Current knowledge of honey adulteration - proof methods

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Abstract

The main aim of the review was to evaluate and emphasize the main current knowledge about honey adulteration and to list possible proof methods. The importance of this issue is best explained by the fact that honey consumption is increasing constantly, same as honey price. This situation on the market is making a template conditions for adulteration due to very possible profit that can be made by non-honest producers or seller. Industrial sugar syrup is used the most often due to its low price. On the other side, there is a constant pressure from the side of consumers for the invention of reliable methods that could be used for the detection of honey adulteration. The review is giving overview of the methods with valuable explanations about methods reliability, price, operationality and time usage.

Consumption, price, sugar syrup, time usage

Introduction

According to Directive 2001/110/CE "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".

Honey is the most complexed mix of sugars that can be found in nature. There have been identified and quantified 16 disaccharides and 12 trisaccharides in honey. It is considered a valuable sweetener due to its high nutritional value and present antioxidants, antibacterial and antimutagenic substances (Sforza, 2013).

Regulations for honey quality and tests for honey adulteration vary from country to country. But honey should not be present on the market if it is adulterated by sugar syrups or water, according to Codex Alimentarius complied by the United Nations Food and Agriculture Organization (Stanway, 2014).

Consumers in Europe are very interested to get more information about food quality as well as for honey quality and especially to be better informed about honey adulteration issues. Probably because some studies showed that it is possible to find on the market honey that consists of more than 80% of corn syrup and at the same time it is labeled as "pure honey" (Alda-Garcilope, et al., 2012, Stanway, 2014).

Honey adulteration

One of the most often falsified or adulterated food product in the world is honey, and it is usually done by sugar adding. Glucose and fructose are the main constituents in honey and they can be provided from cheaper sources. Honey composition makes this product to be easy adulterated. Other reason why honey is very often adulterated is the fact that it is very hard to detect adulteration and at the same time new techniques of honey adulteration have been constantly developing (Cinar et al., 2014).

The most used sugar syrups for honey adulteration are: corn syrups (CS), high fructose corn syrups (HFCS), inverted syrups (IS), sucrose syrups (SS) and high fructose inulin

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syrups (HFIS). These syrups are giving different adulteration markers in adulterated honey, and it is necessary to use specific methodology to detect them. Main adulterant, adulteration marker and used methodology concerning honey adulteration are given in Table 1. (Sforza, 2013).

Table1. Main adulterant, adulteration marker and used methodology concerning honey adulteration

Sample	Adulterant	Adulteration Marker	Methodology
Floral honeys	C3 and C4 sugar syrups	Different sugar concentrations and their relationships	• GC-FID • Column: HP5-MS
			• HPAEC-PAD • Column: CarboPac PA 1
Rosemary honeys	Glucose-fructose-maltose syrups and sucrose syrup (SS)	Carbohydrate profile (13 major sugars)	HPAEC-PAD Column: CarboPac PA 100
Floral honeys	Corn syrup and HFCS with 20%, 40% and 80% of isomerization	Malto-oligosaccharides	• Removal of mono- and disaccharides: activated charcoal • HPAEC-PAD • Column: CarboPac PA 100
Floral honeys	Corn syrup	Malto-oligosaccharides	• Removal of mono- and disaccharides: reversed phase solid phase extraction • HPAEC-PAD • Column: CarboPac PA 100
Nectar and honey dew honeys	(HFCS) and inverted High fructose corn syrup syrup (IS)	(DFAs) Difructose anhydrides	• Enrichment step: yeast (S. cerevisiae) treatment • GC and GC-MS analysis • Column: SPB-1
Floral honeys	High frustose inulin syrups (HFIS)	Inulotriose and DFAs	• GC and GC-MS analysis • Column: SPB-1
Floral honeys HFCS and SS honeys (via bee-feeding)	HFCS	Fructosyl fructoses	• GC and GC-MS analysis • Column: SPB-1
	SS	Sucrose	

Note: from: SFORZA, S. (2013) Food Authentication Using Bioorganic Molecules. *Lancaster, Pennsylvania: DEStech Publications*.

Generally, there are two ways how sugar syrups can be introduced to honey, one method is by the feeding of bees with industrial sugar syrups or sugar syrups can be added to honey when it is already produced. The adding of sucrose is one of the most spread methods for honey adulteration. It is allowed to feed bees with industrials sugar syrups but the question is how this feeding influences the quality of honey especially when all instructions for bee feeding are not followed. Other method is the adding of fructose or glucose to pure honey and changing its fructose/glucose ratio. Fructose/glucose ratio should be 1:1,2 and honey is considered adulterated if this ration is compromised (Puscas et al., 2013, Cordella et al., 2005).

