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GAVIN PUBLISHERS

Food & Nutrition Journal

Review Article

Eshete Y. Food Nutr J 4: 201.
DOI: 10.29011/2575-7091.100101

Review on C-4 Sugar Content of Honey and Adulteration Impact on Commercial Honey

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Citation: Eshete Y (2019) Review on C-4 Sugar Content of Honey and Adulteration Impact on Commercial Honey. Food Nutr J 4: 201. DOI: 10.29011/2575-7091.100101

Received Date: 18 July, 2019; **Accepted Date:** 05 September, 2019; **Published Date:** 13 September, 2019

Abstract

Honey is subjected to fraud by adulteration with low price sugar syrups. As a natural product with a relatively high price, honey has been for a long time a target for adulteration. The adulteration and misrepresentation of honey are major threats to the viability of the apicultural industry. Recently, guaranteeing honey quality is becoming increasingly important for consumers, producers and regulatory authorities. The chromatography tests and others analytical procedures are not so sensitive enough to detect very low concentration of adulteration with c4 sugar plant source syrups. The composition of the adulterant product is very close to the component of pure honey. Carbohydrates and Saccharides in syrups derived from cane, corn or beet sugar are difficult to distinguish from those in pure honeys. Bees collect the nectar most from flowers of C3 plants cycle, and to a lesser extent from the flowers of C4 and CAM plants. The carbon isotope of C3 and C4 plants in photosynthetic cycles are different. The 13C/12C ratios of C3 and C4 plants fall in the range of -28~-23‰ and -15~-9‰, respectively. Isotope Ratio Mass Spectrometry (IRMS) is considered as one of the most powerful analytical techniques for detection of honey adulteration using low cost syrups that often exhibit sugar profiles very similar to authentic honey. Assessment of the quality and authenticity of honey is aiming to protect consumers, and to promote fair trade competition among producers. Traceability of this product is limited to the commitment of each producer, suppliers and processor's recording, documentation and quality control mechanisms. Addition of carbohydrate materials to honey is a type of fraud that should be carefully considered as it would be and difficult to detect as the added sugars are tailored to mimic as those naturally existing in honey. low levels of C4 sugar adulteration and especially addition of C3 sugars is very difficult to detect.

Keywords: C4 Sugar Content; Isotope; Isotope Ratio Mass Spectrometry (IRMS); Stable Carbon Isotopic Ratio Analysis (SCIRA)

Introduction

Honey is defined as the natural sweet substance produced by honeybees. Bees produce honey from the sugary secretions of plants (floral nectar) or from secretions of other insects (such as honeydew), by regurgitation, enzymatic activity, and water evaporation [1,2]. Genuine pure honey is classified as a natural product produced entirely by bees. Honey is subject to fraud by adulteration with low price sugar syrups. Saccharides in syrups derived from cane, corn or beet sugar are difficult to distinguish from those in pure honeys. In 1977 Doner & White [3] established a method for detection of adulteration of honey with syrups using Isotope Ratio Mass Spectrometry (IRMS) [4]. Recently, guaranteeing honey quality is becoming increasingly important for consumers, producers and regulatory authorities. There was an escalation in the practice of adulterating honey in world markets from the 1970s following the introduction of high fructose corn syrup [5]. Nectar, which honey bees collect to create honey, varies considerably in the types of sugars it contains - some nectars are mainly fructose, some mainly glucose, some mainly sucrose [2]. On this regard different types and forms of honey have different levels of sugar concentrating and composition [6,7]. The variety of honey produced by honey bees (the genus Apis) is the best-known, due to its worldwide commercial production and human consumption [8]. Honey gets its sweetness from the monosaccharide's fructose and glucose, and has about the same relative sweetness as sucrose (granulated sugar) [9]. Long shelf life of honey is attributed to an enzyme found in the stomach of bees. The bees mix glucose oxidase with expelled nectar they previously consumed, which then creates two by products: gluconic acid and hydrogen peroxide, partially responsible for honey's acidity and ability to suppress bacterial

