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ARTICLE

Re-evaluating Honey Quality: Key Factors Influencing Its Purity and Excellence

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ABSTRACT

Honey quality is a growing global concern due to its importance in nutrition, health, and the food industry. The purpose of this research is to review the composition, physicochemical characteristics, and variables influencing honey quality. Honey, a natural sweet product produced by bees, contains over 180 essential components, including sugars, minerals, and nutrients that provide energy and enhance immunity. Its physicochemical properties, reducing sugars (>65%), moisture content (<25%), electrical conductivity (<0.8 mS/cm for blossom honey), acidity (<0.2%), and sucrose content (5%), influence its flavor, granulation, nutritional value, and storage. Other indicators, including diastase activity, proline, phenols, and pollen density, are vital for determining honey's quality and origin. Honey is also rich in minerals, with potassium being the most common. The composition and quality of honey are significantly impacted by several factors, including the floral source, climate, beekeeping practices, collection methods, adulteration, processing, agrochemicals, storage conditions, and geographic origin. Standardized quality assessment criteria have been established to evaluate honey quality, ensuring it meets regulatory requirements and consumer expectations. As honey demand continues to rise, understanding these influencing factors is crucial for ensuring that the honey consumed meets high-quality standards and retains its beneficial properties. This review highlights the importance of assessing honey's physicochemical properties and understanding the various factors that contribute to its overall quality, which is essential for both consumer safety and the honey industry's growth.

Keywords: Adulteration; Classification; Composition; Honey; Immunity; Physicochemical; Quality

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1. Introduction

The sweet, viscous food product known as honey is produced by honey bees. Worker bees engaged in foraging visit flowers, gathering pollen for the pollen basket perched on their legs and nectar for the honey stomach. After returning to the hive, the bees regurgitate the nectar onto the mouths of other worker bees, who chew it for approximately 30 minutes, converting complex sugars into simple sugars through enzymes. The bees then cover the cells with bee wax, which permits the enzyme-rich bee feces to finish turning into honey [1].

The global production of honey is estimated at 18,000 metric tons annually, with China leading as the top producer, contributing 28% of the total. Other significant contributors include Turkey (5.9%), Iran (4.5%), and the United States (4.1%), highlighting the international scale of honey production [2]. Ethiopia is currently the greatest honey producer in Africa, having been the home of beekeeping for generations. Ethiopia ranks tenth in the world for honey output and fourth for beeswax production [3].

Phenolics, flavonoids, vitamins (A, B1, B2, B6, C, E, K), fatty acids, and amino acids are among the many bioactive components found in honey that support pharmacologic qualities such wound healing, antidiabetic, anti-inflammatory, antioxidant, antibacterial activity, and antitumoral actions [4]. Sometimes called a "functional food," honey contains a complex mixture of sugars (carbohydrates, including fructose, glucose, and monosaccharides), proteins, vitamins, minerals, enzymes, and organic acid [5]. Honey has gained a lot of attention as a healthy natural sweetener [6]. In vitro, honey has also been associated with decreased inflammation due to its combination antibacterial and antioxidant properties [7]. According to a study by Hossain [8], honey's antiviral properties may benefit COVID-19 patients by boosting the host immune system and managing comorbid conditions. It has also shown effectiveness against a variety of viruses, such as HIV, herpes simplex, influenza virus, and varicellazoster virus. Honey is used in the treatment of several inflammatory conditions, cancer, cataracts, and cardiovascular problems [9].

A major issue with honey and beeswax quality is emerging in many nations, including Ethiopia. Adulteration is one issue with the quality of Ethiopian honey and beeswax. In our nation specifically as well as in Africa in general, adulteration is turning into a significant and pervasive issue [3]. The honey industry's marketing of its products is heavily reliant on the storage quality, granulation, nutritional value, texture, flavor, and medicinal quality, all of which are being adversely affected by this issue. Thus, the current study's goal was to examine the composition, quality, and factors influencing honey and beeswax quality.

2. Methodology

Peers evaluate electronic search tactics as well as the PICOS elements of the present search strategy (population, intervention, comparator, outcome, and study design) [10]. To develop this review study, the literature on adulteration, classification, composition, honey, Immunity, physicochemical, and quality was thoroughly searched for relevant scientific content using PubMed, Web of Science, Ebsco, Scopus, the Cochrane Controlled Register of Trials, ProQuest, and Google Scholar. Likewise, further pertinent information was discovered through a check of the documents' references.

