FOODINTEGRITY HANDBOOK

A GUIDE TO FOOD AUTHENTICITY ISSUES AND ANALYTICAL SOLUTIONS

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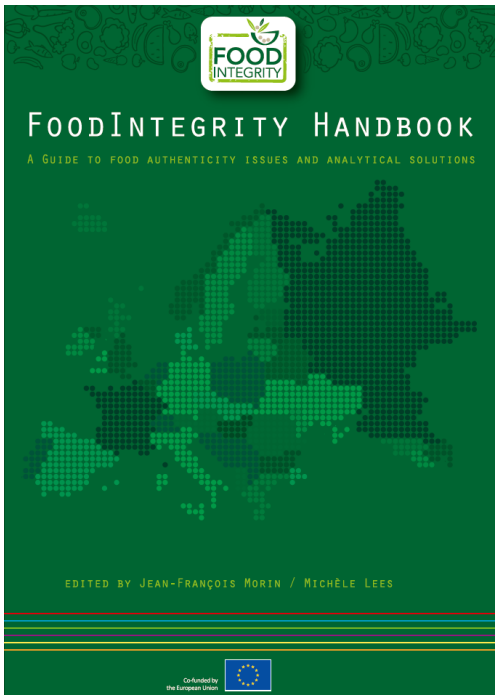

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Honey

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General overview of the products

Honey is a truly natural product traded and consumed all over the world [1]. For thousands of years honey has been produced by bees in the same way and offers a wide spectrum of versatility. Already our ancestors used honey and not only as a sweet food; honey was known as a universal remedy, a valued beauty care product, an effective preservative and was even accepted as a means of payment.

Today honey is used mainly for human consumption either as pure honey or as an ingredient in other food products as a sweetener for juices and cereals. The pure honey available in the market varies from blends favoured for their consistency of colour and flavour to specialist honeys from particular floral, geographical or topological sources. Honey is also commonly used as an additive in beauty care products.

China is the world's largest exporter of honey, with total exports of 128 330 tons in 2016. They are followed by Argentina (81 183 tons), Ukraine (54 442 tons), Vietnam (42 224 tons), India (35 793 tons), Mexico (29 098), Spain (26 874 tons), Germany (25 325 tons), Brazil (24 203 tons), and Belgium (20 816 tons) [2].

The USA is the major importer of honey (166 477 tons in 2016), followed by Germany (81 959 tons), Japan (48 445 tons), UK (41 135 tons), France (35 433 tons) and Spain (27 988 tons). In fact, considered all together, the European Union (EU) of 28 Member States imports more honey than the USA (with a total 283 299 tons) and is a major net importer [3]. The majority of imported honey is blended and labelled as honey from the EU, from non-EU countries or a blend of both, EU and non-EU. These blends are generally sold through supermarkets. Local production offers unifloral and special honeys for the local market.

Whereas bee products royal jelly and propolis are known and accepted as beneficial for human health, pollen is combined and consumed with other food. Wax is less important and used to produce candles or as an ingredient for cosmetic and pharmaceutical products.

Honey received special attention in the US in 2013 when U.S. Immigration and Customs Enforcement and Homeland Security investigations charged five people and two honey-processing

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companies with dumping honey imports from China, including some that were adulterated with unauthorised residues of antibiotics. This incident was considered to be just “the tip of the iceberg” in honey fraud [4].

In the same year in Europe, in the aftermath of the horsemeat scandal, the EU included honey in a top ten list of food products most at risk of food fraud, putting further focus on honey adulteration. However prior to these events, the honey industry sector had been well aware of the concerns of economically motivated adulteration of honey, particularly given the ease with which sugar syrups can be added and premium honey diluted with cheaper types. This has led to considerable efforts being undertaken by various trade bodies such as Apimondia (the International Federation of Beekeepers’ Association), the IHC (International Honey Commission) and the IHEO (International Honey Exporters Organisation) to control the presence of fraudulent product in the market place.

In addition to the diversity of countries exporting honey, often from remote regions with little or no transparency of supply, the practice of beekeeping itself is also under threat. During the last few decades, intensive agriculture and the use of pesticides resulting in a reduction and/or contamination of available areas for bee foraging and the emergence of new bee diseases have all led to a decline in traditional beekeeping activities. The availability of cheap, often fraudulent products in the market resulting in lower prices for domestic honey, has also pushed in the same direction. The ensuing decline in bees, which pollinate a large portion of global food production, poses a serious threat to the food chain. In the EU, it is estimated that pollinators, including honey bees, bumblebees and wild bees, contribute at least EUR 22 billion each year to the European agriculture industry. They ensure pollination for over 80 % of crops and wild plants in Europe [5]. Alarm bells have sounded particularly in Europe and North and South America. An up-to-date review of the current situation on the international honey market is given in reference [6].

1. Product Identity

1.1. Definition of the product and manufacturing process

Honey is primarily a concentrated solution of sugars, composed mainly of glucose and fructose, together with other components such as organic acids, enzymes, vitamins, acetylcholine, flavonoids, minerals is trace quantities [7]. Honey production itself must be considered at two levels, taking into account both the collection and processing of plant fluids by the bees [8], and the extraction and processing of honey by beekeepers and honey packers. The latter includes a number of processing steps which vary according to the unique characteristics of the honey being processed. In general the production process follows six main steps: extraction, dehumidification, liquefaction and blending, heating, pasteurisation, crystallisation, and final packing. Reference [9] provides a detailed description of each of these steps. The INPhO (Information on Post-harvest Operations) of the United Nations FAO (Food and Agriculture Organisation) have produced a Honey Processing toolkit [10] to help in the setting up of a honey infrastructure.

1.2. Current standards of identity or related legislation

Honey, its composition, its specification and related methods are clearly defined and described in international accepted standards such as CODEX, EU, ISO, DIN and guidelines of different trade and beekeeping associations.

