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Honey

Composition and Properties

*Edited by Muhammad Imran,
Muhammad Haseeb Ahmad
and Rabia Shabir Ahmad*



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and Rabia Shabir Ahmad*

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Preface

Even in this age of digitalization and technological advancements, natural food items are highly sought after and consumed. Among various natural products, honey stands alone as a supersaturated solution of sugars with a complex chemical composition prepared by honeybees in a beehive. The chemical composition of honey is dependent on beekeeping practices, climatic conditions, type of honeybee species, and botanical source. It is a complex mixture of numerous types of bioactive components such as phenolics, enzymes, organic acids, peptides, vitamins, minerals, and antioxidants that have beneficial health effects in combatting various diseases and cancers and in wound healing. Honey is considered the world's oldest medicine and is being studied as a therapeutic agent in modern medicine due to its inhibitory effect against microbial infections. Additionally, it acts as a natural preservative in many food applications. There is a plethora of literature that describes the physicochemical, geographical origin, and authentication of honey in this modern era. This book focuses on honey production in beehives and provides information on honey's geographical, entomological, and botanical origins. It also examines modern beekeeping technologies and honey's therapeutic potential to combat various maladies. Furthermore, the book describes the utilization of honey in processed food items.

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Section 1

Honey and Its Authentication
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Chapter 1

Honey Composition, Therapeutic Potential and Authentication through Novel Technologies: An Overview

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Abstract

Honey is acknowledged as a natural functional food with additional health benefits. Due to its medicinal and therapeutic properties, honey is being used in both pharmaceutical and food industries to develop products as a remedy against various types of ailments. Honey contains polyphenols, flavonoids, and other key compounds that play an important role in human health. Honey possesses anticancer and antimicrobial properties as well as contains antioxidant and anti-inflammatory substance. Some studies also highlighted the antidiabetic properties of honey. It supports the respiratory system and contributes beneficial effects to the cardiovascular system. As a functional and nutraceutical food, honey plays a significant role. Due to the modernization and digitalization in this era, the role of novel technologies for characterization and authentication of honey cannot be ignored. Hence, the main purpose of this chapter is to review the latest studies related to honey's advantageous effects on human health and to highlight the novel technologies to detect the impurities in honey.

Keywords: honey, composition, therapeutic potential, novel technologies, authentication

1. Introduction

Honey is an ordinary product that can be found in nature and is the only concentrated sweetener. The composition of honey is thoroughly associated to its environmental conditions and botanical sources. Bees accumulate honeydew and transfer it into the mass, where enzymes act upon the sugars and transform these sugars into

honey. In several countries, it has been used as a treatment of diseases for several centuries. It possesses the great potential or purpose of healing wound and burn injury. In addition to this, it has been recognized to be very effective in virtually all cases of infection [1]. Honey is also known as a natural sweetener with additional health benefits. It is frequently used as an important constituent of herbal medicines and considered as a nutraceutical agent. Since the last 1500 years, honey has been utilized in food and medicinal products [2].

In the past, honey has been utilized for medicinal and therapeutic purposes in a variety of cultures. Ancient Greeks have used it as cure for wound, fever, gout, and pain, and ancient Egyptians used honey for mummifying their deceased and also for the purpose of wound dressing as a topical balm [3]. The medicinal effect of honey is due to the manifestation of different antioxidant compounds, including phenolic acids and flavonoids. Various studies observed the antidiabetic, anticancer, and antimicrobial action of honey. The defensive impact of honey on cardiovascular, gastrointestinal, pulmonary, and nervous systems has also been demonstrated. Due to the excellent bio-functional properties of honey, many industries and local suppliers are adulterating honey by adding artificial sweeteners. In recent decades, the rate of adulteration of honey has increased in both developing and underdeveloped countries. Researchers and scientists have been using various new technologies to test honey adulteration like spectroscopic techniques, electronic tongue, microscopic analysis, immunoassays, and thixotropicity [4].

Honey contains protein contents, carbohydrates, pigments, moisture contents, enzymes, vitamins and minerals, phenols, and minor amounts of bioactive compounds such as carotenoid content, proline level, total flavonoid content, salicylic acid, naringin, and taxifolin. Normally, honey comprises about 80% of carbohydrates and 20% of water [5]. In all religious books, honey has been mentioned as an important food and medicine. It was not renowned as a significant therapeutic agent regardless of its extensive history or as beneficial medicine due to its wide range of activity and inadequate understanding of its properties. Now, honey is rapidly becoming a part of the pharmaceutical industry, and research is being done to check the medicinal properties of honey [6].

2. Sources

Manuka, *Sidr*, *Jelly bush*, *Pasture*, *Jungle*, and *Sumra* are common varieties of honey because of different botanical sources and environmental conditions [5]. Due to its wide range of antibacterial efficiency, *Manuka* honey has had key reputation. This beneficial *Leptospermum* sp. originates from Australia and New Zealand. In the pharmaceutical industry, this medical-grade honey has been effectively used to treat a wide range of diseases [7]. Almost 500 different species of honeybee have been found in Africa, America, Australia, and Southeast Asia. These varieties can be classified into *Melipona* and *Trigona*, the two main genera of honeybees. The *Melipona* genus is generally in majority than the common bee *Apis mellifera* [8].

Apis florae, *Apis cerana*, *Apis dorsata*, and *Apis mellifera* are famous species of honeybee found in Pakistan. *A. mellifera* is found in different parts of Pakistan, especially in KPK, and is one the most common in terms of quality of honey [9]. There are two primary floral sources of honey in Oman: *Ziziphus spina-Christi*, also known as *Sidr* locally, and *Acacia tortilis*, also known locally as *Sumera* [10].

3. Chemical composition of honey

Lim et al. [11] investigated the nutritional makeup of honey from various botanic sources. Results showed that the moisture content of honey ranged from 27 to 31 g/100 g of honey. The ash level of honey samples that were analyzed ranged from 0.15 to 0.90 g/100 g. Protein content was found to range from 0.2 to 0.8 g/100 g of honey. The carbohydrate level of honey samples was analyzed and found between 67.58 and 72.25 g/100 g. Another experiment conducted to examine the nutritional conformation of honey from various botanic sources. Results showed moisture around 18–19%, fructose 45–48%, glucose 29–31%, sucrose 2–4%, total sugar level (glucose, fructose, and sucrose) 77–82%, protein level (mg/kg) 0.76–0.80 mg, and ascorbic acid content 0.22–0.27 mg of honey [12].

Santos-Buelga and González-Paramás [13] hypothesized in 2017 that the chemical makeup of honey is influenced by its botanical source and place of origin. Due to abundance in sugar composition, honey is identified as a naturally sweet product. The composition is different depending on floral sources and processing along with certain natural factors.

3.1 Carbohydrates

The most profuse sugars are monosaccharides, which include fructose and glucose that comprise nearly 70% of the total sugar content in honey. The enzyme invertase present in honey is involved in the hydrolysis of sugars from nectar, resulting in the production of monosaccharide fructose and glucose [14]. Rest of sugars comprise 10–15% of the total sugar content of honey including disaccharides and trisaccharides. A daily dosage of 20 g of honey can provide 3% of one's daily energy needs [15]. In addition to fructose and glucose, honey is also known to contain oligosaccharides, maltose, isomaltose, maltulose, gentiobiose, kojibiose, laminaribiose, nigerose, and kojibiose. Furthermore, honey contains 4–5% fructo-oligosaccharides. Fructo-oligosaccharides are a good source of prebiotics that can support the digestive system, as they are indigestible. They also support the microbiota of intestine [16].

3.2 Proteins

The rough estimate of protein is about 0.5%, which includes amino acids and enzymes; however, the contribution of these proteins in meeting daily requirements is marginal. The main enzymes are amylase, invertase, and oxidase [17]. Proline, which makes up 80–90% of all the amino acids in honey, is the main amino acid found in it. In addition to this, other amino acids are also present (both essential and nonessential) alongside proline in honey [18].

3.3 Vitamins and minerals

Potassium (K) is the main mineral found in honey, with amounts ranging from 0.1 to 1.0%, followed by magnesium (Mg), sodium (Na), sulfur (S), calcium (Ca), and phosphorus (P). Other than these, trace minerals include copper, manganese, iron, and zinc. Some vitamins are also present including vitamin B6, pantothenic acid, B2 complex, thiamine (B1), vitamin C, nicotinic acid, and riboflavin [19, 20].

Bioactive compounds	Amount	Reference
Carotenoid content	0.6–6.2 mg/kg	[25]
Proline level	4.6 mg/kg	[26]
Total phenolic content	1.3–126 mg (GAE)/100 g	[25]
Total flavonoid content	1.9–4.2 mg (QE)/100 g	[26]
Salicylic acid	8.2–94.8 µg/100 g	[27]
Naringin	4–32 µg/100 g	[27]
Taxifolin	12–1920 µg/100 g	[27]

GAE: gallic acid equivalent QE: quercetin equivalent.

Table 1.
Bioactive compounds in honey.

3.4 Polyphenols

Several polyphenols have been found in honey during the last few decades. According to a study, several varieties of honey contain 56–500 mg/kg of polyphenols. The major flavonoid in honey is chrysin, pinobanksin, and pinocembrin, while the minor flavonoids include kaempferol, galangin, quercetin, and isorhamnetin [21, 22]. An average amount of phenylacetic acid, leptosin, methyl syringate, and methoxyphenylacetic acid were found. Other constituents include various 1,2-dicarbonyl compounds [23].

3.5 Other components

Honey also contains polyphenols and organic acids like acetic acid, butyric acid, and citric acid [24]. The key flavonoids in honey are chrysin, pinobanksin, and pinocembrin, while the minor flavonoids include kaempferol, galangin, quercetin and isorhamnetin [21]. Over 600 different volatile compounds have been found in honey as shown in **Table 1**.

Gallic acid, pimaric acid, and pimaric acid isomers were discovered in the cerumen of stingless bees, which demonstrated the powerful antioxidant effect of honey [28]. According to Souza et al. [26], gallic acid is the most prevalent phenolic compound found in the Brazilian species *Apis mellifera*, with lesser levels of cinnamic, protocatechuic, quercetin, and p-coumaric acids being found.

4. Therapeutic properties of honey

4.1 Antioxidant effect

DPPH technique was used to check antioxidants in honey since it is a simple, exact, and precise technique [29]. The IC₅₀ constraint, which addresses the concentration of the material necessary to hinder half of the free radicals, was used to resolve the antioxidant assessment in light of the scavenging activity against the free radical DPPH. As a result, honey's lower IC₅₀ value indicates that it contains more antioxidants and has a greater ability to neutralize free radicals. For Sumer testing, honey samples

had antioxidant levels between 7.8 and 48.6 mg/ml; for *Sidr* tests, between 33.8 and 72.3 mg/ml; and for multiflora tests, between 91.2 and 190.1 mg/ml. According to their more obscure variety and greater phenolic content, *Sumer* honey had the highest antioxidant levels of all the honey samples. A survey described the antioxidants (IC50) in *Acacia* from different countries; their quality ranged between 10.5 and 111.1 mg/ml. Comparative results were published in the study. Also described were the multiflora honey's antioxidant levels, which ranged from 4.4 mg/ml in Croatian honey to 358 mg/ml in Czech honey. Iranian honey had an IC50 of 5.9–89.7 mg/ml compared to 84.9–168.9 mg/ml for Portuguese honey [30].

4.2 Antimicrobial effect

Honey shows antibacterial action against several bacteria in different environments. Honey has exceptional antibacterial effect against MRSA and several varieties of *Pseudomonas*, which are frequently linked with burn and wound infections. Manuka honey shows efficiency against several pathogenic microorganisms, including *S. aureus*, *Salmonella*, *Enterobacter erogen*, and *Escherichia coli* (*E. coli*) [7]. Honey's antimicrobial properties are mostly related to its acidity, osmolality, glucose oxidase enzyme, and production of hydrogen peroxide. Gluconic acid is formed from glucose by this enzyme; hydrogen peroxidase is also produced as a by-product in this reaction. Hydrogen peroxide is responsible for antimicrobial activity against bacteria [31].

4.3 Anti-inflammatory effect

Quantitative analysis of total phenolics, flavanols, and flavonoids was conducted through HPLC for the determination of radical scavenging activity and the anti-inflammatory activity through in vitro studies. The phenolics were found to be 663.22 mg of the gallic acid per 100 g, while the flavanols and flavonoids were found to be 3.16 and 3.61, respectively [32]. An experiment demonstrated the effect of honey on reepithelialization through various cellular responses when loaded with nano-fiber technology, which resulted in it showing antioxidant and anti-inflammatory properties. Further, the assay of markers including interleukins and cyclooxygenases confirmed its role in anti-inflammatory action [33].

4.4 Wound healing

A study was carried out to find the effect of a hydrogel prepared by using honey on the healing of wounds and antimicrobial activity. The gel was used in different concentrations and showed 75% antimicrobial activity and in vivo healing of burns in mice. The result was surprisingly amazing as this gel was 75% more effective than the commercial gel used for burns [34].

In another investigation, the intrinsic production of hydrogen peroxide was used to identify the mechanism involved in wound healing. The human keratinocyte cell lining was used, which showed that H_2O_2 through a specific aquaporin moves to the plasma membrane where it induces the entrance of extracellular calcium through a receptor and the Orai-1 channel due to calcium-ion-channel redox regulation and hydrogen peroxide production. The calcium route is involved in tissue regeneration during wound healing [35].

4.5 Antiulcer

A common disease affecting humans. A study was done to find out how honey affected artificially induced gastric ulcers. The mechanism was determined by using four groups of rat model through the examination of stomach macroscopically. The result showed that there was a reduction in mucosal NO, GSH, lipid peroxide, and superoxide dismutase (SOD). Honey significantly decreased the ulcer index, prevented lesion formation, preserved the stomach's glycoprotein content, and decreased plasma levels of IL-6 and TNF-alpha. Honey exerts its antiulcer effect due to certain enzymatic and nonenzymatic antioxidants and by reducing cytokine levels in the body [36].

Honey has also a protecting effect on oral ulcer. Hence, a study was conducted to determine an effective method for using honey. For that, three groups were created with one as control, while the other two were given honey, to one in adhesive form and to the other in gel form. Although there was complete healing in both of the groups, microscopically a significant difference was observed, with the gel having a higher mean value. So, it could be concluded that the therapeutic value of gel is more than that of the adhesive form in wound healing [37].

4.6 Antidiabetic

To ascertain the impact of honey consumption on individuals with type 1 diabetes, a randomized crossover trial was done. Twenty patients of 4–18 years of age were taken and were given a dietary intervention of honey in an amount of 0.5 ml per kg/day for 12 weeks. The research resulted in significant decline in the skinfold thickness, total cholesterol, fasting serum glucose, serum triglycerides, and LDL and elevation of C-peptide, thereby suggesting that a long-term use of honey may have a great impact on reducing type 1 diabetes [38]. Another randomized trial conducted on type 2 diabetes resulted in increased HbA_{1c} but decreased anthropometric measures [39].

4.7 Anticancer

Human cervical and breast cancer cell lines, as well as normal breast epithelial cell lines, were treated with honey for 72 h to study the anticancer potential of honey. This resulted in elevated lactate dehydrogenase, increased apoptosis in cancerous cells, decreased mitochondrial potential, and the activation of caspases 7 and 9, thus indicating mitochondrial-based apoptotic pathway in human cervical and breast cancer cell lines [40]. A clinical experiment demonstrated honey's effectiveness against head and neck cancer; following 6 weeks of radiation therapy, the honey group saw a lower proportion of oral mucositis than the control group [41].

4.8 Cardioprotective effect of honey

Heart is an important organ of the body. A study determined the effect of chronic intake of honey on cardiac arrhythmias in rat heart; honey was fed to the rats for 45 days; then, after giving anesthesia, their hearts were separated. The result of the ECG test showed that honey significantly declined the ventricular tachycardia and time of reverse ventricular fibrillation [42]. Honey also affects the HDL, LDL, VLDL, and total cholesterol, thus reducing the risk of heart disease. An animal trial showed a decrease in serum LDL and an increase in HDL, VLDL, and TG in comparison with

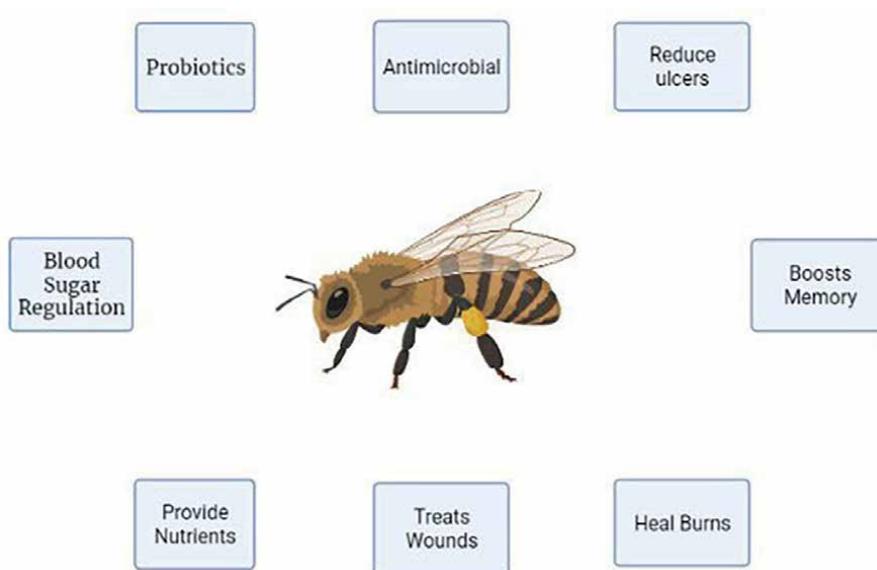


Figure 1.
Nutritional properties of honey.

the control, concluding that the honey can improve lipid profile and decrease the cardiac disease risk (**Figure 1**) [43].

5. Honey as a functional food

Honey can play a useful part in the food industry as functional and nutraceutical substance. Honey-based edible coatings are being utilized in the food industry for fruit preservation because of the antimicrobial and dehydrating properties of honey [44]. Honey can be used as treatment for coughs and sore throat when mixed with lime juice. Research showed the beneficial impact of honey in the food industry. Honey can impart desired characteristics like texture, flavor, and color, especially in pastes, butters, and spreads. Honey is being used in the baking industry as a natural sweetener because of its unique flavor and supportive chemical properties. It would improve the appearance, nutrition, and taste when use in food production [45].

Honey is one of the most important functional foods, which contains protein, minerals, and vitamins. It maintains the face and skin while strengthening memory and the immune system. The sweetness level of honey is higher than sucrose (table sugar) because honey captures its sweetness from glucose and fructose. Honey imparts 33 more calories as compared to sucrose. Due to the presence of oligosaccharides, honey can individually support the gut microbiota by accelerating the growth of probiotic *bifidobacteria* and *lactobacilli*, consequently improving host metabolic relationship [46].

Ramya and Anitha [47] prepared muffins with the addition of honey along with regular sugar. Results showed that there is increase in volume of muffins containing honey as an ingredient. The organoleptic properties of muffins exhibited substantial variation in different parameters like color, flavor, texture, and their overall acceptability. Results showed momentous variation of muffins relieved with honey as linked to control. Spray drying of sweeteners is also an emerging technology in the food sector.

Honey is also produced as spray-dried honey. Using honey as natural sweetener, numerous products are being developed in food industries. Deneesha Madunimani et al. [48] developed a cinnamon-based ready-to-eat drink using honey as sweetener.

6. Novel techniques to detect impurities in honey

For several decades, natural honey of bee has been the topic of research purpose. However, with its biological, prophylactic activity and diversity, and nutritional properties, it still surprises scientists. As a result, techniques for assessing its quality are continuously improved and changed in an effort to dispense with costly and dangerous reagents, speed up analysis, improve accuracy, and lighten the workload. Many scientists are looking for methods that will make it quicker and easier to find contaminants in honey [49].

Adulterants in honey are typically discovered using physicochemical techniques. Chemical analyses using diastase, fructose, sucrose, glucose, and HMF can be used to detect the adulteration of honey by cane sugar syrup, invert sugar syrup, and crystallized cane sugar. Physicochemical characteristics of the honey, including color, moisture, fructose, free acidity, electrical conductivity, glucose, sucrose, and HMF, can be used to classify it geographically [50]. Because detecting honey adulteration is difficult, new adulterant detection technologies are continually being developed.

6.1 Spectroscopic techniques

Many adulterants in food can be detected using infrared (IR) spectroscopy, which is considered superior than other approaches. There is little to no sample preparation required, and just a small number of samples are needed for analysis. Additionally, the technique is regarded as simple-to-perform, nondestructive, quick, and inexpensive. Because of this, the technique may be portable and enable on-site analysis of adulterants in honey. Another alternative for on-site use is Raman spectroscopic analysis, which is nondestructive and requires little sample preparation. The apparatus can be made portable and is comparable to IR spectroscopy in terms of cost, simplicity, and speed. An advantage over IR is that there is no fluorescence interference on the samples [51]. In addition to this, fluorescence spectroscopy may also be an important candidate for the authentication of the honey based on the fluorophores that provide the excitation and emission bands at specific wavelength combination.

6.2 Electronic tongue

Food for mankind is judged by our senses, which assist us in determining the product's acceptability and quality. Emerging technology known as "biomimetics" will further research by simulating human senses to create things like an artificial tongue. Although an electronic tongue has been used in numerous food assessments, only a few research have used it to analyze honey. For instance, electronic tongue was utilized to research the botanical and geographic sources of honey, as well as the physicochemical characteristics of both pure honey and honey adulterants [52, 53].