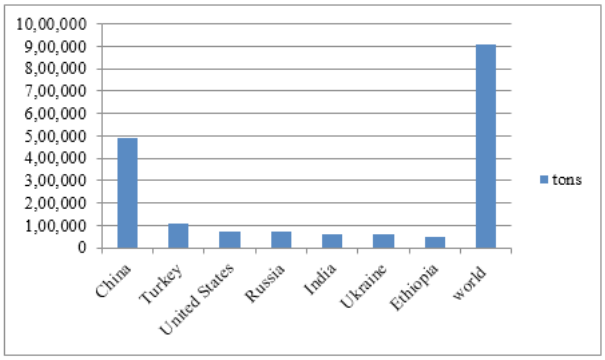
growth [10]. Honey has been associated with fraudulent practices for a long time [3]. Genuine pure honey is classified as a natural product produced entirely by bees. There was an escalation in the practice of adulterating honey in world markets from the 1970s following the introduction of high fructose corn syrup [11]. On the other hand, the authenticity of honey is more difficult to assess since several types of adulterations have already been described for this matrix.

Production

In 2016, global honey production was 1.8 million tons, led by China with 27% of the world total. Other major producers were Turkey, United States, Russia and India [12].

Country	Tons
China	490,839
Turkey	105,532
United States	73,428
Russia	69,764
India	61,335
Ukraine	59,294
Ethiopia	47,706
world	**907,898**

Table 1: Main honey producers in 2016.



Source: FAOSTAT 2017

Figure 1: Main Honey producing countries on 2016 by Graph.

The nectar and honeydew, respectively, are transformed into honey by the bee enzymes diastase (amylases) and invertase (glucosidase) during storage and maturation in the beehive. During this process, diastase and invertase catalyse the conversion of the sugars of nectar and honeydew into fructose and glucose, the main constituents of honey [13].

Classification

Generally, honey is classified by the floral source of the nectar from which it was made. Honey can be from specific types of flower nectars or can be blended after collection. The pollen in honey is traceable to floral source and therefore region of origin [14]. Honey is also classified according to the packaging, processing methods used, and regions of production and as well as color as Pfund scale [15].

Most commercially available honey is blended, meaning it is a mixture of two or more honeys differing in floral source, color, flavor, density, or geographic origin [16]. Generally, honey is bottled in its familiar liquid form, but it is sold in other forms, and can be subjected to a variety of processing methods. In the US, honey grading is performed voluntarily (USDA does offer inspection and grading "as on-line (in-plant) or lot inspection upon application, on a fee-for-service basis.") based upon USDA standards [17].

Quality Indicators and C4 sugar content

Bees collect the nectar most from flowers of C3 plants cycle, and to a lesser extent from the flowers of C4 and CAM plants, which have different 13C/12C ratio. The mean of 13C/12C value determined by SCIRA has been established at -23.5 δ‰, however, the analysis of the carbon isotope ratio yields different 13C/12C values for honeys obtained from different floral sources [18]. Good quality honey can be distinguished by fragrance, taste, and consistency. Accordingly, ripe and freshly collected honey at 20 ˚C (68 ˚F) should flow from a knife in a straight stream, without breaking into separate drops [19]. The compositions of honey can vary among different honeys types due to factors such as the botanical origin, geographic area, season, technology used for honey extraction, and storage conditions, are responsible for conferring specific/individual organoleptic and nutritional properties to honey [20].

If bees collect more nectar from C4 plants such as sugar cane exudates (after the plant is cut-out), or from CAM plants compared to C3 plants, the 13C/12C of honey determined by SCIRA will be higher than -23.5 δ‰. However, this does not mean that the honey is adulterated, as can be determined by measuring the 13C/12C of honey and it associated protein extract using ISCIRA [21]. Much of this questionable honey was officially banned beginning June 2010 by the 27 countries of the European Union and others [22].

Honey authenticity

Consumption of honey and honey products has grown significantly during the last few decades. On the other hand, at the present time, the traceability of this product is limited to the commitment of each producer, suppliers and processor's recording, documentation and quality control mechanisms [23]. In case of doubt or fraud, there is no standardized analysis available that

can discriminate or determine the botanical (floral or vegetable) and geographical (regional or territorial) origin of the honey. A laboratory in Bremen, Germany, founded a half century ago by German beekeepers, can accurately scan honey samples for flower pollen [22]. Now, a day there are recognized labs in Texas and Germany, melissopalynologists using pollen to determine with great accuracy the geographic area where the bees foraged for the nectar. The Chinese have refined methods of masking their contaminated product by ultra-filtration so their honey seems perfect. According to the Codex Alimentarius Commission Standards [24] and European Commission [25] the geographic origin of honey should be the same as the area declared on its label. The most recent AOAC 998.12 sets the upper acceptable limit for C-4 plant sugars in honey at ≤7% [4].