1.2.1. In the European Union

If honey is placed on the market in the EU it must meet the requirements of the Honey Directive 2001/110/EC [11]. The definition of honey is given in the first paragraph of Annex I of the EU honey directive: "Honey is the natural sweet substance produced by *Apis mellifera* 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 origin the main types of honey are blossom or nectar honey, obtained from the nectar of plants, and honey dew honey, obtained mainly from excretions of plant sucking insects on the living part of plants or secretions of living parts or plants.

According to mode of production and/or presentation the following types of honey are defined: comb honey, chunk honey or cut comb in honey, drained honey, extracted honey, pressed honey and filtered honey. These definitions apply to honey which is placed directly on the market. If the honey has a foreign taste or odour, or is beginning to ferment or has fermented, or has been overheated, it is only suitable for industrial use or as an ingredient in other foodstuffs, where it is known as baker's honey.

The processing of honey is limited under the terms of the EU honey directive and exists solely of filtering and homogenisation under controlled temperature. When placed on the market as honey or used in any product intended for human consumption, honey must meet the compositional criteria given in Table 1.

According to Decision 2011/163/EU [12] it is mandatory for non-EU countries that want to export honey to EU Member States to be listed on a third country list in accordance with article 29 of Council Directive 96/23/EC [13].

The general food-labelling rules laid down in Directive 2000/13/EC [14] also apply to honey but are subject to certain conditions. In particular the country of origin where the honey has been harvested should be included on the label. In addition, the labelling of filtered honeys and baker's honeys is mandatory for every transaction on the bulk market.

1.2.2. In the United States

The Center for Food Safety and Applied Nutrition of the US FDA (Food and Drug Administration) has recently published nonbinding recommendations for the "Proper labelling of honey and honey products" as a guidance for industry [15]. It addresses the labelling of honey whether sold as a single-ingredient food, or as a mixture of honey and other ingredients such as sweeteners or flavourings. The document also highlights the FDA's definition of adulteration under the FD&C Act (Federal Food, Drug, and Cosmetic Act, Section 402(b)) which stipulates that *"a food is adulterated if: (1) a valuable constituent has been omitted in whole or in part from a food; (2) if any substance has been substituted wholly or in part; (3) if damage or inferiority has been concealed in any manner; or (4) if a substance has been added to a food so as to increase its bulk or weight, reduce its quality or strength, or make it appear to be better or of greater value than it is".*

Since honey is a commodity that is known to be subject to economic adulteration through addition of cane or corn sweeteners, the FDA regularly monitor imported products labelled as honey to ensure that they contain only honey as the sole ingredient. Results of this surveillance exercise are publicly available at Import Alert 36 [16].

Table 1: Compositional criteria for honey [11]

1. Sugar content	
1.1. Fructose and glucose content (sum of both)	
● blossom honey	not less than 60 g/100 g
● honeydew honey, blends of honeydew honey with blossom honey	not less than 45 g/100 g
1.2. Sucrose content	
● in general	not more than 5 g/100 g
● false acacia (*Robinia pseudoacacia*), alfalfa (*Medicago sativa*), Menzies Banksia (*Banksia menziesii*), French honeysuckle (*Hedysarum*), red gum (*Eucalyptus camadulensis*), leatherwood (*Eucryphia lucida*, *Eucryphia milliganii*), *Citrus spp*.	not more than 10 g/100 g
● lavender (*Lavandula spp*.), borage (*Borago officinalis*)	not more than 15 g/100 g
2. Moisture content	
● in general	not more than 20 %
● heather (*Calluna*) and baker's honey in general	not more than 23 %
● baker's honey from heather (*Calluna*)	not more than 25 %
3. Water-insoluble content	
● in general	not more than 0.1 g/100 g
● pressed honey	not more than 0.5 g/100 g
4. Electrical conductivity	
● honey not listed below, and blends of these honeys	not more than 0.8 mS/cm
● honeydew and chestnut honey and blends of these except with those listed below	not less than 0.8 mS/cm
● Exceptions: strawberry tree (*Arbutus unedo*), bell heather (*Erica*), eucalyptus, lime (*Tilia* spp.), ling heather (*Calluna vulgaris*), manuka or jelly bush (*leptospermum*), tea tree (*Melaleuca* spp.)	no limit defined
5. Free acid	
● in general	not more than 50 milli-equivalents acid per 1000 grams
● baker's honey	not more than 80 milli-equivalents
6. Diastase activity and hydroxymethylfurfural content (HMF) determined after processing and blending	
(a) Diastase activity (Schade scale)	
● in general, except baker's honey	not less than 8
● honeys with low natural enzyme content (e.g. citrus honeys) and an HMF content of not more than 15 mg/kg	not less than 3
(b) HMF	
● in general, except baker's honey	not more than 40 mg/kg (subject to the provisions of (a), second indent)
● honeys of declared origin from regions with tropical climate and blends of these honeys	not more than 80 mg/kg

1.2.3. Codex Alimentarius

Codex Standard 12-1981 [17] was adopted in 1981 with revisions in 1987 and 2001. The Codex definition is not worded identically to the Honey Directive but there is very little, if any, difference in meaning. Additionally it defines Blossom or Nectar Honey as the honey from nectar of the plants, and Honeydew honey as the honey coming mainly from excretions of plant sucking insects (*Hemiptera*) on the living parts of plants or secretions of living parts of plants.

Under its requirements for essential composition and quality factors, the Codex Standard also stipulates that honey sold as such should not have added to it any food ingredient, including food additives, and should 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. And chemical or biochemicals treatments to influence honey crystallisation are not permitted.

The Codex Standard also provides acceptable ranges for moisture, sugars and water insoluble solids contents. It provides guidelines for sampling and analysis, as well as for labelling, with clear recommendations for how the honey should be designated.

1.2.4. Further legislation on honey production and quality

It is important to know that non-EU countries have developed definitions and specifications for honey which are slightly different and can differ from the EU honey directive and as a consequence do not meet EU regulation. A review of the differences that exist in international legislation is given in reference [18].

1.2.5. Other bee products

Recently royal jelly was defined in the standard ISO 12824:2016 [19] which specifies the production and sanitary requirements for royal jelly and establishes a series of organoleptic and chemical test methods to control royal jelly quality. It applies to the production of royal jelly (collecting, preliminary processing and packaging) and trade links but not to royal jelly products in which other foods are mixed.