Some types of honey are more often adulterated than others. Honey produced from acacia plants usually is not so sweet and producers are trying to improve sensory characteristics of this type of honey by adding glucose (Puscas et al., 2013).

Methods for the detection of honey adulteration

Many methods have been developed for the evaluation of adulterated honey. The majority of them include: chromatographic, thermal analysis nuclear magnetic resonance and statistical correlation among sugar composition and following structural and compositional properties: moisture, total soluble solids, nitrogen, apparent viscosity, hydroxymethylfurfural (HMF), ash, sodium, calcium, potassium, proline, refractive index and diastatic activity (Shuifang et al., 2012).

Composition of natural honey is the starting point for the detection of possible adulteration. Sucrose content has been widely used as one of the indicators for honey adulteration, but it is not an appropriate test because worker bees are converting by their digestive enzymes saccharose to glucose and fructose. The ratio between fructose and glucose can also be an indicator for honey adulteration. Maltose is present in honey but in very low quantities, around 30 mg/g. Higher content of maltose in honey can be a sign that sugar syrups or starch hydrolysate were added. The maltose/isomaltose ratio also suggests the adulteration of honey in terms that higher maltose/isomaltose ratio indicates the presence of starch hydrolysate and lower maltose/isomaltose ratio indicates that fructose syrups were added. Other carbohydrates have been also used for the detection of adulterated honey, such as: inulin, inulobiose and inulotriose. Studies showed that inulin is not present in natural honey (Preedy, 2012).

Diastase activity

Natural honey contains a few enzymes which are produced by bees and some of them are present in nectar or pollen. Amylases, invertases, glusidases, catalases and fosfatases are one of the most important enzymes that occur in natural honey. According to Directive 2001/110/CE the activity of diastase (α-, β-, γ-amylase) is very significant parameter of honey quality. Diastase activity should not be less than 8 and for some honey types (citrus honeys) not less than 3. Its activity is used as a marker to determine honey freshness or to evaluate damage of honey caused by heat. Diastase activity can also be a sign for honey adulteration because if diastase activity is low it is possible that honey was adulterated with sugar syrups as they dilute diastase. Many times low level of diastase is hidden by artificially added diastase. (Voldrich et al., 2009, Stanway, 2014).

Hydroxymethylfurfural (HMF)

The content limit of hydroxymethylfurfural (HMF) are also given in Directive 2001/110/CE and it should not exceed 40 mg/kg with the exception for honeys originating from tropical regions in which HMF must not be over 80 mg/kg. HMF is a product of sugar degradation and important factor for the determination of storage period and influence of heat on honey. HMF has been used a lot as indicator for the detection of adulterated honey in the case when sucrose had been added, but HMF level can also increase during exposure of honey for longer period to higher temperatures, after inadequate storage, pH changes and many other factors can influence HMF content in honey (Boffo, et al., 2012).

Detection of microscopic figurative elements

One of the methods used for honey adulteration detection is the evaluation of microscopic figurative elements (epidermal cells, sclerous rings). This method shows unequivocally if the adding of cane sugar syrups had occurred, but its results are not reliable if industrial manufactured micro-filtrated syrups are used (Cordella et al., 2005).

Carbon isotopic ratio analysis

The presence of syrups derived from C4 plants is determined by carbon isotopic ratio analysis (SICRA). The method is based on the difference between $^{12}C/^{13}C$ ratio of C4 plants (monocotyledonous species) and C3 plants (dicotyledons species). Natural honey should have characteristics of C3 plants because bees are gathering nectar from those plants. SICRA is restricted on the evaluation of C4 plants sugar plants and it is not suitable for the detection of sugar syrups obtained from C3 plants (beet, corn, wheat and isoglucose syrups). Other limitation of this method is the fact that it requires the useage of expensive instrumentation (Cordella et al., 2005, Simsek et al., 2012, Ruiz-Matute et al., 2007). Added saccharose, from beet sugar syrup, is easily inverted by acids and enzymes present in pure honey. The consequence is that the level of saccharose is under 5%, and that is considered as standard value for pure honey. That's why carbon isotopic ratio analysis is not efficient method for the determination of adulterated honey by C3 plants sugar syrups, but it is very reliable for the detection of corn and cane sugar syrups which belong to C4 plants. A good feature of this method is that it can measure the percentage of honey adulteration. $\delta^{13}C$ (%) is calculated by equation (Tosun, 2013, Da-Wen, 2008).