Stable carbon isotopic ratio analysis has gained increasing importance in the determination of the geographic origin of honey [26]. The 13C values of honey and protein are strongly influenced by climatic conditions [27] and agricultural practices [28]. In developed country the geographical origin of honey 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 [29].

Adulteration

Honey adulteration has evolved from the basic addition of sucrose and water to specially produced syrups which mimic the sugar composition of natural honey. The addition of fructose or industrial glucose could change the fructose /glucose ratio, which has to be 1 - 1.2 in pure honey. Indirect adulteration by feeding of sugar (syrups) during the main nectar flow period is the second way to adulterate honey. Indirect adulteration is extremely difficult to detect [30]. Over the last few years the practice of adulteration of honey with low-cost sugars has become commonplace in many countries [21]. However, the chromatography tests and others analytical procedures used are not so sensitive enough to detect very low concentration of adulterating sugars, so we are showing the sensibility of this methodology at low % of C4 sugars, added to the pure honey.

Honey has been associated with fraudulent practices for a long time [3] (White 2000). During the last several decades, documents have been developed aiming at proposing tools to assess the quality and authenticity of honey, protecting consumers, and promoting fair competition among producers. To improve the capability of authenticating honey, it is recommended to create a compositional database of authentic honeys and of substances which may be added to increase its volume or bulk ("extenders") or which are used as bee feeding products [13]. Because its especial flavor and attractive price, low-cost sugars from sugar cane syrup or corn syrup glucose are occasionally used to adulterated honey. It has become an international problem and many laboratories all over the world are trying to monitor this adulteration using different analytical techniques to determine the purity or the adulteration of honey. However, the chromatography tests and others analytical procedures used are not so sensitive enough to detect very low concentration of adulterating [21].

Based on the investigation of different studies and laboratory taste result, corn syrup and sugar cane, which are sourced from C-4 plants, both are a cheaper sugar source than honey, are added commonly to honey to increase product volume, which is then traded as a genuine pure honey [31]. By contrast, bees collect nectar and pollen for honey production primarily from the flowers of C-3 plants, and to a lesser extent from the flowers of C-4 plants. Sugar syrups produced by the C-4 metabolic pathway exhibit a 13C/12C ratio (expressed as δ13C) that differs from sugars derived from the C-3 metabolic pathway (-10% to -20% for C-4 plants, and -22% to -33% for C-3 plants) [32,33]. Addition of sugars from C4 plants (sugarcane, maize) can be reliably detected by the EA-IRMS method with a sensitivity of 7%.

Therefore, the difference between the carbon isotope ratio of the protein extract and of honey will provide an exact measure of any minimum level of adulteration. According to White and Winters, 1989, the difference accepts in 13C/12C, between honey and its associated protein extract is -1δ%, (one delta per mil, deviation), which provides the international benchmark of 7% sugar added [18]. In addition, honey with C-4 sugars <-7% can also be classified as adulterated. Although the analysis of δ13C in honey and calculation of the proportion of C-4 sugars is useful for detecting adulteration by the addition of syrups, false positive results may occur if honey is produced naturally from C-4 plants [34]. As well as the addition of sugar to honey, mislabelling of the geographic origin of honey is a growing worldwide problem [35-37] including in the honey market [38]. Sugars are the main components of honey, comprised of mainly glucose and fructose, but also several minor oligosaccharides. Thus, adulteration by the addition of carbohydrate materials is a type of fraud that should be carefully considered as it would be, by principle, difficult to detect if the added sugars are tailored to mimic those naturally existing in honey [39-41].

	Plant Origin	Examples	Range of δ13C values	Methods
1	C4-Plants	Corn Sugar Cane	-8 to -13 ‰	EA-IRMS (AOAC 998.12)
2	C3-Plants	Beet Rice Wheat Cichory	-22 to -30 ‰	**LC-IRMS(new)**

Table 2: Sugar Sources for honey Adulteration and methods of detection.