The definition of the other bee products is more diverse and follows more often industry specifications or guidelines of industry associations. The extraction or production of other bee products is not strictly regulated and follows individual procedures.

2. Authenticity issues

2.1. Identification of current authenticity issues

In general honey needs to meet the given definitions and fixed specifications. Questions of authentication occur on two levels. Firstly, 'pure' honey may have been extended by addition of sugar, syrup and/or water. Secondly, if the honey has a more detailed description indicating botanical, geographical or topological origin, the description may be false even though the product is pure honey. There are other possible incorrect descriptions and information such as health claims, if it is 'organic', has 'antibacterial activity' and so on which are difficult to evaluate.

2.1.1. Intentional addition of cheap sugars and sugar syrups

The main focus regarding honey authenticity is on economically motivated adulteration by the addition of foreign sugars. As honey is more highly priced than sweet substances such as sugar and industrial syrups, extension by addition of these at some stage during processing could be an attractive route to adulteration. Existing and regulated methods to analyse the sugar spectrum of honey can show that honey meets its specification in both qualitative and quantitative sugar

composition. However these methods are limited when required to identify sugar addition by different types of syrups from different botanical sources.

Most bulk sweetening materials are derived from cane sugar, beet sugar or by the hydrolysis of starch. The starch is often derived from maize but new sources such as rice are now easily available on the market. Some forms of rice syrup have even been bio-chemically engineered to make them more difficult to detect.

2.1.2. Feeding hives during a nectar flow

Normal beekeeping practice is to ensure that sugar syrup is not laid down in the combs as if it was honey. Providing syrup at the same time as the honey flow constitutes an easy means of adulteration at the earliest stage of production.

2.1.3. Honey moisture content

Mature honey typically contains 13 to 23 % water [20]. If it is over 18 %, there is a risk of fermentation, roughly related to the level of contamination by yeasts and the water content. The Codex Alimentarius sets an acceptable moisture content of not more than 20 % for all honeys with the exception of heather honey (*Calluna*) for which it is set at not more than 23 %. A certain amount of water is lost during processing prior to final bottling into retail packs and this water is usually replaced.

2.1.3.1. Harvesting of immature honey

In some countries such as China, beekeepers harvest the honey early before the comb is capped resulting in a product that has a moisture content around 30 – 40 %. The product is then dehumidified using a vacuum-activated honey dryer to reach an acceptable moisture content. There are ongoing discussions whether such “water honey”, which has a different compositional profile to mature honey, can be considered as pure honey.

2.1.3.2. Illegal use of resin technology

Some honey producers use resin technology to remove unpalatable tastes and aromas linked to certain floral sources. This process involves bringing moisture level of honey up to 40 % and then reducing it to 18-19 %. Although resin technology is regularly used for a number of food products to remove contaminants, its use in honey production is controversial. In particular the process can remove pollen and thus disguise country of origin or floral source. It also removes certain colour components of the honey, transforming dark honey into lighter, more acceptable product.

The current position of the US FDA as regards the use of resin technology is that “the product should be labelled with a name that sufficiently describes its characterizing properties in a way that distinguishes it from honey which has not been treated with resin technology.” Further scientific efforts are underway to establish analytical methodologies and global databases to better assess the use of this technology.

2.1.4. Mislabelling of botanical source

Individual bees forage from a single species of plant as long as that source lasts but honey, even from a single comb, will not often be entirely from a single source. A judgement has to be made, therefore, about a particular honey crop as to whether it can justifiably be called unifloral and thus command a premium price.

2.1.4.1. Incorrect description of blossom and honeydew honey

Blossom honey is derived from the nectaries of flowers. Honeydew honey is derived from non-floral plant secretions. As a particular crop is unlikely to be entirely from one source or the other, it is necessary to judge the designation of the honey on its physical, chemical and microscopical characteristics. Honeydew honey tends to have a higher pollen count, electrical conductivity and ash, to be darker and to contain soot and mould spores [21].

2.1.4.2. Incorrect description of floral source

Some sources such as orange blossom and acacia (*Robinia pseudoacacia*) command a premium price. Such a description will not be justified if the sample contains too much honey from other floral sources.

Authenticity and quality issues cannot always be separated, as exemplified by the case of acacia honey. This may be collected in association with rapeseed cultivation. The characteristic on which the acacia premium is based is the fact that it remains liquid for a long time due to its high fructose/glucose ratio. The relationship between the rate of crystallisation (resulting in an unpleasant gritty taste) and the fructose/glucose ratio is not well understood and research on honey crystallisation is underway. Rapeseed honey crystallises very readily.

Acacia is considered 'pure acacia' if the pollen consists of 20 % or more *Robinia* pollen. If the other pollen present is from many sources, the honey will probably stay liquid and be of the quality associated with acacia. If, on the other hand, the other 80 % is rapeseed, the honey will crystallise quickly and be of unacceptable quality. In this sense, authenticity and quality issues cannot be completely separated.

Specific case of Manuka honey from New Zealand

Manuka honey is recognised as particularly beneficial to human health due to its exceptional antiseptic properties. Manuka (*Leptospermum scoparium*) is a scrub-type tree that grows only in New Zealand and some parts of Australia. Its nectar contains a specific molecule, dihydroxyacetone (DHA), that converts into methylglyoxal (MGO) during maturation and aging of the honey [22]. It is this latter compound that is primarily responsible for the strong antimicrobial activity of the honey.

To deal with the increasing risk of fraud, the New Zealand Ministry for Primary Industries recently published a science definition of monofloral and multifloral manuka honey [23]. This details a combination of five attributes (4 chemicals and 1 DNA marker from manuka pollen) which are required to authenticate monofloral and multifloral manuka honey. These attributes can be identified using 2 laboratory tests. A chemical test for the 4 marker molecules 3-phenyllactic acid, 2'-methoxyacetophenone, 2-methoxybenzoic acid and 4-hydroxyphenyllactic acid which are determined by liquid chromatography and a DNA test for very specific manuka DNA *leptospermum scoparium* performed by quantitative or real-time PCR (polymerase chain reaction).

