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Review Article

Review on Honey Adulteration and Detection of Adulterants in Honey

Alemu Girma Tura[1], Dechasa Bersissa Seboka[2]

[1]Department of Food Process Engineering, Welkite University, Welkite, Ethiopia

[2]Department of Food Science and Postharvest Technology, Haramaya Institute of Technology, Haramaya University, Haramaya, Ethiopia

Email address:
alemu.girma@wku.edu.et (A. G. Tura), soboqr@gmail.com (D. B. Seboka)

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Abstract: Honey is defined as the natural sweet substance produced by honeybees. Honey consist mainly sugars but also contains some amounts of acids, phenolic contents, HMF (Hydroxyl Methyl Furfural), minerals and water. Honey adulteration is a global concern and developing countries are at higher risk associated with it due to lack of detection methods, awareness of people and policies. Honey adulteration occurs by direct addition of sucrose syrups that are produced from sugar beet, high-fructose corn syrup (HFCS), maltose syrup or by adding industrial sugar (glucose and fructose). Adulterants can be classified as intentional, unintentional, metallic and microbial based on type of adulterants in honey. Nowadays honey is being adulterated in more sophisticated ways that more difficult to detection of adulterants in simple methods. This paper presents a detailed review of common honey adulterants in honey as well as different methods to detect the adulterants both qualitatively and quantitatively. It also gives knowledge on awareness on honey adulterants, how adulterants are identified in quick methods including advanced recent techniques. Types of adulterants, reason and impact of adulterants are also included in this review. Honey composition is based on floral source which bees are collect nectar from and this make more difficult to detect adulterate in different honey.

Keywords: Honey Adulteration, Adulterants, Detection Techniques, Honey

1. Introduction

Honey is a naturally sweet and viscous product produced by honey bees (Apis Mellifera) from the nectar of flowers, from secretions of living parts of the plants, or excretions of plant-sucking insects on the living part of plants that the honey bees collect, transform and combine with specific substances of their own, deposit, dehydrate, store and leave in the honey combs to ripen and mature [23]. Honey consists of a mixture of sugars, mostly glucose and fructose [3]. In addition to water (usually 17-20 percent), honey also contains very small amounts of other substances, including minerals, vitamins, proteins and amino acids. A minor, but important component of most honey is pollen.

Adulteration is the act of intentionally debasing the quality of food offered for sale either by mixing or substitution of inferior substances or by the removal of some valuable ingredient [40]. Honey adulteration occurs by direct addition of sucrose syrups that are produced from sugar beet, high-fructose corn syrup (HFCS), maltose syrup or by adding industrial sugar (glucose and fructose). Adulterants can be classified as intentional, unintentional, metallic and microbial based on type of adulterants in honey.

The detection of honey adulteration is a technical methods used to identify inexpensive sweeteners adulterations such as corn syrups, high fructose corn syrups, invert syrups, sugar cane syrup. Those includes qualitative techniques that are quick methods, isotope ratio ($^{13}C/^{12}C$), thin-layer chromatography (TLC), gas chromatography (GC) are common analytical techniques. Recent advanced techniques such as near infrared (NIR) spectroscopy is also used to identify honey adulteration [37].

Objective of the Review

a. To review on honey adulteration and detection of

adulterants in honey.
b. To review most common adulterants in honey.
c. To review reason for adulteration and identifying detection methods of honey adulterants for consumers.

2. Literature Review

Honey, has been used since ancient times, as an important energy food and is used as an ingredient in a lot of manufactured foods, mainly in cereal-based products as a sweetening agent, color, flavor, caramelisation and viscosity [15]. In the long human tradition honey has been used not only as a nutrient but also as a medicine [22].

According to Food Safety and Standards Authority of India (FSSAI), the technical definition of food adulteration is "the addition or subtraction of any substance to or from food, so that the natural composition and quality of food substance is affected". According to Federal Food, Drug and Cosmetic Act (FFDCA), the primary food safety law administered by the Food and Drug Administration (FDA), food can be declared adulterated if:

a. a substance is added which is injurious to health
b. cheaper or inferior quality item added to food
c. any valuable constituent is extracted from main food article
d. quality of food is below the standards
e. any substance has been added to increase bulk or weight
f. to make it appear more valuable

Honey adulteration appeared on the world market in the 1970s when high-fructose corn syrup was introduced by the industry [30].

