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General studies on honey adulteration: A review

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Abstract
Honey is characterized as a natural and raw foodstuff that can be consumed not only as a sweetener but also as medicine due to its therapeutic impact on human health. It is prone to adulterants caused by humans that manipulate the quality of honey. Although honey consumption has remarkably increased in the last few years all around the world, the safety of honey is not assessed and monitored regularly. Since the number of consumers of honey adulteration have increased in recent years, their trust and interest in this valuable product has decreased. Honey adulterants are any substances that are added to the pure honey. Food adulteration occurs globally and in many facets and affects almost all food commodities. Adulteration not only constitutes a considerable economic problem but also may lead to serious health issues for consumers. As the methods of adulterating foods have become more sophisticated, very efficient and reliable techniques for the detection of fraudulent manipulations are required.

Keywords: Honey adulteration, adulterants, detection techniques, honey

1. Introduction
The standards of Codex Alimentarius [2] defines honey as the natural sweet substance from the nectar of plants or secretions of living parts of the plants that are stored and dehydrated by honey bees to improve its nutritional properties and become consumable for humans. Honey, traditionally, is used for its anti-aging properties, enhancing the immune system, killing bacteria, treatment of bronchial phlegm, and relieving a sore throat, cough, and cold [3]. Moreover, according to literature, honey represents various pharmacological properties such as anti-inflammatory [4], antioxidant [5], anti-cancer activities against breast and cervical cancer [6], prostate cancer [7], and osteosarcoma [8]. The therapeutic effect of honey on human health can be either oral administration or topical application. In this regard, reference [9] revealed the therapeutic properties of oral administration of honey for the treatment of laryngitis, osteoporosis, gastrointestinal ulcers, anorexia, insomnia and constipation, and liver, cardiovascular and gastrointestinal problems. On the other hand, advantages of topical application of honey are prescribed for eczema, lip sores, sterile and infected wounds, genital lesions, burns, surgery scars, and athlete's foot [10]. Adulteration of honey has been a challenge for analytics for decades. Adulteration has been used to increase economic benefits by adding low-price honey or sugars during the production or processing. Furthermore, these food adulterants are often unique, so they avoid getting detected by routine analysis (Moore *et al.* 2012).

1.1 Type of Honey: Reference [6] and Alvarez-Suarez *et al.* [18] classified honey according to its origin as follows:

1) **Blossom honey:** the main source of this honey is the nectar of flowers such as linden, clover, citrus, cotton, thyme, and acacia honey.
2) **Honeydew honey:** the source of this honey is the "honeydew" (Rhynchota genus insects pierce plant cells, ingest plant sap, and then secrete it again) collected by bees. A typical example of honeydew honey is pine, oak, fir, and leaf honey.
3) **Monofloral honey:** named according to the plant that the bees that have produced the honey forage predominantly.
4) **Multifloral honey (polyfloral):** the source of this honey is several botanical flowers, with none of them predominant.

Meadow blossom honey and forest honey are classified in this category. All these classifications indicate the quality and physicochemical properties of honey.

The honey composition and quality varies according to the botanical origin, geographic area, and harvesting season [17]. Moreover, honey can be classified based on the bee species (stingless and honeybee) as mentioned in the next sections.

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 [20]. 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 [21] c) Acidic: free acidity, lactonic acid in including the main acid (gluconic acid) is present in honey in different ranges [22]. d) Aroma compounds: There is a wide variety of honeys with different tastes and colors, depending on their botanical origin [23]. 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 [24]. 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 [25]. 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 [26]. 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 [27]. HFCS is much cheaper than unadulterated honey while its composition is similar to that of honey, which makes detection difficult [28]. Hydroxyl methyl furfural (HMF), a product of acid inversion, can be used as an index to detect the presence of invert syrups in honey [29]. 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 [30]. 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 [31]. 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 [32]. 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 [34, 35, 36]. 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) [37].

