interlaboratory variation.

Fructose, glucose and sucrose can be specifically determined also enzymatically (17) but this method has not been tested collaboratively and it is not clear whether sucrose can be determined precisely in a large excess of glucose.

### ***Water insoluble solids content***

Honey insoluble matter includes pollen, honey-comb debris, bee and filth particles and is thus a criterion of honey cleanliness. It is measured by filtration of a honey solution on a glass crucible. The interlaboratory variation was very high, lying between 26 and 85 %. This should be borne in mind when interpreting results.

### **Proline content**

The main honey amino acid proline is determined by a ninhydrin-based colorimetric method. This method, often used routinely as a criterion of honey ripeness and sugar adulteration, has a satisfactory interlaboratory variation.

### **Specific rotation**

The specific optical rotation of honey solutions is measured with a polarimeter. This measurement has been used most prominently in Italy and Greece and more experience in other countries is necessary before it can be accepted internationally.

### **Revised draft for a Codex Alimentarius honey standard**

The EU Directive and the Codex Alimentarius Standard (1, 2) contain almost identical honey standards, which are presently being revised (3, 4). Table 2 summarises the latest Codex draft for a honey standard. In the following we discuss briefly the different quality factors. The honey laboratories of the authors of this work used these quality factors for the quality determination of the honeys of their respective country. Also, these quality factors have been routinely measured in many thousands of commercial honey samples from all over the world by the Institute for Honey Analysis (IHA) in Bremen, Germany (18), and by Wiertz and Co.; Handelschemiker, Hamburg.

### **Moisture content**

Honey having a high water content is more likely to ferment. A maximum value of 21 g/100 g is suggested in the draft for a new standard. The exception for clover honey is not justified by measurements during recent years. Accordingly, the maximum water content for clover honey should also be 21 g/100 g. In practice, values as high as 21 g/100 g are very seldom attained. In routine honey control carried out by the IHA during the years 1989–97 on ca. 30 000 honey samples 91–95 % of all honeys had a water content of less than 20 g/100 g (18). Also in Switzerland a standard of 20 g/100 g was successfully used in the past 20 years, until the last revision of the Swiss Food Ordinance, where the European Union maximum value of 21 g/100 g had to be accepted. Many national beekeeping organisations (e.g. Germany, Belgium, Austria, Italy, Switzerland) have moisture content maximum values of 18–18.5 g/100 g.

### **Mineral content (ash)**

The ash content is a quality criterion for honey botanical origin, the blossom honeys having a lower ash content than honeydew honeys (14). At present, this measurement is generally replaced by the measurement of electrical conductivity. The ash content could be kept as a quality factor during a transition period, until conductivity is accepted as a world-wide standard.

Table 2  
**Honey Quality Standard according to the draft CL 1998/12-S of the Codex Alimentarius**

Quality Criteria	Value
Moisture Content	
general	≤ 21 g/100 g
heather, clover	≤ 23 g/100 g
Apparent Reducing Sugars Content	
Honeys not listed below	≥ 65 g/100 g
honeydew honey or blends of honeydew honey and blossom honey	≥ 45 g/100 g
<i>Xanthorrhoea</i> pr.	≥ 53 g/100 g
Apparent Sucrose Content	
Honeys not listed below	≤ 5 g/100 g
<i>Robinia</i> , <i>Lavandula</i> , <i>Hedysarum</i> , <i>Trifolium</i> , <i>Citrus</i> , <i>Medicago</i> , <i>Eucalyptus</i> cam., <i>Eucryphia</i> luc., <i>Banksia</i> menz.	≤ 10 g/100 g
<i>Calothamnus</i> san., <i>Eucalyptus</i> scab., <i>Banksia</i> gr., <i>Xanthorrhoea</i> pr.	
Honeydew honey and blends of blossom with honeydew honey	≤ 15 g/100 g
Water-Insoluble Solids Content	
general	≤ 0.1 g/100 g
pressed honey	≤ 0.5 g/100 g
Mineral Content (ash)	
general	≤ 0.6 g/100 g
honeydew or blends of honeydew and blossom honey or chestnut honey	≤ 1.2 g/100 g
Acidity	
Diastase Activity, after processing and blending (diastase number in Schade scale)	≥ 8
general	
honeys with natural low enzyme content	≥ 3
Hydroxymethylfurfural Content	
after processing and/or blending	≤ 60 mg/kg

