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A Review on Honey Adulteration and the Available Detection Approaches

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Abstract: Over the last few decades, the utilization of stingless bees honey product grew significantly due to its natural therapeutic value in honey. These make the demand for honey rises and tends to have a lot of honey product available in the commercial market. However, the purity of honey can be sceptical whenever it turns up to be added or removed with different substances. Recently, adulteration of honey becomes a complex issue because the highly sophisticated adulteration procedures are continually being evolved and the legislative determination of honey quality indicator is incapable to identify most honey adulteration procedures. Therefore, a comprehensive overview of the method of adulterated honey, including direct and indirect adulteration are discussed in this paper. In addition to this, the detection methods approached by most researchers are briefly explained. All these methods contribute to the knowledge about each aspect of honey authenticity and give beneficial information in oncoming works.

Keywords: Adulteration, authenticity, Stingless bee honey, detection method

1. Introduction

Stingless bee honey (SBH) also known as Kelulut honey is a natural sweetener that has millions of therapeutic effect for the human (Abd Jalil, Kasmuri, & Hadi, 2017; Biluca, Braghini, Gonzaga, Costa, & Fett, 2016; Miorin, Levy, Custodio, Bretz, & Marcucci, 2003; Nishio et al., 2016; Nweze, Okafor, Nweze, & Nweze, 2017; Souza et al., 2006; Yaacob, Rajab, Shahar, & Sharif, 2017). The authenticity of honey is defined by the *Codex* standard (Codex Alimentarius Commission, 2001), where it states that the commercial honey is a pure product that does not allow to have any addition of other ingredients nor shall any particular constituent be removed from it. Considering the therapeutic value and high economic, lacking of international standard and limited production of SBH, certain profiteering and unethical individuals have adulterated the pure honey with cheap and easily available sweeteners and selling them at the highest prices in order to gain maximum income. Consequently, it will reduce the nutritional value of honey and thus cause health problem to the human body (Guo, Zhu, Liu, & Zhuang, 2010). Therefore, the honey quality assessment is required in order to detect the adulteration of honey. The purpose of this paper is to summarize information on the adulteration of honey and the technology used for detection.

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Honey is a high-value commercial food product targeted for adulteration. Adulteration of honey can be divided into two categories; direct and indirect, as shown in Fig. 1. Direct adulteration refers to a substance that is directly added to the honey. While indirect adulteration occurs when the honeybees are fed an adulterating substance. The sources of substance used for adulteration are classified as C3 or C4 plants which are based on their carbon metabolism (Zábrodská & Vorlová, 2014). The examples of C3 plants are beet, wheat and rice whereas sugarcane and maize are from C4 plants. Indirect adulteration process, such as the overuse of veterinary drugs, harvesting prior to maturity and overfeeding the honeybees with sucrose or other sugars are performed by irresponsible beekeepers due to the demand of a competitive market (Bogdanov, Ruoff, & Persano-Oddo, 2004; Sahinler, Sahinler, & Gul, 2004). For direct honey adulteration, it can be classified into four main issues; addition of sugar, water content, processing and origin.