3. Composition and Classification of Honey

Honey is a great source of energy since it is mostly composed of 80% carbohydrates (around 40% fructose, 35% glucose, and 5% sucrose) and 20% water [11,12]. Its pH is roughly 4.0 and it contains at least 200 compounds, including enzymes, vitamins, minerals, and amino acids [13]. Many factors, including botanical (flower) sources of nectar, geographical origin, meteorological and environmental conditions, and methods for harvesting, storage, and processing, all affect the content and qualities of honey [14]. Honey bees are subjected to insecticides and industrial pollution. It has been noted that pesticide residues in raw honeys are a sign of environmental contamination and may be used as a biomarker [15]. **Table 1** illustrates how honey has been categorized according to its place of origin, processing method, and grades [12].

Table 1. Honey Classification [12].

Classification of honey	Description
1. Based on the place of origin	
Monofloral (Unifloral honey)	A single plant's honey. The specific plant species' pollen content exceeds 45% of the total pollen content.
Polyfloral (Multifloral honey)	Honey derived from a variety of plants. Different plant species' pollen contents don't surpass 45% of the total pollen load.
Blossom honey	Bees collect nectar from flowering plants to make honey.
2. Processing system	
Comb honey	The bees make honeycomb, which is sold unprocessed and sells for a decent price. Harvesting and preparing this type of honey for sale is one of the simplest processes.
Strained hone	Honey is made by simply straining honeycombs to remove any particles such as pollen, beeswax, or other substances.
Chunk honey	A piece of comb honey, such as honey from Acacia and Robinia psudoacacia, is placed within the jar of liquid honey, which has a very appealing appearance.
Extracted honey	Centrifuging honeycombs yields this type of honey.
Crystallized or granulated honey	Since granulation is a natural process and can be readily returned to its original state, the filtered honey is the one that has crystallized and is safe to consume. The ratio of honey's water and monosaccharide contents is used to calculate its granulation propensity. Honey that contains less water and more glucose granulates more
Creamed honey	It is strained honey that has been churned to create a homogeneous, soft consistency after being seeded to begin crystallization. Since no foreign substance has been added or removed, the technique stabilizes the honey's consistency without sacrificing its originality. About 20% of liquid honey is mixed with fine crystallized honey, and the crystals are then allowed to form at 14 ^{o}C.
3. Based on grading	
Grade A	The highest quality extracted honey with at least 81.4% soluble solids, the best flavor and clarity (it may contain a trace of pollen grains or other finely divided particles in suspension that do not affect appearance), and it is nearly faultless in terms of caramelization, smoke, fermentation, chemicals, and other causes.
Grade B	With a minimum overall score of 80 points, the second-best honey quality has a soluble solids percentage of 81.4, is reasonably free of faults, smoke, caramelization, chemicals, fermentation, and is reasonably free of suspended particles.
Grade C	The lowest grade of extracted honey has a minimum overall score of 70 points, a minimum soluble solids percentage of 80.0, is relatively flaw-free, has a reasonable flavor and aroma, and is reasonably free of dispersed particles. Subpar honey has a flavor and fragrance rating below 70 points and does not meet US Grade C criteria.

4. Physicochemical Characteristics of Honey

The honey industry places a lot of significance on the physicochemical components of honey since they affect its granulation, flavor, nutritional value, and therapeutic qualities as well as its storage quality. Since honey is frequently used as an antibacterial, in the production of various pharmaceuticals and cosmetics, as well as for other therapeutic reasons, it is crucial to standardize its quality from the perspective of the consumer as well as beekeepers in order to properly compensate them [12].

4.1. Hydroxy Methyl Furfuraldehyde

Higher the Hydroxy methyl furfuraldehyde content, the lower the quality of honey. Most honeys naturally include hydroxymethyl furfuraldehyde, an aldehyde produced when fructose breaks down in acidic environments (a process known as the Miaillard reaction, which is a non-enzymatic browning). Extended storage, Improper heating, or adulterating honey with invert sugars are associated with elevated amounts of hydroxymethylfurfuraldehyde [12].

Hydroxymethyl furfuraldehyde levels in freshly extracted honey are less than 5 mg kg^{-1}. Hydroxymethyl furfuraldehyde concentrations in honey are low or negligible when it is held at low temperatures, but they are substantial when the honey is matured and stored at relatively higher or medium temperatures [16]. The use of steel containers and honey floral sources, as well as storage circumstances, have a significant impact on Hydroxymethylfurfuraldehyde concentrations. To ensure that the product has not been subjected to excessive heating during processing and is safe for consumption, the highest amount that the Codex Alimentarius Standard Commission has established for Fiehe's test is used to determine the amount of hydroxymethyl furfuraldehyde: A good reaction is shown by the production of a cherry red color when hydroxymethyl furfuraldehyde and resorcinol combine. On the other side, a negative reaction is indicated by the appearance of a yellow to salmon pink tint or a small pink color that goes away fast. When the test is positive, three milliliters of distilled water are used as a blank to measure the absorbance at 540 nm in a spectrophotometer. The reaction occurs five times faster with a 10°C temperature increase.