6.3 Microscopic analysis

Microscopic analysis is used to find adulterants as well as identify the botanical and geographic origins of honey. It is more accurate to combine microscopic analysis

for adulterants with additional procedures such as PCR, HPLC, and physicochemical analysis. Particularly in developing countries where alternative technologies are prohibitively expensive, microscopic methods may be helpful [54].

6.4 Immunoassays

An analytical method known as an immunoassay relies on the idea of immunology and is based on the interaction of an antigen and an antibody. The body produces an antibody, a glycoprotein, in response to exposure to an antigen, which is a foreign body. In a favorable environment, these antigens trigger the production of antibodies. Immunoassay is a technique for detecting foreign entities (antigens) in a sample matrix, which can be proteins or smaller molecules. A new method of checking contaminants has been developed based on enzymes and honey proteins [54].

6.5 Thixotropicity

Thixotropy, viscoelastic and flow behavior, creep, shear stress, crystal formation, and nitrogen concentration can all be used to identify carbohydrate adulterants like sucrose syrups, fructose, and glucose. The ability to identify adulterants is influenced by the honey's solubility, temperature, and length of storage. The sensitivity of adulterant detection utilizing viscoelastic behavior is unknown, despite the fact that it is effective in identifying the presence and absence of carbohydrate adulterants in honey. More improved detection technologies are necessary for quantification. More research is required to determine the thixotropicity of honey with other adulterants before choosing a method that would be considered viable for a honey adulterant kit. After establishing a carbohydrate adulterant detection tool, glucose, sucrose syrup, and fructose adulterants could be recognized [55].

7. Conclusion

Honey can be considered a natural antioxidant medicine, and it is one such promising nutraceutical antioxidant. The phenolic compounds, which include polyphenols and flavonoids, are involved in preventing or lowering the risk of a number of human disorders, including ulcers, tumors, chronic inflammation, diabetes, cancer, and cardiovascular diseases. Studies show the beneficial effect of honey in wound healing. In the food industry, honey is used as a functional and nutraceutical ingredient in the preparation of novel food products. Some novel technologies like spectroscopy, electronic tongue, microscopy, immunoassays, and thixotropicity may be considered helpful to detect adulterants in honey. This chapter provides significant indications about the use of honey in both food and medical sectors.

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Chapter 2

Brazilian Honey and Its Therapeutic Properties

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Abstract

Honey is an important food and therapeutic product of beekeeping work. In this chapter, our objective is to present different types of Brazilian honey produced in different regions of Brazil by farmers and traditional communities, and how this variety of honey products are used therapeutically by the population to treat some diseases. We mention orange honey, eucalyptus honey, urucu honey, borá honey, vine-grape honey, jataí honey, chestnut honey, and cashew honey. Many of them have been used by the traditional Brazilian community to treat some diseases. According to research, some of these honeys have been shown to have natural compounds that interact with cellular targets and promote therapeutic action.

Keywords: Brazilian honey, therapeutic properties, natural products, bee

1. Introduction

Beekeeping is an activity widely explored by rural people because it is an alternative that generates profit, needs little maintenance, and has a low initial cost compared to other agricultural practices [1]. It also contributes to maintaining and preserving existing ecosystems and does not cause environmental impact [2]. Brazil is considered to have tremendous apicultural potential for being a vast country and for having a diversified flora [3]. It also has a varied climate that enables honey production throughout the year, differing from other countries (China, United States, Argentina, Mexico, and Canada) that only produce once a year [4]. *Honey* is a natural food produced through the nectar of flowers [5]. It has great nutritional value, so it is widely used for medicinal purposes, as it contains essential sources of energy and nutrients beneficial to health [6].

Honey is attributed to several medicinal properties besides its quality as food. Although man has used honey for therapeutic purposes since ancient times, its use as a unique food with special characteristics should be the main attraction for its consumption. Unfortunately, the Brazilian population generally does not see it this way, considering it more as a medicine than as food, consuming it only in the colder seasons of the year, when there is an increase in pathological cases related to respiratory problems. In Brazil, the consumption of honey as food is still small

(approximately 700 g/inhabitant/year), especially when compared to other countries, such as the United States and the European Community and Asia, which can reach more than 1 kg/year per inhabitant.

Among the numerous medicinal properties attributed to honey by folk medicine, many medicinal properties have been proven by scientific studies, and its antimicrobial activity is perhaps its most active medicinal effect [7]. The factors responsible for this antimicrobial ability are physical factors, such as its high osmolarity and acidity, chemical factors related to inhibitory substances, such as hydrogen peroxide, and volatile substances, such as flavonoids and phenolic acids [8].

2. A brief history of honey production in Brazil

Beekeeping was introduced in Brazil by Father Antônio Carneiro in early 1839, who brought some colonies of bees of the species *Apis mellifera* from Portugal to Rio de Janeiro [9]. Soon afterward, other breeds of the same species were food science and technology: contemporary research and 472 practices introduced by European immigrants in some country regions, including *A. mellifera linguistic*, *Apis mellifera carnica*, *A. mellifera scutelatta*, *Apis mellifera caucasica*, [10]. Despite this, in the 1970s, which implemented adequate beekeeping techniques, such as the genetic improvement of bees, periodic replacement of queens, selection of genetic material, and cleaning of equipment, beekeeping has expanded, being explored by large and small producers [11].

Due to its vast territorial expansion and diverse climate, Brazil is the sixth country with the highest honey production globally, behind China, the United States, Argentina, Mexico, and Canada [12]. The Rio Grande do Sul is the top producer of honey in Brazil, with about 7000 tons during the year [13]. The northeast is the second Brazilian region that produces the most honey [14]. It is a region with vegetation resistant to drought, short rainy periods, low soil fertility, and limiting factors for agriculture, contributing to being one of the regions with the greatest beekeeping potential in the world [15]. Brazil produces only 50,000 tons per year but can produce 200,000 tons and could reach high in the market if honey was consumed more in food than medicines (**Figure 1**) [16].



Figure 1.
Honey Brazilian producer situated at Pitangui, Minas Gerais state, Brazil.

3. Biodiversity of bees and kinds of honeys in Brazil

The southern region of Brazil produces 38% of honey, followed by the northeast with 37% and 19% in the southeast.

Honey is considered one of nature's purest products, derived from nectar and other natural plant secretions collected and processed by bees, enabling a new source of potentially nutritious and healthy alternative food. Because it is rich in energy, it enables us to carry out our daily tasks. It has organic acids, flavonoids, hormones, enzymes, water, glucose, fructose, sucrose, maltose, minerals, and vitamins [17].

The composition of honey depends mainly on the plant sources from which it is derived and on different factors, such as soil, the bee species, the physiological state of the colony, the honey's ripeness, and the weather conditions of the harvest, among others [18]. In general, the main characteristic of the honey of meliponid species is the differentiation in its composition, especially the water content (humidity), which makes it less dense than the Honey of Africanized bees (*A. mellifera*).

The color varies from almost transparent to dark amber, and the taste and sugar levels depend on the palate, species, season, region, and especially the bloom. Bee honey, mainly from stingless bees, is widely used by Amazonian Indians for its therapeutic potentials, such as antibacterial, anti-inflammatory, analgesic, sedative, and expectorant effects (**Figure 2**) [18].



COLOR DESIGNATIONS OF EXTRACTED HONEY

USDA Color Standards Designations	Color Range USDA Color Standards	Color Range Pfund Scales Millimeters	Optical Density
Water white	Honey that is water white or lighter in color.	8 or less	0.0945
Extra white	Honey that is darker than water white, but not darker than extra white in color.	Over 8 to and including 17	.189
White	Honey that is darker than extra white, but not darker than white in color.	Over 17 to and including 34.	.378
Extra light amber	Honey that is darker than white, but not darker than extra light amber in color.	Over 34 to and including 50.	.595
Light amber	Honey that is darker than extra light amber, but not darker than light amber in color.	Over 50 to and including 85.	1.389
Amber	Honey that is darker than light amber, but not darker than amber in color.	Over 85 to and including 114.	3.008
Dark Amber	Honey that is darker than amber in color.	Over 114

Source data: USDA

Figure 2. Honey pots from *Apis mellifera* illustrate the variety of colors. The nectar is transported to the hive, changing its concentration and chemical composition. However, during its transport to the hive, secretions from various glands, mainly the hypopharyngeal glands, are added, including invertase (α -glucosidase) and diastase (α - and β -amylase).

3.1 Honey of bees of the genus Apis

Bee honey of the genus Apis is the most consumed in the country and comes from various flowers (multiflora). It has antioxidants, soothing, laxatives, and may positively affect the skin and respiratory tract. It stimulates immunity and is a natural invigorator (**Figure 3a**).

3.2 Coffee flower honey

Coffee flower honey has a mild citrus taste and has an energizing effect. This particular kind of honey is produced from the work of bees during flowering in coffee plantations; the nectar is collected from the flowers. This honey is a rare and seasonal food, and they are produced mainly in the southern region of Minas Gerais (**Figure 3b**).

3.3 Honey from “Assa-peixe”

The “Assa-peixe” (*Vernonia polysphaera* (Spreng.) Less.) is a plant of the genus Vernonia, native to Brazil [1]. The honey from the bees raised near the Assa-peixe plantations is delicious. The Brazilian population uses the Assa-peixe leaf to fight skin diseases, bronchitis, kidney stones, muscle pain, flu, pneumonia, fluid retention, and even coughing.



Figure 3. Brazilian kinds of honey: (a) Multiflora Apis Honey; (b) Coffee (*Coffea arabica*) flower honey; (c) Betônica (*Betonica sp*) flower honey; (d) Pequi (*Caryocar brasiliense*) flower honey; (e) Cipó-uva (*Serjania lethalis*) flower honey; (f) Velame (*Croton heliotropiifolius*) flower honey; (g) Aroeira (*Astronium urundeuva*) flower honey.

It has a pleasant aroma and taste, calming and expectorant effect. Assa-peixe flower honey is extracted from the typical Brazilian flower from the southeast and northeast regions of Minas Gerais, produced during spring and summer.

3.4 Aroeira honey

The Aroeira (*Astronium urundeuva* (M. Allemão) Engl.) is a tree species with great use of its wood. It is found mainly in the north of Minas Gerais and in the northwest region of Brazil. The honey from the Aroeira flower is extracted from the tree's nectar, resulting in unique honey that stands out for its dark color [19, 20]. The difference between this unique honey and the others is the plant's union with an insect, which has sanitary properties in the fight against stomach diseases [21]. Aroeira blossom honey has qualities and specifications comparable to New Zealand manuka honey. Honey de Flor de Aroeira rarely crystallizes and has demonstrated a powerful antioxidant and bactericidal effect produced mainly in the north and center of Minas (Figure 3g).

3.5 Eucalyptus honey

One of the most popular types, eucalyptus honey, is widely used to relieve sore throat, sinusitis, and colds because of its expectorant action. It is produced in the south and southeast regions, and, unlike orange blossom honey, it has a darker color and a solid and refreshing taste [22].

It has a more pungent taste and dark coloration. It is recommended that additional treatment relieve intestinal infections [23], urinary tract, and respiratory diseases. Eucalyptus flower honey is found all over Minas Gerais, mainly between March and June.

3.6 “Velame” honey

“Velame” (*Croton heliotropiifolius* Kunth) is endemic to the Brazilian northeast. The “Velame da Caatinga” is a trendy, fragrant plant with thick leaves and clusters of white flowers. Natural medicine is used for all kinds of infections [24]. The honey extracted from this plant has a very light color and is very tasty.

“Velame” flower honey is crystalline and is also called white amber. It is incredibly soft and produced from October to January. Found throughout the entire territory of Minas Gerais (Figure 3f).

3.7 “Candeia” flower honey

In Brazil, “Candeia” is a popular plant in the Asteraceae family. However, it is of challenging identification. The honey, known as “morrão de candeia” or “Candeia” honey (*Croton* sp.), is one of the most popular kinds of honey in folk medicine has a dark color and is used to treat respiratory system diseases, such as cough in general, whooping cough, laryngitis, hoarseness [24]. Very good as an adjuvant in cases of asthma attacks and gastric ulcers. Found in the central region of the state.

3.8 “Capixingui” flower honey

The “Capixingui” (*Croton floribundus* Spreng) is a small, fast-growing tree that can be exploited for its wood or honey.

“Capixingui” flower honey has a light amber color, with a pleasant and characteristic taste. Found mainly in the areas of influence of the Atlantic forest of eastern Minas.

3.9 “Cipó-uva” honey

“Cipó-uva” (*Serjania lethalis* A.St.-Hil.) is a plant because it exudes an aroma of pink grapes when in bloom. Cipó-uva honey has a light amber color and a pleasant flavor. Cipó-uva is known for its medicinal properties, extolled as a balm for intestinal cramps and kidney pain. Parts of the Cipó-uva plant, such as the roots, leaves, and stems of this plant, are indicated for topical use and treatment of pain. It is a natural detoxifier for liver and blood sugar control [25]. Although the pharmacological and cultivation characteristics of *S. lethalis* have already been widely researched scientifically, its therapeutic virtues are still incipient. The pollen honey of this plant has been used and appreciated in folk medicine for many decades.

3.10 “Periquiteira” flower honey

“Periquiteira” (*Trema micrantha* (L.) Blume) is a pioneer species belonging to the cannabis family, previously considered to belong to the Ulmaceae family. It can be found in Brazil’s south, southeast, central-west, and northeast regions.

Its small fruits are widely eaten by birds, giving the species a high ecological value. Its most loyal consumers are Psittaciformes, a family which includes parakeets and parrots.

This honey is indicated for rickets, acting as an emollient in the respiratory tract, used against catarrhal coughs, with effects on lung disorders, bronchitis, pharyngitis, and asthma, presenting a concrete expectorant action and anti-inflammatory and antiarthritic [26]. Present in the northern region of Minas Gerais.

3.11 Orange blossom honey

More precisely, produced in the southeast in Minas and São Paulo orange blossom honey has a light color, mild aroma, and flavor with a light citrus touch. It can be a good ally for those who have trouble sleeping because it helps fight insomnia and improves bowel function.

3.12 “Uruçu” honey

The northeast region produces this yellowish honey with a propolis-like flavor. It stands out for its medicinal functions and for having plenty of water. The *Melipona scutellaris* “uruçu” bees are native to northeastern Brazil, widely found in forests on the coasts of the northeast, preferring humid places to make their hives, usually in trees [27].

The word uruçu derives from the Tupi word “eiru su,” which means giant bee. This is due to the various bees of the same genus, also found in north Brazil. It is possible to find the yellow uruçu and the uruçu bees. The honey is produced by the “uruçu” bee, a stingless bee with high-water content and antibacterial properties.

3.13 “Borá” honey

The “Borá” honey is produced by the bee Borá (*Tetragona clavipes*), a native stingless bee species of South America [28]. It is a bee of the Meliponidae family, and the original name comes from the Tupi Heborá, which means “that one which has to have

honey.” “Borá” bee is popularly known as “Jataizão” and “Vorá.” The Indians also know it in Xingu, which is found in abundance. “Borá” is a yellow, bitter substance found in the nests of this bee, possibly because of a large amount of “Samora,” “saburá” (pollen) stored by this bee. Although honey is known for its sweet taste, “Bora” honey is different because it has a salty and slightly acidic flavor. It is often used to accompany cheese, fish, and other more elaborate dishes. It is produced in southeastern Brazil.

3.14 “Jataí” honey

Tetragonisca angustula, the jataí bee, is a little Brazilian bee, measuring approximately 5 mm, golden yellow, with prominent corpuscles (or pollen baskets) on its hind legs. The morphology of the entrance to the nest is a characteristic of the species: a tube of wax or cerumen with small holes along its length and an opening that allows several bees to pass simultaneously. At night, this entrance is closed to protect the nest. The Jataí bee is recorded in the five regions of Brazil. In the northeast region, it is found in Bahia, Maranhão, Ceará, Paraíba, and Pernambuco [29]. This species differs from other *Melipona* bees for its ability to nest and survive in urban areas, which positively influences the species’ evolutionary success, given the threats they have been experiencing in rural areas.

“Jataí” honey is produced throughout Brazil and has a light color and slightly acidic taste. It is excellent to ease flu symptoms by having decongestant action and works as an ally to increase the body’s immunity.

3.15 Cashew honey

The cashew tree (*Anacardium occidentale* L.) is a plant of the Anacardiaceae family, native to the northeast region of Brazil, with a twisted crown architecture and of different sizes [30]. In nature, there are two types: the common (or giant) and the dwarf. This species can reach a height of 5–12 m, but can reach 20 m in very favorable conditions.

Honey from this plant has six times more vitamin C than the fruit itself—an essential nutrient to solve the problem, helping the body absorb iron.

3.16 Bracatinga honey

Bracatinga honeydew (*Mimosa scabrella* Bentham) is relatively darker. Bees make it from the collection of a sweetened liquid (honeydew) produced by other insects called non-floral cochineal honey; once, the bee uses the molasses produced by the cochineal to produce the Bracatinga honey.

Produced in southern Brazil, it has recently gained prominence in the news in the beekeeping world.

4. Therapeutic usage by traditional communities in Brazil

The medicinal properties of bee honey have been mentioned for various medicinal and nutritional purposes [26]. Honey, by definition, is a natural product of bees obtained from the nectar of flowers (floral honey), from secretions of living parts of plants, or from excretions of insects sucking on living parts of plants (honeydew honey). Honeydew is a biological term that refers to the excretions in the form of sugary liquids of many species of Homopteran that live as parasitic suckers of the elaborate sap of plant phloem [31].

The bees sought and collected these sugary liquids as if they were nectar undergoing the same enzymatic processes. The bees, in turn, will use the resources available as a source of sugar to elaborate it. Therefore, the most common occurrence is floral honey mixed with honeydew honey. Honey consists of various sugars, predominantly D-fructose and D-glucose, and other components and substances, such as organic acids, enzymes, and solid particles collected by bees [32]. The appearance of honey varies from almost colorless to dark brown, and it can be fluid, dense, or even solid. Its taste and aroma vary according to the origin of the plant. Varieties of honey can be identified by their color, taste, flavor, and manner of crystallization.

The honey sediment is analyzed for its pollen grain content in exceptional circumstances. Alternatively, in honeydew honey varieties, another characteristic of components presents in honeydew honey varieties, such as spores, mycelium fragments, or leaf fragments, is determined. Other characteristics helpful in identifying the type of honey include specific conductivity and variety-specific flavor components. Honey is considered the most accessible beekeeping product to exploit, the best known and the one with the most significant potential for commercialization. Besides being a food, it is also used in the pharmaceutical and cosmetic industries for its therapeutic actions.

Honey is a rich food with high energetic value, consumed worldwide, and extremely important for human health when pure. It has several properties: antimicrobial, curative, soothing, tissue regenerative, and stimulant.

The medicinal properties of bee honey and other hive products, for example, pollen, royal jelly, propolis, and bee larvae, have been mentioned for their variety of medicinal and nutritional purposes. Honey is undoubtedly the best known and most widespread of the products provided by bees. It was one of man's first foods, and practically all ancient civilizations have used it as food and as a medicinal resource. Nowadays, man uses honey abundantly as food without ignoring its medicinal qualities and nutritional value.

One and three hundred sixty kilos of honey, by definition, is a natural product of bees obtained from the nectar of flowers (floral honey), secretions of living parts of plants, or excretions of sucking insects of living parts of plants ("honey de melão"). Honey is a biological term that refers to the excretions in the form of sugary liquids of a large number of species of Homopteran, which live as parasites and suck the elaborate sap from the phloem of plants. Honey is a complex matrix due to the interference of variables not controlled by man during its production, such as climate, flowering, and the presence of sucking insects, among other factors.

The bees, in turn, will use the available resources as a source of sugar to produce it. Therefore, the most common occurrence is floral honey mixed with honeydew honey. Honey consists of various sugars, predominantly D-fructose and D-glucose, and other components and substances, such as organic acids, enzymes, and solid particles, collected by bees. The appearance of honey varies from nearly colorless to dark brown, and it can be fluid, dense, or even solid. Its taste and aroma vary according to the origin of the plant. Varieties of honey can be identified by their color, taste, flavor, and manner of crystallization.

The honey sediment is analyzed for its pollen grain content in exceptional circumstances. Another characteristic of components presents in honeydew honey varieties, such as spores, mycelium fragments, or leaf fragments, is determined. Other valuable characteristics in identifying the type of honey include specific conductivity and variety-specific flavor components. Honey is considered the most specific beekeeping product to exploit, the best known, and the one with the most significant potential

for commercialization. Besides being a food, it is also used in the pharmaceutical and cosmetic industries for its therapeutic actions.

Honey is a vibrant food with high energetic value, consumed worldwide, and extremely important for the health of the human body when pure because it has several properties, such as antimicrobial, curative, soothing, tissue regenerative, and stimulant, among others [33]. It consists of simple sugars, such as glucose and fructose. Its passage from the digestive tract to the bloodstream and from the bloodstream to the interior of cells, where it is metabolized, does not require many transformations, and its entry into cellular metabolism is relatively fast.

Honey has an energizing action on the human body, mainly due to the enzymes, vitamins, the presence of chemical elements that are critical for the proper functioning of the body, and the trace elements [34]. Honey has essential mineral elements for the human body, especially selenium, manganese, zinc, chromium, and aluminum.

5. Natural products on Brazilian honey

Among the raw materials extracted from beekeeping, honey is considered the most specific product to be exploited. It is also the one with the most significant possibilities for commercialization. Besides being a food, it can also be used in the pharmaceutical and cosmetic industries due to its therapeutic actions. It is known that there are several medicinal properties of honey. In this sense, this review sought to evaluate the scientific literature from the last decade regarding the biological properties associated with honey. It was verified that this product had been used in several therapeutic lines, primarily due to its antimicrobial, antioxidant, and healing activities. In addition, there are also studies demonstrating its antiproliferative and antimetastatic effects in brain tumors, suggesting a synergistic effect in the use of different types of honey from different origins with different biological activities [17].