1.2 Antioxidant Capacity

The generation of reactive oxygen species (ROS) and other free radicals during metabolism is an essential and normal process that ideally is compensated through the antioxidant system. However, due to many environmental, lifestyle, and pathological situations, free radicals and oxidants can be produced in excess, resulting in oxidative damage of biomolecules (e.g., lipids, proteins, and DNA). This plays a major role in the development of chronic and degenerative illness such as cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular, and neurodegenerative diseases (Pham-Huy *et al*. 2008; Willcox *et al*. 2004). The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally synthetized in situ, or externally supplied through foods, and/or supplements (Pham-Huy *et al*. 2008). Research indicates that foods rich in antioxidants such as honey can protect from the damaging effects of free radicals and ROS and thus exhibit beneficial effects on human health; such as cardiovascular protection by preventing ROS-induced low

density lipoprotein (LDL) oxidation (Schramm *et al*. 2003); cell death in some cancer cell lines (Jaganathan *et al*. 2015); enhance the human antioxidant defense system (Schramm *et al*. 2003) among others (Ajibola 2015). For instance in animal models, honey showed a protective effect against damage and oxidative stress induced by cigarette smoke in rat testis (Mohamed *et al*. 2011); honey supplementation exhibited a hepatoprotective and nephroprotective effect in rats with experimental aflatoxicosis due to its antioxidant activity (Yaman *et al*. 2016). The antioxidant capacity (or antioxidant activity) of honey is commonly attributed to its phenolic compounds. These compounds exhibit several preventive effects against different diseases like cancer, cardiovascular diseases, inflammatory disorders, neurological degeneration, wound healing, infectious diseases and aging (Khalil *et al*. 2010). The main antioxidant phenolic compounds in honey are: (a) phenolic acids: gallic acid, caffeic, ellagic, ferulic and p-coumaric acids, syringic acid, benzoic acid, cinnamic acid; chlorogenic acid, and (b) flavonoids: apigenin, chrysin, galangin, hesperetin, kaempferol, pinocembrin and quercetin (Rao *et al*. 2016; Erejuwa *et al*. 2014). While some of these bioactive compounds are found in most honey samples, others such as hesperetin and naringenin are found in few honey varieties (Erejuwa *et al*. 2012). The amount and type of the phenolic antioxidants depend largely upon the honey's floral source and/or variety of the honey (Gheldof *et al*. 2002). Generally, darker honeys have been shown to have a higher total phenolic content (TPC) and consequently a higher antioxidant capacity than lighter honeys (Eteraf-oskouei and Najafi 2013). Beside this, Ferreira *et al*. (2007) found that the dark honey contained the highest concentration of other antioxidants such flavonoids, ascorbic acid, and β-carotene compared to the light and amber honeys. In addition, some *in vivo* studies have shown that the antioxidant compounds of honey are bioavailable to the human body. Schramm *et al*. (2003) observed that honey fed at 1.5 g/kg body weight increased both phenolic antioxidants and plasma antioxidant capacity inhealthy human subjects. These results supported the concept that phenolic antioxidants from honey are bioavailable and that these compounds may augment oxidative defense in the human body. Similar evidence has was observed by (Gheldof *et al*. 2003). The antioxidant activity of phenolic compounds is related to a number of different mechanisms, such as free radical-scavenging, hydrogen-donation, singlet oxygen quenching and/or metal ion chelation (Eteraf-oskouei and Najafi 2013). Therefore, in order to obtain more accurate and representative results, the antioxidant capacity of honey is generally measured by use of various *in vitro* assays such as: in the form of antiradical activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay; 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay; oxygen radical absorbance capacity (ORAC) assay; and commonly used ferric reducing antioxidant power (FRAP) assay, that measures the conversion by antioxidants of the oxidized form of iron (Fe3+) to the reduced form (Fe2+) (Erejuwa *et al*. 2012). Several *in vitro* studies showed that the antioxidant capacity is strongly correlated with the content of the total phenolics in honey (Chua *et al*. 2013; Sagdic *et al*. 2013). For instance, a positive correlation was found between antioxidant capacity (ORAC assay) and TPC of various commercial honeys contributed to their antioxidant properties. However, Gheldof *et al*. (2002) stated that the levels of single phenolic or other compounds in honey are too low to have a major individual antioxidant significance. Hence, the total antioxidant capacity of honey has been associated to the result of the combined activity and interactions of a wide range of compounds, including both enzymatic: catalase, glucose oxidase, peroxidase and non-enzymatic substances: ascorbic acid, α-tocopherol, carotenoids, amino acids, proteins, organic acids, Maillard reaction products, and other minor components (Nayik *et al*. 2016; Eteraf-oskouei and Najafi 2013; Ferreira *et al*. 2007). Erejuwa *et al*. (2012) described the synergistic antioxidant effect of honey and thus considered the advantage of honey over other antioxidants, such as vitamins C and E. In fact, these vitamins in their antioxidant action do not end with scavenging or elimination of free radicals. Instead, they can become themselves pro-oxidants which can require other antioxidants for their regeneration into the active or antioxidant form. The advantage of honey is that it comprises several antioxidant constituents and if any of them exhibit pro-oxidant properties, there would be sufficient other antioxidants, which can protect the one against oxidative destruction, and thus lead to the regeneration into the antioxidant form. In fact, honey contains both aqueous and lipophilic antioxidants and thus can act at different cellular levels as an ideal natural antioxidant (Oryan *et al*. 2016). Moreover, the quantity of honey consumed in the diet is low compared with the quantity of many of the food sources of antioxidants. According to (Erejuwa *et al*. 2012), if honey would be used instead of refined sugars as a sweetener for food and drinks it could make a substantial difference to the quantity of antioxidants consumed in the diet.