Food is mainly adulterated to increase the quantity and make more profit. The food is sucked of
its nutrients and the place where the food is grown is often contaminated. For example; milk is
mixed with water, ergot is used as an adulterant for cereals, chalk powder is used as an adulterant for flour, roasted barely is used as an adulterant for coffee powder, papaya seeds are used as an adulterant for black pepper, brick powder is used as an adulterant for chili powder, argemone seeds are adulterant for edible oils [40].

2.1. Constituents of Honey

Honey consists of a mixture of sugars, mostly glucose and fructose [3]. In addition to water (usually 17-20 percent), honey also contains very small amounts of other substances, including minerals, vitamins, proteins and amino acids. A minor, but important component of most honey is pollen. Pollen is carried to the bees' nest (hive) and stored inside it quite separately from nectar, but a few pollen grains find their way into nectar, and eventually into honey. The 'ash' content of honey is mainly mineral trace elements. Minerals present are calcium, copper, iron, magnesium, manganese, potassium, sodium, and chlorides, phosphates, silicates and sulphates. Dark honeys are often very rich in minerals, but variation in the mineral content of different honeys is great. These trace amounts of minerals may be important for human nutrition.

Other constituents of honey

a) HMF: is hydroxyl methyl furfural, a breakdown product of fructose (one of the main sugars in honey) that is formed slowly and naturally during the storage of honey, and much more quickly when honey is heated. The amount of HMF present in honey is the reference used as a guide to the amount of heating that has taken place. The higher the HMF value, the lower the quality of the honey. Some countries set an HMF limit for imported honey (sometimes 40 milligrams per kilogram), and honey with an HMF value higher than this limit will not be accepted. However, some honeys have a naturally high HMF level. HMF is measured by laboratory tests [28].

b) Enzymes: in honey (invertase, glucose oxidase, amylase, etc.) come from the bees or from the plant where the bee foraged. The levels of enzymes present in honey are sometimes assayed and used as a guide to honey quality. They are present in very small quantities, but may still have a nutritional importance in the human diet. The enzymes are very sensitive to overheating (above 35°C) or storage at too high a temperature. Because they are destroyed by heating, a low enzyme level may mean that honey has been heated, but many honeys of good quality are naturally low in enzyme content [19]

c) Acidic: free acidity, lactonic acid in including the main acid (gluconic acid) is present in honey in different ranges [26].

d) Aroma compounds: There is a wide variety of honeys with different tastes and colors, depending on their botanical origin [13]. In the past decades extensive research on aroma compounds has been carried out and more than 500 different volatile compounds were identified in different types of honey. Indeed, most aroma building compounds vary in the different types of honey depending on its botanical origin [9]. Honey flavor is an important quality for its application in food industry and also a selection criterion for the consumer's choice. Polyphones are another important group of compounds with respect to the appearance and the functional properties of honey. 56 to 500 mg/kg total polyphones were found in different honey types [2].

2.2. Types of Food Adulteration

Adulterants in food can be categorized into following categories:

a) Intentional adulteration: is the inclusion of inferior substances having properties similar to the foods in which they are added. They are thus difficult to detect. The adulterant could be physical or biological in nature. Some examples of intentional adulteration include addition of water to liquid milk, sugar syrup to honey, extraneous matter to ground spices, or the removal or substitution of milk solids from the natural product etc. [7].

b) Unintentional adulteration: is inclusion of unwanted substances due to ignorance, carelessness or lack of proper facilities and hygiene during processing of food. This can be of acquired type like contamination of foods by bacteria or fungi, spoilage of food by rodents, entry of dust and stones, harmful residues from packing material, etc. or inherent adulteration *e.g*. presence of certain chemicals, organic compounds or radicals naturally occurring in foods like toxic varieties of pulses, mushrooms, green and other vegetables, fish and sea foods [7]

c) Metallic contamination: is the intentional or unintentional inclusion of different types of metals and metal compounds in food. Out of all, lead, arsenic, mercury and cadmium are considered most toxic as their intake is highly chronic. If they accumulate in body they can cause organ damage [36].

d) Microbial contamination: is the spoilage of food due to infusion of different microbes through various sources. Foods may be contaminated by microorganisms at any time from several sources during food processing like during harvest, storage, processing, distribution, handling, or preparation [7].