The exact chemical composition of any honey depends mainly on the plant sources from which it is derived and on climate, soil, and the bee species that produce it. Therefore, honey varies significantly in pollen content physicochemical, sensory, and aromatic characteristics from one region to another. An essential point in honey production is the certification of its botanical origin. Although there is no specific legislation for this parameter, and it is not mandatory for its commercialization, this knowledge allows inferring which bees explored plant species to make honey and, consequently, inferring the product characteristics, such as color and flavor medicinal properties.

Finally, several articles demonstrate that honey may have associated biological properties, such as healing activity, antifungal, antioxidant, antiviral, antiparasitic, anti-inflammatory, and antimicrobial properties [33].

In Brazil, phytotherapeutics and homemade syrups with honey are widely used in popular therapies, mainly in indigenous and rural areas, due to their healing properties. Its topical application on the skin, including treating wounds and burns, is fascinating among its medicinal properties. Honey can extract moisture from the environment and thus dehydrate bacteria with the help of its hyperosmolar properties. In addition, honey also plays an essential role in the rapid autolytic debridement and deodorization of deep wounds.

This is due to the low makeup ideal for wound acidification, which accelerates the healing process. Honey promotes angiogenesis, granulation, and epithelialization, which helps to accelerate the wound healing process. In addition to all these

properties, honey also stands out for its antimicrobial activity numerous characteristics of its numerous characteristics.

This enzyme, secreted by bee glands, is responsible for converting glucose, in the presence of water and oxygen into gluconic acid and hydrogen peroxide, both considered solid antioxidant agents that attack the envelope of microorganisms, preserving and maintaining the sterility of honey during its maturation [35]. The iron and copper minerals in honey, associated with hydrogen peroxide, can lead to the generation of hydroxyl radicals with antimicrobial properties.

Finally, other factors that can contribute to the antimicrobial property of honey are high osmotic pressure, low water activity, low protein content, low redox potential due to the high content of reducing sugars, high viscosity that limits oxygen solubility, and other chemical and phytochemical agents [18].

6. Reported therapeutic actions of different kinds of Brazilian honey

To exemplify the properties of a kind of Brazilian honey on therapeutic usage, we can cite the Aroeira honey with the demonstrated antibacterial ideal concentration for inhibiting *S. aureus* and *E. coli*. The use of honey as antimicrobials has shown advantageous for reducing the microbial load since it could inhibit the growth of pathogenic bacteria. Moreover, the antibacterial activity of the honey observed in this work reinforces the potential for the therapeutic use of Aroeira honey produced in the north of Minas Gerais, thus contributing to the aggregation value of this honey [19].

Bracatinga Honey's benefits are associated with antioxidant and antimicrobial activity and the presence of highly bio-accessible minerals [20]. Another kind of Brazilian honey-like orange and eucalyptus honey, has been found to inhibit the formation of edema and infection and render the presence of crusts transient. The assa-peixe, eucalyptus, or orange honey reduces inflammation and necrosis and optimizes granulation tissue growth, fibroplasia, and reepithelization [36].

In vitro antimicrobial potential of these honey was verified in different dilutions through antibiograms against strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. It was observed that this pure monofloral honey prevents the growth of the main bacterial agents present in the skin, emphasizing orange honey in the control of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli*. Therefore, the assa-peixe, orange, or eucalyptus honey has healing and antimicrobial potential [36].

Some antibacterial and antioxidant properties have been attributed to honey of velame (*Croton argyrophylloides*). Chemical analysis of *C. argyrophylloides* leaves revealed the presence of compounds with antioxidant activity. The antioxidant activity can be confirmed by the DPPH method and demonstrating a significant content of phenolic compounds and total flavonoid content in the species, which corroborates the activity in the plant sample. The foliar extracts had an antimicrobial effect, tested on plates that showed growth inhibition halos of 10 and 12 mm on *Staphylococcus aureus* [37, 38].

7. Conclusions

The incredible biodiversity of flora in Brazil can provide a diverse source for bee productivity, obtaining a diversity of types of honey in Brazil. These bee products

have a high content of natural compounds with great potential for use in the treatment of human health. There are different effects already studied as antimicrobial, anti-inflammatory, antioxidant, and immunological. However, more research is still needed, with pharmacological and medicinal use in the larger population, and with a scientific method, mainly related to monofloral honey to verify its phytotherapeutic potential. However, the potential of natural compounds produced by honey as a by-product of nectar, flowers, and another source of the high biodiversity of Brazilian flora has been demonstrated.

Some studies are conclusive, but given the incredible biodiversity, there is still a lot of work to be done on the natural products of Brazilian honey.

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Conflict of interest

The authors declare no conflict of interest.

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Section 2

Honey Production and
Beekeeping Practices

Chapter 3

Geographical, Entomological and Botanical Origins of Honey

Robin E. Owen

Abstract

The Codex Alimentarius Commission defines honey as: "... the natural sweet substance produced by honey bees from the nectar of plants ... which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature". Honey, produced in all regions of the world varies widely in its chemical and physical properties, which depend on the plants the bees visit and on the species of *Apis* themselves. The Codex sets standards for the composition of honeys, levels of contaminants permitted, and the correct labelling according to floral source and geographic origin. The growth of stingless bee (*Meliponidae*) domestication in Central and South America, Asia and Australia has led to another significant source of honey, which is very variable in its properties. Here I review of the properties of honeys and the techniques used to analyze the geographical, entomological and botanical origins of honey, discuss some of the properties and features of the honeys made by the stingless bees, and discuss unusual honeys, the so-called "mad honeys", made from nectar containing toxic compounds, and the effect of toxic nectar on bees (bumble bees) and humans.

Keywords: honey, honey composition, honeybees, *Apis*, stingless bees, meliponid bees, toxic honey

1. Introduction

All bees (*Apoidea*) collect pollen and nectar from plants for food for themselves and their brood, and the eusocial bees in particular the honeybees (*Apini*, *Apis*), and the stingless bees (*Meliponini*) often store considerable quantities of nectar in their hives. This nectar has been processed by the bees, by addition of enzymes, etc. and when stored is now classified as honey. Bumble bees (*Bombini*, *Bombus*) also store nectar in their colonies, but in much smaller amounts which they generally use quite rapidly, and although it does thicken it is not processed and does not count as honey as such. Both honeybees and stingless bees produce honey in amounts that can, and have been, profitably harvested by humans of many different societies for thousands of years [1–3]. The Codex [4] standard for honey adopted by the Codex Alimentarius Commission in 1981, and revised in 1987 and 2001 and amended in 2019 defines honey as:

“... the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature”.

Therefore, strictly speaking honey, as such, is a product of honeybees (*Apis*). Honey is produced in all regions and in most countries of the world, and thus varies widely in its chemical and physical properties, which depend on the plants the bees visit and on the species of *Apis* themselves. The Codex sets standards for the composition of honeys, levels of contaminants permitted, and the correct labelling according to floral source and geographic origin. Recently the growth of stingless bee (Meliponid) domestication in Central and South America, Asia and Australia has led to another significant source of honey, but which is not regulated to the same extent as *Apis* honey and is much more variable in its physicochemical properties.

I will (1) provide an overview of the physicochemical and biochemical properties of honeys and the techniques used to analyze the geographical, entomological and botanical origins of honey, (2) discuss some of the properties and features of the honeys made by the stingless bees – the Meliponidae, and (3) discuss unusual honeys, the so-called “mad honeys”, made from nectar containing toxic compounds, and the effect of toxic nectar on bees (bumble bees) and humans.

2. Characterization of honey and international standards

The recognized standards are that of the International Honey Commission (IHC) [5] and the Malaysian Standards (MS) [6]. Although most honey consumed worldwide is undoubtedly *Apis* honey (and mainly from *A. mellifera*) the Codex does differentiate between Blossom or Nectar Honey which comes from the nectars of plants, and Honeydew Honey which comes from the excretions of plant sucking insects (Hemiptera) or the secretions of living parts of plants [4]. The standards cover both sources of honey. The standards set by the IHC and the European Union (EU) are given in **Table 1**. However, there is considerable inconsistency between legislation national in the legislation of many countries¹ applying to the Codex and the standards (**Table 1**) [15]. Many countries maintain out of date quality criteria, in particular there is variation regarding moisture content, HMF, diastase activity, electrical conductivity, and sugars [15].

In recent years there has been a considerable increase in the consumption and commercial production of honey by stingless bees (Meliponini) in the Neotropical countries such as Mexico [2] and in tropical parts of Asia, particularly Malaysia [16]. This has led the Malaysian Department of Standards to set standards for stingless bee honey produced in Malaysia. The Malaysian Standard [6] defines *kelulut* or stingless bee honey as:

“A natural sweet with certain acidity substance produced by stingless bees of Meliponini tribe from the nectar of plants or from secretions of living parts of plants, which the stingless bees collect, transform by combining with the specific substances of their own, deposit, dehydrate, store and leave in the natural honey pots to ripen and mature.”

¹ Argentina, Belgium, Brazil, Canada, China, Colombia, Czech Republic, Ethiopia, India, Germany, Greece, Japan, Poland, Russia, Serbia, Slovakia, Turkey [15].

	Component	Determination method	Standard		Use	Refs.
			IHC	MS		
1	Moisture, M (g/100 g i.e. %)	Refractometer	≤21	≤35	QA/QC	[6]
2	Free acidity, FA (meq/100 g)	Titration	≤50	n/a	QA/QC	[7]
3	pH	pH meter	n/a	2.5–3.8	QA/QC	[6]
4	Ash content (g/100 g)	Heated to 600°C, residue weighed	≤0.5	≤1.0	QA/QC	[6]
5	HMF content (mg/kg)	SPEC-UV/HPLC	≤40	≤30	QA/QC	[7]
6	Diastase activity, DN	Schade/phadebas	≥ 8	n/a	QA/QC	[7]
7	Sugars (g/100 g)					
	(Fructose + glucose) = TRS	GC, HPLC-RID	≥60	≤85	QA/QC	[7]
	Sucrose	GC, HPLC-RID	≤5	≤7.5	QA/QC	[7]
	Maltose	GC, HPLC-RID	n/a	≤9.5	QA/QC	[7]
	Others (e.g. erlose)	GC, HPLC-RID	–	–	Characterization	[8]
8	Plant phenolics	FCM/AlCl ₃ /HPLC/UHPLC	n/a	Present	QA/QC	[9]
9	EC (mS/cm)	Conductimeter, (lower range 10 ⁻⁷ S)	≤0.8 [*]	–	QA/QC	[7]
10	Amino acids + proteins	HPLC, UHPLC	–	–	Characterization	[10]
11	Vitamins	SPEC/COL, HPLC	–	–	Characterization	[10]
12	Lipids	GC-MS	–	–	Characterization	[10]
13	Minerals	AAS/OES/ICP/MS	–	–	Characterization	[10]
14	Organic acids	HPLC/IC ¹ H-NMR	–	–	Authentication	[10]
15	Hydrocarbon composition	HPLC/ ¹ H-NMR/GC	–	–	Toxin ID; Bee ID	[11, 12]
16	C ₃ /C ₄ sugar ratio	Δδ ¹³ protein/honey & C ₁₄ %	–	–	Authentication	[13]
17	DNA	DNA metabarcoding	–	–	Plant ID, bee ID	[14]

Table 1.
 Some components and properties measured in raw honey from *Apis* and *meliponid* bees.

Notes: TRS = total reducing sugars; QA/QC = required to meet standard; *EU standard; HFM hydroxymethylfurfural; FCM/AlCl₃ = Folin–Ciocalteu method, AlCl₃ colorimetric assay; BSWWhite = bisulfite White method, Winkler = Winkler method; HPLC/UHPLC = high-performance liquid chromatography, ultra-high-performance liquid chromatography; GC = gas chromatography, HPLC-RID = HPLC coupled to a refractive index detector; SPEC/COL = spectrophotometric/colorimetric analysis; GC–MS = GC & mass spectrometry; ¹H-NMR = nuclear magnetic resonance; DNA

metabarcoding = sequencing of plastid *rbcLa*, mt COI, nuclear internal transcribed spacer 2 (ITS2) region of nuclear ribosomal DNA.

Furthermore, the MS defines raw *kelulut* honey as that collected from natural sealed honey pots, while processed *kelulut* honey is raw honey which undergoes drying at a temperature not more than 40°C to reduce moisture content, to not more than 22.0% [6]. The Malaysian standards are also given in **Table 1**. A notable difference between *Apis mellifera* honey and stingless bees honey is that the latter is more acidic contributing to its unique sour taste [9] and the requirement that plant phenolic compounds must be present [6].

The various components of honey, are given in **Table 1**, together with the most common chemical and biological methods used to analyze these. The first nine are requirements of international or national standards to certify honey as genuine, and these consist of physicochemical and biochemical properties of the honey. Moisture, free-acidity, pH and ash content (1–4, **Table 1**) are all basic physicochemical properties of honey specified by the standards to fall within prescribed limits. The next two following, Hydroxymethylfurfural (5-(hydroxymethyl)-furan-2-carbaldehyde) or HFM content and diastase activity (5–6, **Table 1**) indicate if the honey has been subject to undue heating and/or improper storage [17].

Honey, composed of 60–80% monosaccharides and disaccharides, is the most concentrated sugar source found in nature, and so sugars from the nectar collected by the bees are, of course, the essential components of honey. Both the standards specify the sum of Fructose and Glucose to be at a specified minimum or maximum, and sucrose to be at a specified maximum (7, **Table 1**). To further characterize other sugars are often also measured (see **Table 2**) the honey, but are not required by the standards.

Next, phenolic compounds (8, **Table 1**) produced by plants, and present in nectar are required to be present in Meliponid honey but not in *Apis* honey. Phenolic compounds are made up of either one or more aromatic rings with hydroxyl groups,

Plant	<i>n</i>	EC	pH	FA	F	G	S	M	I	E
Acacia	36	0.185	3.9	14.5	34.65	21.60	8.8	3.6	1.2	2.8
Rhododendron	29	0.300	4.1	15.0	37.60	30.65	2.6	8.6	2.5	3.7
Chestnut	60	1.160	5.4	17.0	40.60	25.70	3.6	5.6	2.4	4.3
Dandelion	31	0.505	4.6	10.0	35.90	32.60	0.3	5.7	1.7	0.8
Heather	22	0.860	4.5	28.0	37.95	28.85	0.6	1.9	1.3	0.2
Lime	39	0.665	5.1	12.5	37.25	34.55	4.5	5.7	2.2	0.9
Rapeseed	36	0.210	4.1	12.0	37.05	35.75	2.0	2.2	1.5	1.4
Fir honeydew	132	1.015	4.7	31.5	30.15	23.15	2.7	4.9	3.4	4.5
Metcalfa honeydew	14	2.025	5.2	31.0	29.95	23.55	0.1	6.2	2.65	0.6

Note: *n* = sample size, EC = electrical conductivity ($mScm^{-1}$), pH = pH-Value, FA = free acidity (meq/kg), F = fructose (g/100 g), G = glucose (g/100 g), S = sucrose (g/100 g), M = maltose (g/100 g), I = isomaltose (g/100 g), E = erlose (g/100 g).

Table 2.

Summary of physical characteristics of honey or honeydew from nine species of plants. The data are derived from Ruoff *et al.* [18], and are the midpoint of the ranges of values given in their **Table 1**. A generalized distance matrix was calculated using these data to give **Figure 2**.

and are classified as either flavonoids (e.g. flavonols, anthocyanidins, flavanones) or others (e.g. phenolic acids) [10]. They work as primary antioxidants of free radical scavengers [9] with supposed health benefits. The phenolic composition in honey depends on many factors, including plant preferences by bees, geographic origins or weather conditions [10]. Ramly et al. [9] compared total phenolic content (TPH) and total flavonoid content (TFC) in honey from four species of meliponid bees from Banggol Peradong, Malaysia: *Heterotrigona itama*, *Geniotrigona thoracica*, *Lepidotrigona terminata* and *Heterotrigona erythrogastra*. As shown in **Figure 1** from Ramly et al. [9] there is considerable difference between species for both TPC and TFC, and this can probably results from different floral preferences of the bees foraging in the same geographical area [9]. Electrical Conductivity (9, **Table 1**) is positively correlated with ash and acid content of the honey, is technically easy, and is a good indication of the botanical origin of the honey [7].

The next set of components (10–13), Amino acids & Proteins, Vitamins, Lipids and Minerals, have all been measured in a wide variety of honeys to further characterize the honey for specific purposes, often related to benefits for human health, and using an extensive variety of methods [10].

Organic acids (14, **Table 1**) are often metabolic intermediates or result from microbial metabolism, and some have antidiabetic, antimicrobial, or antioxidant activity, and they can also be good markers of honey authenticity [10]. For example, Seraglio et al. [19] used aliphatic organic acids to differentiate Brazilian *Mimosa scabrella* or bracatinga honeydew honey from blossom honeys and adulterated honeydew honey.

Two types of hydrocarbons (15, **Table 1**) have been isolated from various honeys.

Grayanotoxins are a class of diterpenoids produced as secondary metabolites produced by plants and occur in the nectar of some species. When honey made from this nectar is ingested by humans these can have extreme toxic effects (see Section 5 below). Grayanotoxins can be detected and identified by reversed phase HPLC column using a water–methanol gradient [11]. The entomological origin of honey can be determined by the presence and the analysis of fragments of beeswax, which consist of various hydrocarbons and other organic compounds that differ between species of bees. Zuccato et al. [20] differentiated between various stingless bee honeys on thjis basis using nuclear magnetic resonance, similarly Zhang et al. [12] used gas

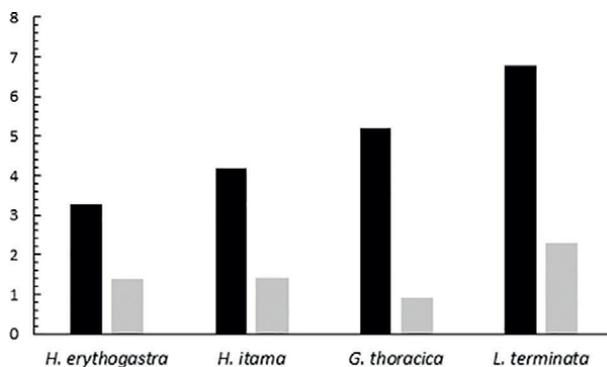


Figure 1. Total phenolic content, (TPC; mg of gallic acid equivalents (GAEs) per gram of honey) and total flavonoid content (TFC; mg catechin equivalents (CE) per gram of honey) in honey of four stingless bee species (*Heterotrigona itama*, *Geniotrigona thoracica*, *Lepidotrigona terminata* and *Heterotrigona erythrogastra*). TPC = unshaded, TFC = shaded. From: Ramly et al. [9].

chromatography to distinguished species specific hydrocarbon profiles in honey from *A. mellifera* from *A. cerana*.

Pure honey which meets the standards (**Table 1**) tends to be relatively expensive, and there is a temptation to produce and sell fraudulent honey, and this is often produced by adulterating pure honey by adding extra sugar solution. One cheap way of doing this is to add commercially available syrups such as High Fructose Corn Syrup 55, etc. [13]. However, although these are readily available, these syrups generally are derived from C₄ plants, which use the carbon isotopes ¹³C and ¹²C in a different ratio than C₃ plants from which most nectar and honey is derived by bees [13]. The difference in the proportions of the C₃ and C₄ sugars (16, **Table 1**), and in relation to total protein the honey, thus indicates honey adulterated by this means [13].

Finally (17, **Table 1**) DNA in the honey coming from the plants or the insect visitor, can be used to identify in many instances exact species of each [14]. The method involves the sequencing of the barcoding regions of the nuclear, internal transcribed spacer 2 (ITS2) region of nuclear ribosomal DNA, and the chloroplast Ribulose-1,5-bisphosphate carboxylase-oxygenase gene (*rbcLa*) to identify plant species, and the barcoding region mitochondrial Cytochrome Oxidase I gene (*mt COI*) to identify bee visitors [14]. Prosser and Hebert [14] analyzed seven different honeys (Produced in Canada - Light, Dark, Blended, Pasteurized, Medium; France – Creamed; Mexico - Meliponine). They detected a total of 72 botanical sources in the Light honey, 16 of which could be identified as to species, and 63 botanical sources in the meliponine honey, but only two of these could be identified to species, the rest only to Family level [14]. The bees were *Apis mellifera* and *Melipona beechii* for the Canadian and Mexican samples respectively [14].

As can be seen honey is an extremely variable biological product, and its composition depends on many factors – the plant from which the nectar was gathered and its geographical location, the type of bee, how the honey has been collected and stored, being the most obvious ones. Some components may be present in very small quantities (minerals for example), and some are only relevant for identification and are not relevant to the person consuming the honey.

3. Characterization of pure honey

Although the IHC and Malaysian Standards ensure consistence among honeys, producers and regions, there can still be considerable variation among unifloral honeys. Most honey is polyfloral with the nectar being a mixture gathered by bees from many different plant species, but unifloral honeys are those where the nectar has come from a single plant species [18]. Unifloral honey is produced by placing the honeybee hives for a limited amount of time by a single crop species, ideally somewhat isolated to force the bees to forage solely from that crop. Ruoff et al. [18] obtained and analyzed honey from nine different species of plant, and in **Table 2**, I have given the main components they measured which I have summarized from their **Table 1**. As an easy way to visualize the similarities and differences between the honeys I performed an Unweighted Pair Group Mathematical Average (UPGMA) cluster analysis on the data in **Table 2** using Mesquite [21], and the results are shown in **Figure 2**. Unsurprisingly the two honeydew honeys are very similar and cluster together, but what is interesting and perhaps surprising is that honeys from three very different



Figure 3. A colony of *Trigona hypogaea* in Malaysia. Image by Mohamad Izham M.A. used from Wikimedia under the Commons Creative Commons Attribution-Share Alike 3.0 Unported license.