$\delta^{13}C$ (%) = $[(^{13}C/^{12}C_{sample}) / (^{13}C/^{12}C_{standard}) – 1] \times 10^{-3}$
Adulteration, % = $[(\delta^{13}C_{protein} - \delta^{13}C_{honey}) / (\delta^{13}C_{protein} - \delta^{13}C_{sugar})] \times 100$ (C4 sugar ratio)

Good indication for honey adulteration is if honey has $\delta^{13}C$ (%) more negative than -23,5%. Likewise, the difference between stable carbon isotope ratios of honey and its fraction of proteins should not be more than 1%. The usage of isolated honey proteins as an internal standard improved the method in the way that lowered the limit of C4 sugar detection from 20% to 7% (Simsek et al., 2012, Elflein and Raezke, 2008).

High performance anion-exchange chromatography-pulsed amperometric detection (HPAEC-PAD)

High performance anion-exchange chromatography-pulsed amperometric detection (HPAEC-PAD) method is one of the most used methods for the evaluation of adulterated honey. HPAEC-PAD method provides the possibility to analyze malto-oligosaccharides profile of honey and it detects the adding of corn syrups and high fructose corn syrups. The method is performed fast and it is less expensive than other methods (Morales et al., 2008).

Difructose anhydrides (DFAs)

The determination of difructose anhydrides using gas chromatography can represent an easy inexpensive method for the evaluation of honey quality. The method was proven effective in the case of adding 10% of glucose but not when 5% of glucose was added. Likewise, it is easy to detect 5% addition of fructose and sucrose with this method. It is suggested that if DFAs are used as markers for honey adulteration, concentration of DFAs in samples should be concentrated with yeast treatment (Montilla et al., 2006, Ruiz-Matute et al., 2007).

Proline and erlose content in honey

Due to fact that sucrose level in honey is decreasing during storage other parameters should be used as indicator for honey adulteration. Proline content is a good indicator because its content is stable even after 20 years of storage. Erlose can be found in honey even when really low percentage of bee-inverted sucrose is present, but erlose is also found in nectar with naturally higher sucrose concentrations (Piotr, 2004).

Site-specific natural isotope fractionation-nuclear magnetic resonance (SNIF-NMR)

In 1980's site-specific natural isotope fractionation-nuclear magnetic resonance (SNIF-NMR) was introduced. SNIF-NMR method is maybe the most sophisticated method for revealing adulterated honey. It is based on the determination of deuterium/hydrogen (D/H) ratio at precise sites in a molecule. When D/H ratio is below specified level the sample of honey can be considered as adulterated. The problem with SNIF-NMR method is that it requires a large number of data to be efficient (Cordella et al., 2005).

High-performance thin-layer chromatographic method (HPTLC)

High-performance thin-layer chromatographic method (HPTLC) method is used for the determination of fructose, glucose and sucrose content in honey. The method gives the information about fructose/glucose ratio which is a good indicator for adulterated honey. The method is considered to be faster and cheaper than many other used methods (Puscas et al., 2013).

Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectroscopy is also used for the detection of adulterated honey, because by this method is possible to detect inverted beet sugar. Beet sugar belongs to C3 plants and many times it is very difficult to estimate the presence of C3 sugars in honey (Bertelli et al., 2007).

Raman spectroscopy

Raman spectroscopy combined with partial least squares-linear discriminant analysis (PLS-LDA) represents a good method for the detection of maltose syrup but it is not so efficient for the detection of high fructose corn syrup. It is easy to perform this method, it is efficient method and it doesn't require special preparation of sample. These characteristics make the method suitable also for the evaluation of honey sample on site/in field (Shuifang et al., 2012).

Conclusion

The consumption of honey has been increasing constantly due to its beneficial properties for human health. The price of honey is also increasing constantly and at the same time there are many possibilities for honey producers to enlarge their profit by industrial sugar syrup addition to pure honey. Industrial sugar syrups are very cheap and by adding them to pure honey producers are selling cheap product for high price. That is probably the main reason why honey is the most often adulterated food in the world. There is a big demand from consumers for the invention of method which would clearly distinguish adulterated honey from pure honey. Right now, adulterated honey can be detected with the usage of two or more methods. Future work has to be focused on the finding of unique method for honey adulteration which would covered the identification of sugar syrups, both from C3 and C4 plants, and at the same time it has to be reliable and not time consuming.

Good advice for consumers who want to be sure that they are consuming pure honey without added adulterants is that they should buy honey from trustworthy and reliable producer which cannot permit the reputation ruin of his brand by any kind of adulteration.

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