4.2. Viscosity

Many factors, such as temperature during processing and storage, moisture content, and chemical composition, affect viscosity, a critical physical property of honey. With a modular compact rheometer, it is measured. Temperature and water concentration (14–24%) affect honey's viscosity [12]. The viscosity of honey is its main rheological property. Most commonly, honey is utilized in a highly viscous liquid form. Simply said, viscosity and flow ease are correlated: the higher the viscosity, the more difficult it is for a fluid to flow. The viscosity of honey is thousands of times that of water, making it difficult to flow through. Temperature, water content, and honey composition are the primary factors influencing honey viscosity [17].

4.3. pH

No matter where it comes from, honey has an acidic natural state because it contains organic acids (especially malic, gluconic, citric acids and pyruvic), esters, lactones, and some inorganic ions like chloride and phosphate that give it flavor and stability from microbes deterioration [12]. Variations in pH can result from a variety of factors, including the floristic content and diversity of flowers, bee saliva, enzymatic and fermentative conversion of raw materials, and improper harvesting techniques. Honey with a pH of 3.5 – 4.5 is considered to be blossom honey, whereas honey with a pH of more than 5 is thought to be of lower quality [12]. A pH meter is used to measure it.

4.4. Colour

Honey's color, which ranges from light white to dark amber and adds to its beauty and quality, is a key attribute that determines its market worth. The color of honey is a feature of its floral source and is correlated with its mineral content. Honey hue was divided into two portions by Fellenberg and Rusiecki [18]: water soluble (light colored honey) and fat soluble (darker honey). Tryptophan and tyrosine are absent from pale honey compared to black honey [12]. After granulation, honey appears lighter. The USDA has classified honey into seven hues, ranging from 0.0945 to 3.008. With a maximum optical density of 0.3 at 600 nm, it fluctuates from light to dark brown in accordance with Indian norms and Agmark specifications. Darker honey has a stronger flavor than lighter honey (National Honey Board). Heat has an impact on honey's color as well as the quantity of suspended particles like pollen. When natural honey is cooked, its color darkens [12].

4.5. Pollen Density

Bee pollen is a concoction of bee digesting enzymes and pollen. One of the key physical features of honey is its pollen density. Compared to processed honey, raw honey has a somewhat higher pollen content. Honey pollen analysis is crucial for quality control since it can detect adulterated honey and help Melissopalynologists determine the geographic and botanical origin of a certain variety of honey. A gram of pollen should have 25,000 counts [12].

4.6. Moisture Content

The amount of moisture in honey determines its quality, stability, and resistance to deterioration as well as its ability to withstand granulation and yeast fermentation. Fermentation during storage results from the extraction of immature and unprocessed honey, as well as from collecting during wet seasons. It is dependent upon the geographic origin during, relative humidity, and temperature. It is ascertained by either an oven drying method or a refractive index measurement using a refractor at 20 degrees Celsius [12,19].

4.7. Electrical Conductivity

The conduction of electrical current is impacted by the mineral elements, amino acids, and organic acids (such citric acid) found in bee honey. These compounds produce ions in honey aqueous solutions. Honey's EC is influenced by its levels of proteins, complex sugars, minerals, organic acids, and inorganic salts. Minerals burn honey and then become ash. It is said that colonies fed sugar syrup yielded honey with a reduced ash level. The EC increases with increasing ion and organic acid content [20]. EC is used to describe unifloral honey and to distinguish between blossom and honeydew honey. Blossom honey is defined as honey with an EC <0.8 mS/cm, while honeydew honey is defined as honey with an EC >0.8 mS/cm [21]. The color also affects conductivity; black honey has a higher conductivity.

4.8. Carbohydrates or Sugars

Honey's primary sugars are fructose and glucose, despite the fact that it contains 25 other oligosaccharides, such as sucrose, maltose, trehalose, turanose, panose, 1-ketose, 6-ketose, and palatinose. primary sugars found in honey are fructose and glucose, although it also contains 25 different oligosaccharides, such as sucrose, maltose, trehalose, turanose, panose, 1-ketose, 6-ketose, and palatinose [22]. The primary carbohydrates in honey, fructose and glucose, are rapidly absorbed into the bloodstream and utilized by the body for energy purposes; a daily intake of 20% honey is comparable to roughly 3% of daily energy requirements [23]. The types of sugars and their contents can be used to differentiate honey. For instance, Metcalfa honey, a new variety of honeydew honey made mostly in Italy, stands out from other honeydew honeys because of its high maltotriose content and distinct concentration of oligomers called dextrins.