(honeybees), Bombini (bumble bees) and the Euglossini (orchid bees) [22]. The meliponids are a very diverse tropical group of bees with over 500 species identified and of these there are over 400 species in the Neotropics, 50 in the Palearctic, 60 in the Indo-Malaysian region and 11 in northern Australia [3, 16, 22]. The diversity is the highest in the Neotropics, but some of the most important species economically and culturally in Latin America are in the large genus *Melipona* (74 species) which only found in the Neotropics [2]. *Trigona* is the other large genus which is global in distribution occurring from the Neotropics to the Indo-Malaysian region. **Figure 3** shows a typical colony of *Trigona hypogaea* in Malaysia. The honey is stored in extensive wax pots surrounding the brood comb.

Like *Apis* honey, stingless bee honey has been extensively measured for its physicochemical and biochemical properties, but there are far more species used for producing honey than with honeybees. One would expect much more variation and this is what has prompted the Malaysian government to implement standards [6]. In **Table 3** I have compiled comparable data where available from the sources given in Nordin et al. [16] for the Malaysian Standards for stingless bees, and I have also included comparative data from five populations of honeybees, *A. mellifera*, from different geographical regions. Again as a way to visualize the similarities and differences between the honeys produced by different species of bees, I performed an Unweighted Pair UPGMA cluster analysis on the data in **Table 3** using Mesquite [21], and the results are shown in **Figure 4**. As can be seen, in some cases there is clustering of closely related species, i.e. those within the genus. Also with *A. mellifera*, the populations from different geographical locations tend to cluster together, i.e. make similar honey suggesting a possible phylogenetic effect for this species. Turning now to the stingless bees, **Figure 5** shows the same analysis now run just with the meliponid species from **Table 3**, and without *Apis*. Superficially there does appear to be some clustering according to genus however it appears that geographical location is of primary importance. Species of the different genera have produce very similar honeys when in the same region, whereas species of the same genus produce very different honeys when in different regions. This is what one would expect given that stingless bees are mostly generalist foragers.

Taxon	Ref.	M	FA	pH	HMF	Ash	EC	TRS	F	G	DN
<i>Homonotrigona fimbriata</i>	[23]	41.0	52.0	3.30	46.00	1.00	2.60	22.00	74	15.0	?
<i>Lepidotrigona doipaensis</i>	[23]	31.5	1975	3.50	2.30	0.51	1.20	38.50	12.0	11.9	1.60
<i>Lepidotrigona flavibasis</i>	[23]	28.0	168.0	3.70	8.50	0.51	1.30	68.00	16.0	13.0	3.10
<i>Lisotrigona furva</i>	[23]	28.0	53.0	3.60	0.21	0.18	0.34	62.50	33.5	26.5	?
<i>Melipona beecheii</i>	[24]	28.6	41.5	3.20	9.23	0.46	0.58	69.21	?	?	1.30
<i>Melipona paraensis</i>	[25]	26.4	30.4	4.29	3.40	0.14	1.37	60.80	?	?	2.90
<i>Melipona quadrifasciata 1</i>	[26]	32.5	42.5	3.71	5.20	?	0.33	61.77	34.7	27.4	23.00
<i>Melipona quadrifasciata 2</i>	[26]	30.0	28.0	3.74	1.45	0.15	0.22	60.24	?	?	1.72
<i>Melipona scutellaris</i>	[27]	28.0	40.4	3.55	1.77	0.18	0.27	55.45	?	?	2.16
<i>Melipona scutellaris latrelle</i>	[28]	25.5	42.7	3.83	?	0.16	0.52	67.38	54.3	42.4	?
<i>Melipona sp.</i>	[29]	38.7	35.7	3.60	8.60	0.38	0.39	49.40	?	?	15.63
<i>Melipona subnitida</i>	[30]	24.8	32.5	?	7.56	0.02	0.10	50.97	29.2	21.8	0.0
<i>Scaptotrigona mexicana</i>	[31]	23.9	?	3.75	12.61	0.49	0.28	56.48	?	?	?
<i>Tetragonisca angustula</i>	[32]	24.4	45.2	4.10	9.93	0.39	0.13	55.46	?	?	32.28
<i>Tetragona carbonaria</i>	[33]	26.5	128.9	4.00	1.20	0.48	1.64	?	17.5	24.5	0.40
<i>Tetragonilla collina</i>	[23]	28.0	25.0	3.90	5.90	0.24	0.43	52.00	26.0	26.0	0.40
<i>Tetragonula fuscobalteata</i>	[23]	26.0	96.5	3.70	22.0	0.67	1.35	32.50	21.0	31.5	?
<i>Tetragonula laeviceps</i>	[34]	27.0	81.4	3.62	1.07	0.27	0.62	47.87	27.1	20.8	?
<i>Tetragonula laeviceps-pogdeni</i>	[23]	28.0	76.0	3.60	5.40	0.22	0.59	29.00	17.0	12.0	0.63
<i>Tetragonula testaceitarsis</i>	[23]	30.5	70.5	3.60	2.95	0.20	0.59	41.00	22.0	19.0	0.22
<i>Trigona apicalis</i>	[23]	42.0	495.0	3.20	0.26	1.40	2.60	12.50	6.7	5.90	4.90
<i>Trigona melanoleuca</i>	[23]	43.0	592.0	3.40	28.0	3.10	2.80	15.00	6.0	8.90	0.15
<i>Trigona angustula latrelle</i>	[35]	24.3	39.2	4.20	1.30	0.20	0.66	?	23.5	30.1	16.70

Taxon	Ref.	M	FA	pH	HMF	Ash	EC	TRS	F	G	DN
<i>Trigona sp.</i>	[36]	13.0	78.1	3.35	3.18	0.20	0.57	29.34	?	?	16.67
<i>Trigona laevipiceps</i>	[36]	15.7	50.8	?	3.32	0.14	0.57	27.37	?	?	13.64
<i>Trigona pagdenis</i>	[36]	14.7	20.0	4.01	3.97	0.22	0.45	41.64	?	?	11.11
<i>Apis mellifera 1</i>	[37]	18.3	26.5	?	10.82	0.18	0.28	62.28	23.5	38.8	42.87
<i>Apis mellifera 2</i>	[38]	20.1	17.6	4.28	0.58	0.23	0.26	75.92	41.0	34.9	10.89
<i>Apis mellifera 3</i>	[39]	16.7	41.3	4.52	2.01	?	0.95	61.92	34.9	27.0	38.53
<i>Apis mellifera 4</i>	[39]	16.9	29.2	3.48	13.12	?	0.25	66.56	37.2	29.3	35.24
<i>A. mellifera 5</i>	[39]	13.6	23.6	4.18	1.98	?	0.62	60.28	36.6	23.7	15.78

Note: Categories as defined in Tables 1 and 2; ? = missing data.

Table 3.

Data from the sources given in Nordim et al. [16] for the Malaysian Standards for stingless bees. Also included are data from five populations of honeybees, *A. mellifera*, from different geographical regions.

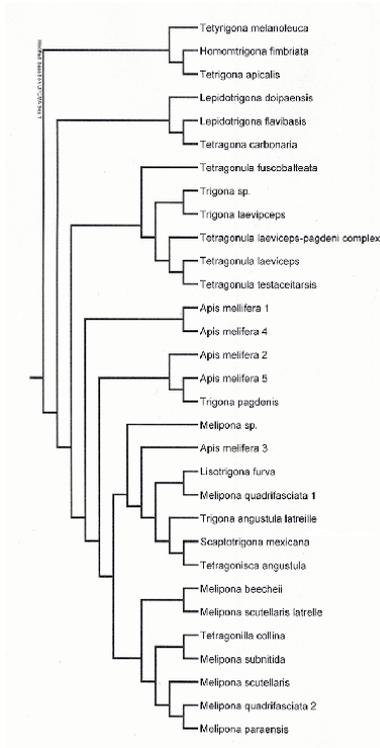


Figure 4. Result of the Unweighted Pair Group Mathematical Average (UPGMA) cluster analysis on all the data in Table 3 using Mesquite [21]. Geographic origins of *A. mellifera*; 1 = Brazil; 2 = Thailand; 3, 4, 5 = Spain.

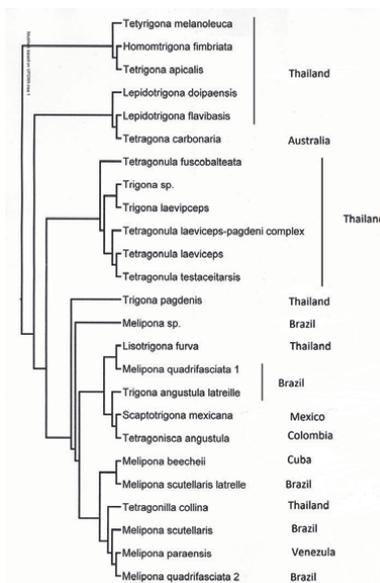


Figure 5. Result of the Unweighted Pair Group Mathematical Average (UPGMA) cluster analysis on only the data in Table 3 from the meliponid species using Mesquite [21]. The geographical origin of each species is also indicated.

5. Nectar and toxic honey

Not all nectar contains only beneficial compounds. Some plants secrete nectar containing toxic compounds to which pollinators may or may not be tolerant [40]. These toxins are secondary metabolites of plants and include various alkaloids (e.g. caffeine), diterpenoids (e.g. grayanotoxins), and cyanogenic glycosides, all of which are involved in plant defense [41]. Thus it appears paradoxical as to why they are found in nectar (and pollen in a few species) which is a reward for pollinators [41, 42]. Many of these compounds are moved via the phloem and may just diffuse into the nectar rather than being actively transported in, as are sugars [42, 43]. Thus it is most reasonable to suppose that these metabolites are initially “unintentionally” deposited in the nectar and then subsequently selection and co-evolution with pollinators occurs [41]. This may be for reduced metabolite concentrations in nectar in some cases or in others for the retention of these metabolites [41]. For example caffeine, which occurs naturally at low concentrations in *Coffea* and *Citrus* species, improves the memory of honeybees when presented with a sucrose reward containing caffeine [44], therefore one would expect that bees would return more often to these plants.

Honeybees have fascinated humans for millennia, originally perhaps, just as a source of food as prehistoric cave paintings suggest, and then taking on a mystical and a religious significance. Indeed the Kulung and Gurung people of eastern Nepal still collect honey in this ancient precarious fashion by climbing down bamboo rope ladders hung from the top of granite cliffs to reach the colonies of the giant black honeybee, *Apis laboriosa*² [1]. These bees build single combed nests in aggregations sometimes of 50 or more under rock ledges high on cliffs in deep river valleys [45]. The honey is especially prized at certain times of the year because of its intoxicating properties; this is the so-called “Mad Honey” [1]. One class of toxins which has been extensively studied are the grayanotoxins, which are the active ingredient in the notorious “mad honey” mainly produced in the region around the Black Sea especially in Turkey [46], and also in Nepal [47]. In humans, low doses of mad honey cause hypotension, dizziness, nausea, excessive sweating and vomiting, but at high doses can cause serious cardiac problems including atrioventricular block, syncope and asystole (cardiac arrest), however there are no reported human deaths [48]. Grayanotoxins are polyhydroxylated cyclic hydrocarbons neurotoxins which block sodium channels in cell membranes thus nerve and muscle cells remain in a state of depolarization [48]. Although grayanotoxins (GTX) occur in a variety of Ericaceae, *Rhododendron ponticum* is of particular interest due to its invasive nature and it has been extensively studied [49–51]. In *Rhododendron* GTX I, II and III are the types of GTX (of the 25 different kinds) which commonly occur [48]. Tiedeken *et al.* [50] tested the toxicity of GTX I and GTX III on three native species of bees in Ireland where *R. ponticum* is invasive: *Apis mellifera*, a solitary bee *Andrena carantonica* and the bumble bee *B. terrestris audax*. Honeybee mortality increased by 20% when fed solutions containing GTX I, although the survival of the solitary bees and the bumble bees was not affected, however solitary bees did exhibit sublethal toxic effects including aversion to feeding and abnormal behaviors.

² This species is now recognized as a high altitude cliff bee endemic to the pan-Himalayan region and distinct from the giant honeybee *Apis dorsata* [45]. These bees build single combed nests in aggregations of 50 or more under rock ledges high on cliffs in deep river valleys [45].

Also neither bumble bee survival nor behavior was affected after consumption of GTX I even when the bees were subjected to the additional stressors of parasite infection or food reduction [50]. Previously Tiedeken et al. [49] had found that in paired-choice experiments *B. terrestris dalmaninus* did not preferentially avoid the toxic compounds nicotine, amygdalin, caffeine and GTX when presented at naturally occurring concentrations in sucrose solution. Again this subspecies did not suffer increased mortality when fed GTX but experience significant mortality with amygdalin and caffeine [49]. Some bumble bees are adapted to plants with toxic nectar. For example *Bombus gerstaeckeri* exclusively visits *Aconitum* spp. in Europe and is tolerant to aconitine in the nectar [51]. Egan et al. [52] examined geographic variation in the concentrations of GTXI and GTX III in nectar of *Rhododendron ponticum* in its native (Spain & Portugal) and introduced populations (Ireland). Interestingly, the nontoxic (to bees) GTXIII occurred at only low levels ($\sim 0.2 \mu\text{g mg}^{-1}$) in both populations but GTXI was on average just more than half the concentration in the introduced populations in Ireland as compared to that in its native range (mean 0.81 vs. 1.46 $\mu\text{g mg}^{-1}$ respectively) [52]. Egan et al. [52] found that 13 environmental and climatic variables did not significantly affect GTX levels in nectar, and suggest that pollinator-mediated selection for lower GTX concentration and/or release from nectar robbers in the invasive populations could account for this difference.

Toxic nectar and honey is certainly somewhat of an oddity, but nevertheless of great interest and importance for human health and behavior, and for understanding patterns of selection and coevolution between bees and plants.

6. Conclusions

Honey has been consumed in all regions of the world for millennia and continues to be an important component of many peoples' diets and used as a sweetener. However, as a sweetener it is relatively expensive [53] but is often preferred because of its health benefits and because honey is generally perceived as being "environmentally friendly" [53]. Even in medieval China (circa 400 BCE) honey was expensive, although apiculture was quite well developed, honey production was limited [54]. Honey, along with honeydew and cane sugar, was one of the most prestigious sweeteners that the wealthy could afford, as opposed to the malt sugars consumed by most of the population [54].

Most honey was and still is, *Apis* honey, with the exception of stingless bee honey produced by the Mayans [2], and by Australian aboriginal groups [3]. As analytical techniques have improved our understanding of the physicochemical and biochemical components and properties of honey have increased tremendously. This has led to the more exact characterization of the chemical, physical and biological properties of honeys. Some of these techniques are also used forensically. Since many specialized honeys are relatively expensive there is documented fraud whereby, for instance genuine C_3 honey can be differentiated from C_3 honey that has been diluted by the addition of sugars from C_4 plants. The techniques are also extensively used to characterize the different types of honey produced by stingless bees. The domestication of meliponid bees has undergone a resurgence in Mexico [2] and Australia [3] and has been extensively developed in Malaysia since 2012 as an alternative to honeybee keeping [16]. Many species of stingless bees have been domesticated to produce honey, which therefore varies much more than *Apis* honey which comes from only a few species, and mostly the western honeybee *Apis mellifera mellifera*. The Malaysian

government has taken the very progressive step of setting out their own set of standards for meliponid honey [6], and what is particularly significantly different from the honeybee standards [4] is that the Malaysian standard [6] includes plant phenolic content to be required. This emphasizes the potential health benefits of using stingless bee honey.

Honey is generally regarded as an “ethical” sweetener as compared to refined sugar, etc. and so some work has been done using life cycle assessment (LCA) to estimate the carbon footprint and greenhouse gas (GHG) emissions in the production of honey [55–57]. Depending on the size of the operation the emissions are quite variable [55], also large honey producers the main use of their hives is to provide pollination services [55]. When pollination services are also accounted for by an LCA then it appears production has the greatest impact (e.g. use of glass for the honey jars and electricity used for refrigeration), and then distribution the next [56]. In fact, the study by Arzoumanidis et al. [56] found that transport of the orange-blossom honey from Italy to the U.S.A. had the most impact in the distribution phase. However, in many countries (e.g. Romania, [53]) people generally prefer to buy and consume locally produced honey which is obviously the most environmentally sustainable strategy. Nonetheless, some countries export most of their honey, an example being Argentina where almost 95% of all honey produced is exported adding to the carbon footprint [57], although within Argentina GHG emissions are comparable to those in other countries [57].

Honey is, and always will be, one of the most important naturally produced foods used by humans. It is essential that we continue to learn more about it, continually develop better methods of analysis and to promote environmentally friendly ways of production.

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Summer Gifts from the Hive: Botanical Origin, Antioxidant Capacity, and Mineral Content of Hungarian Honeys

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Abstract

Although Hungary is one of the biggest honey producers in the EU, there is little information on diagnostic traits, nutritional value, and potential health benefits of the honeys produced in this Central European country. The aim of this study was to perform a complex analysis of eight Hungarian summer honeys, focusing on melissopalynology, antioxidant measurements with three different assays, and the macro- and microelement profile. Light-colored honey types included a multifloral honey and unifloral phacelia, milkweed, and linden honeys; dark-colored honeys were represented by unifloral goldenrod, sunflower, and chestnut honeys and a dark multifloral honey. Pollen analysis and sensory traits confirmed the botanical origin of each unifloral honey, while the dominance of *Tilia*- and Lamiaceae-pollen was observed in the light- and dark-colored multifloral honeys, respectively. The total reducing capacity (TRC) assay and the microelement content clearly separated the light- and dark-colored honeys. The oxygen radical absorbance capacity (ORAC) assay highlighted the strong antioxidant activity of linden honey, comparable to that of dark-colored honeys. Multivariate statistical analysis revealed correlations between antioxidant assays, color, and mineral content of honeys. The results contribute to establishing unique character sets for each honey type, aiding proper identification and quality control of these natural products.

Keywords: *Phacelia*, *Asclepias*, *Tilia*, *Solidago*, *Helianthus*, *Castanea*, melissopalynology, antioxidant activity, macroelement, microelement, correlation analysis, principal component analysis

1. Introduction

Honey, as a complex food, offers several nutritional and health benefits, due to its favorable composition and high levels of antibacterial and antioxidant activity. In addition to sugars which constitute the largest part of honey, polyphenolic substances, vitamins, and minerals are also essential for the beneficial effects.

The quality of honey is largely determined by the floral source, which can be a single or multiple plant species [1, 2]. The correct identification of honey types is based on their physicochemical properties, pollen composition, and marker compounds [3]. However, it is difficult to find reliable chemical markers, since the chemical composition of honey depends on several factors, like geographical origin, season of harvest, and storage conditions. Although melissopalynology is considered as one of the most effective tools in checking the identity of honey [4], and detailed pollen analyses are available for different honey types from various countries [5–7], it is acknowledged that determining the pollen quality and quantity in honey samples requires considerable time and botanical expertise [8]. Thus, the need arises to establish a set of quality traits that are suitable to unequivocally identify and characterize each honey type.

One of the most intensively researched health benefits of honey is its antioxidant capacity, attributed to high levels of plant-derived compounds, like flavonoids and carotenoids that are efficient against reactive oxygen species [3, 9]. The antioxidant activity of honey has been evaluated recently in several countries, e.g. Turkey [10], Poland [11–13], Greece [14], Serbia [15], Romania [16], and Hungary [17, 18]. Total antioxidant capacity (TAC) of honeys can be determined with several assays. The most frequently used methods are based on single electron transfer (SET), such as total reducing capacity (TRC) also known as Folin-Ciocalteu assay, Trolox Equivalent Antioxidant Capacity (TEAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, and the Ferric Reducing Antioxidant Power (FRAP). In honey research, the hydrogen atom transfer (HAT)-based assays like the oxygen radical absorbance capacity (ORAC) assay are used less commonly [19]. Maurya et al. [20] and more recently Martinello and Mutinelli [21] provided a comprehensive overview of the antioxidant parameters in different honey types, involving several multifloral honeys from different floral and geographical origin. The close relationship between the color and antioxidant activity of honey has been confirmed by several research groups [13, 22–24]. Other factors that can have an impact on the antioxidant capacity of honey include environmental and climatic conditions, harvesting treatment, and storage [25]. Floral origin of honey was found to play a decisive role in its biological activities, regarding both antioxidant and antibacterial properties [4, 15]. In turn, Džugan et al. [26] reported that antioxidant capacity can be a useful indicator of honey's botanical origin.

Although minerals are only minor constituents of honey, they contribute significantly to its quality [27]. In addition, minerals can be used for honey identification [28], since the composition of elements has strong botanical specificity and may reflect the mineral content of the soil, too [29–31]. The mineral composition of honeys has been studied in various countries: Altun et al. [32] analyzed Turkish honeys, Sager et al. [33] analyzed Austrian honeys, Conti et al. [34] analyzed Italian honeys, and Czipa et al. [35] and Sajtos et al. [36] analyzed Hungarian honeys. Solayman et al.'s [27] review compared the mineral content of honeys from different countries, revealing that the mineral composition of honey can vary within wide ranges, influenced by both botanical and geographical origin throughout the world. The mineral content of honey was found to correlate with its color: dark honeys contain higher amounts of certain minerals when compared to pale-colored ones.