2.1.4.3. Mislabelling of geographical origin

A honey from a particular geographical origin may also command a premium compared with the bulk blend. For example, Greek Hymettus honey and certain 'forest' honeys fetch a higher price.

A number of specific honey origins have been recognised under the EU quality labels (PDOs and PGIs) that guarantee that the product is from a specific region and follow a particular traditional production process [24]. These include honeys from France: miel des Cévennes, de Provence, d'Alsace (PGI); miel de sapin des Vosges, de Corse (PDO); from Italy: miel Varesino, delle Dolomiti

Bellunesi, della Lunigiana (PDO); from Spain (Mel de Galicia (PGI); Mel Villuercas-Ibores, de Liébana, de Tenerife, de Granada, de la Alcarria (PDO). These special labels are recognised by the consumer and command a premium price.

2.2. Potential threat to public health

Honey is recognised as a healthy product. For people with a pollen allergy nectar honey can have a low potential threat to their health. Also a contamination by bee feedings like milk proteins can potentially cause an allergic reaction. In any case such allergic reactions are very seldom.

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

There are numerous publications devoted to analytical methods to test for honey quality and authenticity, including physical parameters (electrical conductivity, rheological properties, specific rotation, colour and water activity) and chemical components (moisture, sugars, enzymes, HMF, acidity and pH, formol index, insoluble solids, organic acids, proteins, amino acids, vitamins, minerals, volatile and semi-volatile compounds and polyphenols). A comprehensive and recent review of these analytical methods is provided in reference [25].

3.1.1. AOAC methods

The AOAC International Compendium of methods provides details of AOAC methods for the main physical and chemical parameters of honey. These have also been included in Codex Standard 12-1981 and its subsequent revisions. Some of the main parameters and related methods are:

- AOAC 969.38B Determination of moisture content
- AOAC 980.23 Hydroxymethylfurfural (HMF)
- AOAC 958.09 Diastase activity
- AOAC 998.12 Detection of C_4 sugar in honey (more details provided below)

3.1.2. IHC methods

In addition to officially-recognised methods, the International Honey Commission (IHC) has collaboratively tested a wide range of different methods to test for honey authenticity [26]. Harmonised methods for which precision criteria are available include:

- Moisture (refractometric method), electrical conductivity, ash content, pH and free acidity (titration),
- Hydroxymethylfurfural (HPLC, or White/Winkler methods),
- Diastase (Schade method, Phadebas α-amylase assay),
- Sugars (by HPLC or GC),
- Insoluble matter, invertase activity, proline and specific rotation.

3.1.3. Focus on specific methods

3.1.3.1. Melissopalynology

Melissopalynology, or pollen analysis, is an essential part of honey authenticity testing. Pollens grains from different types of plants have a distinctive morphology that can be identified by microscopic examination [7]. The technique, which requires expert judgement, is used to determine the country of origin by linking pollen type to the characteristic flora of the geographical source, or to verify authenticity when a particular botanical origin is claimed.

The pollen count can be used to estimate the proportions of nectar present. However the method does have some limitations, mainly due to the natural variability of amounts of pollen from botanical sources. For example, in some cases the specific pollen may be 'under-represented' such as for citrus and lavender, whereas for others, such as forget-me-not, the pollen is 'over-represented'.

Pollen is also available as a product and the determined adulterator could filter out all the pollen and add back pollen of choice.

Despite its limitations however, pollen analysis is still a useful method to control country of origin. A review of harmonised methods of melissopalynology is given in reference [27].

3.1.3.2. HMF as an indicator of freshness or excessive heating

All honeys contain some amount of hydroxymethylfurfural (HMF) which is formed by from the action of the acidity in honey on reducing sugars through the Maillard reaction. Excess heating during processing or unsuitable storage conditions can HMF content, making it a useful indicator of honey quality. Both the EU Directive and Codex Standard 12-1981 and its subsequent revisions have fixed a limit of 40 mg/kg for HMF in honey after processing and/or blending, with a higher limit of 80 mg/kg in the case of honey of declared origin from countries or regions with tropical ambient temperatures, and blends of these honeys. Reference [28] provides an overview levels of HMF in honey and its effect on bee and human health.

Reversed phase HPLC with UV detection is the most commonly used method for the determination of HMF in honey. The sample should be a clear, filtered aqueous solution of honey. Details on sample preparation are available under AOAC 920.180 or in the IHC description of methods of analyses.

3.1.3.3. Determination of C_4 sugars in honey

Maize and sugar cane metabolise by the Hatch-Slack or C_4 metabolic pathway. As a result, syrups derived from them exhibit a $^{13}C/^{12}C$ ratio, expressed as a δ-value close to -10 ‰ compared with a value for honey which on average is around -25.4 ‰. This difference has been used very successfully to detect adulteration in honey [29,30], and is an official AOAC Method 998-12. In this method the $^{13}C/^{12}C$ ratio, expressed as $\delta^{13}C$ of the whole honey is measured by SIRA (stable isotope ratio analysis) and compared to the $\delta^{13}C$ value of the protein isolated from the honey. The difference between these values is a measure of the C_4 sugar content of the honey, provided that both honey and protein have been analysed on the same instrument [31,32].

Addition of syrups derived from beet and other plants utilising the Calvin or C_3 metabolic pathway remains a considerable analytical challenge. Further solutions are described in the following sections.

3.2. Other commonly used methods

3.2.1. Chromatographic techniques

A variety of chromatographic methods have been developed in order to authenticate honey from different floral origins and to detect added sugar and sugar syrups. This section will provide a few examples of methods available in the literature.

High performance liquid chromatography (HPLC) and gas chromatography (GC) are commonly used to quantify the major carbohydrates, glucose, fructose, sucrose [33]. A method using anion-exchange chromatography in conjunction with pulsed amperometric detection (HPAEC-PAD) has been used to provide a qualitative and quantitative analysis of the minor oligosaccharides present in honey [34]. This method has also been successfully used to detect the addition of certain starch-derived sweeteners in fruit juices.