1.3 Antibacterial Activity

The treatment of bacterial infections is being increasingly complicated by the ability of bacteria to develop resistance to current available antimicrobial agents. This evidence leads to the need of less and better use of antibacterials and antifungals, improved infection control and research on new therapeutic compounds (Feás *et al*. 2013). Antibacterial activity of honey is one of the most important findings that were first recognized in 1892 by the Dutch scientist Van Ketel (Eteraf-oskouei and Najafi 2013). The recent research indicates that the effectiveness of honey in many of its medical uses is due to its antibacterial activity that is capable of inhibiting Grampositive and Gram-negative bacteria, including multidrug resistant strains (Kwakman *et al*. 2008), and some species of fungi and viruses (Irish *et al*. 2006; Naama 2009). For instance, Junie *et al*. (2016) compared *in vitro* antibacterial activity of several types of honey of different origins against the bacterial resistant strains isolated from patients, including Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Salmonella enterica serovar Typhimurium, Bacillus cereus, Bacillus subtilis, and Listeria monocytogenes. The results showed that all the honey samples presented antibacterial activity against the studied strains and that all the honey samples inhibited bacterial growth. This evidence was similar to other studies conducted elsewhere (Huttunen *et al*. 2013). The quantitative determination of the reduction of microbial colonization against a representative panel of bacteria is generally analyzed by *in vitro* tests including (a) determination of minimum inhibitory concentration (MIC) using broth tube dilution methods through visual inspection and (b) by determination of minimum bactericidal concentration (MBC) by sub-culturing tubes showing no visible sign of growth/ turbidity (Wasihun