### Acidity

The old standard fixed a maximum of 40 milliequivalents/kg, which has been increased to 50 milliequivalents/kg in the Codex draft, as there are some honeys, which have a higher natural acidity (19).

### Hydroxymethylfurfural content

This major honey quality factor is an indicator of honey freshness and overheating. In fresh honeys there is practically no hydroxymethylfurfural (HMF), but it increases upon storage, depending on the pH of honey and on the storage temperature. Some European bee federations (Germany, Belgium, Italy, Austria) market a major part of their honey as a «quality honey», having a maximum of 15 mg/kg. In

international trade, a maximum value of 40 mg/kg has proven satisfactory. In long term routine honey control at the IHA during the last 10 years, more than 90 % of the raw honey samples ( $n = 30\,000$ ) and more than 85 % of the retail honey samples ( $n = 2000$ ) had less than 30 mg HMF/kg (18). The Codex proposal is a maximum of 60 mg/kg. The proposal for a higher maximum value is based on the experience that HMF increases on honey storage in warm climate countries. The latest EU standard proposal demands a maximum of 40 mg/kg (3). A possible solution of this contradiction is the introduction of a regional standard for this quality factor.

### ***Diastase activity***

Honey diastase activity is a quality factor, influenced by honey storage and heating and thus an indicator of honey freshness and overheating. Although there is a large natural variation of diastase (20, 21), the present standard of a minimum DN value of 8 has proven to be useful. In long-term routine honey control at the IHA more than 92 % of the raw honey samples ( $n = \text{ca. } 20\,000$ ) and more than 88 % of the retail honey samples ( $n = \text{ca. } 1000$ ) had a DN greater than 8 (18). When interpreting diastase results one should take into consideration that certain unifloral honeys have a naturally low diastatic activity (20).

### ***Apparent sugar content***

In most blossom honeys apparent reducing sugars represent the great majority of honey sugars, but in honeydew honeys, the situation is often very different. Indeed, many honeydew honeys have high amounts of non-reducing oligosaccharides such as melezitose, maltotriose and raffinose. Because of these findings, the standard for apparent sugars has been modified compared with the previous one: a minimum of 45 g/100 g has been proposed, compared to the old standard with a minimum of 60 g/100 g.

The measurement of reducing sugars detects only the difference between blossom and honeydew honeys, but this difference can be determined much easier by other methods, e.g. by electrical conductivity determination. There are many arguments for replacing the measurement of the reducing sugars with that of specific sugars (see next section).

### ***Water insoluble solids content***

The measurement of insoluble matter is an important means to detect honey impurities that are higher than the permitted maxima. It was set in the times, when a significant portion of world honey was harvested by pressing the combs. However, nowadays almost all commercial honey is harvested by centrifugation. It seems to us that the permitted maximum of 0.1 g/100 g is very high. Mostly lower values, in the range of 0.005 to 0.05 g/100 g are found. Wax, which is not determined by the Codex method, is a major source of water-insoluble contamination. For this purpo-

se other filtration technique can be used, e.g. with paper filter, but such a method has not been officially proposed yet.

### **Additional criteria for the international honey standard**

The conductivity and sugar data, gathered from the analysis in the laboratories of the authors of this review is compiled in tables 3 and 4. About 50 % of the analytical data (mostly mixed blossom honeys and honeydew honeys) come from the IHA, Bremen, where honeys from Europe, Asia (mostly China), South and North America and Australia are routinely examined. The data for the unifloral honeys come from the laboratories of the other authors of this work, where honeys of different countries were analysed (18-30).