2. Honey Adulteration

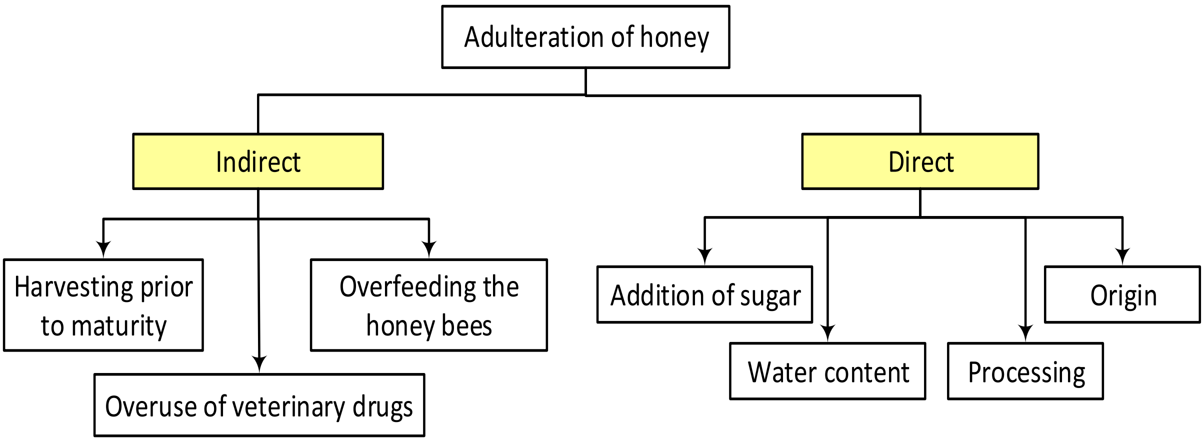

Fig. 1 – Categories of honey adulteration

2.1 Addition of Sugar

Addition of sugar is the main authenticity issue (Bogdanov & Gallman, 2007). Honey is adulterated with cheaper and easily available sweeteners, e.g. glucose syrup, cane sugar and corn syrup, where the percentage of adulteration is varied from 2% until 27% (Sivakesava & Irudayaraj, 2001) in order to increase the honey sweetness as well as to cater higher market demand. However, the honey adulterated by sugar addition can change the biochemical and chemical properties, e.g. electrical conductivity, enzymatic activity and other specific compounds i.e. glucose, sucrose, fructose and hydroxymethyl furfural (HMF) (Soares, Amaral, Oliveira, & Mafra, 2017).

2.2 Water Content in Honey

In addition to this, water is the second most significant component in honey. The content of water in honey is critical since it affects the shelf life of honey. The water content in honey depends on the storage, harvesting and climatic condition in which can spoil the physical properties of honey such as crystallization and viscosity. The higher water content in SBH compared to honey of sting bees (*Apis mellifera*) (Maria et al., 2016) has facilitated the proliferation of yeast that can cause a fermentation process. Fermentation occurs when the honey is harvested prematurely, hence affect the quality of honey due to the higher water content (Zábrodská & Vorlová, 2014). This makes the honey product unsuitable for human consumption and inhibits its marketing.

2.3 Processing

According to the Codex standard [8], the water content in honey should be less than 20%. However, the honey from the high relative humidity in the geographical origin usually contained more than 20% of water content. Therefore, the dehumidification or vacuum-evaporation process (Ramli et al., 2017) is done by the suppliers to remove an excess of water content until the Codex's requirement is achieved.

Besides that, certain suppliers have applied some processing techniques including thermal treatment, refrigeration and filtration in order to lengthen the honey's shelf life (Blidi, Gotsiou, Loupassaki, Grigorakis, & Calokerinos, 2017; Turhan, Tetik, Karhan, Gurel, & Tavukcuoglu, 2008). Thermal treatment of honey is applied to prevent or postpone the honey crystallization process. Meanwhile, the filtration is a process of removing some foreign matters that might appear in the honey. Since the consumers have related a natural process of crystallization honey to be adulterated honey, some suppliers apply high temperature or uncontrolled heating to remove air bubbles and liquefy the glucose microcrystals. The process of altering the composition of honey may affect the quality and its biological value, at the same time reduce the amount of vitamins and enzymes in the honey.

2.4 Botanical Origin

The specific characteristic of honey and its quality can be determined by the specific botanical origin in the area from which the honeybees collected the nectar. Usually, honey can be classified as a mono-floral and multi-floral honey. Mono-floral honey is the most demanded owing to their biological properties and particular taste (Maria et al., 2016). In addition to this, mono-floral honey is composed at least 45% of pollen grains from a single species, compared to multi-floral honey that produced pollen grains from several plant species but none of which being considered predominant (Soares et al., 2017). Because of this reason, botanical source of honey is greatly related to its price, thus the suppliers possibly will be interested to describe different nectar sources to increase their profits. The mono-floral honey which has high economic value is subjected to be adulteration through incorrect labeling and deceitful mixing with the honey of lower quality and value.

3. Detection Method

In order to check the authenticity of honey, numerous analytical methods have been developed to identify and detect the adulterants present in the honey, as shown in **Error! Reference source not found.**. The quality of honey can be identified from their physical, sensorial, chemical, and microbiological characteristics (Pilizota & Nedic Tiban, 2009).