and Kasa 2016); these determinations allow a distinction between whether a honey is just stopping the bacteria from growing (bacteriostatic action) or is killing the bacteria (bactericidal action), respectively (Molan 1992). It has been stated that honey possessed a significant antibacterial activity against some bacteria which are resistant to antibiotics (Junie *et al*. 2016; Mohapatra *et al*. 2011). In a study by Wasihun and Kasa (2016) the antibacterial activity of honey was evaluated against multidrug resistant human pathogenic bacterial isolates (Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, coagulase-negative Staphylococcus, Streptococcus pyogenes and Klebsiella pneumoniae). The MIC and MBC values indicated that the tested honeys had potential bacteriostatic and bactericidal activities against the tested bacteria. Unlike most conventional antibiotics, honey dose may not lead to development of antibiotic-resistant bacteria and it may be used continuously (Eteraf-oskouei and Najafi 2013). According to Alandejani *et al*. (2009), antibiotics tested (cefazolin, oxacillin, vancomycin, azithromycin, fusidic acid, gentamicin, and linezolid) were not bactericidal to methicillin-sensitive Staphylococcus aureus, methicillin-resistant Staphylococcus aureus (MRSA), or Pseudomonas aeruginosa (PA) biofilms. But the bactericidal rates for the Sidr and Manuka honeys were significantly higher than those seen with the single antibiotics. Thus, the use of honey in a medical setting is considered to be helpful in combating bacterial resistance (Kwakman *et al*. 2008). The bacterial strains differ in their sensitivity to honeys. Due to the different floral source, locations, bee species, storage (time and temperature), and processing, the antibacterial potency of different honeys can vary (Grego *et al*. 2016; Sousa *et al*. 2016), for some by more than 100-fold (Lusby *et al*. 2005). Thus it is difficult to standardize honeys and assess their usefulness in a medical application (Sousa *et al*. 2016). In spite of this, there are medical grade honeys like Revamil source (RS) honey and Manuka (i.e., Medihoney). Having reproducible antibacterial activity, these honeys are produced under controlled conditions in greenhouses and each batch is analyzed individually to assess the Unique Manuka Factor (UMF) that gives a number based on its bactericidal activity (Knight 2013). However, the mechanism by which honey exerts the activities against a broad spectrum of organisms is still under debate. There are some factors that are closely related to the antibacterial capacity of honey, including the level of hydrogen peroxide (H_2O_2), which is formed when honey is diluted (Knight 2013). According to Hadagali and Chua (2014), enzymes convert sucrose into a simple and soluble mixture of monosaccharides. The sugar molecules in the honey solution bind to free water molecules, which means that there is no water available for microbes to use, preventing their survival. The enzyme glucose oxidase (produced by bees) converts glucose into gluconic acid, making the honey too acidic for microbes to grow and survive. The H2O2 produced as a by-product of this reaction acts as a sporicidal antiseptic that sterilizes the honey. Further, the osmolarity of honey, due to about 80% of its composition being sugars, is another important factor to prevent growth of bacteria (Kwakman and Zaat 2012). Different concentrations (or dilutions) of honey used in the *in vitro* tests have been associated with a different antibacterial response (Steinberg *et al*. 1996). Beside the sugar content, the low pH (between 3.2 and 4.5 for undiluted honey) is inhibitory to many pathogenic bacteria. However, when consumed orally, the honey would be so diluted by body fluids that any effect of low pH is likely to be lost (Molan 1995). In spite, honeys have substantial antibacterial activity due to non-peroxide components including methylglyoxal and the antimicrobial peptide bee defensing-1. For instance, these compounds have been identified in Manuka and RS honey as antibacterial compounds (Kwakman and Zaat 2012). In addition, factors such as phenolic compounds (i.e. flavonoids and phenolic acids) (Kwakman and Zaat 2012; Sousa *et al*. 2016) and some unknown floral or bee components are being considered as contributing to the antibacterial activity of honey as well (Nishio *et al*. 2016).

1.4 Anti-inflammatory Capacity

Honey possesses quite a large number of therapeutic properties, including antioxidant and antimicrobial properties, as well as anti-inflammatory activity (Vallianou *et al*. 2014). The anti-inflammatory property of honey is mainly related to its antiseptic nature that works by removing infectious bacteria stimulating the inflammatory response, and reduction of the amount of bacteria present in the wound (Hadagali and Chua 2014). In fact, that honey can remove bacteria that cause inflammation, a decrease in wound inflammation after applying honey gauze has been associated to its direct anti-inflammatory properties, such as antioxidant capacity (Yaghoobi *et al*. 2013). In particular, some of the antioxidant phenolic compounds (i.e. flavonoids) are deeply related to anti-inflammatory effects as previously reported in the literature (González *et al*. 2011). However, beside the wound inflammation (Tomblin *et al*. 2014), the correlation of antioxidant capacity of honey with its anti-inflammatory action has been observed in other inflammation models as well (Owoyele *et al*. 2011). For instance, the potential protective effect of a honey flavonoid extract (HFE) has been studied on the production of pro-inflammatory mediators by lipopolysaccharide stimulated N13 microglia. It has been shown that the HFE (containing luteolin, quercetin, apigenin, kaempferol, isorhamnetin, acacetin, tamarixetin, chrysin, and galangin) can inhibit microglial activation and thus be considered as a potential preventive−therapeutic agent for neurodegenerative diseases involving neuroinflammation (Candiracci *et al*. 2012). The main evidence that considers the antioxidant activity as the anti-inflammatory factor is the ability of antioxidants to inhibit ROS production during the inflammatory process. A number of drugs are available for the treatment of ulcerative colitis. Manuka honey has been shown to specifically decrease the inflammatory response associated with ulcerative colitis, an inflammatory bowel disease characterized by an over-expression of inflammatory cells, possibly byincreasing antioxidant activity (Prakash *et al*. 2008). In a study by Borsato *et al*. (2014), honey extract decreased edema, reduced leucocyte infiltration, and inhibited the production of ROS during the inflammatory process induced chemically in mice ear. The anti-inflammatory activity has been associated with a synergetic effect of the honey phenolic compounds, including kaempferol and caffeic acid. In general, the transcription factor nuclear factor-kappa beta (NF-KB) plays a key role in pathogenesis of inflammation, being known as marker of inflammation (Vallianou *et al*. 2014). It enhances pro-inflammatory activity, thereby contributing to an amplified inflammatory response, and activates genes encoding pro-inflammatory cytokines – interleukin (IL)-6, IL-8, and tumor necrosis factor-α (TNF-α). These proinflammatory cytokines stimulate nitric oxide