As advised by CAC, 2001, adulteration of honey with syrups (such as maple syrup or sugar cane syrup) should be identified. The glucose to fructose ratio can be used to determine whether honey has crystallized because crystallization is characterized by a lower fructose concentration and a higher glucose content [24]. Honey samples that have been tampered with or heated excessively have a higher sugar content—eight percent. Sugar levels are found using the Fehlings test and the HPLC Carbohydrate Profile [25]. The honey's crystallization state is indicated by the fructose to glucose ratio. The honey has high rates of fluidity [26]. Honey crystals grow quickly when the ratio is equal to or less than 1.14, slowly when the ratio is larger than 1.3, and their value is decreased when the proportion is higher than 1.58 [27].

4.9. Diastase Content

One of the main enzymes in honey is diasterase (alpha and beta amylase), which is added by bees as they gather and allow blossom nectar to ripen. Honey's diasterase breaks down starch into short-chain sugars, and the enzyme's activity suggested that the honey may have been heated or stored improperly. Diastase activity is dependent on the product's freshness, floral origins, and geographic location, and it decreases as temperature and heating time rise [28]. Honey with low natural enzyme concentration, such as lemon honey, must have a minimum activity of 3 DN. One unit of diastase activity is defined as the amount of a-amylase necessary to convert 0.01 grams of starch to the required level in an hour at 40°C. This value is calculated using a spectrophotometer at 620 nm and given in Schade units per gram of honey (Diastase number DN).

4.10. Compositions of Minerals

Honey contains a variety of macro and microelements, including potassium, magnesium, calcium, iron, phosphorus, sodium, manganese, iodine, zinc, lithium, cobalt, nickel, cadmium, copper, barium, chromium, selenium, arsenic, and silver. These minerals will vary depending on the plant used to make the honey [12]. The most common mineral in honey is potassium, which accounts about one-third of the total [29]. Heavy metals (cadmium, lead, mercury, and arsenic) become harmful once the upper limit is exceeded.

is a rich source of macro and microelements, including potassium, magnesium, calcium, iron, phosphorus, sodium, manganese, iodine, zinc, lithium, cobalt, nickel, cadmium, copper, barium, chromium, selenium, arsenic, and silver. Depending on the honey's plant origin, these minerals will differ [12]. Potassium is the most common element and usually accounts for one-third of the minerals in honey [29]. Heavy metals (arsenic, mercury, lead, and cadmium) turn toxic if the upper limit is exceeded.

The WHO and FAO propose tolerable levels of 15 µg kg^{-1} arsenic, 25 µg kg^{-1} lead, 5 µg kg^{-1} mercury, and 7 µg kg^{-1} cadmium. Quantifying the hazardous mineral components in honey is essential for the environment, human health, and safety because there has been evidence of elevated mineral concentrations near industrial areas.

4.11. Amino Acids

While pollen is the primary source of honey's proteins, other sources include plant or animal debris, as well as fluids and nectar released by honeybee salivary glands. Therefore, honey's amino acid composition may reflect its botanical and geographic origins. Honeydew honey has a protein content of 3.0%, whereas flower honey has a protein content of 0.1% to 1.5%. Proline is the most prevalent amino acid in honey, accounting for 50–80% of all amino acids [30]. When honeybees convert nectar to honey, their salivary secretions contain proline.

Since proline levels in honey gradually decrease throughout storage, they may be an indicator of honey maturity. Tyrosine, glutamic acid, phenylalanine, alanine, leucine, and isoleucine are the other amino acids that are most commonly found in honey [31]. Other amino acids, such as glutamine, histidine, glycine, arginine, threonine, valine, methionine, cysteine, tryptophan, lysine, and serine, are in lower levels.

4.12. Vitamin-C

Honey has very little vitamin C and very little vitamin B complex. Nearly all varieties of honey contain vitamin C, and the amount and antioxidant power of this nutrient are determined by how the honey is processed, stored, and where it comes from botanically. Freshness of the honey sample is indicated by a low acidity value, whereas the fermentation of sugars into organic acids is indicated by a high acidity value. There are additional reports of variations in Vit. C resulting from plant sources [32]. Vitamin C measurement is an unstable indicator because it changes rapidly in reaction to light, air, and heat and is very vulnerable to chemical and enzymatic oxidation.

4.13. Phenols

Total phenolic content, which scavenges free radicals and has antibacterial and antioxidant properties, is a useful metric for evaluating honey's quality [33]. Folin Ciocalteu can be used as the reagent in the calorimetric method to determine phenols. Folin-Ciocalteu reacts with phenolic chemicals to generate a blue color, which can be quantified using gallic acid as a standard. Changes in the composition of honey through biochemical processes, climate, and beekeeping practices can all cause variations in the amount of phenolic content.