#### **Electrical conductivity**

Conductivity is a good criterion of the botanical origin of honey and today it is determined in routine honey control instead of the ash content. This measurement depends on the ash and acid content of honey; the higher their content, the higher the resulting conductivity (14). There is a linear relationship between the ash content and the electrical conductivity (29):

$$C = 0.14 + 1.74 A$$

where  $C$  is the electrical conductivity in milli Siemens  $\text{cm}^{-1}$  and  $A$  the ash content in g/100 g.

The conductivity data of different unifloral, blossom and honeydew honeys are summarised in table 3. Based on this data we propose that blossom honeys, mixtures of blossom and honeydew honeys should have less than 0.8 mS/cm and honeydew and chestnut honeys should have more than 0.8 mS/cm (see table 5). Exceptions are *Arbutus*, *Banksia*, *Erica*, *Leptospermum*, *Melaleuca*, *Eucalyptus* and *Tilia* honeys as well as their blends, having an extremely high variation in their conductivity (table 3).

Specific standards for honeys with different botanical and geographical origin could be elucidated when a further characterisation of honey is required. The conductivity measurement is easy and fast and needs only inexpensive instrumentation. It is very widely used for discrimination between honeydew and blossom honeys and also for the characterisation of unifloral honeys. Thus an introduction of an international conductivity standard is recommended as urgent.

#### **Specific sugar content**

In table 4 the data of the sum of fructose and glucose and the sucrose content of about 3500 unifloral and mixed honeys are compiled. These data are compiled mostly from measurements in the laboratories of the authors with two exceptions, where the sucrose data were compiled from reference 31. Based on these data a general standard for a minimum content of the sum of fructose and glucose of 60 g/100 g for all blossom honeys and 45 g/100 g for all honeydew honeys can be proposed (table

Table 3  
Electrical conductivity of honey

Honey-Type Species (common name)	Origin	n	mS/cm
<i>Arbutus</i> (arbutus)	E	46	0.63–0.90
<i>Banksia</i> (heath)	Au	4	0.74–1.02
<i>Brassica</i> (rape)	E	442	0.09–0.27
<i>Castanea</i> (chestnut)	E	297	0.80–2.07
<i>Centaurea</i> (ventaurea)	E	27	0.21–0.75
<i>Citrus</i> (orange, lemon)	E	150	0.10–0.35
<i>Erica</i> (heather)	E, A	291	0.42–1.40
<i>Eucalyptus</i> (eucalyptus)	E, Am, Au	181	0.19–1.33
<i>Eucryphia</i> (leatherwood)	Au	6	0.46–0.89
<i>Gossypium</i> (cotton)	E	20	0.45–0.76
<i>Hedysarum</i> (honeysuckle)	E	57	0.09–0.30
<i>Helianthus</i> (sunflower)	E	174	0.20–0.60
<i>Leptospermum</i> (manuka, jelly bush)	A	25	0.31–1.07
<i>Lavandula</i> (lavender)	E	304	0.12–0.60
<i>Maleuca</i> (tea tree)	Au	5	0.40–1.12
<i>Medicago</i> (lucerne)	E	16	0.11–0.23
<i>Phacelia</i> (phacelia)	E	46	0.09–0.44
<i>Robinia</i> (false acacia)	E, A	685	0.09–0.30
<i>Rhododendron</i> (alpine rose)	E	112	0.15–0.45
<i>Rosmarinus</i> (rosemary)	E	81	0.10–0.35
<i>Taraxacum</i> (dandelion)	E	67	0.29–0.65
<i>Thymus</i> (thyme)	E	154	0.24–0.72
<i>Tilia</i> (lime)	E, A	199	0.33–1.15
<i>Trifolium</i> (clover)	E	65	0.13–0.25
Blossom, blend	E, A, Am, Au	2052	0.10–0.70
Honeydew	E, A, Am, Au	1623	0.80–2.11

The data is compiled from measurements in the laboratories of the authors.

n = number of samples

Min (minimum) and Max (maximum) values for more than 99 % of the measured samples.