2.3. Honey Adulterants

Food Safety and Standards Act of India (FSSA) defined "adulterant" as any material which could be employed for making the food unsafe or sub-standard or misbranding or containing extraneous matter. Honey adulteration occurs by direct addition of sucrose syrups that are produced from sugar beet, high-fructose corn syrup (HFCS), maltose syrup or by adding industrial sugar (glucose and fructose), syrups obtained from starch by heat, enzyme or acid treatment, or by feeding the bee colonies excessively with these syrups during the main nectar period `[32]. Fructose and glucose are the two key indicators for qualitative analysis of honey. Addition of small amounts of invert syrup does not change fructose and glucose levels beyond the normal ranges found in honey [28]. HFCS is much cheaper than unadulterated honey while its composition is similar to that of honey, which makes detection difficult [10]. Hydroxyl methyl furfural (HMF), a product of acid inversion, can be used as an index to detect the presence of invert syrups in honey [28]. However, the possibility exists that HMF levels increase as a result of heating, or even storage of, honey. The validity of HMF as an adulterant indicator is therefore questionable [29]. Cane sugar is also commonly used adulterants in honey in Ethiopia [40].

Indirect adulteration has often occurred in recent years by excessive supplementary feeding of bee colonies during the main nectar flow period, a huge injustice for both consumers and pure honey producers [20].

Plant syrups, obtained by heat concentration of vegetable juices or plant sap, can also act as adulterants. Three types of plant syrups, namely palm syrup or honey, must syrup and sugar cane syrup are reported from Spain [33].

The presence of sugars as adulterants in honeys can be related to the direct addition of syrups, at certain ratios after production, to increase honey sweetness or to overfeed the bees during the main nectar period in order to recover more honey from hives. Inexpensive sugars or industrial syrups are generally used for this purpose, with well‐known adulterants being sugar syrups, such as corn syrup (CS) and high‐fructose corn syrup (HFCS), glucose syrup (GS), sucrose syrup or inverted syrup (IS) which are produced from sugar cane or sugar beet [3, 20, 38]. Honeys adulterated by sugar addition can present, in fact, changes in some chemical and/or biochemical parameters, such as enzymatic activity, electrical conductivity, and contents of specific compounds (HMF, glucose, fructose, sucrose, maltose, isomaltose, ash) [1].

2.4. Reasons of Adulteration

As indicated by different authors, reasons for adulteration of food products are to fetch higher cash income and/or to increase shelf life. It is difficult to get a food items, may be flour, pulse, oil, fruit, vegetable, milk, sweet, spices, tea, coffee, honey, bakery item, chocolate, fruit juice, etc. which is free from one or the other adulterants. The main reason that attracts adulteration is for boosting their cash income by increasing its volume. Even though increasing their profit margins initiated adulteration done by some selfish producers, processors and retailers, the main cause for adulteration is dishonesty and lack of accidental quality assessment on products suspected [4].

Table 1. *Some common foods items and extraneous substances added into them.*

Food and drink items	adulterants	Reason of adulteration
Milk	Water, skim milk	To increase volume
Honey	Can sugar, molasses	To increase volume
Common salt	White powder stone, chalk	To increase amount
Coffee powder	Roasted barely powder	To add bulk and color
Butter	Vegetable oil, banana	To increase volume and make yellowish
Sugar	Chalk powder	To increase amount

Sources:- [4].

2.5. Impacts of Adulteration

The problems of adulteration makes the food items used in our daily life unsafe and unhygienic for use due to poor handling [4]. In the past few decades, honey adulteration of food has become one of the serious problems and consumption of adulterated honey causes serious diseases like cancer, diarrhea, asthma, ulcers. In general, adulteration of food items has a very serious impact on producers/farmers, processors or manufacturers/enterprises, consumers and government. More recently, adulterant use for example, in the People's Republic of China (Chinese milk scandal case with melamine) in which some children killed and thousands were harmed has inspired much public attention [4]. So it is indicative that food adulteration is fast growing worldwide as an industry and global market of adulteration and fake goods is more than several hundred billion dollars which constitutes more than 10 percent of total trade [6].

2.6. Detection Methods

Traditional analyses of chemical composition and physical properties of honey are commonly used to detect direct adulteration. They are routinely applied in the honey trade but these analytical methods are relatively time-consuming and require tedious preparation of the samples as well as complex analytical equipment [12]. Honey adulteration can also be detected using several modern techniques.