Table 1 – Types of detection methods

Honey Adulteration	Detection Method
Addition of sugar	Isotope analysis, chromatography, spectroscopy, 1D and 2D NMR
Water content	RI determination, drying method, KFT
Processing	HPLC method, MECK
Origin	Melissopalynology, physico-chemical analysis, DNA analysis, chromatography, spectroscopy

Abbreviation; NMR: Nuclear Magnetic Resonance, D: Dimensional, RI: refractive index, KFT: Karl Fischer Titration, HPLC: high-performance liquid chromatography, MECK: Micellar Electro Kinetic Capillary Chromatography, DNA: deoxyribonucleic acid.

Stable carbon isotope ratio analysis (SCIRA) has been suggested for a long decade (González Martín, Marqués Macías, Sánchez Sánchez, & González Rivera, 1998) to detect the honey adulteration with sugar and evaluate indirect adulteration (Padovan, Jong, & Rodrigues, 2003). It is determined by the $^{13}C/^{12}C$ isotope ratio, which is different in C4 and C3 plants. Nevertheless, this method is not suitable for the addition of C3 plant sugar due to the similarities in isotopic composition. Hence, an analytical chromatographic approaches have been employed to analyze sugar in honey, i.e. gas chromatography (GC), HPLC, liquid chromatography (LC), high performance anion exchange chromatography with Pulsed Amperometric Detection (HPAEC-PAD) and gas chromatography coupled with mass spectrometry (GC-MS) (Cordella, Militao, Clement, Drajnudel, & Cabrol-Bass, 2005; Ruiz-Matute, Weiss, Sammataro, Finely, & Sanz, 2010; Rybak-Chmielewska, 2007; Wheeler & Robinson, 2014). The HPAEC-PAD and GC-MS also have been used to detect bee-feeding adulterations as well as for the characterization of the honey botanical and geographical origin. Besides that, the HPLC method was proposed to determine HMF content for the evaluation of honey freshness and the overheating process. Other than the HPLC method, MECK also was suggested as an alternative method for HMF determination due to its short time analysis and low cost (Rizelio et al., 2012).

In the meantime, a comprehensive survey in honey analysis using spectroscopy techniques with chemometric was presented by authors (Noviyanto, Abdulla, Yu, & Salcic, 2015) through observation of 61 peer-reviewed journals. Spectroscopy is a study associated with the interaction of electromagnetic radiation and matter. The responses from this interaction provide information on the total chemical composition of a sample. According to several authors, the development of spectroscopic techniques based on NMR, Raman and infrared (IR) are considered as useful methods to quantify the sugar profile (Rajalakshmi, Gopal, Kumar, & Dinesh Kumar, 2017) and determine the geographical origin (Lekova & Tsankova, 2017). The Fourier transform infrared (FTIR) spectroscopy in combination with multivariate statistical techniques such as attenuated total reflectance (ATR) (Sivakesava & Irudayaraj, 2001), principle component analysis (PCA) and partial least square (PLS) techniques (Kumaravelu & Gopal, 2015) are possible to discriminate between the pure and adulterated honey simultaneously in a reliable, direct and rapid way.

The chromatographic and spectroscopic are new analytical methodologies for botanical and geographical origin distinction due to the difficulties associated with the traditional melissopalynology method. Melissopalynology is the traditional microscopic analysis in identifying the pollen grains contained in the honey. It has been extensively used (Ponnuchamy et al., 2014) to determine the botanical and geographical origins of honey. However, the effectiveness of this technique depends on the method of extracting the pollen from honey and the skills of palynologist in interpreting the results (V.M. Bryant, 2018). Besides that, melissopalynology is not applicable to the cases of adulteration by pollen addition or insufficient honey filtration by beekeepers.

On top of that, the chemical composition such as phenolic acids, volatiles, sugars and other constituents of honey can be influenced by the environmental conditions and climatic changes as well as the beekeeping techniques (Madesis, Ganopoulos, Sakaridis, Argiriou, & Athanasios, 2013). Moreover, honey has a very complex matrix in terms of the pollen origin since the bees are visiting different plant species to produce honey. Hence, the use of physico-chemical analysis and DNA analysis for pollen identification (Guertler, Eicheldinger, Muschler, Goerlich, & Busch, 2014) were approached in determining the origin of honey.