E = Europe, A = Asia, Am = America, Au = Australia

Table 4  
Sugar content of honey

Honey-Type Species (common name)	Origin	n	G+F g/100 g	S g/100 g
<i>Arbutus</i> (arbutus)	E	23	63.1–74.3	0.1–3.3
<i>Brassica</i> (rape)	E	310	68.2–83.9	0.0–1.0
<i>Banksia</i> (heath)	A	51	nd	1.6–10.7*
<i>Castanea</i> (chestnut)	E	158	62.0–81.4	0.0–1.3
<i>Centaurea</i> (ventaurea)	E	27	68.8–79.5	0.0–4.5
<i>Citrus</i> (orange, lemon)	E, A	104	63.7–77.9	0.0–8.4*
<i>Erica</i> (heather)	E	199	64.0–82.6	0.0–1.5
<i>Eucalyptus</i> (eucalyptus)	E	26	63.3–77.0	0.1–2.8
<i>Hedysarum</i> (honeysuckle)	E	29	67.0–74.1	0.0–8.3
<i>Helianthus</i> (sunflower)	E	117	68.7–84.8	0.0–1.8
<i>Lavandula</i> (lavender)	E	159	60.1–73.2	0.0–15.0
<i>Medicago</i> (lucerne)	E	16	63.8–75.6	0.0–7.0
<i>Phacelia</i> (phacelia)	E	39	64.9–80.4	0.0–4.0
<i>Robinia</i> (false acacia)	E, A	1474	60.6–83.8	0.0–10.0
<i>Rhododendron</i> (alpine rose)	E	91	60.8–79.4	0.9–4.3
<i>Rosmarinus</i> (rosemary)	E	43	64.8–84.1	0.0–4.6
<i>Taraxacum</i> (dandelion)	E	40	67.4–84.2	0.0–5.0
<i>Thymus</i> (thyme)	E	40	64.0–80.3	0.0–0.6
<i>Tilia</i> (lime)	E	150	54.7–79.3	0.0–4.5
<i>Trifolium</i> (clover)	E	23	66.6–78.5	0.0–3.1
Blossom, blend	E, A, Am, Au	880	60.0–83.0	0.0–4.8
Honeydew	E, A, Am, Au	442	45.1–71.8	0.0–4.8

The data is compiled from measurements of specific sugar content in the laboratories of the authors, excepted the sucrose data marked by \*, which was compiled also by using reference 31.

n = number of samples, nd = not determined; G = glucose, F = fructose, S = saccharose

Min (minimum) and Max (maximum) values for more than 99 % of the measured samples.

E = Europe, A = Asia, Am = America, Au = Australia

5). This standard could be fulfilled in more than 99 % of the analysed honeys. For sucrose the situation is more complex. Here the general standard of 5 g/100 g could be fulfilled in more than 99 % of the analysed honeys, with the exception of some unifloral honeys like *Banksia*, *Citrus*, *Hedysarum*, *Medicago* and *Robinia* honeys with up to 10 g/100 g and *Lavandula* honeys with up to 15 g/100 g sucrose. The sum

Table 5

**Sugar content and electrical conductivity: proposal for a new international standard**

<i>Suggested New Quality Criteria</i>	<i>Proposed Value</i>
Sugar Content	
<i>Sum of fructose and glucose</i>	
blossom honeys	$\geq 60 \text{ g / 100 g}$
honeydew honey or blends of honeydew honey and blossom honey	$\geq 45 \text{ g / 100 g}$
<i>Sucrose</i>	
honeys not listed below	$\leq 5 \text{ g / 100 g}$
<i>Banksia, Citrus, Hedysarum, Medicago, Robinia</i>	$\leq 10 \text{ g / 100 g}$
<i>Lavandula</i>	$\leq 15 \text{ g / 100 g}$
Electrical Conductivity	
Blossom honeys excepted the honeys listed below and blends with them; blends of honeydew and blossom honey	$\leq 0.8 \text{ mS/cm}$
Honeydew and chestnut honey, excepted the honeys listed below and blends with those	$\geq 0.8 \text{ mS/cm}$
Exceptions: <i>Arbutus, Banksia, Erica, Eucalyptus, Eucryphia, Leptospermum, Melaleuca, Tilia</i>	