Carbohydrate profiles quantified by HPAEC-PAD and chemometrics have been used to characterise the botanical origin of honey from a single geographical area [35]. The same technique together with an integrated chemometric approach has been described as an improved COFRAC (COmité FRançais d'ACréditation) method for the evaluation of honey quality and the characterisation of floral source [36].

Chromatographic methods have also been extensively used to analyse chemical components other than sugars as markers of honeys from specific floral sources. Examples the content of phenolic acids, including caffeic, chlorogenic, p-coumaric, ferulic, homogentisic, p-hydroxybenzoic and vanillic acids, and flavonoids, such as apigenin, genistein, hesperetin, kaempferol, luteolin, rhamnetin, rutin, tricetin and quercetin [37]. Amino acid analysis of honey by HPLC together with statistical treatment of the resulting data has also been used to discriminate different botanical origins and to detect the addition of sugar syrup [38].

Methyl anthranilate is a good indicator for orange blossom honey [39–41], which contains very little citrus pollen. Synephrine has been described as another biomarker for orange honey authenticity and can be determined following a LC-MS/MS method [42]. Numerous other biomarkers have been described in the literature.

3.2.2. Stable isotope analysis

3.2.2.1. Detection of sugar and sugar syrups

As described above, stable isotope analysis is the official AOAC method for the detection of sugar addition in honey. The method cannot, however, detect all sugar sources. A new technique developed by Elflein and Raezke [43] combines Liquid Chromatography with Isotope Ratio Mass Spectrometry. This method enables the separation of the individual sugars including their individual $^{13}C/^{12}C$ ratios and is a considerable improvement in the detection of honey adulteration [44,45].

3.2.2.2. Verification of geographical origin

The stable isotope ratios of the light bio-elements have been successfully used to verify country of origin of a number of foodstuffs. An investigation was undertaken as part of the European product

TRACE[1] in which the stable isotope ratios of the elements carbon, nitrogen, sulphur and hydrogen were measured in the protein fraction extracted from honey produced in 20 European areas [46]. The honey protein fraction was specifically chosen since it is part of the preparation in the method to detect added C_4 sugar (as described above) and less easy to manipulate. The study demonstrated that both hydrogen and carbon isotopes in honey protein are correlated to precipitation and climate. The sulphur stable isotope composition of the honey protein is clearly influenced by the geology of the rock underlying the soil in which the flora grew, and from which the bees foraged nectar and pollen. Despite the natural variability of the product and the similarity of geological and climatic conditions across the countries investigated, the study concluded that the four stable isotope ratios considered here, measured on honey protein can be applied to verify the origin of honey.

3.2.3. DNA-based techniques

Biomolecular methods are becoming more frequently used in the authentication of honey since it contains intrinsic DNA markers that can be used to identify origin. One of the most important examples is the use of a DNA marker for manuka honey, required under the New Zealand government's definition for authentic manuka honey, as described above.

Several studies applying DNA-based techniques have been proposed in the literature. Sobrino-Gregorio et al. [47] use both conventional and real-time PCR DNA amplification techniques to the detection and quantification of rice molasses in honey. In another study, PCR primers have been used to amplify specific fragments from the informative mitochondrial DNA region of *Apis mellifera* [48]. Soares et al. [49] have exploited DNA barcoding combined with high resolution melting (HRM) analysis to establish the botanical origin of honey, using lavender honey as a case study.

Honey can be produced by different species of honeybees, of which there are two main species of economic importance. These are *Apis mellifera* (known as the European honeybee) and *Apis cerana* (known as the Asian honeybee). Due to the decline of the wild populations of the Asian honeybee, this honey generally attains much higher market value, being prone to adulteration. A novel real-time PCR method with high resolution melting analysis has been developed to target the 16S rRNA gene of both bee species, which was then further successfully applied to the authentication of Asian and European honey samples [50].

3.2.4. Spectroscopic methods

3.2.4.1. FT-MIR – NIR

FT-NIR (Fourier Transform Near Infrared) and FT-MIR (Fourier Transform Mid-Infrared) spectroscopies have been proposed as rapid methods for honey authentication. They provide simultaneous determination of sugars and other physicochemical parameters and can be used in routine quality control of honey.

Pita-Calvo et al. [51] have used FT-NIR and FT-MIR spectroscopy to distinguish between honeydew and blossom honey. Two characteristic markers of honeydew honey, the trisaccharide melezitose and a diacylglycerilether, made it possible to classify honeydew and blossom honeys correctly.

[1] TRACE Project. Tracing the origin of food. 2005-2009. Funded by the European Commission under the 6th Framework Programme.

NIR spectroscopy is particularly useful as a rapid and non-destructive method for the detection of honey adulteration. Combined with chemometric data treatment the technique has been used to discriminate honey adulterated with high fructose corn syrup (HFCS) demonstrating its potential as a screening method for quality monitoring [52].

3.2.4.2. ^{1}H NMR screening

An innovative analytical approach using proton-NMR (Nuclear Magnetic Resonance) profiling coupled to suitable quantification procedures and statistical models has been developed to tackle the most common adulterations and quality deviations in honey [53]. The NMR technique has a number of advantages: it is highly reproducible and requires a very simple sample preparation. In addition the NMR spectra can be used as “fingerprints” to compare, discriminate or classify samples while its structural elucidation power can be used to characterise novel or unknown biomarkers.

Having a wide screening potential, based on a global observation of all soluble components of honey, NMR profiling is now also widely used for authenticity checks. Since it is independent from potential manipulations of pollen, it also provides a complementary tool to check the declared botanical and geographical origin, beyond the detection of sugar addition and the fast monitoring of many honey quality parameters. This spectroscopic technique also produces a unique fingerprint for each sample, which can be used to check the traceability along the supply chain.