4.14. Acidity

Honey's acidity is caused by organic acids (citric, oxalic, tartaric, acetic, etc.), nectar, or bee secretions; a higher acidity value indicates the beginning of the fermentation process, which converts the alcohols it contains into organic acids; it also enhances honey's flavor, stability against microorganisms, chemical reaction, and antibacterial and antioxidant activity; a lower acidity value indicates the freshness of honey samples [34]. As honey ages, gets removed from combs that contain propolis, and especially deteriorates as a result of fermentation, its natural acidity may rise. Furthermore, the acidity of honey tampered with with sugar syrup is very low (less than 1), but the acidity of honey tampered with with inverted sugar syrup is higher. To determine how acidic honey is, titrate it against sodium hydroxide equivalents.

5. Factors Influencing the Quality and Composition of Honey

5.1. Seasonal Variations in Honey's Composition and Quality

The season, the biological type of plants, and the bees' selection behavior all influence their foraging activities [35]. Analysis of pollen and nectar from flowers, which are essentially the primary food supply for bees from larval to adult stages, can be used to manage bee colonies seasonally. Seasons affected the hue of the honey. The rainy-season honey was more diverse, with five distinct colors: black red, light brown, amber, and black, in contrast to the dry-season honey's black amber, amber, and dark brown hues.

5.2. Impact of Beehive Technology on Composition and Quality

Bee keepers often use both traditional and modern bee hives to produce honey [36]. Low-technology hives are designed to achieve the benefits of movable frame hives without the drawback of expensive manufacturing, and they can be stored close to home for inspection. The collected honey is of excellent quality and isn't contaminated by pollen or brood [12].

5.3. Harvesting's Impact on the Content and Quality of Honey

There are essentially two ways to gather honey: traditional (honey hunting) and modern procedures, which affect the honey's acceptability and quality and make it appear tampered with when displayed [37]. A mixture of ripe and unripe honey, dead bees, beeswax, and other detritus may be the consequence of honey hunting, but this does not always mean that the product is of low quality. Therefore, it is crucial to investigate the actual impacts of these harvesting techniques on the acceptability and quality of honey.

5.4. Impact of Storage and Processing on the Content and Quality of Honey

Honey treated with heat or thermal processing gets rid of microbes, slows down the fermentation process [38], makes processing and bottling easier, lowers the water content, dissolves the sugar crystal nuclei to delay granulation, and evens out the color of the honey. When honey is warmed, aged, or improperly stored, its HMF content—a measure of freshness—increases and its diastase activity falls. It is not recommended to heat honey above 70 degrees Celsius because this changes the honey's flavor, color, and granulation and breaks down its bioactive ingredients and oxidants [39].

5.5. Geographical Origin's Impact on the Quality and Content of Honey

The chemical composition of honey is significantly influenced by the type of nectar flow. The composition of honey may differ if it comes from different places but has the same flower origin. The geographical floral origin, season, ambient conditions, and beekeeper care all affect the properties and components of honey. Thakur et al. discovered that zone 2 (mid hills, sub humid zone) had the highest pollen density, pH, sucrose, HMF, amino acid, phenols, Ca, and K, while zone 4 (high hills, temperate dry zone >2200 m amsl) had the highest fructose, F:G ratio, acidity, vitamin C, and diastase [32]. According to research, honey from four distinct agroclimatic zones in Himachal Pradesh was well within tolerances, and pH was positively correlated with pollen density, moisture, and electrical conductivity.

5.6. Impact of Honey's Various Plant Origins

Plant based on honey quality and quality One of the main elements affecting the composition and quality of honey is its botanical origin [40]. Bees store the mixture in honeycomb to mature after combining the gathered plant chemicals with salivary gland secretions. The composition and quality of honey from different plants vary depending on their botanical origin (uniflora, multiflora, and honeydew honey). According to their physico-chemical properties, Schiassi et al. evaluated several types of laranjeira (Citrus sinensis), coffee (coffee spp.), silvestre and vassourinha (Baccharis spp.), bracatings (Mimosa scabrella), polyfloral, extrafloral, and honeydew honeys (Brazil) [41]. Every honey met the requirements for quality parameters. Compared to other honeys, sugarcane honey was more orange in color. The highest viscosity was shown by assa-peixe, laranjeira, and coffee monoflower honey, demonstrating the impact of moisture on this metric. The strongest antioxidant property is found in sugarcane, while the highest phenolic content is found in bracatinga. Extrafloral honey smelled of burn, and honey dew honey tasted tannic, boozy, and bitter.