production (NO), an important mediator of inflammation (Tomblin *et al*. 2014). The anti-inflammatory effect of honey has been observed in numerous reports, stating that honey can inhibit the release of pro-inflammatory cytokines, expression of nitric oxide synthase (iNOS), production of ROS (Candiracci *et al*. 2012), and can decreases prostaglandin levels, one of the major players in the process of inflammation (Al-Waili and Boni 2003). According to an *in vivo* study by Owoyele *et al*. (2011), honey caused inhibition of NO release in acute and chronic inflammation. Further, Gelam honey has been investigated in an acute inflammation model system showing the reduction of edema in inflamed rat paws. The mechanism was associated with the inhibition of cyclooxygenase (COX-2) and iNOS, which resulted in suppressed levels of pro-inflammatory mediators such as NO, PGE2, TNF-α, and IL-6 (Hussein *et al*. 2012). In general, acute inflammation is the body's primary response to injurious stimuli, and some of the body's responses are characterized by pains (Hadagali and Chua 2014). Side effects of the available drugs for the treatment of inflammatory pain can sometimes limit the use of these drugs (e.g., NSAID, Indomethacin) (Owoyele *et al*. 2014). It has been shown that honey significantly decreased production of proinflammatory cytokines, which was similar to the effect of the anti-inflammatory drug Indomethacin (NSAID) (Hussein *et al*. 2012), and also could modulate muscarinic receptors to produce its analgesic effect (Owoyele *et al*. 2014), thus being potentially useful for treatment of inflammation.