Less attention has been paid to the possible relationships between mineral content of honeys and their antioxidant activity. Such studies included Hungarian, Indian, Italian, and Turkish honeys, establishing some correlations between the composition of macro- and microelements and the antioxidant capacity of respective honeys [37–39]. Principal component analysis (PCA) allowed differentiation of honeys based on factors like mineral, physicochemical, and enzymatic analysis, antioxidants and physicochemical

properties, metal content and contamination (antibiotic and pesticides residues), and browning index and antioxidant activity [16, 37, 40, 41], respectively.

Based on the observation that the color of honey can be an indicator of antioxidant capacity and mineral content, eight Hungarian honey types from light- to dark-colored repertoire were selected for the purposes of the present study. The honey types chosen for this study included representatives of cultivated plants with high nectar production, to relatively rare specialty honeys, which are produced by few beekeepers in Hungary and worldwide. The comprehensive analysis included melissopalynology, antioxidant measurements with three different assays, and the macro- and micro-element profile. An additional goal was to reveal correlations among the analyzed parameters. Although Hungary is one of the biggest honey producers in the EU, little is known about the diagnostic traits, nutritional value, and potential health benefits of various Hungarian uni- and multifloral honeys. The results of the current study are intended to facilitate comprehensive characterization of eight Hungarian summer honeys, aiding their proper identification and quality control.

2. Botanical sources, pollen analysis, sensory characteristics, and color of honey samples

2.1 Plant sources of honey samples

The botanical sources of the eight honey samples (**Figure 1**) included cultivated plants like phacelia and sunflower, natural flora elements like linden and sweet chestnut, as well as invasive plants like milkweed and goldenrod.

The flowers of phacelia (*Phacelia tanacetifolia* Benth., Hydrophyllaceae) provide an excellent nectar source for honeybees, and their strong odor attracts large numbers of bees throughout the summer. The flowers cluster in scorpioid cymes, the long stamens protruding from the corolla tube (**Figure 1a**). Phacelia is frequently used outside its native North American range as a cover crop and bee plant, being among the best honey plants worldwide, performing well particularly in temperate climates [42, 43].



Figure 1. Botanical sources of the honey samples. (a) phacelia—*Phacelia tanacetifolia*, (b) milkweed—*Asclepias syriaca*, (c) linden—*Tilia platyphyllos*, (d) goldenrod—*Solidago gigantea*, (e) sunflower—*Helianthus annuus*, (f) chestnut—*Castanea sativa* (photos: A. Farkas, E. Zajácz).

Common milkweed (*Asclepias syriaca* L., Apocynaceae) is native to North America. Having been introduced to Europe as early as the seventeenth century, today in Hungary it is considered an invasive weed species, widespread on sandy soils of the Great Hungarian Plain. The plants provide a good nectar flow, and the sweet-scented, white to purple flowers are very attractive for honeybees (**Figure 1b**), even though they get occasionally trapped in a blossom and their motion may be hindered by pollen masses (pollinia) sticking to the bee's body [44].

Representatives of the linden genus (*Tilia* spp., Malvaceae) that are common in Hungary include small-leaved linden (*T. cordata* Mill.), large-leaved linden (*T. platyphyllos* Scop.), and silver linden (*T. tomentosa* Moench). The flowers of these deciduous trees (**Figure 1c**) can provide good honey flow in favorably hot and humid weather [44].

Goldenrod species (*Solidago* spp., Asteraceae) of North American origin, today widespread in Europe, include giant goldenrod (*S. gigantea* Ait.) and Canadian goldenrod (*S. canadensis* L.). Similar to milkweed, they are treated as invasive species in Hungary, which, however, are valued as medicinal and bee plants. Together with European goldenrod (*S. virgaurea* L.), the yellow flowers of *Solidago* species (**Figure 1d**) are good nectar and pollen sources in late summer and early fall, providing ample food for overwintering bees.

Sunflower (*Helianthus annuus* L., Asteraceae) is the second most important honeybee plant in Hungary, following black locust (*Robinia pseudoacacia* L.). The plant is cultivated on large areas, being exploited primarily as an oil plant. Blooming from June through July, the disk florets of the capitulum (**Figure 1e**) provide both pollen and nectar for the bees [44].

Sweet chestnut (*Castanea sativa* Mill., Fagaceae), native to Southern Europe and Asia Minor, is a deciduous tree with edible fruits. The flowers serve as abundant pollen source, and the staminate flowers (**Figure 1f**) provide nectar, as well. Although single flowers yield low volumes of nectar, the total nectar production of a tree can be significant, due to the large number of flowers in an inflorescence [44].

2.2 Pollen profile and sensory traits of honey samples

The botanical origin of honey samples was determined with a combination of microscopic pollen analysis and spectrophotometric color determination (**Table 1** and **Figure 2**), following the methodology of Polish and Spanish research groups [22, 45]. Furthermore, to confirm the floral sources of honey samples, the sensory traits odor and consistency were evaluated. In case of multifloral honeys, a more detailed melissopalynological analysis was carried out to reveal the pollen spectrum of the samples and to determine their floral origin (**Table 2**).

Linden (*Tilia*) honey belongs to honey types with under-represented specific pollen. In the comprehensive study of Oddo et al. [46], mean linden pollen percentage was 23%, with extreme values 1–56%, while Kuš et al. [22] found only 22–26% *Tilia* pollen in linden honeys. The 46% *Tilia* pollen in our linden honey sample support the unifloral origin of this honey. Similar to our characterization, light amber color was reported for certain Polish linden honeys, although most of them were described as extra white [22]. In accordance with our linden honeys, Polish linden honey was characterized by solid, fine granulated consistency. The 47% *Helianthus* pollen in our sunflower honeys fits nicely within the range of 12–92% *Helianthus* pollen in European sunflower honeys [46], similar to Turkish sunflower honey samples, which contained *Helianthus* pollen as their dominant pollen type (45–70%), proving their

Nr.	Honey type plant name	Dominant pollen (%)	Sensory characteristics (color, odor, and consistency)	ABS ₄₅₀ (mAU)
1	Phacelia <i>Phacelia tanacetifolia</i>	<i>Phacelia tanacetifolia</i> (74.1%)	Light beige, moderately intense odor, fine granulated, semisolid	247 ± 11 ^a
2	Milkweed <i>Asclepias syriaca</i>	Brassicaceae (45.3%)	Light yellowish amber, moderately intense flower-like odor, liquid, viscous	248 ± 12 ^a
3	Linden <i>Tilia</i> spp.	<i>Tilia</i> spp. (45.9%)	Light amber, strong odor, fine granulated, semisolid	285 ± 8 ^b
4	Multifloral- <i>Tilia</i> (MF- <i>Tilia</i>)	See Table 2	Light amber, intense odor, semisolid, fine granulated	306 ± 8 ^b
5	Goldenrod <i>Solidago gigantea</i>	<i>Solidago gigantea</i> (45.3%)	Amber, moderately intense odor, semisolid, fine granulated	531 ± 15 ^c
6	Multifloral-Lamiaceae (MF—Lamiaceae)	See Table 2	Brownish amber, intense malt odor, semisolid, fine granulated	606 ± 18 ^d
7	Sunflower <i>Helianthus annuus</i>	<i>Helianthus annuus</i> (47.4%)	Bright yellow, moderately intense odor, coarsely granulated, solid	719 ± 5 ^e
8	Chestnut <i>Castanea sativa</i>	<i>Castanea sativa</i> (90.8%)	Dark amber with reddish tone, strong odor, liquid, viscous	920 ± 10 ^f

Each code number in the first column represents three biological replicates (n = 3) of honey types. Means in the same column with different superscripted letters are significantly different according to Student's t-test (p < 0.05).

Table 1.
 Identification and sensory characteristics of analyzed honey samples.

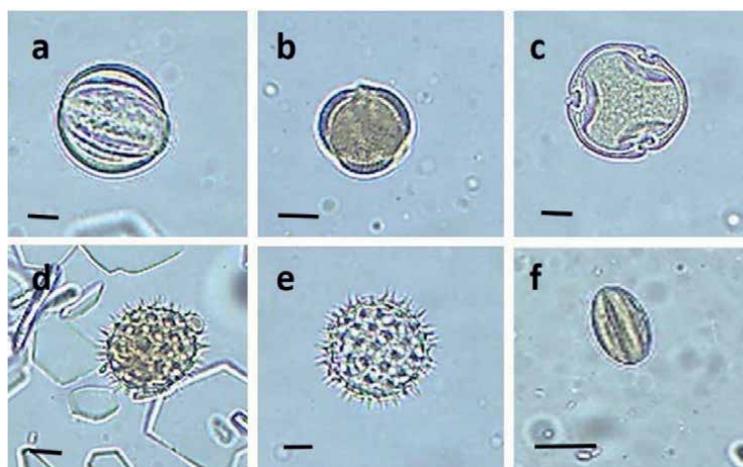


Figure 2.
 Honey samples—observed pollen types (a) *phacelia*—*Phacelia tanacetifolia*, (b) *milkweed*—*Brassicaceae* pollen, (c) *linden*—*Tilia* spp., (d) *goldenrod*—*Solidago gigantea*, (e) *sunflower*—*Helianthus annuus*, (f) *chestnut*—*Castanea sativa*. Scale bar = 10 μm (photo: K. J. Csetneki).

unifloral origin [47]. Turkish sunflower honeys were characterized by similar color, texture, and crystallization traits as our Hungarian samples, being bright yellow, fragrant, creamy, crystallizing quickly. From the eight honey samples in this study, phacelia and chestnut honeys belong to honeys with over-represented pollen, containing more than 60% *Phacelia* and at least 90% *Castanea* pollen, respectively [46, 48].

Pollen type relative frequency (%)	MF- <i>Tilia</i>	MF-Lamiaceae
Brassicaceae	—	15.6
<i>Tilia</i>	21.5	1.6
<i>Solidago</i>	—	6.8
Lamiaceae	—	30.8
<i>Robinia</i>	12.1	1.6
Rosaceae	10.1	4.0
Asteraceae	10.7	5.6
Caryophyllaceae	4.7	—
Poaceae	1.3	—
Apiaceae	3.4	—
<i>Fagopyrum</i>	—	3.6
<i>Trifolium</i>	—	1.2
Fabaceae	—	1.2
Others	29.5	23.2

Dominant pollen >45%, secondary pollen 16–45%, important minor pollen 3–15%, minor pollen <3% of the pollen grains counted.

Table 2.
Pollen spectrum of the studied multifloral honeys.

The 74% *Phacelia* and 91% *Castanea* pollen content in our phacelia and chestnut honey samples, respectively, met these requirements. In case of milkweed honey, we cannot rely on melissopalynology to determine if the nectar was collected from *Asclepias* flowers, since the honeybees are not able to collect the large-size pollinia. However, sensory traits like odor, taste, and color can aid identification. The color of milkweed honeys can range from yellowish green [44] to somewhat darker amber color [49]. The color of our milkweed honey sample was in the darker range, between the light beige phacelia and the light amber linden honeys [18]. According to our microscopic analysis, *Brassica* pollen was the most abundant in this sample; however, 45% relative frequency of *Brassica* pollen is far from the values required in unifloral rape honeys (minimum 60% [50], and in some regions above 80%) [17]).

The unifloral origin of our goldenrod honey samples was supported by the dominance of *Solidago* pollen, as well as sensory characteristics. The amber color of our samples was darker than the extra light amber color of Polish (138–205 mAU) [51, 52] and Croatian goldenrod honeys (287 mAU) [53]. This might be explained by a difference in their floral origin: the botanical source of goldenrod honey in Poland and Croatia can be the native European species, *Solidago virgaurea*, while our samples with darker color (535 mAU) originated from the North American invasive species, *S. gigantea*. Similarly, dark, rich amber color characterized goldenrod honey from the US, the native region of *S. gigantea* [54].

The light and dark-colored multifloral honeys (MF-*Tilia* and MF-Lamiaceae) in our study showed remarkable differences in their pollen spectrum, as expected. The most abundant pollen type of the lighter colored multifloral honey was *Tilia*, while the darker colored one was dominated by Lamiaceae pollen (Table 2). Multifloral honeys show great variability, with diverse features from all aspects, among others pollen composition, antioxidant parameters, or mineral content [20, 21, 34].

Color represents an important characteristic of honey, referring to its botanical origin and also its composition [55]. Based on the color, the studied honeys were divided into light- and dark-colored groups, exhibiting color intensity from 200 to 300 mAU (phacelia, milkweed, linden, and MF-*Tilia* honeys) and from 500 to 1000 mAU (goldenrod, MF-Lamiaceae, sunflower, and chestnut honeys), respectively. Color intensity distinguished the dark honeys from each other, while in the light-colored group, phacelia and milkweed honeys and also linden and MF-*Tilia* honeys did not differ significantly in their absorbance. The reported color intensities were similar to the Romanian honeys, which were in the range of 210 mAU (acacia honey) to 1228 mAU (forest honey) [56]. Net absorbances of Slovenian and Croatian linden honeys were four times higher than that of chestnut honey [57, 58]; however their color values were lower than those of the same honey types in our study. Beretta et al. [59] reported lower color parameter for chestnut honeys (610 mAU), Cimpoi et al. [56] measured lower color intensity for sunflower honey (512–556 mAU) compared to our results. Based on the Pfund scale, according to which honey can be water white, extra white, white, extra light amber, light amber, amber, and dark amber [60], the color of sunflower honey samples was extra light amber [16].

3. Total antioxidant capacities of honeys

In order to provide the most reliable results, the combination of nonenzymatic antioxidant assays is required. In the current study, three different TAC methods were used to determine the antioxidant behavior of the honey samples. Light-colored honeys clearly separated from dark-colored honeys based on the results of TRC, whereas ORAC assay did not distinguish these two groups from each other (Table 3).

Nr.	Honey types	TRC (mg GAE kg ⁻¹)	DPPH (IC ₅₀ mg mL ⁻¹)	ORAC (μmol TE g ⁻¹)
1	Phacelia	91.67 ± 19.03 ^a	55.78 ± 1.95 ^a	13.79 ± 0.58 ^a
2	Milkweed	144.72 ± 17.17 ^{ab}	37.61 ± 0.41 ^b	22.67 ± 0.97 ^b
3	Linden	119.14 ± 13.80 ^b	35.86 ± 0.62 ^c	71.68 ± 5.43 ^c
4	MF- <i>Tilia</i>	195.44 ± 9.87 ^c	37.16 ± 1.57 ^{bc}	63.00 ± 4.43 ^c
5	Goldenrod	255.27 ± 22.44 ^d	33.65 ± 2.20 ^c	19.50 ± 1.69 ^a
6	MF-Lamiaceae	475.71 ± 40.63 ^e	28.52 ± 0.81 ^d	32.41 ± 2.41 ^d
7	Sunflower	230.25 ± 8.35 ^d	26.62 ± 0.49 ^e	34.32 ± 3.57 ^e
8	Chestnut	232.82 ± 24.97 ^d	17.37 ± 0.57 ^f	75.20 ± 4.71 ^c
Total				
Light-colored honeys (nr. 1–4)		137.74 ± 44.15 ^a	41.60 ± 9.48 ^a	43.42 ± 29.33 ^a
Dark-colored honeys (nr. 5–8)		298.51 ± 118.67 ^b	26.54 ± 6.80 ^b	40.16 ± 24.51 ^a

TRC—total reducing capacity; DPPH—antiradical power; ORAC—oxygen radical absorbance capacity; data are means ± standard deviations of three independent determinations (n = 3). Means in the same column with different superscripted letters (a-f) are significantly different according to Student's t-test (p < 0.05).

Table 3.
 Total antioxidant capacities of selected honey samples.

Phacelia honey displayed the lowest antioxidant activity with each assay, while the highest values were measured in MF-Lamiaceae and chestnut honeys with the SET-based methods such as TRC and DPPH, respectively. Remarkably, the light-colored linden and MF-*Tilia* honeys showed as high antioxidant capacity as the dark-colored chestnut honey, according to the HAT-based ORAC assay.

In addition, TRC distinguished the light-colored members from each other, except milkweed honey. In the dark group, only MF-Lamiaceae separated from the others, using this SET-based method. A high variation of TRC was reported for various honeys from different parts of the world. Chestnut honey was reported to have high antioxidant potential in several studies [24, 38, 39]. Our results on this honey type are in accordance with values reported for Italian [59] and Slovenian [57] chestnut honeys, but in case of linden honey the values of the above research groups were slightly lower than ours. Flanjak et al. [58] measured somewhat lower values for these two types of honeys from Croatia, while Kus et al. [22] obtained higher TRC parameters for linden honey (192.5 ± 17.8 mg GAE kg⁻¹) compared to our results. Furthermore, Gül and Pehlivan [10] measured even higher reactivities for linden and chestnut honeys, respectively (268.81 mg GAE 100 g⁻¹ and 327.60 mg GAE 100 g⁻¹). TRC values of sunflower honey in this study were in line with those reported by Pauliuc et al. [16]. Sari and Ayyildiz [47] measured TRC of 50 sunflower honeys in Turkey, with results in a broad range (6.9 – 23.2 mg GAE 100 g⁻¹). Our samples approached the upper limit reported by them. Similar to linden and chestnut honeys, much higher values were calculated by Gül and Pehlivan [10] for Turkish sunflower honeys (77.64 ± 0.86 mg GAE 100 g⁻¹). The TRC values of our goldenrod honey, which originated from *S. gigantea*, were higher than those of Polish goldenrod honeys derived from *S. virgaurea* (147 – 199 mg GAE kg⁻¹ [51], and 210.3 mg GAE kg⁻¹ [52]), but lower than those measured in Croatian goldenrod honey, also originating from *S. virgaurea* (492 mg GAE kg⁻¹) [53]. Goldenrod honey is unique in the sense that its color does not necessarily correlate with its antioxidant capacity, which might reach the level measured in dark-colored honeydew honey [26]. The TRC of multifloral honeys can range between 32 and 147 mg GAE kg⁻¹ [20]. Our multifloral honeys were characterized by higher TRC values than multifloral honey from Serbia (87 mg GAE kg⁻¹) [15]. TRC values of our MF-Lamiaceae honey were in the same range as those of Polish multifloral honeys [26, 61], but were much lower compared to the values (325 – 937 mg GAE kg⁻¹) measured in 18 multifloral honeys from Burkina Faso (Africa) [62].

The results of the DPPH assay, which was used to determine the free radical-scavenging activity of the honey samples, showed parallel tendency with the darkness of honeys. In this assay, the lower is the IC₅₀ value, the higher is the antioxidant activity. In this study, the highest radical scavenging activity was identified for chestnut honey and the lowest for phacelia honey. In case of dark honeys, the IC₅₀ values varied significantly ($p < 0.05$) depending on the botanical origin of the honey samples, while the values of the light-colored honeys were only partially different from each other. The assay has been frequently used to characterize the antioxidant activity of honeys, e.g. Polish honeys [11, 12, 22], Czech honeys [63], Romanian honeys [16], Indian honeys [37], or Lithuanian honeys [64]. The activity of linden honey in this study was in the upper range of values measured by Bertoneclj (28.8 ± 5.4 mg ml⁻¹) and in the lower range of values measured by Flanjak (42.77 ± 10.32 mg ml⁻¹) [57, 58]. The activity of our chestnut honey was lower compared to data obtained by the above-mentioned researchers. The multifloral honeys from Greece exhibited a broad range of DPPH activity from 7.5 to 109.0 mg ml⁻¹ [14]. The IC₅₀ values of the DPPH analysis were significantly lower for the dark-colored honeys in this study, which means

that their antiradical power was significantly higher than that of the lighter honeys, except the goldenrod honey. Its DPPH value was close to those of linden and MF-*Tilia* honeys. Slovenian and Turkish linden (*Tilia*) honeys showed similar DPPH values as our above-mentioned honeys. Beretta et al. [59] reported lower antioxidant values in unifloral dandelion and acacia honeys, while their multifloral honey was much more active ($IC_{50} = 5.32 \pm 0.2 \text{ mg mL}^{-1}$) compared to our honeys in this study.

Compared to other assays used for characterizing the antioxidant capacity of honeys, ORAC is thought to be the most biologically relevant method, which is based on hydrogen atom transfer [21]; thus, it may evaluate different groups of antioxidants than the SET methods. This assay distinguished the linden and MF-*Tilia* honeys from the light-colored group with similarly high ORAC values as those of the dark-colored chestnut honey. Similar exceptional cases were reported by Bogdanov [65], who measured particularly high antioxidant power in the relatively lighter arbutus and sourwood honeys, similar to dark honeys. Goldenrod honey in the present study provided similarly low ORAC value as acacia honey in a previous Hungarian study, while the antioxidant activity of our multifloral honey was significantly higher compared to the dark-colored fennel and the amber-colored sunflower honeys [18]. The ORAC value of our milkweed honey was comparable to that of strawberry tree honey, while the values measured in our other honey samples were significantly higher than those of the dark African or buckwheat honeys [59].

4. Multi-element analysis of honeys

The macro- and microelement contents measured in our honey samples are shown in **Tables 4** and **5** and **Figures 3** and **4**. The samples in our study were harvested in the Western part of Hungary, the mineral content of which was compared with honey samples originating from South and East Hungary [35, 36, 66].

4.1 Macroelements

Expectedly, K was found to be the most abundant mineral in all studied honeys. Linden honey had significantly higher K content than the other light-colored honeys, similarly high as the darker MF-Lamiaceae honey. The mean K level in linden honey was comparable to that reported for this honey type in Croatia [67]. Relatively high amounts of K and Ca were measured also in linden and chestnut honey samples from other parts of Hungary [35, 36]. Previous results are in accordance with our observation regarding the K content of honeys, increasing in the order: phacelia < sunflower < linden < chestnut. MF-*Tilia* and MF-Lamiaceae honeys had similar K content as sunflower and linden honeys, respectively, in the previous Hungarian studies. Significantly lower K content was measured in our milkweed and goldenrod honey samples, the values being similar to those of acacia, phacelia, and rape honeys from the Eastern part of Hungary. Comparing Ca to P and S to Mg contents within the same honey provided differences ($p < 0.05$) among honey types as follows: linden, MF-*Tilia*, goldenrod, sunflower, and chestnut honeys had higher Ca content than P, while the pale phacelia, milkweed, and the dark MF-Lamiaceae honeys showed reverse relation. The macroelement values obtained for the above-mentioned honey types were variable among the previously studied Hungarian honeys [35, 66]; however, the relation between their average amount of Ca and P was in agreement with each other. Similarly, the ratio of S and Mg was different in various honey types.