of the fructose and glucose content is very close to the sum of all reducing sugars, as fructose and glucose represent mostly more than 90 % of all reducing sugars. Indeed, the proposed minimum standard for the sum of glucose and fructose of 45 and 60 g/100 g for honeydew and blossom honeys is almost identical to the proposed standards for apparent reducing sugars of 45 and 65 g/100 g respectively. On the other hand, the proposed standard for true sucrose is very similar to the one for apparent sucrose (table 2). Exceptions are differences for honeydew honeys, where the «apparent sucrose standard» is 15 g/100 g, while the specific sucrose standards is only 5 g/100 g and for some Australian and New Zealand honeys, which figure in the standard for reducing sugars (table 2), but not in the proposed standard for specific sugars (table 5) as no specific sugar data is available for these honeys.

The introduction of a standard for specific sugar contents will have other positive consequences for routine honey control. Presently the apparent sugar content of commercial honey samples is checked for standard compatibility, but it does not yield much information on honey quality. On the other hand, the sugars of honey samples are analysed to get information on different aspects of honey quality. Thus, the fructose/glucose ratio and the sucrose concentrations are good criteria for differentiating between different unifloral honeys (20, 22, 25, 26). Also, the content of melezitose (32, 33), maltotriose (34) and 2 other unidentified oligosaccharides (35) is a good indicator of the honeydew content of honey. The specific sugar spectrum

yields also information on honey authenticity and sugar adulteration (see reference 36 and further references therein).

### **Additional quality factors outside the standards**

There are some useful quality criteria, used for the determination of honey quality outside the international honey regulations.

#### *Invertase activity*

Invertase activity is particularly sensitive to heat and storage damage and is used as a freshness indicator. It was proposed that fresh and unheated honeys should have an invertase number (IN) more than 10; for honeys with low enzymatic activity a IN of more than 4 is recommended (37). Although, like honey diastase, the activity of invertase has a great natural variation (38) its use has been proven in honey quality control. A freshness invertase standard is also used in the honey standards of the beekeepers associations of Germany and Belgium.

#### *Proline content*

The honey proline content is a criterion of honey ripeness and in some cases, also of sugar adulteration (39). A minimum value for genuine honey of 180 mg/kg is accepted in honey control laboratories. However, it should be taken into account that there is considerable proline variation, depending on the honey type (40).

#### *Specific rotation*

The overall value for the optical rotation is a resultant of the values of the different honey sugars. The measurement of specific rotation is currently used in Greece; Italy and UK to distinguish between blossom and honeydew honeys. In Italy it was found that blossom honeys (20, 41) have negative values of optical rotation, while honeydew honeys have a positive one (20). Whether this method is capable of differentiating these honeys in other geographic regions remains to be examined in future studies.

### **Conclusions**

The present review summarises the latest information on the main methods and quality factors used in the international honey regulations for the determination of honey quality. We enumerate many arguments to support the introduction of new standards for specific sugars and electrical conductivity. Indeed, during the last 30 years there are very few publications, where reducing sugars and ash content are used as quality factors. On the other hand, specific sugars and electrical conductivity are mostly used instead. Thus the latter quality criteria should be introduced as international honey standards.

Besides the criteria, enumerated in this review, the Codex Alimentarius prescribes also hygienic, authenticity and contamination tests. Apart from these, the honey

specialists use also a number of other quality criteria to determine the botanical and geographical origin of honey, especially the characterisation of unifloral honeys. In its further work the IHC is going to compile and harmonise the methods and criteria, used for this purpose. Indeed, up to now chemical quality criteria for unifloral honeys are valid only in separate countries, but they are not officially recognised in the international honey trade.