The NMR method has been used to characterise known manuka honey markers, methylglyoxal and dihydroxyacetone. Together with a newly identified NMR marker, leptosperin. The technique can be used to discriminate manuka honey from other floral honey types from Oceania [54].

The NMR technique is now considered as one of the most powerful methods to detect the various forms of adulteration that were described earlier [55].

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Analytical technique	Indicative data, analyte or parameter	Authenticity issue or information
Microscopy	Pollen analysis	Botanical and geographical source
Refractometry	Moisture content	Compliance with regulated limits
Conductimetry	Electrical Conductivity	Distinguishes blossom honey & honeydew
Colorimetry	Diastase	Detects heat abuse
HPLC, Colorimetry	Hydroxymethylfurfural (HMF)	Detects heat abuse
Photometry	Heat-stable α-amylase (diastase surrogate)	Foreign enzyme as marker for foreign syrup additions
Photometry	Foreign α-amylases (diastase surrogates)	Foreign enzyme as marker for foreign syrup additions
HPLC, GC	Sugars	Sugar profile, detects abnormal sugar profile
IRMS	^{13}C ratios of whole honey and extracted protein	Detects C_4 sugar addition
IRMS	Isotope ratios of H, C, N and S	Geographical origin
^{1}H-NMR	Untargeted and targeted screening against reference data base, sugars	Syrup additions, quality deviations, mannose, botanical/geographical origin
IRMS	Isotope ratios of H, C, N and S	Geographical origin
LC-UV	Foreign ß-/-y-amylases (diastase surrogates)	Foreign enzyme as marker for foreign syrup additions
LC-ELSD	ß-fructofuranosidase (invertase surrogate)	Foreign enzyme as marker for foreign syrup additions
LC-ELSD	Syrup-specific oligosaccharides	Marker molecule for foreign syrup additions
ICP-MS	Arsenic	Trace marker for rice syrup additions (TMR)
LC-MS	Marker molecule	Specific marker for rice syrup / cassava syrup additions
LC-MS	Colorant E150d	Addition of dyes or colourings
LC-ELSD	Mannose	Marker molecule for foreign syrup additions
LCMS	Psicose	Marker molecule for foreign syrup additions

5. Conclusion

Today honey and bee products are continuously recognised as pure natural products. The importance of these products is confirmed by the extent of current controls on honey authenticity at every stage of the global supply chain. The composition of honey, the treatment with veterinary drugs and contamination by pesticides and other contaminants are closely monitored.

With the daily news reporting repeatedly about food fraud, adulteration of honey and other bee products will still draw significant attention in the future. It is the responsibility of all stakeholders of the honey supply chain to optimise existing control mechanisms.

As mentioned earlier, the European Commission started an action plan to tackle food fraud late in 2013. As a follow-up in 2015, the European Commission launched a coordinated control plan on honey authenticity in which its Joint Research Centre carried out analyses to detect honey adulteration with exogenous sugars (Commission Recommendation C(2015) 1558 [56]). The aim of the control plan was to establish the prevalence on the European Union market of: (a) honey mislabelled with regard to its geographical and/or botanical origin and (b) products declared or presented as honey although containing exogenous sugars or sugar products. 2 264 honey samples were collected at all stages of the supply chain; the majority of the samples came from retailers. More than 10 % of the honey samples checked by EA/LC-IRMS did not conform to published benchmark purity criteria indicating that foreign sugars may have been added. Around 20 % of honey either declared as blends of EU honeys, or unblended honeys bearing a geographical reference related to an EU Member State or a third country were suspected to contain added sugar.

The published report demonstrates the need for further investigation by all stakeholders of the honey industry to ensure authentic honey and to justify the trust of the consumer in this natural product.

An outlook is given in the Meeting Report of the Technical Round Table on Honey Authentication [57]. The participants agreed on:

- A critical review of the current definition of identity and purity criteria of honey is necessary.
- Acceptance / rejection criteria for authenticating honey are needed.
- An appropriate analysis of the vulnerability of the honey supply chain should be done and an improved traceability system implemented.
- Screening methods should be developed to economise testing.
- Analytical methods to detect emerging fraud cases should be developed and already existing methods should be validated.
- A mechanism for providing quality assurance tools should be established.
- Chemical and biological characteristics of genuine honeys (including blends), bee feeding products, and products from inappropriate practices should be generated and stored in a publicly available database.

In addition to those methods that are already regulated, it will be important to regulate methods that are more suitable to tackle food fraud. One idea will be the evaluation of EA/LC-IRMS as an official method for honey authenticity controls in future. If this happens authorities should be able to claim “non-authentic” honey more often which will have a significant impact on the whole production chain of honey.

With its wide screening potential, the NMR profiling technique is now also widely used for authenticity checks. More generally, non-targeted methods will be a major add-on to the existing approaches for anticipating new fraudulent practices in the future.