A DNA analysis has been developed (Manivanan, Rajagopalan, & Subbarayalu, 2018) as an alternative tool in molecular biology for pollen identification. In the context of plants, the selective DNA strands are barcoded and the screening process is done to distinguish the pollen types in the honey bee. Meanwhile, physico-chemical properties of honey essentially depend on the climatic and environmental conditions, the botanical source of the nectar and the species of bees that produce the honey. Determination of the physico-chemical analysis of stingless bee honey has discussed in (Nascimento et al., 2015) and (Ij, Ab, Salwani, & Lavaniya, 2017). Physico-chemical is beneficial not only for the honey characterization, but it also acts as an indicator to ensure the product quality on the market. The types of the indicator are including the pH, colour intensity, electrical conductivity, HMF, moisture content, ash content and reducing sugar.

The identification of water content in honey is very important to establish the marketability and the quality of honey. Generally, the RI measurement using a relative conversion table or an empirical formula (Soares et al., 2017) is applied for the water content determination. The RI measurement is carried out with an Abbe-type refractometer or hand-held refractometer as shown in Fig. 2. However, it requires heat pre-treatment in creamy (crystallized) honey before the analysis using a refractometer approach is executed. This may result in a loss of water and the empirical formula or conversion table is therefore incorrect for different types of honey (Gallina, Stocco, & Mutinelli, 2010). For that reason, an alternative method known as KFT has been suggested (Isengard, Schultheiß, Radović, & Anklam, 2001).

Fig. 2 – Types of refractometer

KFT is a technique based on the reaction between the water molecule and the chemical substance. The authors (Gallina et al., 2010) have made a comparison between the KFT method with the RI determination for more than 100 samples of honey. The results have proved that KFT was the most reliable method compare to RI technique. In addition to this, the authors (Isengard et al., 2001) have compared the different methods used in determining the water content in honey i.e. RI determination, KFT and drying method. The drying method is the heat-controlled process in which the volatile compounds in the sample is evaporated. The mass loss in the sample is then calculated so that the percentage of moisture contained in the sample can be achieved. However, other volatile compounds presented in the honey also may evaporate together with the water, unless the drying parameters are well-chosen.

Besides that, the dielectric properties or permittivity of honey also have been suggested as a potential tool to determine the water content in honey (Guo et al., 2010; Yang, Gao, Liu, Fan, & Zhao, 2018). The relative permittivity which is influenced by pH and temperature can be useful for the detection of honey microbial contamination, while the effect of HMF content in dielectric loss coefficients is potentially to be an indication of honey freshness.

Recently, a lot of optical sensors have been demonstrated and published for honey adulteration measurement (Noviyanto et al., 2015). Fiber optic sensing is the most successful applications of both fiber optics and sensing technology. For example, the authors in (Hida, Bidin, Abdullah, & Yasin, 2013) has been demonstrated the honey purity detection in distilled water by using intensity modulated fiber optic displacement sensor (FODS), as shown in Fig. 3. In addition to this, the authors (Bidin et al., 2016) has been proposed a similar adulterated honey detection based on the transmission technique of the FODS. The detection of sugar contents in the adulterated honey by using the fiber optic sensors has been published in (Kingsta, Shamilee, & Sarumathi, 2018) as illustrated in **Error! Reference source not found.** 4. In the meantime, Fig. 5 depicted an optical microfiber sensing that has been approached by (Irawati et al., 2017) to detect the adulterated honey. These alternative methods are fabricated by using inexpensive components, simple techniques and small in size in order to detect the adulteration of honey based on the addition of sugar.

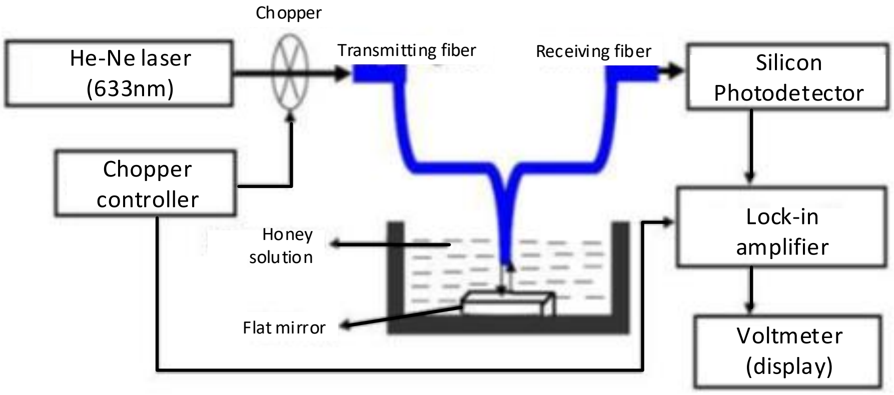

Fig. 3 – Honey purity detection in distilled water (Hida et al., 2013)