Some techniques are specific for particular adulterants, such as when the chemical composition of the adulterant is similar to honey. The C-isotope approach, relying on carbon isotope ratio ($^{13}C/^{12}C$) differentiation between plant groups, is one of the oldest methods to detect adulterants in honey and is still an effective tool (Croft, 1987). The analytical techniques that are commonly used are thin-layer chromatography (TLC), gas chromatography (GC) and high-performance liquid chromatography (HPLC). Advanced techniques, such as near infrared (NIR) spectroscopy and Raman spectroscopy, each having particular advantages widely in quantitative and qualitative analysis of food products because it is rapid, low-cost, nondestructive and user friendly.

The detection of adulteration is a technical problem and such adulterations with inexpensive sweeteners such as corn syrups, high fructose corn syrups or invert syrups.

Unfortunately when sugar concentrate is added to honey, laboratory assessments are found to be ineffective in detecting this adulteration. Unlike tracing heavy metals in honey, sugar adulterated honey is much trickier and harder to detect, and traditionally it has been very challenging to come up with a suitable method to prove the presence of adulterants in honey products.

2.6.1. Quick Test Methods

a. *Disperse:* To detect sugar solution in honey transparent glass of water was taking and after a drop of honey to the water adulteration is known. If it is pure honey it is not disperse, however, if it disperses in water, it tells the presence of added sugar. But in this method it is difficult to identify which adulterants is added to honey.

b. Firing: This is done by dip cotton in honey and lights it up with matchstick. If the honey is pure, it will burn. Adulterated honey will produce a cracking sound due to the presence of water in it [18].

2.6.2. Isotopic Ratio ($^{13}C/^{12}C$)

In the last decade, the C-isotope approach has become commonplace for the detection of adulterants in honey. It is based on carbon isotope ratio ($^{13}C/^{12}C$) differentiation between plant groups, which results from the photosynthetic pathways in plants. The $^{13}C/^{12}C$ isotope ratio is different in monocotyledonous plants (such as cane and corn) compared to di cotyledons (where bees collect nectar) [27]. Based on the photosynthetic pathway, plants can be divided into C_3 (Calvin and Benson cycle), C_4 (Hatch–Slack cycle) and CAM (Crassulacean acid metabolism). The $\delta^{13}C$ value reflects the $^{13}C/^{12}C$ ratio of the plant, and all plants have their own standard values vary from 22% to 33% for C_3 plants, 10% to 20% for C_4 plants, from 11.0% to 13.5 for CAM plants and beyond this value the honey is adulterated [10]. Stable carbon isotope ratio analysis (SCIRA) has been used to detect adulterated honey, and the results are expressed as $^{13}C/^{12}C = \delta^{13}C$ (%). The SCIRA method is much more useful for detecting C_4 (cane and corn) than C_3 (beet) sugars [17]. C_4 sugar syrups (corn and cane sugars) change the $\delta^{13}C$ ratio of honey when added, but beet sugar syrups do not affect the $\delta^{13}C$ ratio when added to honey. In this case the bound galactose analysis method is recommended. The average amount of galactose found in honey is 3.1 mg/100 g, whereas in beet sugar it is 30.1 mg/100 g [10]. A honey sample is considered to be adulterated with beet sugar if more than 80 mg/kg of galactose is detected in the tested samples (White *et al.,* 1986). The formula for $\delta^{13}C$ is given in the following equations (1) according to, [27].

$$\delta^{13}C\% = \frac{R\ sample}{R\ standard-1} \times 10^3, \text{ where } R = {}^{13}C/{}^{12}C \qquad (1)$$

Determining the $\delta^{13}C$ value of different pure honey samples of each variety is highly essential as a reference before testing for adulterated honey of that variety. The main limitation in the application of this procedure is the requirement for highly expensive instrumentation [33].