The addition of rice syrup has recently emerged as an increasing adulteration in the honey market due to its very difficult detection [42]. Being a C3 plant, the use of common SCIRA method of detection is not feasible to detect it in this syrup [43].

In recent years, there has been a major adulteration problem in the world, concerning mainly Chinese honey. Presently these sweeteners are mainly bee feeding syrups, produced by the hydrolysis of maize, cane and beet sugar [3].

	Percentage of corn syrups addition										
corbon 13 percentage in honey	0	10	20	30	40	50	60	70	80	90	100
	-10 -12 -14 -16 -18 -20 -22 -24 -28	corn syrups pure honey									

Table 3: addition of corn syrup to honey and 13 c composition change.

Sugar profile

Sugars are one of the biggest and most common families of molecules in the life lining things. They serve as energy resources, the backbone of DNA and RNA, the carrier of biologically active compounds and as structure building material of plants. Sugars can carry information of their origin and processing. If isotopically labeled they tell us about their pathways and metabolism. Honey contain a mixture of sugars and other carbohydrates, honey is mainly fructose (about 38%) and glucose (about 32%), [44], with remaining sugars including maltose, sucrose, and other complex carbohydrates [2]. Its glycemic index ranges from 31 to 78, depending on the variety [45]. Accordingly, specific composition, color, aroma, and flavor of any batch of honey depend on the flowers foraged by bees that produced the honey [46].

According to [47] mixed floral honey from several United States regions typically contains:

- Fructose: 38.2%
- Glucose: 31.3%
- Maltose: 7.1%
- Sucrose: 1.3%
- Water: 17.2%
- Higher sugars: 1.5%
- Ash: 0.2%
- Other/undetermined: 3.2%

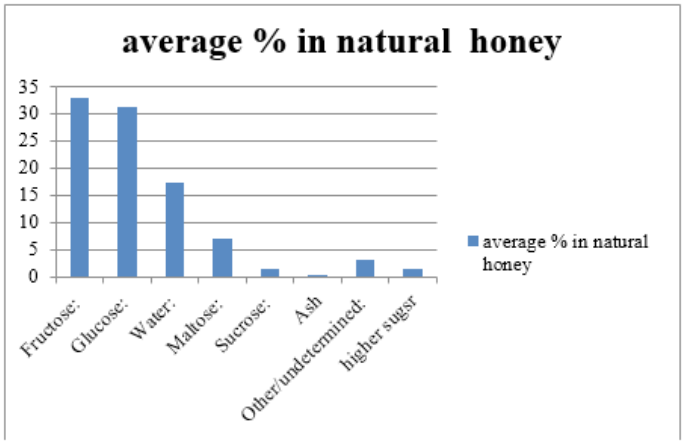


Figure 2: Major Compositions of Natural Honey.

Honey Adulteration Detection Methods

At present, a variety of analytical techniques have been developed to detect adulteration of honey, such as isotopic (stable isotope methodology), chromatographic, spectroscopic, trace elements techniques and thermal analysis. Some of these methods are time-consuming, and some are expensive. Although there are powerful methods to prove honey adulteration, they have to be further improved in order to ensure honey quality [48]. Regarding to honey adulteration becoming an international concern different chromatographic techniques have been developed for the detection of adulterated honey including thin-layer chromatography and gas chromatography-mass spectrometry [49,50]. Besides, high-

performance liquid chromatography with electrochemical and evaporative light scattering detection have been developed [51,52].

Isotope Ratio Mass Spectrometry (IRMS) is considered as one of the most powerful analytical techniques for detection of honey adulteration using low cost syrups that often exhibit sugar profiles very similar to authentic honey. Isotopic ratios are measured relative to a working reference gas calibrated using internationally accepted standards and they are reported using the delta notation (d) and expressed in units per mill (%). In the case of carbon stable isotope analysis, the delta notation is defined as:- 13C (%) = [R (Sample) / R (Standard) - 1] x 1000. C3 plants exhibit d13C values ranging from -23 to -28‰, whereas C4 plants have isotopic ratios ranging from -9 to -15% [29].