1.5 Wound Healing Activity

Several animal studies and clinical trials have examined the application of honey for acute and chronic wounds (Moore *et al*. 2001) including burn injuries (Bangroo *et al*. 2005), and have demonstrated that it limits the amount of edema, improves granulation and epithelization in the proliferative phase while decreasing total wound healing time, reduces scarring and contractures in patients with burn wounds (Mohamed *et al*. 2015), without adverse effect (allergy or toxicity) at all (Yaghoobi *et al*. 2013). Due to its low adherence in wound surface, honey causes minimal pain during application and upon removal preserving the newly forming granulation tissue (Mohamed *et al*. 2015). There is evidence that honey can heal partial thickness burns more quickly (around 4–5 days) than conventional dressings; and post-operative infected wounds can be treated by honey more effectively than by use of antiseptic or gauze (Jull *et al*. 2008). In a study by (Mohamed *et al*. 2015), a total of 12 patients with chronic foot ulcers utilized natural honey as an effective alternative to more expensive, advanced wound products. After the wound rinsing with normal saline, natural honey was applied and the wound was covered by glycerin-impregnated gauze. Patients were followed on a daily basis for an average of 4 weeks. The results showed that all ulcers healed with no contractures or scars with a mean healing time of 3 weeks. Moreover, there was a 75% reduction in the dressing budget of the health center and a high level of satisfaction among both health professionals and patients. Also, patients' pain levels were reduced significantly after using natural honey. Similar evidence has been observed when Manuka honey gel was used for treatment of partial-thickness facial burns. The healing time was congruent with or better than what would been expected with standard treatment. No abnormal bacterial growth was reported and the patients reported overall satisfaction with the treatment and cost of the treatment. It has been suggested that Manuka honey is a clinically and economically valuable treatment for partial-thickness facial burns (Duncan *et al*. 2016). In addition, a recent study by Aziz *et al*. (2017) showed that honey dressings can promote better results for burn wounds than the silver-based dressings (i.e., silver sulfadiazine), the currently extensively used method used to treat a variety of acute and chronic wounds. The presence of antibiotic resistant S. aureus in wounds is a cause for concern due to its capacity to acquire resistance to multiple antibiotics that make the treatment of wounds difficult. Jenkins *et al*. (Jenkins *et al*. 2012) showed that Manuka honey effectively inhibited the strains of vancomycin-intermediate S. aureus (hVISA, VISA) and the clinical strains of vancomycin-sensitive S. aureus (VSSA) in the clinical setting. It has been indicated that Manuka honey at low concentration (≤6% (w/v)) can inhibit the growth of clinical isolates of S. aureus and thus can be used as a treatment option to help decontaminate wounds infected with antibiotic-resistant organisms like S. aureus. Besides that, clinical and laboratory data indicate that natural honey is effective against a variety of common pathogens. Honey facilitates wound healing by its ability to create an effective viscous barrier on the wound surface, thus preventing the invasion of microorganisms (Aziz *et al*. 2017) present in the wounds and can remove any dead tissue that may provide a favourable environment for the growth of microorganisms (Zbuchea 2014). The acidic pH of honey (3.2 to 4.5) inhibits growth of most pathogenic bacteria within wounds, and increases production of hydrogen peroxide from the enzyme glucose oxidase at 1:1000 concentration. This is less than the conventional rinse solutions but enough to inhibit bacterial growth without compromising the new granulation tissue (Mohamed *et al*. 2015). Thus, when applied topically, honey is capable of cleaning infection from a wound and improving healing (Al-waili *et al*. 2011). Nevertheless, the wound healing capacity of honey is not only through its antiseptic nature, but also through its immunomodulatory effects, which boost the immune system to fight infection. The components in honey related to its immunomodulatory properties have not been yet fully identified, but are being attributed to lipopolysaccharide (LPS), a 5.8 kDa component, major royal jelly protein 1, arabinogalactants, polyphenols, and antioxidants (McLoone *et al*. 2016). Different types of honey have been shown to act with different mechanisms, and moreover that some of these mechanisms are more efficient than others (Ranzato *et al*. 2013). For instance, buckwheat honey is used in wound healing products because of its high-polyphenolic content, which make this honey effective in reducing ROS levels causing cell damage and inhibition of wound healing; Manuka honey has notable antibacterial and healing activities, which directly originate from the methylglyoxal it contains, and make this honey useful for treating problematic wounds (Ranzato *et al*. 2013). The Manuka honey has been claimed to have therapeutic advantages over other honeys and is thus the type of honey most often studied in controlled wound healing studies (Majtan 2011). The honey for wound healing is being commonly used as a base for ointments, gels, and in surgical dressings (Shenoy *et al*. 2012) and some studies successfully demonstrated its healing effect when applied directly in a raw form (Mohamed *et al*. 2015). However, natural honey from the comb is not medical grade and should not be used in wound care. Medical grade honey is filtered; gamma irradiated to kill Clostridium spores, and produced under