6. Bibliographic references

1. Ltd. Research and Markets (2018). – World: Honey - Market Report - Analysis and Forecast to 2025.
2. García N.L. (2018). – Honey quality and the international honey market. , Abu DhabiAvailable at: http://innovationsinagriculture.com/exhibition/smart-honey-production/.
3. European Comission (2017). – Honey Market Presentation. Available at: https://ec.europa.eu/agriculture/sites/agriculture/files/honey/market-presentation-honey_en.pdf.
4. Seattle F.S.N. 1012 F.A.F.F. & Washington 98104-1008 (2013). – "Honeygate" Sting Leads to Charges for Illegal Chinese Honey Importation. *Food Saf. News*. Available at: https://www.foodsafetynews.com/2013/02/honeygate-sting-leads-to-charges-for-illegal-chinese-honey-importation/.
5. Honey bees - Food Safety - European Commission (2018). *Food Saf.* Available at: /food/animals/live_animals/bees_en.
6. García N.L. (2018). – The Current Situation on the International Honey Market. *Bee World*, **95** (3), 89–94. doi:10.1080/0005772X.2018.1483814.
7. Molan P.C. (1996). – Authenticity of honey. . In *Food authentication1st ed* (P.R. Ashurst & M.J. Dennis, eds), Blackie Academic & Professional, London ; New York. pp 259–303
8. Crane E. (1991). – Honey from honeybees and other insects. *Ethol. Ecol. Evol.*, **3** (sup1), 100–105. doi:10.1080/03949370.1991.10721919.
9. Baglio E. (2018). – Honey: Processing Techniques and Treatments. . In *Chemistry and Technology of Honey Production*, Springer International Publishing, Cham. pp 15–22doi:10.1007/978-3-319-65751-6_2.
10. Honey harvesting and transport Available at: http://www.fao.org/3/a-au122e.pdf.
11. Council Directive 2001/110/EC of 20 December 2001 relating to honey (2001). *Off. J. Eur. Union*, **L10**, 47–52.
12. 2011/163/EU: Commission Decision of 16 March 2011 on the approval of plans submitted by third countries in accordance with Article 29 of Council Directive 96/23/EC (2011). *Off. J. Eur. Union*, **L70**, 40–46.
13. Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC (1996). *Off. J. Eur. Union*, **L125**, 10–32.
14. European Commission (2000). – Directive 2000/13/EC of the European Parliament and of the Council of 20 March 2000 on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs. *J Eur Comm*, , 29–42.
15. Nutrition C. for F.S. and A. (2018). – Labeling & Nutrition - Guidance for Industry: Proper Labeling of Honey and Honey Products. Available at: https://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/labelingnutrition/ucm389501.htm.
16. Import Alert 36-01 (2018). Available at: https://www.accessdata.fda.gov/cms_ia/importalert_108.html.
17. Codex Alimentarius Commission (1981). – Revised Codex Standard for Honey Codex Stan 12-1981, Rev. 1 (1987), Rev. 2 (2001). . pp 1–7
18. Thrasyvoulou A., Tananaki C., Goras G., Karazafiris E., Dimou M., Liolios V., Kanelis D. & Gounari S. (2018). – Legislation of honey criteria and standards. *J. Apic. Res.*, **57** (1), 88–96. doi:10.1080/00218839.2017.1411181.
19. ISO 12824:2016 - Royal jelly -- Specifications (2018). Available at: https://www.iso.org/standard/65648.html.
20. White, J.W. (1975). – Composition of Honey. . In *Honey: a comprehensive surveyCrane, E.*, Heinemann, London. pp 157–206
21. Crane E. (ed. . (1975). – *Honey. A comprehensive survey.* Heinemann, London.
22. The origin of methylglyoxal in New Zealand manuka (Leptospermum scoparium) honey - ScienceDirect Available at: https://www.sciencedirect.com/science/article/abs/pii/S0008621509001220.

23. The Ministry for Primary Industries (2018). – Manuka Honey Science Definition.

24. DOOR (2018). Available at: http://ec.europa.eu/agriculture/quality/door/list.html?locale=en&recordStart=0&filter.dossierNumber=&filter.comboName=miel&filterMin.milestone__mask=&filterMin.milestone=&filterMax.milestone__mask=&filterMax.milestone=&filter.country=&filter.category=PDOPGI_CLASS_14&filter.type=&filter.status=REGISTERED.

25. Pascual-Maté A., Osés S.M., Fernández-Muiño M.A. & Sancho M.T. (2018). – Methods of analysis of honey. *J. Apic. Res.*, **57** (1), 38–74. doi:10.1080/00218839.2017.1411178.

26. Bogdanov S. (2009). – *Harmonised Methods of the International Honey Comission*. Available at: http://ihc-platform.net/ihcmethods2009.pdf.

27. Ohe W. von der, Persano Oddo L., Lucia Piana M., Morlot M. & Martin P. (2004). – Harmonized methods of melissopalynology. *http://dx.doi.org/10.1051/apido:2004050*, **35**. doi:10.1051/apido:2004050.

28. Shapla U.M., Solayman M., Alam N., Khalil M.I. & Gan S.H. (2018). – 5-Hydroxymethylfurfural (HMF) levels in honey and other food products: effects on bees and human health. *Chem. Cent. J.*, **12**. doi:10.1186/s13065-018-0408-3.

29. Roßmann A., Lüllmann C. & Schmidt H.L. (1992). – Massenspektrometrische Kohlenstoff-und Wasserstoff-Isotopen-Verhältnismessung zur Authentizitätsprüfung bei Honigen. *Z. Für Lebensm.-Unters. Forsch.*, **195** (4), 307–311. doi:10.1007/BF01187904.

30. White J.W.J. & Doner L.W. (1978). – Mass spectrometric detection of high fructose corn sirup in honey by use of 13C/12C ratio: collaborative study. *J Assoc Anal Chem*.

31. White J.W. & Winters K. (1989). – Honey protein as internal standard for stable carbon isotope ratio detection of adulteration of honey. *J. - Assoc. Off. Anal. Chem.*, **72** (6), 907–911.

32. White J.W. (Honeydata C. (1992). – 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. AOAC Int. USA*.

33. ihcmethods2009.pdf Available at: http://www.ihc-platform.net/ihcmethods2009.pdf.

34. Swallow K.W. & Low N.H. (1990). – Analysis and quantitation of the carbohydrates in honey using high-performance liquid chromatography. *J. Agric. Food Chem.*, **38** (9), 1828–1832. doi:10.1021/jf00099a009.

35. Nozal M.J., Bernal J.L., Toribio L., Alamo M., Diego J.C. & Tapia J. (2005). – The use of carbohydrate profiles and chemometrics in the characterization of natural honeys of identical geographical origin. *J. Agric. Food Chem.*, **53** (8), 3095–3100. doi:10.1021/jf0489724.

36. Cordella C.B.Y., Militão J.S.L.T., Clément M.C. & Cabrol-Bass D. (2003). – Honey characterization and adulteration detection by pattern recognition applied on HPAEC-PAD profiles. 1. Honey floral species characterization. *J. Agric. Food Chem.*, **51** (11), 3234–3242. doi:10.1021/jf021100m.