5.7. Agrochemicals' Effects on the Quality of Honey

Because honeybees acquire pesticide residues, including acaricides, insecticides, organic acids, fungicides, herbicides, and bactericides, along with some metals and other chemicals, they are used to monitor environmental contamination [12]. Despite being sprayed on crops, these pesticides, herbicides, and fungicides get to bees through pollen, nectar, and the air, water, or soil. Since many of these pollutants are hazardous, they are outlawed. Bees instantly die when insecticides are sprayed on flowers while they are there. Other insecticides let the bees go back to their homes and perish. All ages of worker bees can be impacted by pesticides, but broods can be harmed by contaminated bee wax. The main source of toxicity is grayanotoxins, often referred to as andromedotoxins [42]. According to a study of pesticide residues, certain honeys had arsenic levels between 0.1 and 0.2 parts per million during the 75 years that inorganic pesticides were used. Remains of honey float from nearby locations. Numerous death bee incidents and ongoing honey contamination were caused by insecticide application, which led to the production of honey of poor quality. Every pesticide poses a risk, and some of them may cause chromosomal abrasions and are suspected carcinogens.

Pesticides are also known to alter the neurological, endocrine, and reproductive systems. To guarantee the product's quality and safety, pesticide residues must be regularly monitored. The concentrations of all pesticide residues were below the EU's suggested maximum residual limits, or limit of quantification (LOQ), which is 0.05 mg kg^{-1} in honey (0.01 PPM).

6. Adulterants

6.1. Impact of Adulteration on Honey's Composition and Quality

Adulteration can be achieved in a number of ways, including by incorporating commercial sugar syrups into honey or by overfeeding honey bee colonies with these commercial industrial sugars during the major nectar flow season. Excessive sugar use affects honey's proline, mineral matter, and sugar concentration, according to multiple studies [12].

6.2. Adulterants Found in Ethiopian Honey

Adulteration of honey with foreign compounds, including starch solution, molasses, glucose, water, inverted sugar and sucrose, occurs worldwide. In Ethiopia, many writers have reported various adulterant materials from various regions, as indicated in **Table 2**. By heating sugar with honey and then adding sugar powder to the honey, honey can be adulterated. The most common way to tamper with honey is to mix, homogenize, heat, or melt adulterant materials with pure honey [43]. Additionally, Meseret Gemeda and Taye Negera's studies revealed that the main ways to adulterate honey and beeswax are by boiling sugar with honey, adding crushed or powdered sugar directly to the honey, and melting animal tallow with wax [3].

6.3. Harmful Effect of Adulterated Honey

For many people who are unable to digest ordinary cane sugar, pure honey, sometimes referred to as natural honey, is the greatest substitute sweetener because it is a predigested food. It might come from plant sap that is digested in the bee's stomach or nectar found in flowers. Eighty percent of it is made up of simple sugars that the digestive system can easily break down, such fructose and glucose. As a result, it enters our bloodstream directly and is immediately transformed into energy. About 200 compounds, including water, minerals, vitamins, proteins, enzymes, phenolic acids, and antioxidant qualities, were identified in pure honey in addition to simple sugar [43].

The primary components of all honeys are nearly the same, however the precise makeup may differ depending on the type of plant the bee forages on [44]. Numerous studies demonstrated that the honey's various compositions worked in concert to provide a number of health benefits rather than just one [43]. Honey's safety and quality are changed by adulteration. For example, honey that has been chemically tampered with reduces its therapeutic efficacy and may be harmful to users [45].

Rats given adulterated honeys satisfied all the requirements for obesity and hyperglycemia, including higher body weight gain, fat pads, BMI, and levels of triglycerides, cholesterol, and glucose, in contrast to rats given pure honey, which markedly reduced these parameters [43]. This suggests that consuming adulterated honey causes obesity and possibly diabetes. In addition to obesity and diabetes, prolonged use of tainted honey has been shown to cause serious toxicity, as seen by elevated levels of the kidney and liver marker enzymes total bilirubin, urea, total protein, and creatinine, as well as anomalies in the weight of the organs.

Numerous studies demonstrate that pure honey has remarkable therapeutic benefits by lowering excess body weight increase and other indicators of diabetes and obesity, rather than being poisonous. Adulterated honeys, on the other hand, exhibit opposing effects that are detrimental to the body and may cause a number of illnesses [43]. The reason is that in addition to simple carbohydrates like fructose and glucose, pure honey also contains additional nutrients that are vital to our health, like proteins, antioxidants, and minerals. One issue that has a detrimental impact on the treatment of human folk medicine is the adulteration of honey. Therefore, contaminated honey poses a risk, particularly to diabetics. Additionally, adulteration undermines consumer confidence, which hinders industry expansion.

Table 2. Typical Adulterant Components in Ethiopia by Area [43].