Nr.	Honey types	K (mg kg ⁻¹)	Ca (mg kg ⁻¹)	P (mg kg ⁻¹)	S (mg kg ⁻¹)	Mg (mg kg ⁻¹)	Na (mg kg ⁻¹)
1.	Phacelia	145.62 ± 4.23 ^a	19.88 ± 3.11 ^a	33.68 ± 2.46 ^a	13.04 ± 0.88 ^a	6.04 ± 0.33 ^a	6.38 ± 1.38 ^a
2.	Milkweed	340.28 ± 11.12 ^b	19.06 ± 2.67 ^a	39.19 ± 3.56 ^a	15.98 ± 1.88 ^{ab}	11.78 ± 0.10 ^b	3.56 ± 3.09 ^a
3.	Linden	1278.08 ± 18.97 ^c	67.85 ± 8.01 ^b	41.52 ± 4.46 ^a	15.89 ± 4.46 ^b	16.51 ± 0.30 ^c	9.29 ± 1.03 ^b
4.	MF- <i>Tilia</i>	845.88 ± 35.67 ^d	53.71 ± 11.05 ^b	37.73 ± 2.69 ^a	14.22 ± 0.94 ^{ab}	15.74 ± 1.30 ^c	37.02 ± 8.49 ^c
5.	Goldenrod	342.73 ± 12.29 ^b	75.79 ± 10.44 ^b	40.74 ± 3.85 ^a	16.67 ± 1.15 ^b	24.30 ± 0.11 ^d	8.69 ± 0.36 ^b
6.	MF-Lamiaceae	1264.73 ± 70.79 ^c	73.78 ± 12.22 ^b	127.04 ± 4.20 ^b	52.31 ± 0.67 ^c	34.54 ± 2.07 ^c	8.80 ± 1.43 ^b
7.	Sunflower	758.95 ± 18.69 ^e	126.37 ± 14.93 ^c	76.25 ± 8.22 ^c	26.53 ± 8.22 ^c	33.26 ± 1.28 ^e	13.23 ± 1.70 ^e
8.	Chestnut	1815.79 ± 20.69 ^f	153.01 ± 12.60 ^c	79.04 ± 5.41 ^f	35.55 ± 5.41 ^e	45.38 ± 17.32 ^e	20.94 ± 0.80 ^f

Data are means ± standard deviations of three independent measurements (n = 3). Means in the same column with different superscripted letters (a-f) are significantly different according to Student's t-test (p < 0.05).

Table 4.
Macroelement content of the studied honey samples.

Nr.	Honey types	B (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Zn (mg kg ⁻¹)
1.	Phacelia	4.10 ± 0.52 ^a	<0.10	<0.05	<0.10	1.17 ± 0.25 ^a
2.	Milkweed	3.79 ± 0.63 ^a	0.13 ± 0.01 ^a	0.73 ± 0.00 ^a	0.12 ± 0.03 ^a	0.44 ± 0.00 ^b
3.	Linden	2.70 ± 0.09 ^b	0.12 ± 0.02 ^a	<0.05	1.01 ± 0.03 ^b	0.15 ± 0.08 ^c
2.	MF-Tilia	2.41 ± 0.52 ^b	0.13 ± 0.01 ^a	0.62 ± 0.08 ^a	0.62 ± 0.05 ^c	0.63 ± 0.00 ^d
3.	Goldenrod	4.90 ± 1.02 ^{ac}	0.13 ± 0.01 ^a	1.80 ± 0.60 ^b	0.16 ± 0.01 ^a	2.15 ± 0.20 ^e
4.	MF-Lamiaceae	6.49 ± 0.43 ^c	0.77 ± 0.03 ^b	1.53 ± 0.29 ^b	0.77 ± 0.01 ^d	3.32 ± 0.04 ^f
5.	Sunflower	4.90 ± 0.61 ^a	0.23 ± 0.02 ^c	0.75 ± 0.09 ^a	0.45 ± 0.01 ^e	4.87 ± 0.15 ^g
6.	Chestnut	4.51 ± 0.44 ^a	0.34 ± 0.20 ^{ac}	1.16 ± 0.85 ^{ab}	8.45 ± 2.81 ^f	3.88 ± 1.23 ^{fg}

Data are means ± standard deviations of three independent measurements (n = 3). Means in the same column with different superscripted letters (a-g) are significantly different according to Student's t-test (p < 0.05).

Table 5.
 Microelement content of honey samples.

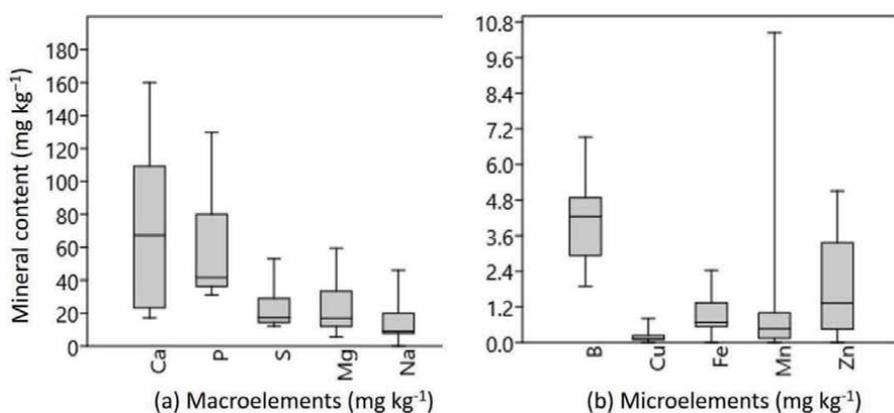


Figure 3.
 The range of the measured elements in our honeys. (a) Macroelement content (Ca, P, S, Mg, Na, K not shown due to values differing by one order of magnitude) and (b) microelement content (B, Cu, Fe, Mn, and Zn).

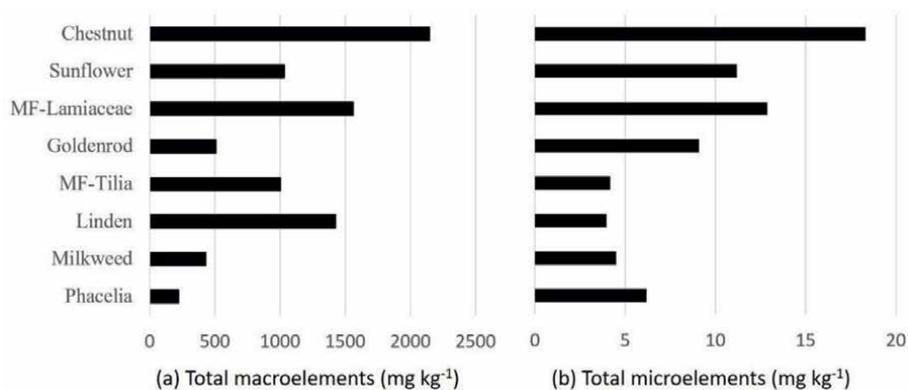


Figure 4.
 Reported average concentrations of (a) macroelements (K, Ca, P, S, Mg, and Na) and (b) microelements (B, Cu, Fe, Mn, and Zn) in the studied honeys.

S content was higher than Mg in the light-colored phacelia, milkweed, and MF-Lamiaceae honeys, while in the goldenrod honey Mg exceeded the S. Linden, sunflower, and chestnut honeys contained similar amounts from both elements. Similar to our results, S content of phacelia was significantly higher than its Mg content in an earlier Hungarian study [35]. The range of Mg content was similar to that reported for honeys from Bulgaria, France, Italy, and Poland [27]. Among the macroelements, the amount of Na was the lowest, except in MF-*Tilia* honey, where Na proved to be the third main macroelement together with P, after K and Ca. Nayik et al. [37] measured higher amount of Na than P content in Indian acacia honey. High Na level characterized also the avocado honey, in which Na was the second most abundant mineral [68]. The Na content of honeys can show differences between continents: European (Bulgarian, Italian, Polish, and Spanish) honeys were characterized by lower Na levels (7.2–152 mg kg⁻¹), compared to those measured in Asian (Indian and Malaysian) honeys (83–732 mg kg⁻¹) [27]. The range of macrominerals (**Figure 3a**) clearly shows the decreasing amount of the measured elements after K (Ca > P > S > Mg > Na). Compared to our results, the macromineral content of Italian multifloral honeys decreased in different order (K > S > Ca > P > Na > Mg) [34].

Regarding the total macroelement content (**Figure 4a**), the dark-colored chestnut honey was found to be particularly rich in minerals, while the light-colored phacelia honey was found to be poor in elements with average values of 2150 mg kg⁻¹ and 222 mg kg⁻¹, respectively. The total macroelement content did not differentiate the dark- and light-colored group from each other. The light milkweed and the twice darker goldenrod honeys represented similarly low values (430 mg kg⁻¹ and 500 mg kg⁻¹, respectively), while the macroelement content of the light-colored linden and MF-*Tilia* honeys was similarly high as that of the dark-colored MF-Lamiaceae and sunflower honeys (around 1500 mg kg⁻¹ and 1000 mg kg⁻¹, respectively).

4.2 Microelements

The amounts of microelements determined in our honey samples are summarized in **Table 5** and **Figure 3b** and **4b**. The majority of the measured microelements was present in each honey, whereas Cu, Fe, and Mn were under detection limit in phacelia honey and Fe in linden honey. The range of Zn and B was the broadest among the selected microelements, while the Cu content was under 1 mg kg⁻¹ in all of the samples (**Figure 3b** and **Table 5**). The dark-colored group contained significantly higher amount of microelements than the light-colored group (**Figure 4b**), but there were differences in the ranking of each element. Chestnut honey was the richest and linden honey the poorest in microelements, with average amounts of 18.3 mg kg⁻¹ and 3.9 mg kg⁻¹, respectively. Regarding the dark-colored honeys, MF-Lamiaceae was the richest in B and Cu, goldenrod in Fe, and chestnut in Mn, while high Zn content characterized the sunflower honey.

Based on the microelements, phacelia was distinguishable from other light-colored honeys due to its significantly higher B and Zn content; and chestnut honey stood out from dark-colored honeys due to its extremely high Mn content. Among the studied microelements, B content showed the highest amount in all honey types, except chestnut honey. However, Amtmann et al. [66] measured lower B content in sunflower, chestnut, and even phacelia honey (0.98 mg kg⁻¹), compared to our results, while that of linden honey was similar in the two studies. In contrast, Sajtos et al. [36] measured higher B content in the above-mentioned honeys, compared to our and to

Amtmann et al.'s [66] results. Linden and MF-*Tilia* honeys contained similar amount of this microelement. Similar to our results, Cu content of phacelia was under detection limit in the study of Czipa et al. [35]. They reported similar average amount of Cu in sunflower, while higher Cu content in linden honey ($0.320 \pm 0.073 \text{ mg kg}^{-1}$). Low Cu content was measured in milkweed, linden, MF-*Tilia*, and goldenrod honeys in this study, while MF-Lamiaceae showed the highest Cu content. The microelement Fe was missing or present only in low amounts in the light-colored honeys and the dark sunflower honey. The highest Fe content was measured in goldenrod and MF-Lamiaceae honeys, but much higher upper limit of Fe content had been detected in honeys from France (86.76 mg kg^{-1}) and Malaysia (233 mg kg^{-1}) [27]. Regarding microelements, chestnut honey is exceptional due to its particularly high Mn content, in accordance with the results of Sajtos et al. [36], who measured even higher level of Mn in this honey type (11.9 mg kg^{-1}). The high Mn content of chestnut honey was confirmed by several authors, being similarly high as that of fir honey, but slightly lower than that of oak honey [29, 38]. Light-colored honeys contained significantly lower Zn content than the dark-colored ones. However, the light acacia and rape honeys from the eastern part of Hungary had significantly higher Zn content, similar to our darker goldenrod and MF-Lamiaceae honeys [35]. The highest Zn level was found in our sunflower honey samples, supporting the observations of Sajtos et al. [36], who compared the Zn content of sunflower honey to that of linden, phacelia, and chestnut honeys. Regarding the linden honey in Bogdanov et al.'s study [29], it showed similar Cu and Fe content, and higher Zn content than chestnut honey, while in our study linden honey contained lower amount of each microelement than chestnut honey. Compared to our results, higher Cu and Fe levels have been reported for linden and chestnut honeys in Croatia, while the Zn content of chestnut honey was higher in our samples [67].

The results of multi-element analysis in Egyptian and Italian honeys proved that mineral content can serve also as a marker of geographical origin [34, 69, 70]. However, the minor components in honeys cannot be considered as a reliable biomonitor of environmental pollution [34]. Mohammed et al. [31] showed evidence that minerals in some honeys were not in strict relation with the soil mineral content. Bogdanov et al. [25] and Nayik et al. [37] concluded that the mineral composition in honey has been primarily attributed to the botanical origin rather than geographical and environmental exposition of nectar sources.

5. Correlation analysis

The data matrix of color (absorbance), antioxidant values, and mineral contents was analyzed by Pearson's correlation (**Table 6**). The following significant relationships were found. Correlation was obtained between color and the SET-based antioxidant capacities (TRC and DPPH), as well as between these methods. As expected, color and TRC did not show correlation with ORAC. Most of the minerals correlated with color, except Na. TRC and DPPH were in strict correlation with the macrominerals P, S, and Mg and the microminerals Cu and Mn. TRC was the strongest predicting factor regarding B and Fe, while DPPH was related to K, Ca, and Mn content. ORAC showed strict correlation with the K content of honeys. Ca, Na, B, and Mn also correlated with this assay.

Similar to our results, many studies reported that darker honeys had higher antioxidant capacities, while lighter honeys were characterized by relatively low

Variable	Color	TRC	DPPH	ORAC
TRC	0.5388**			
DPPH	-0.8429***	-0.5429**		
ORAC	0.2596	-0.0583	-0.5091*	
K	0.6191**	0.3845	-0.7529***	0.8176***
Ca	0.9199***	0.3372	-0.8373***	0.4906*
P	0.6572***	0.8834***	-0.6082**	0.0798
S	0.6611***	0.8673***	-0.6113**	0.1078
Mg	0.9287***	0.6273***	-0.8742***	0.3506
Na	0.1546	0.0280	-0.3005	0.6241**
B	0.5404**	0.7541***	-0.3309	-0.4405*
Cu	0.5152**	0.9021***	-0.5415**	0.1266
Fe	0.4714*	0.6496***	-0.2368	-0.3112
Mn	0.6913***	0.1063	-0.6322***	0.5895**
Zn	0.8847***	0.5437**	-0.646***	-0.0104

TRC, total reducing capacity; DPPH, antiradical power; ORAC, oxygen radical absorbance capacity.
*Significant at $p < 0.05$.
**Significant at $p < 0.01$.
***Significant at $p < 0.001$.

Table 6. Correlation matrix (Pearson's correlation coefficients) of color, antioxidant, and mineral parameters in Hungarian honeys.

antioxidant values [15, 24, 51, 52, 71]. The DPPH assay showed the strongest correlation with the color of honey samples, in accordance with the results of Džugan et al. [11], Flanjak et al. [58], and Gorjanovich et al. [72]. The used SET-based methods gave positive linear correlation in this study, consistent with the results of other authors [15, 22, 24, 37]. However, ORAC as an exception showed correlation only with DPPH, in contrast to the results reported by Beretta et al., Gorjanovich et al., and Bodó et al. [18, 59, 72]. Similarly, ORAC and TRC, known also as total polyphenolic content (TPC), were found to correlate with each other [13]. However, TPC does not always correlate with antioxidant activity [73]. Our study highlights the fact that in a comprehensive study on antioxidant activity of honeys, it is essential to include HAT-based methods as well, besides the SET-based assays.

All of the studied macro- and microelements were in good correlation with color, except Na. The contribution of minerals to the color of honey has already been established [74]. Perna et al. [39] proved that color intensity is positively correlated with metal content in honey; furthermore, with an increase in metal content, there is an increase in antioxidant capacities of honeys.

The above-described correlations could be clearly interpreted in the light of principal component analysis (PCA). Three groups of parameters—antioxidant assays, macroelements, and microelements—were selected to establish their identification power in terms of honey types. The results are presented in the biplots of **Figure 5**.

The first principal component, PC1, included most of the information with 95.3% for the antioxidant results, 99.4% for the macroelements, and 65.9% for the microelements of the total variance, while the second principal component, PC2, explained

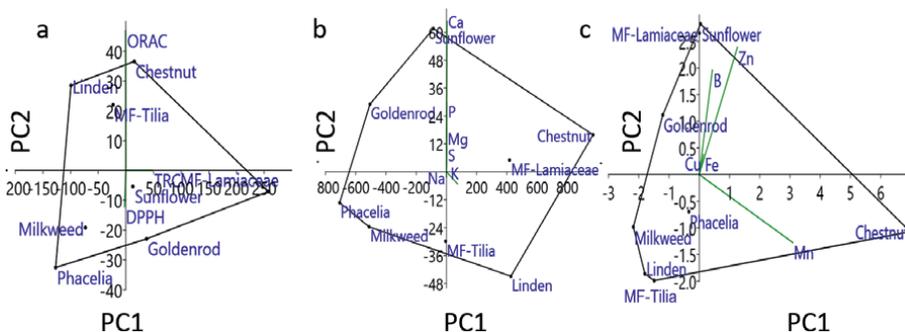


Figure 5. Principal component analysis (PCA) of datasets consisting of (a) antioxidant parameters, (b) macroelements, and (c) microelements analyzed from the honey samples.

4.43%, 0.37%, and 28.7%, respectively. The light-colored phacelia and milkweed, furthermore the linden and MF-*Tilia* honeys, were located close to each other in each biplot. Most of the analyzed parameters of phacelia and milkweed honeys showed low variability, locating them always on the negative PC1 and PC2 quarter. The relation between linden and MF-*Tilia* honey types suggested the influence of their linden (*Tilia*) plant origin.

Regarding antioxidant variables, MF-Lamiaceae, sunflower, and goldenrod honeys were located on the negative PC2, while ORAC activity clustered linden, MF-*Tilia*, and chestnut on the positive PC2 coordinate. The analysis of macroelements revealed a different picture, separating the honeys from each other according to their color. The light-colored honeys represented negative, the dark-colored ones positive PC2 value. K played a key role in clustering of linden, MF-Lamiaceae, and chestnut honeys on the positive PC1 coordinate. The third group, the microelements, separated clearly the Mn-rich chestnut honey. Similarly to the macroelement analysis, the biplot separated the light- and dark-colored honeys from each other.

Several attempts have been made to identify and differentiate honey types from each other by means of PCA. For example, PCA of three Indian honeys based on antioxidant properties and minerals was able to separate different honey types, but analyzing the same honeys based on their sugar content was not so powerful [37]. In a Turkish study, antioxidant capacity, mineral contents, and vitamin B₂ values were useful in distinguishing honeys from various botanical origin [38]. Romanian honey types could be separated with PCA based on mineral content, color, antibiotic, and pesticide residues [40], while the analysis of physicochemical parameters was only partly able to identify various honey types [16]. PCA working with nine variables (browning index, color parameters, and antioxidant values) successfully distinguished light- and dark-colored Polish honeys [41].

However, in this study, PCA was applied not only to identify honey types but also to interpret the relationships of the studied parameters, namely the antioxidant activities, macroelement contents, and botanical origin of honeys.

6. Future prospective

The complex approach applied in this study can be used in further investigations aimed at identification of various honey types and establishing their nutritional

values and biological activities. Since the issue of honey adulteration poses a great challenge internationally [75, 76], it is essential to recognize the unique traits of each honey type, which can aid proper identification of these beehive products. As there is a growing interest in applying natural agents, such as the products of the beehive, for health maintenance [21, 77–79], the differences in antioxidant power, antibacterial property, and mineral content should be revealed for each honey type by further studies.

7. Conclusions

Our complex analysis of four light- and four dark-colored Hungarian summer honeys revealed the characteristic traits and unique values of each honey type. Melissopalynological analysis combined with the sensory traits color, odor and consistency, and spectrophotometric color determination was found to be suitable for confirming the botanical origin of honey samples. Our study supported the general view that light-colored honeys possess lower antioxidant activity compared to darker colored ones, except for linden honey, which acted as strong antioxidant despite its light color. The color of honey can be a good predictor of its mineral content. The PCA based on macro- and microelements composition, successfully separated the light- and dark-colored honeys, the latter typically containing more of both macro- and microelements. The honeys with a dominance of *Tilia* pollen were exceptions, with macroelement contents comparable to those of dark-colored honeys. The SET-based antioxidant assays TRC and DPPH were found to correlate with the macrominerals P, S, and Mg and also with the microminerals Cu and Mn. Chestnut honey can be a good source of Mn, whereas goldenrod honey and multifloral honey with Lamiaceae pollen dominance can supply Fe. The unique character sets based on the above analyses can aid the botanical authentication of each honey type and reveal their individual nutritional values and health benefits.