## **Summary**

International honey standards are specified in a European Honey Directive and in the Codex Alimentarius Standard for Honey, both of which are presently under revision. In this paper the present knowledge on the different quality criteria and on the methods used for their determination is reviewed. The standard drafts, mentioned above include standards and methods for the determination of the following quality factors: moisture, ash, acidity, hydroxymethylfurfural, apparent reducing sugars, apparent sucrose, diastase activity and water-insoluble matter. However, during the last 30 years there are very few published works on reducing sugars and ash content of honey. Instead, specific sugars and electrical conductivity are mostly used. In this review we present analysis data of these quality factors, measured in many thousands of honeys from all over the world. Based on this data, international honey standards for the sum of fructose and glucose content, the sucrose content and electrical conductivity are proposed. Besides, the use of other quality factors, like invertase activity, proline and specific rotation, additionally used in many countries, is also discussed.

## **Zusammenfassung**

Die internationalen Honignormen sind spezifiziert in der Europäischen Honig-Richtlinie und in der Codex Alimentarius Honignorm, beide gegenwärtig in Revision. In dieser Veröffentlichung wird der gegenwärtige Stand des Wissens über die wichtigsten Honigqualitätskriterien und die entsprechenden Bestimmungsmethoden vermittelt. Die erwähnten Normentwürfe beinhalten Qualitätsstandards und entsprechende Bestimmungsmethoden für folgende Qualitätskriterien: Wasser, Asche, Säure, Hydroxymethylfurfural, scheinbare reduzierende Zucker, scheinbare Saccharose und wasserunlösliche Stoffe. In den letzten 30 Jahren sind jedoch wenig Publikationen und Untersuchungen über scheinbare reduzierende Zucker, scheinbare Saccharose und Aschegehalt erstellt worden. Dafür wurde das spezifische Zuckerspektrum sowie die elektrische Leitfähigkeit gemessen. In der vorliegenden Arbeit wird umfangreiches Datenmaterial über die Messungen an einigen tausend Honigproben aus vielen Teilen der Welt präsentiert. Auf diesem Datenmaterial basierend werden internationale Normen für die Summe des Glucose- und Fructosegehaltes, des Saccharosegehaltes und der elektrischen Leitfähigkeit vorgeschlagen. Zusätzlich werden die in vielen Ländern genutzten Qualitätsparameter wie Invertaseaktivität, Prolinegehalt und optische Rotation diskutiert.

## Résumé

Les normes internationales concernant le miel sont spécifiées dans les directives européennes en la matière et dans les normes du Codex Alimentarius qui font tous deux actuellement l'objet d'une révision. La présente publication donne l'état des connaissances au sujet des critères de qualité les plus importants pour le miel de même que les méthodes de détermination correspondantes. Les projets de normes mentionnés contiennent des normes de qualité et des méthodes de détermination pour les critères de qualité suivants: eau, cendres, acide, hydroxyméthylfurfural, sucres réducteurs apparents, saccharose apparente et substances non solubles dans l'eau. A noter que peu de publications et d'analyses ont été réalisées ces 30 dernières années au sujet des sucres réducteurs apparents, de la saccharose apparente et de la teneur en cendres. En revanche, on a mesuré le spectre des sucres ainsi que la conductibilité électrique. Les présents travaux présentent de nombreuses données par rapport aux mesures effectuées sur quelques milliers d'échantillons de miel provenant de différentes régions du globe. Sur la base de ces données, des normes internationales concernant le total de la teneur en glucose et en fructose, en saccharose et la conductibilité électrique ont été proposées. De plus, les paramètres de qualité utilisés dans beaucoup de pays tels que l'activité de l'invertase, la teneur en proline et la rotation optique font l'objet d'une discussion.

## Key words

Honey, Quality standard, Analysis method, Codex Alimentarius, EU

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