Location/area	Adulterant substance names
Arsi	Pollen, empty combs, molasses, sugar, matured bananas, wheat flowers, potatoes, maize flowers, melting sweets, an d hot water
Bahirdar	sugar or invert
Gedo	sugar syrup, sweet potato, banana, and/or corn and/or wheat flour syrup
Adigrat and surrounding area	Water, sugar, and banana
Eastern Tigray	Banana, sweet potato, corn and/or wheat flour syrup, and sugar syrup
Oromia region	Molasses, candy, and sugar Cumber, orange, banana, and sugar

7. Toxicological Implications of Honey Contaminants

Honey contamination or adulteration with harmful substances such as hydroxymethylfurfural (HMF), heavy metals, and pesticide residues poses toxicological threats that compromise consumer safety. These compounds can induce genotoxic, neurotoxic, or carcinogenic effects, especially when ingested chronically or at concentrations exceeding regulatory limits.

7.1. Hydroxy Methyl Furfural (HMF)

HMF is a thermal degradation product of fructose that forms during prolonged storage or overheating. Elevated HMF levels in honey are indicative of excessive heating or adulteration with invert sugar syrups. Toxicologically, HMF has demonstrated cytotoxic, genotoxic, and hepatotoxic effects in animal and cell line studies [46,47]. Zhang et al. showed that HMF causes oxidative stress and mitochondrial dysfunction in hepatocytes [48]. Additionally, El Sohaimy et al. emphasized the risk of HMF for infants due to underdeveloped detoxification pathways [49].

The Codex Alimentarius prescribes a maximum of 40 mg/kg HMF in general and 80 mg/kg for tropical honeys. However, some commercial honeys surpass these limits due to poor processing or storage [50].

7.2. Heavy Metals (Pb, Cd, Hg, As)

Heavy metals may enter honey through environmental pollution, contaminated equipment, or beekeeping practices. Lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) are particularly concerning due to their bioaccumulative nature. Chronic Pb exposure can impair neurodevelopment in children, and Cd is a recognized human carcinogen according to IARC [51,52]. Abbas et al. found Pb levels exceeding 0.2 mg/kg in 12% of honey samples from urbanized areas in South Asia [53].

The EU and Codex Alimentarius recommend maximum levels of 0.10 mg/kg for Pb and 0.05 mg/kg for Cd [8]. Studies by Rao et al. and Mohammad et al. have shown elevated levels of these metals in honey from industrialized regions [51,54].

7.3. Pesticide Residues

Honey can be contaminated with pesticides when bees forage in treated areas. Neonicotinoids (e.g., imidacloprid), organophosphates (e.g., chlorpyrifos), and pyrethroids are commonly detected. These compounds may induce endocrine disruption, neurotoxicity, and carcinogenicity with repeated exposure [55,56]. Alves et al. reported imidacloprid concentrations near or above the MRLs in 22% of samples from high-intensity agricultural zones [57].

MRLs for pesticides in honey range from 0.01–0.05 mg/kg, depending on the compound and region [56]. Bera et al. found that 8% of children consuming honey in their study had a hazard quotient greater than 1, suggesting potential non-carcinogenic risk [58].

8. Analytical Techniques for Detection of Adulteration and Contaminants

The detection of adulterated or unsafe honey has become increasingly sophisticated with advancements in analytical chemistry and food authenticity testing. Modern techniques provide higher specificity, sensitivity, and reliability in identifying both intentional adulterants and unintentional contaminants.

8.1. Liquid Chromatography–Mass Spectrometry (LC-MS/MS)

LC-MS/MS is widely used for the detection of antibiotic residues, pesticide contamination, and sugars from non-honey sources. Its high sensitivity and selectivity enable multi-residue screening in complex honey matrices [59,60]. For example, Rezende et al. successfully applied LC-MS/MS to detect neonicotinoid pesticides in honey with limits of quantification below MRLs [61].

8.2. Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR-based profiling has emerged as a robust non-targeted method for honey authentication. It allows for simultaneous quantification of sugars and detection of atypical compounds introduced through adulteration. Recent studies show that ^1H-NMR, combined with chemometric models, can differentiate floral origins and identify adulterated samples with over 95% accuracy [62,63].

8.3. Stable Isotope Ratio Analysis (SIRA)

SIRA, particularly using ^13C/^12C ratios, is an established technique to detect C4 plant sugars (e.g., corn syrup) added to honey. The method is endorsed by international standards (AOAC 998.12) for authenticity testing. Isotope ratio mass spectrometry (IRMS) is commonly used to perform this analysis [64].

8.4. Fourier Transform Infrared Spectroscopy (FTIR) and Raman Spectroscopy

FTIR and Raman spectroscopy are rapid, non-destructive tools gaining popularity for screening adulterated honey. When combined with multivariate statistical techniques such as PCA or PLS-DA, they can detect adulteration levels as low as 5% [65]. These methods are increasingly favored in regulatory and field settings for their minimal sample preparation requirements [66].