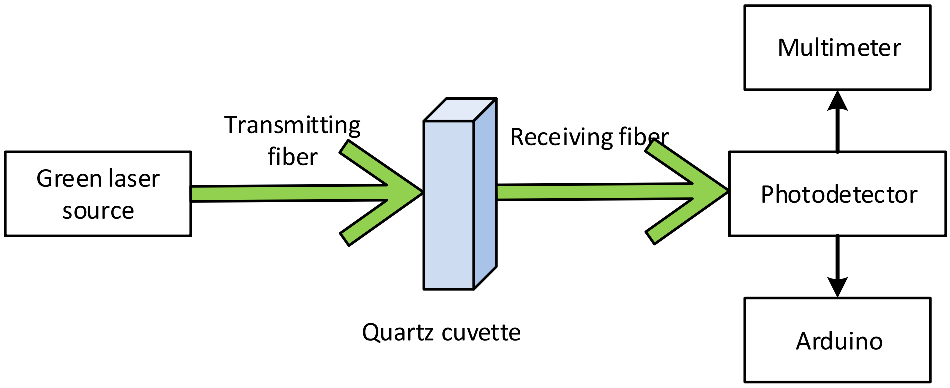

Fig. 4 - Sugar content detection by using fiber optic sensor (Kingsta et al., 2018)

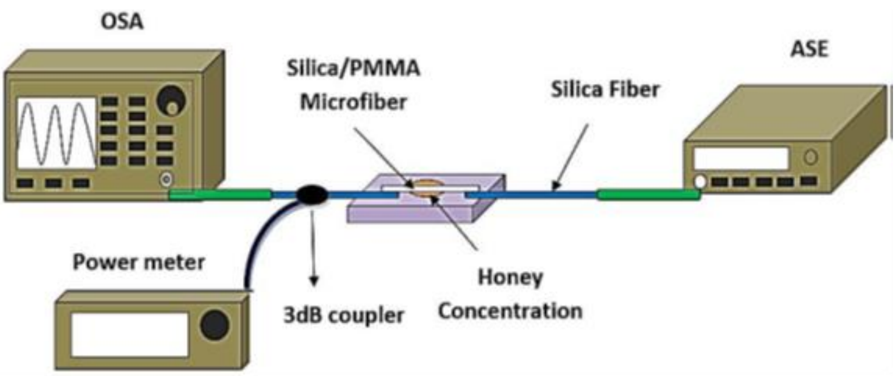

Fig. 5 - Optical microfiber sensing of adulterated honey (Irawati et al., 2017)

4. Discussion

Adulteration of honey is a critical issue which is determined by the numerous methods, e.g. spectroscopy, KFT, HPLC, chromatography and isotope analysis in order to get the information from each aspect of it. Due to the expensive devices, time-consuming methods and require high-skill knowledge to handle the devices, several authors have introduced the fiber optic sensing as an alternative method to determine the adulteration of honey. Fiber optic sensing is a simple and low-cost technique, in which it is convenient to be used by consumers to detect adulterated honey. However, other applied methods need to be taken into account since different methods will describe the different type of adulterants.

5. Conclusion

In a nutshell, the ideas on the adulteration of honey and the methods used for detection have been discussed in this paper. For unethical economic gain, the pure honey has been adulterated with the inverted sugar, water, processing as well as mislabelling the origin of honey. Therefore, there are huge of techniques have been proposed and demonstrated to detect the honey adulteration, including isotope analysis, chromatography, spectroscopy, KFT, drying method, refractometry, melissopalynology, physico-chemical and DNA analysis. All of these techniques contribute to the knowledge relating to each aspect of honey authenticity. An overall review that has been prepared by researchers regarding the applied methods in detecting adulterated honey would give beneficial information in oncoming research works.

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