37. Sergiel I., Pohl P. & Biesaga M. (2014). – Characterisation of honeys according to their content of phenolic compounds using high performance liquid chromatography/tandem mass spectrometry. *Food Chem.*, **145**, 404–408. doi:10.1016/j.foodchem.2013.08.068.

38. Cotte J.F., Casabianca H., Giroud B., Albert M., Lheritier J. & Grenier-Loustalot M.F. (2004). – Characterization of honey amino acid profiles using high-pressure liquid chromatography to control authenticity. *Anal. Bioanal. Chem.*, **378** (5), 1342–1350. doi:10.1007/s00216-003-2430-z.

39. Serra Bonvehi J. (1988). – Determination of methyl anthranilate in Citrus honey from eastern Spain and its effects on the diastase activity of honey. *Alimentaria*, **25** (197), 37–40.

40. Viñas P., Campillo N., Córdoba M.H. & Candela M.E. (1992). – Simultaneous liquid chromatographic analysis of 5-(hydroxymethyl)-2-furaldehyde and methyl anthranilate in honey. *Food Chem.*, **44** (1), 67–72. doi:10.1016/0308-8146(92)90260-9.

41. White J.W. (1966). – Methyl Anthranilate Content of Citrus Honey. *J. Food Sci.*, **31** (1), 102–104. doi:10.1111/j.1365-2621.1966.tb15421.x.

42. Tette P.A.S., Guidi L.R., Bastos E.M.A.F., Fernandes C. & Gloria M.B.A. (2017). – Synephrine – A potential biomarker for orange honey authenticity. *Food Chem.*, **229**, 527–533. doi:10.1016/j.foodchem.2017.02.108.

43. Elflein L. & Raezke K.P. (2008). – Improved detection of honey adulteration by measuring differences between13C/12C stable carbon isotope ratios of protein and sugar compounds with a combination of elemental analyzer - isotope ratio mass spectrometry and liquid chromatography - isotope ratio mass spectrometry (δ13C-EA/LC-IRMS). *Apidologie*, **39** (5), 574–587. doi:10.1051/apido:2008042.

44. Krummen M., Hilkert A.W., Juchelka D., Duhr A., Schlüter H.J. & Pesch R. (2004). – A new concept for isotope ratio monitoring liquid chromatography/mass spectrometry: New concept for isotope ratio monitoring LC/MS. *Rapid Commun. Mass Spectrom.*, **18** (19), 2260–2266. doi:10.1002/rcm.1620.

45. Cabañero A.I., Recio J.L. & Rupérez M. (2006). – Liquid Chromatography Coupled to Isotope Ratio Mass Spectrometry: A New Perspective on Honey Adulteration Detection. *J. Agric. Food Chem.*, **54** (26), 9719–9727. doi:10.1021/jf062067x.

46. Schellenberg A., Chmielus S., Schlicht C., Camin F., Perini M., Bontempo L., Heinrich K., Kelly S.D., Rossmann A., Thomas F., Jamin E. & Horacek M. (2010). – Multielement stable isotope ratios (H, C, N, S) of honey from different European regions. *Food Chem.*, **121** (3), 770–777. doi:10.1016/j.foodchem.2009.12.082.

47. Sobrino-Gregorio L., Vilanova S., Prohens J. & Escriche I. (2019). – Detection of honey adulteration by conventional and real-time PCR. *Food Control*, **95**, 57–62. doi:10.1016/j.foodcont.2018.07.037.

48. Utzeri V.J., Ribani A. & Fontanesi L. (2018). – Authentication of honey based on a DNA method to differentiate Apis mellifera subspecies: Application to Sicilian honey bee (A. m. siciliana) and Iberian honey bee (A. m. iberiensis) honeys. *Food Control*, **91**, 294–301. doi:10.1016/j.foodcont.2018.04.010.

49. Soares S., Grazina L., Costa J., Amaral J.S., Oliveira M.B.P.P. & Mafra I. (2018). – Botanical authentication of lavender (Lavandula spp.) honey by a novel DNA-barcoding approach coupled to high resolution melting analysis. *Food Control*, **86**, 367–373. doi:10.1016/j.foodcont.2017.11.046.

50. Soares S., Grazina L., Mafra I., Costa J., Pinto M.A., Duc H.P., Oliveira M.B.P.P. & Amaral J.S. (2018). – Novel diagnostic tools for Asian (Apis cerana) and European (Apis mellifera) honey authentication. *Food Res. Int. Ott. Ont*, **105**, 686–693. doi:10.1016/j.foodres.2017.11.081.

51. Pita-Calvo C. & Vázquez M. (2017). – Differences between honeydew and blossom honeys: A review. *Trends Food Sci. Technol.*, **59**, 79–87. doi:10.1016/j.tifs.2016.11.015.

52. Ferreiro-González M., Espada-Bellido E., Guillén-Cueto L., Palma M., Barroso C.G. & Barbero G.F. (2018). – Rapid quantification of honey adulteration by visible-near infrared spectroscopy combined with chemometrics. *Talanta*, **188**, 288–292. doi:10.1016/j.talanta.2018.05.095.

53. Spiteri M., Jamin E., Thomas F., Rebours A., Lees M., Rogers K.M. & Rutledge D.N. (2015). – Fast and global authenticity screening of honey using 1H-NMR profiling. *Food Chem.*, **189**, 60–66. doi:10.1016/j.foodchem.2014.11.099.

54. Spiteri M., Rogers K.M., Jamin E., Thomas F., Guyader S., Lees M. & Rutledge D.N. (2017). – Combination of 1H NMR and chemometrics to discriminate manuka honey from other floral honey types from Oceania. *Food Chem.*, **217**, 766–772. doi:10.1016/j.foodchem.2016.09.027.

55. Phipps R. (2018). – International Honey Market. *Am. Bee J.*, **January**, 23.

56. DG SANTE Health and Food Safety (2015). – Commission Recommendation C(2015) 1558 of 12.3.2015 on a coordinated control plan with a view to establishing the prevalence of fraudulent practices in the marketing of certain foods. , 1–4.

57. JRC-Geel (2018). – Technical Round Table on Honey Authentication - Meeting report. , 1–19.