8.5. DNA-Based Methods and ELISA

DNA barcoding has recently been used to identify plant and pollen origin, providing insights into honey authenticity. ELISA-based kits are employed for rapid screening of specific antibiotic or pesticide residues and are useful in large-scale monitoring programs [67,68].

8.6. Method Validation and Regulatory Use

Most of these techniques undergo validation per international standards such as ISO/IEC 17025, ensuring accuracy, precision, and reproducibility. Their application in food control laboratories is critical for risk assessment and regulatory enforcement.

9. Regulatory Oversight and Global Standards on Honey Safety

Regulatory frameworks are essential for safeguarding public health by ensuring that honey products meet safety and quality criteria. These frameworks set compositional standards, establish residue limits, and prescribe validated testing protocols to prevent unsafe or fraudulent honey from reaching consumers.

9.1. Codex Alimentarius

The Codex Alimentarius, developed by the FAO and WHO, provides international benchmarks for honey quality and safety under the Codex Standard for Honey (CXS 12-1981, revised 2021). It specifies maximum limits for moisture (≤20%), HMF (≤40 mg/kg for most honey; ≤80 mg/kg for tropical honey), and impurity levels. It also endorses isotope ratio methods to detect adulteration with C4 sugars [69,70].

9.2. European Union (EU)

The EU regulates honey under Directive 2001/110/EC, revised in 2022, which stipulates that honey must be free from additives and external sugars and should preserve natural enzymes. Residue limits are defined under Regulation (EC) No. 396/2005. The EU also limits heavy metals such as lead (≤0.10 mg/kg) and cadmium (≤0.05 mg/kg) in honey [71,72]. A 2023 update has reinforced analytical testing using NMR and DNA-based methods to enhance authenticity verification [73].

9.3. United States – Food and Drug Administration (FDA)

Although the U.S. does not have a binding federal standard of identity for honey, the FDA has issued draft guidance to define honey and prevent misbranding. It also oversees contaminant levels under the Pesticide Residue Monitoring Program and enforces action levels for toxic compounds such as lead and chloramphenicol [74,75].

9.4. India – Food Safety and Standards Authority of India (FSSAI)

The FSSAI defines honey standards under the Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 2011 (updated in 2021 and 2023). These include thresholds for sucrose (≤5%), reducing sugars (≥65%), moisture (≤20%), and HMF (≤80 mg/kg) [76]. The agency now mandates isotope ratio mass spectrometry (IRMS), nuclear magnetic resonance (NMR), and sugar syrup markers to detect adulteration [77]. Following high-profile cases of adulteration, FSSAI has introduced batch traceability, third-party lab accreditation, and stricter export rules [78].

9.5. Global Harmonization and Challenges

Despite significant regulatory advances, challenges persist due to variability in analytical standards and detection thresholds across countries. Inter-laboratory validation and harmonization of analytical methods such as LC-MS/MS and IRMS remain ongoing priorities [69,73].

9.6. Prospective research and suggestions

Major issues brought on by adulteration include loss of market share, health issues, consumer attrition, price reductions, and decreased satisfaction. While breakability, homogeneity, and scent are concluded to be the physical identification techniques of adulterated beeswax, dissolving in water and continuous flow demonstrate good results for the physical identification methods of adulterated honey. The body in question should get the information provided by this work, and more research on the methods and procedures utilized to identify adulterated honey and beeswax physically is advised. In particular, the suggestions that were sent along are as follows: strict national laws enacted pertaining to the apiculture industry to prevent the needless mixing of adulterants with honey; Reducing honey adulteration can be achieved by teaching beekeepers and other stakeholders on acceptable manufacturing techniques, monitoring, and how to handle and identify adulterated honey; It should be encouraged to conduct more study on the effects of adulterating honey on humans and alternative control systems. Creating straightforward, reasonably priced methods that may be applied with a high degree of repeatability to identify adulterated honey; additionally, to ensure the safety of honey, health authorities worldwide must enact strict laws and regulations governing its production, handling, and analysis.

10. Conclusions

Honey, with its nutritional benefits and immune-boosting properties, is an essential product, especially during the current pandemic. Its quality is influenced by various physical and chemical components, making them critical to the honey industry. Improving awareness and capacity among beekeepers is key to enhancing honey quality. As honey demand grows, understanding these quality factors ensures that the honey consumed meets necessary standards. However, honey is susceptible to contamination and adulteration, often due to poor beekeeping practices and inadequate training. This issue is particularly prominent in developing countries like Ethiopia, where honey adulteration with substances such as sugar, molasses, and syrups is common. Therefore, collaboration between scientific communities and regulatory bodies is vital to develop, implement, and promote better detection methods for adulterated honey, ensuring its quality and protecting consumer health.

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Conflicts of Interest

The author declares no conflict of interest.

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