2.6.3. Gas Chromatography

Gas chromatography (GC) is a technique used to analyze monosaccharide, disaccharides and trisaccharides in honey with a relatively high resolution and sensitivity, which aids detection of adulterants, especially HFCS [16]. GC has been used to detect honey adulterated with HFCS, maltose and isomaltose. The quantities and ratios of the adulterants can be determined. GC is a first choice technique to detect HFCS adulteration, because of its high sensitivity. The detection of HFCS is mainly based on DFAs, but the drawback is that the honey samples require yeast treatment to concentrate the di- and trisaccharides, a process that is not essential in other advanced techniques [33]. DFAs are pseudo disaccharides containing two fructose residues that are formed during caramelization [34]. This reaction occurs during heating of sugars, or food products rich in sugars, and leads to a smaller volatile fraction and higher level of non-volatile components (90%–95%), including DFAs. The DFA content in food is dependent on food composition and processing. The presence of DFAs in HFCS and invert sugar syrups was first detected by GC. However, studies have not detected the presence of DFA in honeys stored at room temperature for three years [33].

2.6.4. Thin-layer Chromatography (TLC)

Thin-layer chromatography can be used to monitor the progress of a reaction, identify compounds present in a given mixture, and determine the purity of a substance. Thin-layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide (alumina), or cellulose. This layer of adsorbent is known as the stationary phase [21].

After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved. The mobile phase has different properties from the stationary phase. For example, with silica gel, a very polar substance, non-polar mobile phases such as heptane are used. The mobile phase may be a mixture, allowing chemists to fine-tune the bulk properties of the mobile phase. After the experiment, the spots are visualized, to quantify the results; the distance traveled by the substance being considered is divided by the total distance traveled by the mobile phase. (The mobile phase must not be allowed to reach the end of the stationary phase). This ratio is called the retardation factor (Rf). So based on this pure honeys yield only 1 or 2 blue-grey or blue-brown spots at Rf values above 0.35. The adulterated samples show an additional series of spots or blue streaks [25]. This was the first official method for detection in early years recently, a HPTLC method to detect adulterants in honey based on the fructose/glucose (F/G) ratio and the sucrose content [31].

2.6.5. NIR Measurement System

When honey from one country is sold in another country to increase its sales it can have an effect on the sales of other honeys in that country. To prevent this, detection of the honey origin and adulterants are to be determined by near infrared (NIR) technique. NIR spectroscopy is a useful technique to evaluate adulteration of honey samples and it is rapid and non-destructive which may be suitable as a screening technique in the quality control of honey. NIR system is used under reflectance mode to get NIR spectra in the range of 400nm - 2500nm. This instrument utilized to get the spectra sample to samples, composition to compositions to detect and quantify the content of adulteration in honey samples [37].

2.6.6. Calorimetric Method

Application of DSC showed the possibility of using the glass transition temperature to distinguish between honeys and syrups and is a powerful technique for characterizing the thermal behavior of honeys and for detecting the effect of adulteration on physicochemical and structural properties of samples [11].

2.6.7. Microscopic Detection

Microscopic analysis of adulterated honeys with cane sugar exhibited parenchyma cells, single ring vessels and epidermal cells. Overall the microscopic procedure is a good screening method for the detection of adulteration of honey with cane sugar products [24]. The pollen in honey can be identified using this methods by determine the geographical origin of honey by the pollen it contains and gives a guide to the plants from which bees has been collecting nectar and pollen. In many countries, pollen analysis of the locally produced honeys is regularly carried out and the pollen specialists have a precise knowledge of the pollen spectrum of the honeys of their region.

3. Conclusion

Honey adulteration one of common food adulteration such as milk, coffee powder, butter and pepper powder in Ethiopia. There are many reasons why honey is adulterated more often. Some of the consumers are unaware of the problem, others have no access to methods of identification and the rest are due to carelessness. Adulteration in the honey items can cause unexpected affect on health without consumer knowledge. The consumer should avoid buying honey from unknown local traders. Both local and branded honey handling, stores, transportation should be inspected by government bodies. In Ethiopia sugar is the most adulterant in honey. Consumers or merchants traditionally identify this added sugar by taste and solidification whereas government checks through laboratories. There are also some people who identify adulterated their local honey by their experience. This traditional method has limitation because some honey is crystallized naturally. According to the obtained data from different literatures some of physicochemical characteristics of honey depend on floral source and difficult to detect in simple methods. The pollen in honey can be identified using a microscope, and gives a guide to the plants from which bees has been collecting. Experts are able to determine the geographical origin of honey by the pollen it contains before detection. Honey adulteration is the issue of all aspect of honey chains to maintain its quality and safety. That is government by develop and implement policy on honey adulterate; private food industries by monitoring and moral responsibility on produced honey; traders by selling or buying quality honey through good handling and consumers by understand adulteration issue.

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