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Adoption of Modern Hive Beekeeping Technology: Evidence from Ethiopia

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Abstract

Ethiopian farmers have a variety of livelihood alternatives, including beekeeping. This book chapter discusses the extent to which farmers have adopted modern Hive Beekeeping Technology as well as the factors that influence adoption. Better beehive technology has a significant positive impact on honey output. Farmers are encouraged to adopt or continue using the technology because the return on investment, that is productivity increases, has been realized. Because improved beehive technology adoption has multiplier effects ranging from increased beekeeping productivity to economic growth and poverty reduction, the study's findings highlight the importance of appropriate policy implementation that promotes the adoption of productivity-enhancing technologies in general and improved beehive technology in particular. As a result, it is recommended that policies be developed to take advantage of the factors influencing farmers' adoption of improved beehive technology. Furthermore, efforts should be made to increase access to improved beehives by introducing substitutes that can be built from locally available materials while lowering production costs, such as *chefeka*.

Keywords: adoption, beekeeping practices, beekeeping technology, modern hive

1. Introduction

Ethiopia, with its diverse biological and climatic circumstances, is one of Africa's leading honey producers [1]. Ethiopia has some of the most diverse flora and wildlife in Africa, providing surplus nectar and pollen to foraging bees, and the country has more than 10 million beehives, and around 2 million individuals involved in honey production [2, 3]. This massive disparity is attributed to the country's conventional production methods, which result in low output. The favorable climatic conditions of the country and the wide range of floral resources allow it to sustain approximately 10 million colonies of honeybees, 7 million of which are kept in local bee hives by farmers [4].

Furthermore, in all parts of the country, several million colonies of bees are managed using traditional beekeeping methods [5]. Traditional beehives are basic cylindrical containers in which bees and their combs are housed. They are hives with permanent honeycombs that are typically housed in hollow logs or clay or Wicken vessels. Traditional beekeeping does not use contemporary equipment or practices [6]. As a result, collecting fruits and vegetables kills or seriously weakens the colony.

The Ethiopian government attempted to offer alternative beekeeping technologies to beekeepers after discovering the potential of the apiculture subsector and the problems related to conventional beehives [7]. For example, in 1965, demonstration stations of beekeeping were established in various areas, such as Holleta, Nekemte, and Jimma to introduce improved beekeeping technologies (box hives, casting molds, honey extractors, honey presser, smokers, water sprayers, veils, gloves, etc.) imported from abroad to beekeepers and to offer beekeeping training for farmers and experts [8].

However, in recent years, the well-known type of movable frame hive, the modern hive (MH), has been introduced and is now actively advocated. It is a modern beekeeping technique that provides an alternative system for frame hives [9]. The main claimed benefits of a moveable frame hive are that it is significantly more productive and easier to manage bee colonies than a regular hive, which requires people to examine. Furthermore, it is easier to open, remove, and harvest than a regular hive.

Furthermore, despite efforts by numerous organizations to increase the adoption and production of beekeeping, some social, ecological, and climatic aspects have been identified as barriers that prevent farmers from adopting existing beekeeping technologies [10, 11]. However, there are no comprehensive data on the adoption status and determinants of modern beehive technology.

2. Adoption of modern hive beekeeping technology

2.1 Definition and concept of adoption

Romanelli et al. [12] defined adoption as a farmer's degree of use of new innovation by a farmer after receiving complete knowledge of the new innovation and its potential. The individual and aggregate adoption of new technologies was classified by the authors. Individual adoption was described as the farmer's decision to incorporate new technology into the production process, while aggregate adoption was defined as the process of dissemination of new technology within an area or population.

The first type of adoption is discussed in the study of enhanced hive adoption. At the farm level, the adoption pattern of technology development in agriculture is not uniform. It is a complex process driven by numerous socioeconomic elements [13]. Farmers' socio-psychological systems, as well as their level of readiness and exposure to improved practices and ideas, that is, changes, such as farmers' awareness and attitudes toward improved agricultural technologies, institutional factors that act as incentives/disincentives to agricultural practices, and farmers' resource endowments, such as land holding size and labor, are all important factors in bringing about technological change.

Adoption is a mental process that an individual farmer (a decision maker or a group of family members of the decision maker) goes through to decide [14]. Specific economic, technological, and institutional conditions must be met to accept new innovations. Farmers should find that the new technology is more profitable than the previous options. Furthermore, smallholders must have access to the new technology and all other necessary inputs at the proper time, place, quantity, and quality [15].

2.2 Background of the adoption study

Technology creation and development are collaborative efforts, and the demand for new technologies must be driven by user demand. The following are some of the reasons why adoption studies are important: to quantify the number of technology

users over time to assess impacts or determine expansion requirements; to provide information for police reform; and to provide a foundation for analyzing the impact [16]. Rural sociological research on the diffusion of agricultural innovation began in the United States in the 1920s when the United States Department of Agriculture started to evaluate the process of adopting improved agricultural practices among farmers [17].

In the 1950s and 1960s, the sociological study of agricultural innovation spread rapidly in the United States, influencing the start of similar studies in other countries [18]. The adoption of agricultural technology has attracted the interest of development economists because the majority of the population of developing countries relies on agricultural produce for their living, and new technology appears to offer opportunities to improve production and productivity [19].

Adoption research in Ethiopia began three decades ago. In terms of the adoption of beekeeping technology, the study is mostly limited to the adoption of agricultural, live-stock, and soil and conservation technologies [20]. As a result, this research contributes significantly to the lack of knowledge about the adoption of beekeeping technology.

2.3 Farmers' adoption decision

Adoption is a decision-making process in which a person goes through a series of mental processes before deciding to adopt new technology [21–23]. The process by which an individual progresses from first learning about an invention to developing an attitude toward it, making a decision to adopt or reject it, putting the new concept into action, and receiving confirmation of the decision is known as decision-making.

The first was a lack of awareness of the technology or its potential benefits, which could include misunderstandings about the associated costs and benefits [24]. The second issue was that the technologies were not profitable, given the complicated decisions that farmers have to make about how to distribute land and labor between agricultural and nonagricultural enterprises [25]. This could be because farmers selected traits found only in local varieties or because acceptable varieties for their agro-ecological settings were unavailable. It could also be attributed to institutional variables, such as the policy environment, which have an impact on the availability of inputs (land, labor, seeds, and fertilizer), as well as credit and production markets [26].

These institutional considerations also have an impact on input prices. It is also possible that enhanced technologies could raise production risks: if crops fail, the financial losses will be greater. Specific economic, technological, and institutional criteria must be met to effectively use technology [27]. From the perspective of farmers, the new technology should be more profitable than the old options. Smallholder farmers should be able to use the new technology as it is technically possible and easy to administer, as well as flexible to the surrounding sociocultural settings. Similarly, smallholders should have access to new technologies and all other necessary inputs at the correct time and location, in the right quantity and quality [28, 29].

2.4 Beekeeping in Ethiopia

The diverse agro-climatic conditions of the country generate a climate that supports the cultivation of more than 7000 types of flowering plants, the majority of which are bee plants [30]. Demissew et al. [31] also confirm that Ethiopia is home to several indigenous flowering plant species. The ideal environment of the country

allows the existence of approximately 10 million honeybee colonies, of which 7 million are housed in various man-made hives and the rest are wild colonies [32].

In Ethiopia, beekeeping has a long history. As a result, beekeepers have developed indigenous technical knowledge on traditional hive construction from a variety of locally available materials, as well as honey bee management practices, such as honey season identification, swarm catching and attracting ant methods, swarm control, honeybee enemy protection, and traditional sting protection and pain reduction methods [33]. Traditional forest, traditional backyard, transitional, and enhanced or modern hive beekeeping are the four types of beekeeping techniques in Ethiopia, according to Holeta Bee Research Center [34].

Traditional Forest Beekeeping: It involves the establishment of hives in the woods on very tall trees to catch swarms. It is often practiced in areas of the country where there are large populations of honeybees [35]. The benefit of forest beekeeping is that bees do not hurt domestic animals or humans, and they have access to a variety of forage plants in their immediate surroundings. Its downsides include a lack of close monitoring and injury to the honey bee colony when the beekeeper removes the hive from the tree during honey harvesting. Climbing a tall tree at night is extremely dangerous for the beekeeper [36].

Traditional Backyard Beekeeping: It is done in a protected environment for honey bees, usually in a homestead. The advantages of such practices are that they are simple to construct, do not require improved beekeeping equipment, and do not require skilled manpower; on the other hand, they are inconvenient to conduct internal inspection and feeding, in some cases the size is too small and causes swarming, there are no superpositions options, and there is no partition to differentiate the brood chamber from the honey chamber [35, 37].

Transitional Beekeeping: It is one of the most advanced beekeeping techniques. The Kenyan Top Bar Hive (KTBH) and Tanzanian Top Bar Hive (TTBH) are the two types of hives (TTBH). The hives can be built from wood, mud, or other locally accessible materials. Honeybees attach their combs to 27–30 top bars in each hive [38]. The top bars are 3.2 cm wide and 48.3 cm long, with a width and length of 3.2 cm and 48.3cm, respectively.

The hive can be opened easily and quickly, the bees are guided to build parallel combs by following the line of the top bars, the top bars are easily removable, and this allows beekeepers to work quickly, honeycombs can be removed from the hive for harvesting without disturbing combs containing broods, the hive can be suspended with wires or roosts, the hive can be suspended with wires or roosts, the transitional beekeeping has its own drawbacks, such as the fact that top bar hives are more expensive than traditional hives, and that combs dangling from the top bars are more likely to fall apart than combs built within frames [39–42].

Modern Hive Beekeeping Practices: It uses different types of frame hives. The Zandar and Langstroth hives are the most common that exist in the country. Dadant, Revised Zandar, and foam hive are uncommon. The number and size of frames in these hives vary. In Ethiopia, the most common type of hive is type. The Zandar Brood chamber, the super (honey chamber), the inner, and exterior cover are all components of improved beekeeping hives. The improved hive has an advantage over the others in that it produces a high quality and quantity of honey. Another advantage of upgraded hives is their ability to prevent swarming by transporting bees from one location to another in search of honeybee flowers and pollination services [43, 44]. On the other hand, its downsides are that the equipment is somewhat expensive, that professional labor is required, and that the equipment requires extremely precise precautions.

2.5 Overview of modern hive beekeeping technology development

According to the Holeta Bee Research Center, the cornerstone of current beekeeping technology development can be traced back to Langstroth's practical use of the bee space concept in 1851 [45]. Four significant discoveries have contributed to the rapid development of contemporary beekeeping:

1. In 1806, the first moveable frame hives were built.
2. Langstroth's 1851 application of "bee space" and the subsequent invention of the modern movable frame hive. Bees respected the bee space, which is a 9.5mm air gap between the frame or comb and the walls and coverings of the hive. In the creation of enhanced box hives, the room for bees is quite important. An unwanted comb is created when the bee area is larger, making it difficult to transfer frames easily [46].
3. The invention of the beeswax foundation press in 1857, which produces sheets of beeswax for cell basis identification.
4. In 1865, the centrifugal honey extractor was invented. In the same year, the queen excluder was designed. By preventing the queen and drone from entering the honey chamber, it helps to keep the brood out of the honey storage frames [47, 48]. In general, the pattern of better beekeeping emerged in the half-century between 1851 and 1900.

2.6 Role of beekeeping

2.6.1 Central role of beekeeping

Beekeeping is a sustainable form of agriculture that benefits the environment, provides economic reasons for the preservation of native habitats, and can increase food and forage crop yields [49].

- A. Bees are cosmopolitan, which means that they can adapt to a wide variety of environments. They may survive below 400 m altitude, where cattle production may be severely limited due to tsetse flies or other factors [50, 51].
- B. Beekeeping is a viable option for small-scale farmers and landless peasants. The hive occupies a relatively little area and bees can gather nectar and pollen from all over [52].
- C. Beekeeping does not compete for resources with other agricultural operations and can be managed along with them. In the absence of bees, man cannot harvest and use nectar and pollen [53].
- D. Beekeeping does not disrupt the ecological balance, as do crop production and animal husbandry methods [54].
- E. Initial investment and ongoing costs are low, with little risk. Because bees can be collected from the wild, beekeeping is possible even for those with limited

resources. Equipment can also be built locally, and bees rarely require the help of beekeepers [55].

- F. Honeybee pollination is provided throughout the world. This is an essential plant biological activity in the development of crops and fruits. As a result, beekeeping contributes significantly to the agricultural economy as a whole [56].
- G. Honey, beeswax, and propolis are all produced by honeybees. These commodities have a long shelf life and can be increased according to beekeeper interests and available time [57], without the need for special storage and shipping infrastructure, such as dairy.
- H. The whole family can participate because most work can be done at home by men, women, or older children [42, 58, 59].
- I. A beekeeper might gain satisfactory knowledge and skills that help to build self-reliance [60].
- J. Other local trade profit from the production of hives and equipment, as well as from the use and sale of value-added products. Honey, beeswax, pollen, and propolis can be used in a variety of foods, cosmetics, ointments, and other products that can be created and marketed locally, increasing livelihood options [61].
- K. Apitherapy, that is, medicine employing bee products. All communities have a wealth of traditional knowledge about the curative benefits of bee products [62].

2.6.2 Economic participation of beekeeping

Since ancient times, beekeeping has been an essential part of Ethiopia's agricultural system. It has been a tradition for a long time, long before other farming systems were developed. Beekeeping is a long-standing and deeply ingrained tradition in rural communities throughout the country, with an estimated one million farmers keeping bees [63]. Beekeeping has played and continues to play an important role in the country's national economy, as well as in smallholder farming [64]. Although difficult to quantify, the contribution of bees and hive products is likely one of the most important small-scale income-generating activities for hundreds of thousands of farmer beekeepers [65].

Beekeeping has a number of benefits that help farmers improve their quality of life [66–69]. The following is a summary of the socioeconomic impact of beekeeping, as well as the primary hive products and the relevance of beekeeping.

2.6.3 Honey production

Honey, a natural product of the honeybee, has been dubbed “man's sweetest food” on numerous occasions. It is a great energy source because it is made up of simple sugars that are ready to absorb as soon as they enter the intestine. Honey comprises around 180 components and has a variety of applications [70]. Honey is in high demand in the area since it is used to make the traditional “Tej” (honey) mead. Furthermore, beekeepers are expected to earn approximately 360–480 million Birr per year from the total honey produced in the country [42, 45].

2.6.4 Bees wax production

Beeswax collecting is insignificant in some parts of the country, and beeswax generated by bees, which could be retrieved by beekeepers, is wasted. Because wax has no practical utility for beekeepers, it is typically left or discarded by beekeepers [71]. Ethiopia is now the world's fourth largest producer of beeswax, behind China, Mexico, and Turkey. Beeswax contributes to the national economy by earning foreign exchange. Beeswax is currently one of the most important agricultural exports. Ethiopia is Africa's third largest exporter of beeswax, with annual beeswax exports valued at around 125 million Birr [72]. Beeswax, like honey, is a multifunctional natural bee product that is used in the production of over 300 different products. Honey and beeswax also play an important role in the cultural and religious life of the country [73].

2.6.5 Crop pollination

Bees play an important role in the agricultural system. Although the importance of honeybees in agricultural pollination is undervalued, they play an important role in enhancing the national food supply and the regeneration of plant species. Honeybees are the world's most important pollinators [74, 75]. Although it is far more difficult to calculate their usefulness, its pollination service is estimated to be worth almost 15 times the value of all hive products combined [64, 76].

2.6.6 Source of immediate cash income

Beekeeping is believed to play an important role and is one of the solutions available to small farmers to sustain their livelihood. It is not just a source of additional money, but a large number of individuals rely exclusively on beekeeping and honey sales for their living [77]. Honeybees and their products generate direct cash income for beekeepers, according to Amulen et al. [78]. Beekeepers can sell their colonies to the market in locations where honey production is not profitable. Honeybees, in this case, serve as "near cash" capital that generates attractive money [50].

Because one of the goals of this study is to identify the characteristics that influence the adoption of improved hive beekeeping technology, and because studies on this topic are scarce, a review of studies on technology adoption in the agricultural area, in general, is offered. This proved to be useful in hypothesizing determinants of technology adoption in beekeeping and in developing models for in-depth research of the postulated components. Adoption, according to Ugochukwu et al. [79], is defined as the long-term incorporation of innovation into farmers' usual farming practices. Adoption was divided into two categories by the authors: farm-level adoption and aggregate adoption.

The degree of use of new technology in long-term equilibrium when the farmer has full information on the potentiality of new technology is described as the final adoption at the farm level of the individual farmer [79]. The diffusion process is described as the spread of technology within an area by the same authors in the context of aggregate adoption behavior. Some ideas have received widespread acceptance, while others have been rejected or embraced by a limited number of farmers.

Although the OECD [80] has a similar notion, it emphasizes the value of information. He stated that few early adopters obtain complete knowledge due to the fear of the hazards associated with the introduction of new technologies. Munguia et al. [14] define the adoption process as a series of mental phases that an individual goes through after first hearing about innovation and ultimately selecting whether to adopt or reject it. There are

five stages to the process: awareness, interest, evaluation, trial, and adoption. The time between being aware of an innovation and adopting it is known as the adoption period, and it varies not only from person to person but also from practice to practice [14, 81].

2.6.7 Technology adoption

The contribution of new technology to economic growth can only be appreciated when it is extensively disseminated and utilized. According to Mwangi [82], decisions to begin using new technology are often the result of a comparison of the uncertain benefits of the new invention with the uncertain costs of adopting it, while in the case of consumers, the benefits are the increased utility of the new good, but may also include such “noneconomic” factors as the pleasure of being the first on the block with a new good, the availability of complementary skills and inputs, and the strength of the new technology. The authors pointed out that knowing the reasons that influenced this decision was critical for both economists investigating growth determinants and designers and producers of such technologies. Various scholars have conducted several empirical technology adoption studies during the last few years. In this section, we will try to demonstrate the reviews that have been done and the inferences that have been made based on the findings of the studies.

Feder et al. [81] looked at theoretical and empirical research on agricultural innovation adoption in developing nations. They demonstrated that the observed diffusion patterns depend on complicated interactions between elements, such as new technology risks, farmer attitudes toward risk, fixed adoption costs, and financial availability. Farm size, risk and uncertainty, human capital, labor availability, credit restrictions, and landownership and rental arrangements are among the characteristics that have been theorized by a number of empirical researchers to influence farmers’ adoption decisions.

Munguia et al. [14] and Batz et al. [83] conducted a study in Kenya’s Meru district to assess the influence of technology characteristics on the rate and speed of adoption of dairy technologies and found that relative investment was less influential than relative complexity and relative risk, despite the fact that the strong influence of relative complexity and relative risk of technologies on adoption can be explained by the characteristics of farmers and farming circumstances. The study concluded that the development of low complexity risk reduction technologies would be preferable to the technologies that should be replaced.

According to Adesina et al. [84], the farmer’s sex extension contacts, farmers’ group participation, and places facing fuel wood scarcity all showed higher adoption of alley farming, whereas locations with very high population pressure showed lower adoption. On the other hand, farmer household characteristics are rarely observed to have a significant impact on adoption. Importantly, household income did not appear to be a factor in adoption, implying that minimum tillage is a low input technology acceptable for resource-poor households. They stated that the findings of research like this are valuable in identifying low-input technologies and strategies that promote them among agricultural households.

2.7 Factors affecting adoption

The introduction of the new technology to smallholder farmers does not guarantee that it will be widely adopted and used efficiently. Farmers’ adoption decisions are influenced by a variety of factors. Farmers’ decisions about the adoption of agricultural innovation can be influenced by factors, such as economic, institutional,

demographic, and physical aspects. Some demographic and socioeconomic characteristics that influence the adoption of different technologies among small farmers in developing countries have been identified in previous research.

The plot size, previous experience with fertilizer, fertilizer supply, farm size, amount of rainfall, household size, and the ratio of the price of the main crop to the cost of fertilizer, as well as access to credit, were identified as factors that restrict the demand for fertilizer among arable crop farmers by ref. [67] in Ethiopia and [85] in Sub-Saharan Africa. Credit, farm size, risk, labor availability, human capital, land tenure, and education, according to Feder et al. [81] in their research report, are the main determinants determining technological adoption. Personal, economic, institutional, and intervening (psychological) factors of the family are variables recognized as having a relationship with adoption for the simplicity of grouping [46].

The size of the farm, the area of the potato farm, extension contact, and attitudes toward improved practices were found to be strongly associated with the adoption of improved potato practices in the research conducted by ref. [57] using correlation analysis in Bangladesh. However, adoption was not related to the farmer's age, education, or organizational involvement. Honeybee pests, which harm honeybees and hive products, can stymie the adoption of improved beekeeping technology. This is not to say that pests do not affect traditional beekeeping methods (**Figure 1**) [86].

2.7.1 Personal factors

Age is an essential household feature that influences the adoption behavior of subsistence farmers. It is commonly assumed that older farmers will have greater farming expertise and skill, allowing them to more quickly appreciate the technology's benefits than younger farmers. According to the research conducted by Gebiso [87], the increase in technology adoption with age may be related to the fact that

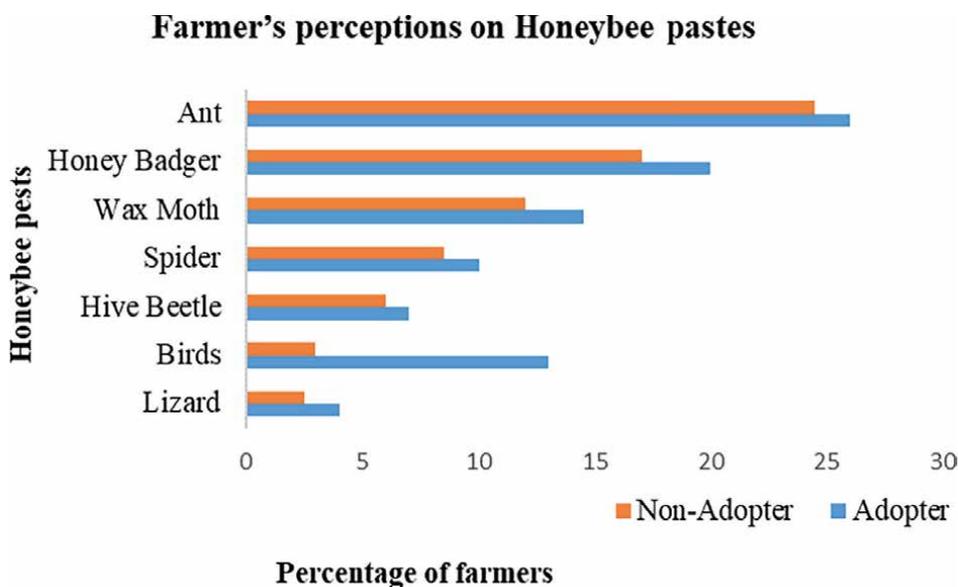


Figure 1.
Honeybee pastes [86].

most resources are in the hands of older people and most young farmers do not have adequate backyards for beekeeping and live in the town.