exacting standards of hygiene. There are some commercially available sterile honey products like Revamil source (RS) and Manuka honey, the two major medical-grade honeys. Other Uses of Honey Gastroenterology Honey is reported to have e֜ects of preventing and treating gastrointestinal disorders such as peptic ulcers, gastritis, and gastroenteritis. Honey is a potent inhibitor of the causing agent of peptic ulcers and gastritis, Helicobacter pylori [5]. Honey is natural and will not raise blood-sugar levels; a mix of honey and water is a good cure for colic [50]. Honey has prebiotic ejects increasing the population of bacterial mLcroflora important for the health of gastrointestinal tract. According to Ustunol [51], the consumption of honey increases the population of normal flora called bLfidobacterLa, where its constitutents were found to pose prebiotic e֜ect that resembles the e֜ect of fructooligosaccharides (FOS). Honey and diabetics Honey contains a good proportion sugars with: dextrose (31%), levulose (38%), and about 1.3% sucrose. On a weight basis, honey is approximately as sweet as granulated sugar; hence more sweetening power might be considered available to the diabetic at a lower dextrose "price" from honey than from granulated sugar [42]. Studies have shown that honey consistently produces a lower glycemic index when compared to glucose and sucrose in normal volunteers and type I diabetics, and that honey or sucrose at breakfast do not have additional acute hyperglycemic e֜ects over an isoglucidic amount of bread in type II diabetics [52]. Compared to glucose and sucrose, honey has lower glycemic and incremental indices in type I diabetic patients [53]. Sports nutrition Carbohydrate consumption prior to, during and after exercise enhances performance and speeds recovery. Honey is a natural source of readily available carbohydrates and is as e֜ectLve as glucose for carbohydrate replacement during endurance exercise [20]. It helps maintain muscle glycogen, also known as stored carbohydrates, which are the most important fuel source for athletes to help them keep going. Honey serves as an athletic aid. Pre-exercise, as with many carbohydrates, pure honey may be an e֜ectLve form to ingest just prior to exercise.

When honey is eaten before a workout or athletic activity, it is released into the system at a steady rate throughout the event. During exercise, consuming carbohydrates, such as honey, during a workout helps muscles stay nourished longer and delays fatigue, when compared to not using any aid or supplement. Post-exercise, ingesting a combination of carbohydrates and protein immediately following exercise (within 30 minutes) is ideal to refuel and decrease delayedonset muscle soreness. Herefore, honey is a great source of carbohydrate to combine with post-workout protein supplements. In addition to promoting muscle recuperation and glycogen restoration, carb-protein combinations sustain favorable blood sugar concentrations after training National Honey Board [20].

2. Honey Adulteration

2.1 Analysis methods

1. Gas Chromatography (GC) and Liquid Chromatography (LC) analysis: This method may be considered as a replacement of isotopic analysis, which has some limitations. Near Infrared Transflectance Spectroscopy (NIR): It is a rapid, non-destructive and relatively inexpensive method which may be suitable for use as a screening technique in the quality control of honey [12].

2. Fourier Transform Infrared (FTIR) spectroscopy with Attenuated Total Reflectance (ATR): In contrast to the time-consuming carbon isotope ratio analysis techniques, these FTIR spectroscopic procedures can be performed in very short time [13].

3. Protein characterization: The major proteins in honey have different molecular weights depending upon the honeybee species. Therefore, the measurement of major proteins in honey is a useful method to discriminate the honey that produced from different honeybee species [19].

4. High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD): It is an efficient tool for the characterization of the honey floral species. This method is less time consuming and less expensive than other methods [14].

5. Liquid Chromatography Coupled to Isotope Ratio Mass Spectrometry (HPLC-IRMS): The new procedure has advantages over existing methods in terms of analysis time, sensitivity, lack of sample preparation, reduced consumption of reagents, and simplicity of the operative procedure. In addition, it is the first isotopic method developed that allows beet sugar addition detection [15].

6. Calorimetric methods (Application of DSC): 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.

7. 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 are 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].

8. 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) [40]. Based on the photosynthetic pathway, plants can be divided into C3 (Calvin and Benson cycle), C4 (Hatch–Slack cycle) and CAM (Crassulacean acid metabolism). The δ13C 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 C3 plants, 10% to 20% for C4 plants, from 11.0% to 13.5 for CAM plants and beyond this value the honey is adulterated [39]. Stable carbon isotope ratio analysis (SCIRA) has been used to detect adulterated honey, and the results are expressed as $^{13}C/^{12}C$ = δ13C (%). The SCIRA method is much more useful for detecting C4 (cane and corn) than C3 (beet) sugars [38]. C4 sugar syrups (corn and cane sugars)

change the δ13C ratio of honey when added, but beet sugar syrups do not affect the δ13C 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 [39]. 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 δ13C is given in the following equations (1) according to, [40].

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

Determining the δ13C 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 [41].

9. Fourier Transform (FT) Raman spectroscopy: FT-Raman spectroscopy is successfully applicable to detect beet and cane invert syrups. This method can also be used to discriminate between the types of adulterants irrespective of its floral origin [16].

10. 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.

11. 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 [33].

12. 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 [42]

13. 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 [43]. 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 India. 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. 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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