In 2008 Italian study determined nuclear magnetic resonance spectroscopy can be used to distinguish between different honey types, and can be used to pinpoint the area where it was produced [2]. For the detection of honey adulteration with C4 sugars the limits of reliable detection of adulteration with this method is 7% of C4 sugar addition. Another technique used is the pattern recognition of sugars by HPLC. But low levels of C4 adulteration and especially addition of C3 sugars is very difficult to detect by bulk analysis.

Due to the development of new and more sophisticated fraud practices and the limitation of officially accepted analytical techniques can make difficult to detect honey adulteration. The use of EA-IRMS (Elemental Analysis - Isotope Ratio Mass Spectrometry) is the only official detection method for addition of C-4 sugar in honey [43]. Honeys that are tested with a $\delta^{13}C$ value of -23.5 and lower are deemed to be pure. Honeys that have a $\delta^{13}C$ value between -23.5 and -21.5, fall into a grey area. Honeys that have a $\delta^{13}C$ of -21.5 or higher are deemed to be adulterated.

Guide to C4 screen results and how they compare to the AOAC 998.12 method	
C4 Screen Result	**Interpretation**
0 - 5.5%	Very likely to pass AOAC 998.12
5.6 - 7.0%	Some risk of failing AOAC method
7.1 - 8.5%	High risk of failing AOAC method
Over 8.6%	Sample will almost certainly fail AOAC method

Table 4: Honey C4 Sugar analysis result and Interpretation.

The adulteration of honey with invert sugar or syrup may not readily be detected by direct sugar analysis because its constituents are the major natural components of honey and the adulterated product would also have similar physical properties to natural honey. Both researchers and Regulatory Authorities are still searching for newer, simpler, and more sensitive and economical procedures [1,53-55]. Based on numerous studies Consumer demands for authentic and natural products with beneficial health properties have positioned honey as an important food commodity [56]. On the other hand, some genuine unadulterated honey may sometimes fail the test depending on which flowers the bees have visited. On the contrary other C3 plants - like rice - can also be used for sugar production [42]. So, honey adulterated with rice syrup won't be detected by the C4 test as "adulterated". Most researchers and food fraud experts point out that honey is one of the most commonly mislabelled foods around the world.

Conclusion

If bees collect more nectar from C4 plants such as sugar cane exudates (after the plant is cut-out), or from CAM plants compared to C3 plants, the 13C/12C of honey determined by SCIRA will be higher than -23.5 δ%, but this does not mean that the honey is adulterated, as can be determined by measuring the 13C/12C of honey and it associated protein extract, by ISCIRA, (White, 1992).

low levels of C4 adulteration and especially addition of C3 sugars is very difficult to detect. Honeys that are tested with a $\delta^{13}C$ value of -23.5 and lower are deemed to be pure. Honeys that have a $\delta^{13}C$ value between -23.5 and -21.5, fall into a grey area. Honeys that have a $\delta^{13}C$ of -21.5 or higher are deemed to be adulterated. Although the analysis of δ13C in honey and calculation of the proportion of C-4 sugars is useful for detecting adulteration by the addition of syrups, false positive results may occur if honey is produced naturally from C-4 plants. Most bees produce honey from plant nectar derived from the Calvin (C3) photosynthetic cycle and these honeys have a relatively uniform $\delta^{13}C$ value of - 25 (using the PDB scale). Sucrose produced from cane sugar etc., is derived from the Hatch-Slack (C4) cycle and is heavier in $\delta^{13}C$ with values ranging from -10 to -16. The adulteration of honey with invert sugar or syrup may not readily be detected by direct sugar analysis because its constituents are the major natural components of honey and the adulterated product would also have similar physical properties to natural honey. Both researchers and Regulatory Authorities are still searching for newer, simpler, and more sensitive and economical procedures.

Regarding adulterations with sugar addition to increase honey production or overfeed the bees, SCIRA has been used as an official analytical method in many countries based on the fact that monocotyledonous plants (C4), such as sugar cane or corn, and dicotyledonous (C3), the main plants used as nectar sources, have distinct carbon isotopic ratios from different photosynthetic cycles. However, the addition of C3 plant sugars, such as sugar beet or rice syrups is not feasible to detect based on such analysis.

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