Farmers with large families may be more likely to use technology to meet the needs of their families. As a result, households with large families were expected to be more likely to adopt technology [33]. The ability of a farmer to receive, understand, and use knowledge related to the adoption of new agricultural technologies should improve as a result of exposure to education. According to Zegeye et al. [88], education level influenced the adoption of improved wheat cultivars and chemical fertilizers. Technical applicability is important for improving the use of box hive technology usage; Feder et al. [81] stated that education improves decision making, and thus, influences the level and/or composition of the input.

As a result, education is expected to improve the understanding of technology and, as a result, adoption. Literate farmers are more exposed to the outside world and information, which makes it easier for them to connect to technological sources [79]. Similarly, educational attainment and technological adoption have a strong and direct association [58]. Another major element related to the household that has an impact on adoption is the experience of farming. Longer farming experience means acquired farming knowledge and ability, which contributes to adoption [89]. These farmers' characteristics, such as participation in field days and demonstrations, confirmed that farm technology adoption is aided by participation in field days and demonstrations. Visiting other beekeepers' apiaries or demonstration locations might help a beekeeper have a better understanding of beekeeping. Visits between farmers to share their experiences also help establish a positive attitude toward an invention or new technology [79].

Training is critical to increasing beneficiary productivity, while also raising knowledge of technology. According to Wodajo [90], the training may have instilled technical competencies, increased exposure to the subject, and persuaded farmers to embrace new agriculture technologies. The participation of beekeepers in demonstrations and training of modern beehives was one of the most important predictors of adoption [87]. The proportion of adopters who were trained was substantially higher than those of nontrained adopters [77], indicating that farmer training on the technology presented in this case on the current beehive technology has a favorable influence on adoption. Farmers' adoption decisions were likely to be influenced favorably by the acquisition of technical skills and knowledge of beekeeping through training [47].

2.7.2 Economic factors

The amount of livestock a family owns is a good indicator of their financial situation. Livestock is also major source of income for farmers, allowing them to invest in the adoption of modern agricultural methods. It has varied effects on different people in different regions in regard to adopting improved technologies. It has a beneficial impact on the adoption of agricultural technologies by households in most cases [88]. Many adoption studies have found that having livestock has a beneficial impact on adoption [58, 66].

Farmers' adoption behavior is influenced by land-related characteristics, as land holding is an important unit where agricultural operations occur. Many adoption studies found that the size of a farm was positively associated with the adoption of upgraded technologies [47, 67]. Another element is the availability of credit for the acquisition of agricultural technologies. Those farmers who have access to financing will be able to purchase contemporary beekeeping equipment at a lower cost than those who do not. Farmer participation in off-farm/non-farm occupations will alleviate financial constraints, allowing them to purchase inputs like contemporary beehive

equipment. As a result, financing has a beneficial and considerable impact on the adoption of current beehive technology [30].

2.7.3 Institutional factors

Extension agents offer farmers with information on agricultural technology on a regular basis, based on the frequency with which they interact with them. Extension efforts, according to Feder et al. [81], raise the likelihood of new technology by growing the store of information about current production increases. Extending the use of improved box hive technology requires close monitoring by extension personnel. Many adoption studies have found that farmers who have access to extension services are more likely to embrace improved agricultural technologies [34, 57].

Attendance in extension activities such as demonstrations, training, and field days are also important to improve farmers' experience, capacity, and confidence in the benefits of better agricultural technologies. Yigezu et al. [91] found that participation in field days had a positive and significant impact on the adoption of agricultural technology. On the other hand, ref. [77] reported that farmers' adoption decisions were positively influenced by participation in demonstrations and training on farms. Participation in extension activities was found to have a favorable and substantial link with adoption in other research [88].

Equipment for honeybees is available: Improved hives and working tools for the rural community are beyond the reach of most farmers, and even for those who can afford them, they are not readily available [27]. Smallholders should have access to new technology and all other relevant inputs at the right time and location, in the right amount and quality [47]. Other key issues include the lack of cash and lack of modern beehives and their accessories (honey harvesting and processing equipment, such as wax scrubbers, queen excluders, honey extractors, bee smokers, and others) available to beekeepers [87]. To operate the hive with honey, beekeepers must wear protective clothing (overall suit, bee veil, and gloves) and use equipment, such as a smoker. The availability of these materials influences the adoption of the technology [15].

2.7.4 Psychological factors

Beekeeper Perceptions: Farmers' opinions on innovation features influence adoption rates [33]. The positive attitude of beekeepers toward technology influences their decision to adopt it. Beekeepers' positive attitude about technology influences their desire to adopt it. The respondent's perception of technological characteristics, such as (I) grasp of relative advantages and (II) awareness of or concern about disadvantages. The package's overall perceived attribute is then determined by the differences between the two [58, 92].

Knowledge of technology: Knowledge is the process by which a person is made aware of the existence of innovation and a comprehension of how it works. Knowledge of technology is essential to be effective and efficient.

3. Conclusions

Since ancient times, beekeeping has been an essential part of Ethiopia's agricultural system. It has been a tradition for a long time, long before other farming systems were developed. Beekeeping is a long-standing and deeply ingrained tradition in rural areas throughout the country, with an estimated one million farmers keeping bees.

Several factors, including the educational level of the respondents, the size of the land, extension contact, access to funding, and the market, had a significant impact on the adoption of modern hive beekeeping technology.

It is advised that the Livestock and Fish Resource Development office adopt ways to help illiterate people of the community benefit more from modern hive beekeeping technologies, and should develop a strategy to benefit farmers with large land holdings with modern hive beekeeping technology; extend contact with farmers prior to technology innovation leads to better adoption of technology; strongly advised to link a strategy with a microfinance institution, so that farmers who do not have access to credit can benefit more from technology adoption; and must develop strategies to promote technology adoption among farmers with no market access. Promotional operations focused on respondents' lack of market access, which necessitated their active participation in adoption.

The adoption of contemporary beehive technology has a substantial impact on hive productivity, as beekeeping may be used as a source of income diversification or even as the primary activity for most rural residents. However, the number of beekeepers who have switched to contemporary beehives (adopters) are small and continues to use traditional beekeeping methods. Despite the fact that practically all beekeepers are aware of the existence of current beehive technology, they have chosen not to accept it for a variety of reasons, including personal, economic, institutional, and psychological issues. The frame size, form, and color are the most important considerations, and they should be differentiated according to the agro-ecological conditions. It is tough for bees to adjust and generate their goods, while remaining alive because most beekeeping hives are the same across the country.

Conflict of interest

The authors declare no conflict of interest.

Notes/thanks/other declarations

Thank you, God! And I had like to thank my father, Ato Bojago Dado, for his advice and sharing of evidence, and I'd like to add, "Thank you for being there; I honestly don't know what I would have done without you."

1. *Chefeka* is a sort of enhanced beehive constructed with locally accessible materials at the lowest possible cost.
2. Adopters are beekeepers who have utilized at least three improved box hives for at least two years, whereas non-adopters are beekeepers who have not used improved box hives throughout the research period.

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Section 3

Application of Honey
in Food Products

Chapter 6

Utilisation of Honey in Processed Food Products

Neha Babbar, Parv Bansal, Poonam Aggarwal, Kulwant Singh and Sukhpreet Kaur

Abstract

Honey is a unique food owing to its rich composition. Honey consumption in the diets dates back to older times where it was used as a remedy for stomach aches, dehydration, allergies, intercellular damage, skin and hair problems, as well as for its astringent. Honey is used in several food formulations these days. The majority of population is demanding partial or complete replacement of sugar with some healthy sweeteners. Honey is one of the replacers offering so many benefits from being sweet to therapeutic. The unique healing properties of honey such as antiseptic, antibacterial and antiviral are well recognised. By harnessing the unique benefits of honey for formulating healthier products is very beneficial for overall nutrition and well-being. Many honey-containing products such as honey candy, honey spreads, honey bread, honey yoghurt and honey flakes have been prepared which showed increased therapeutic potential. Also, honey-containing beverages are becoming popular owing to its natural sweetness. Honey offers great scope for the development of value-added products or as an ingredient in several formulations.

Keywords: antiviral, therapeutic, astringent, nutrition, formulation

1. Introduction

Modern fast living has directly influenced eating habits, which has had a number of detrimental effects on health. Unambiguously stating how food is connected to both good and bad health, the quote 'Food is associated to gratification, thus the compromise between pleasure and health is a dilemma' and is highly intriguing [1]. Growing consumer knowledge has resulted in a shift towards functional food consumption and an expansion of the market for these health foods. As a result, there are now more and healthier dietary options available. Honey is a wonder food that not only tastes good but also has many health advantages. It is well recognised that honey contains bioactive substances that have antioxidant capabilities, either on their own or in combination with other food ingredients. Honey is nature's gift to the mankind. It is a superfood with distinct qualities, a delicious flavour and health advantages. One of the most well-known uses for honey is as a remedy for stomach aches, dehydration, allergies, intercellular damage, skin and hair problems, as well as for its astringent, cosmetic and antiseptic, antibacterial and preservative effects

Honey added products	Characteristics	References
Gummy candies	Sucrose was replaced with honey Developed using honey and propolis extract Higher antioxidant capacity	Rodríguez-Zevallos et al. [2]; Rivero et al. [1]
Honey hard candies	Honey as a source of sugar and nutrients Optimal amount of honey was 29% to prepare honey candies	Sahlan et al. [3]
Beetroot candies	Developed using honey and sugar syrup of 30°Brix A progressive increase in TSS was attained up to 70°Brix	Bhattarai and Kusma [4]
Honey-fruit candies	Prepared by the process of osmotic dehydration Temperature: 60°C Sucrose concentration: 100:13 Time: 420 min Solution to fruit ratios: 2:1 Obtained better results by using sucrose and honey mixture as an osmotic solution.	
Guava honey spread	Vital ingredients are sugar, acid, pectin, guava and honey Prepared by using 1:2 honey: guava pulp Shelf life of more than 150 days Increased nutritional value	Parihar and Kumar [5]
Goat meat spread	Higher protein content Increased spread ability Boosted cooking yield Obtained best results on the addition of 3% honey in terms of flavour, texture, appearance, adhesive power, aftertaste, spread ability and overall acceptability	Raziudin et al. [6]
Honey malt spread	Prepared by using different levels of honey	Dianat et al. [7]
Honey flakes	An effective substitute for liquid honey Almost perfectly matched the flavour, colour, and aroma of real honey. Shelf life of more than a year when stored at room temperature. No observable flavour or colour loss if the product clumped during storage	Umesh Hebbar, Rastogi and Subramanian [8]
Honey osmo-dried apple pops and rings; apple choco shots and apple pie	Better antioxidant activity Excellent energy value Better quality	Sharma, Vaidya, and Gupta [9]
Addition of honey to beverages	Enhanced the nutritional content Better phenolic compound profile Boosted good sensory acceptability Increased catechin contents Enrichment of quercetin was observed	da Silva et al. [10]; Leite et al. [11]
Addition of honey to yoghurt	The average protein content increased Increased nutritional value Synthesis of more amino acids	Shuwen et al. [12, 13]
Addition of honey to bread	Enhanced nutritional, sensory and preserving qualities of bakery products Boosted the dough's ability to ferment or combine Improved the freshness of bakery products Prevented them from ageing 5-10% honey powder was used for best results	Tong et al. [14]

Honey added products	Characteristics	References
Addition of honey to muffins	Improved volume Significant variation in colour, texture and flavour	Ramya and Anitha [15]
Addition of honey to biscuits	Increased adhesiveness of dough Improved organoleptic properties	Conforti and Lupano [16]

Table 1.
Development of different honey-enriched products and their characteristics.

on people and food. Modern consumers are choosing to switch to healthier and tastier products as they become more and more aware of the underlying reasons of bad health. The consumer prefers processed food products that completely or partially replace sugar with honey over those that only contain sugar. The baking, beverage and confectionary industries use honey extensively. Many processed food items that include honey are discussed in the chapter, along with their health advantages. **Table 1** gives a detailed information on the utility of honey in different processed products.

2. Confectionary foods containing honey

Confectionary items are all time and everyone's favourite. To increase the nutrition through confectionary, many researchers have included honey as an ingredient in confectionary food items especially candies and toffees. Rodríguez-Zevallos et al. [2] created gummy candies with an intention to decrease the glycemic index wherein the sucrose was replaced with honey. Rivero et al. [1] developed gummy candies using honey and propolis extract. A higher antioxidant capacity was observed in gummy candies prepared with honey when compared with commercial counterparts. Sahlan et al. [3] prepared honey hard candy using honey as a source of sugar and nutrients along with glucose syrup, water and sucrose as the main components. The addition of honey by 29% was found to be optimal for the development of honey candy. In another study, beetroot was candied using 30°Brix sugar syrup and honey. After boiling and draining the syrup for a few days, a progressive increase in TSS was attained up to 70°Brix, and it was eventually dried at $55 \pm 3^\circ\text{C}$ [4].

Osmotic dehydration was used to prepare herbal gooseberry candies with honey as the main ingredient [17]. Several researchers have found better results by using sucrose and honey mixture as an osmotic solution. Bawa and Gujral [18] examined the impact of sucrose and honey solution on solid growth and water loss. It was discovered that the agents' concentrations in the soak solutions at room temperature as well as the rate of moisture loss in the fruit vary. The sensory evaluations revealed that samples treated with honey had better flavour while samples treated with sugar had higher colour and general appeal. In comparison to other samples treated with honey and sucrose separately, pineapple sample cubes treated with honey sucrose solution (1:1) at 50°C temperature were shown to have better rehydration features and the lowest moisture content value [19]. The sample that contained both sucrose and honey (50°C) received the highest scores after sensory analysis of all the samples. After osmotic dehydration, samples with sugar and honey had the highest ascorbic acid level. As a result, the sample treated at 50°C with a solution of sucrose and honey turned out to be the best in terms of nutritional value, shelf stability and all other measured characteristics [19].

3. Honey spreads

The creation of honey spreads is a result of rising consumer demand for items containing honey. When making honey spreads, different vital ingredients such as sugar, acid and pectin are mixed together and heated under controlled conditions. Sandwiches and bread are where honey spreads are most commonly used. The addition of fruits to honey-based spreads increases the value of the product and promotes greater product variety [8]. Parihar and Kumar [5] prepared guava honey spread using 1:2 honey:guava pulp, and the spread had a shelf life of more than 150 days and also showed increased nutritional value. Goat meat spread is another example of a honey spread; it demonstrated a significantly higher protein content, spreadability and cooking yield with the addition of more honey. In terms of flavour, texture, appearance, adhesive power, aftertaste, spreadability and overall acceptability, spread containing 3% honey scored much higher than other spreads [6]. Dianat et al. [7] prepared honey malt spread using different levels of honey. Tekiki et al. [20] prepared honey spread by using olive oil and honey.

4. Honey flakes

It has been demonstrated that a variety of honey-based products, such as honey flakes, function effectively as substitutes for liquid honey in a variety of circumstances [8]. Turkot et al. [21] described a method for creating cold-roller-based honey flakes. In this process, a liquid honey was rapidly concentrated in an agitated thin-film evaporator, either in its pure form or with the addition of sucrose syrup, in a continuous manner. Based on an organoleptic analysis of the product, the inventors assert that the regenerated honey almost perfectly matched the flavour, colour and aroma of real honey. The item had a shelf life of more than a year when stored at room temperature. Although the product clumped during storage, no observable flavour or colour loss was noted [8].

5. Honey candy

Another well-liked consumer good is honey candy. Honey candies are typically made with numerous additional components and are used as health foods or as cough and asthma treatments [22, 23]. However, as many of the inventions are secret in nature, there is not much information available about how honey candy is made. In one of the often used techniques, the honey, sugar, water and butter mixture is boiled until it hits the hard crack stage, and then it is poured over a glazed surface to make honey candy [8]. Tadao [23] registered a method for making honey candies that involved heating (between 120 and 150°C) a mixture of honey, granulated sugar and corn syrup to obtain a uniform viscous liquid, which was then rolled to make a rod with royal jelly in the middle. Li et al. [22] developed a technique for making a honey candy that could be used for the treatment and prevention of cough and asthma. Before being packed in pre-sterilised bags, a mixture of plant materials (29 items), containing turnip, peach kernel, walnut kernel, pear and almond, was combined with honey and condensed to achieve the appropriate viscosity. There are number of ingredients, which can be added to produce candy with different tastes. These ingredients included milk powder, soy milk, cinnamon, peanuts, corn starch, oats, dry fruits, nuts, pecans, lemon rinds, coconut gratings, vanilla and corn flakes [8].

6. Bread containing honey powder

One of the most popular foods consumed worldwide is bread, which is also one of the ancient techniques ever discovered [14]. As new methods, instruments and devices are developed, this technology has in fact been continuously changing [24]. The idea of incorporating honey in bread preparation is not new. Many research studies had concluded that the nutritional, sensory and preserving qualities of bakery products are enhanced by adding honey powder to the dough when making them. The rheological characteristics of dough were also significantly impacted. Honey powder made the crust golden brown and the crumb yellow, boosted the dough's ability to ferment or combine and kept baked goods moister and fresher for longer. Therefore, utilisation of honey powder improved the freshness of bakery products and/or prevented them from ageing [14]. In an experiment, it was discovered that only a specific range was considered excellent for the softening of dough. For example, 5–10% is a desired range of honey powder to be used in dough to generate higher-quality bread with looseness and tenderness. Any deviation from this range deformed the bread quality. For instance, less than 5% caused a reduction in softening, and more than 10% weakened the intention of the dough and resulted in problems with stickiness during kneading, made the dough difficult to work with [14].

7. Honey as a functional ingredient in yoghurt

The emergence of functional dairy products, which essentially offer health advantages in addition to their basic ingredients, is a result of the recent public interest in healthier diets. Due to its positive connotations with health, yoghurt is the most widely consumed fermented milk product [25]. The addition of honey increases the nutritional value of yoghurt. 4.49% fibre, 3.15% protein, 0.32% ash, 2.73% fat, 20.54% total solids and 5.3108 Cfu/ml of lactic acid bacteria were found in 10% honey-infused maize yoghurt [26]. Because of the synthesis of amino acids brought about by the interaction of honey components with yoghurt cultures during fermentation, the average protein content of yoghurt increased from 3.15% to 4.34% with increase in concentration of honey from 0 to 10% [12, 13].

8. Other products

Ramya and Anitha [15] incorporated honey in muffins from coconut in various concentrations. The resulted muffins had improved volume and organoleptic evaluation of the muffins revealed a significant variation in the characteristics of colour, texture, flavour and overall acceptability. Conforti and Lupano [16] developed biscuits with incorporation of lemon juice, honey and two concentrations of whey protein and studied the effect of these on structural and functional properties of biscuits. The results showed the increased adhesiveness of dough in sample made from lemon juice and whey protein concentration as well as improved organoleptic properties of biscuits. In another study by Sharma, Vaidya and Gupta [9], Golden Delicious apples were osmo-dried and utilised to make apple rings and pops, which were then used to make premium products such as apple chocolate shots and apple pie. Honey being rich in phenols and flavonoids resulted in better antioxidant activity with excellent energy value of the developed products. The apple choco shots

and apple pie prepared from honey osmo-dried pops and rings were found better in quality with energy value of 430.09 kcal/100 g and 260.74 kcal/100 g, respectively. Thus, the developed technology can be commercially explored at industry level for the production of quality osmo-dried apple pops, apple rings, apple choco shots and apple pie.

Honey enhances the nutritional content of the beverage and makes it taste sweeter since it includes vitamins, minerals and bioactive chemicals that are nutritionally superior to sugar and sweeteners such as p-coumaric and caffeic acids [10, 27, 28]. Honey has lately demonstrated promise antiviral activity against infections that cause severe respiratory syndromes as well as possible molecular action against coronavirus. It may also improve the inflammatory state of obesity [29, 30]. Although there was no discernible change in colour, the inclusion of honey increased the total soluble solids contents, DPPH (62%), TEAC (72%) and FRAP (20%) values. Comparing the honey mixed beverages with the control mixed beverage, the honey-mixed beverages showed a better phenolic compound profile, with an increase in catechin contents and an enrichment of quercetin, as well as boosting good sensory acceptability [11].

9. Conclusion

With the increase in awareness among the consumers, the demand for functional foods is also increasing. The full or partial replacement of sugar with honey results in a healthier processed product. Many products substituted with honey have shown considerable increase in nutritional value. There is a great scope of using honey in the food processing industry.

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Honey is a natural food product with health benefits due to its numerous functional components that help to prevent and cure various diseases. This book provides a comprehensive overview of honey and honey production. The chapters discuss beekeeping practices, the geographical, entomological, and botanical origin of honey, the characteristics and properties of honey, the therapeutic and medicinal potential of honey, and honey's utilization in processed food products.

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