Chapter

Medical Grade of Honey: Ecology of Production, Botanical Origin, Authenticity and Safety

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Abstract

Providing medicinal honey involves a unique process based on scientific regulations and guidelines. Little attention has been paid to the integrative and comprehensive criteria for medical grade honey (MGH) production and evaluation. Because of the high importance of this valuable natural product and its use as a medicinal supplement, treatment aid, and even a therapeutic agent, the guidelines and criteria for identifying and authenticating medical grade honey (MGH) must be reviewed and analyzed. Medicinal grade honey is achieved through a continuous chain from the location of colony establishment to the production process to storage and screening. Any disruption in this chain will disrupt the entire process. Furthermore, numerous geographical zones lack the ability to produce medicinal honey. Accordingly, the production of natural honey for medicinal use requires harsh conditions so as to guarantee the health of consumers. Medical grade honey covers a limited range of naturally produced honey in the world.

Keywords: bee products, medical grade, ecology, authenticity, botanical origin

1. Introduction

The US Food and Drug Administration (FDA) has distinguished between food and drug classes, requiring medicine to obtain pre-market approval as well as efficiency and safety reports. Medical foods, however, are not controlled as drug supplies under the Federal Food Drug and Cosmetic Act but should be regulated under the food rules. Food classes comprise conventional foods, medical foods, dietary supplements, and foods for special dietary uses (FSDUs) [1]. Food is a combination of chemicals or nutrients that support diverse functions in the body (e.g., energy, growth, and protection against diseases). The nutritive element (primary metabolites) covers macronutrients (e.g., proteins, lipids, and carbohydrates) and micronutrients (e.g., minerals, vitamins, and water). Additionally, secondary metabolites cover bioactive or phytochemical components that have significant effects on human health as well as disease control (hypertensive, cardiovascular, inflammatory, and cancer diseases) and are classified as functional components [2].

Some prominent human disorders can be managed by medical foods, including Alzheimer's disease, diabetes, inherited metabolic disorders, gastrointestinal disorders, and cancer. Such foods can also aid in targeted nutrition, improved compliance, and reduced side effects [3].

A medical food is one formulated specifically for physician-supervised administration and dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation [4].

The Codex standard for the labeling of and claims for Foods for Special Medical Purposes defines foods for special medical purposes as those specially processed or formulated and dispensed for the dietary management of patients; they may be used only under medical supervision [5].

The American Society for Parenteral and Enteral Nutrition (ASPEN) has developed special standards for medical foods used for hospitalized patients, which are classified as either oral nutrition (ON) or enteral nutrition (EN-gastrointestinal tract via a tube, catheter, or stomach) foods. Accordingly, foods are designated as medical grade (MG) based on specific special considerations, guidelines, and regulations.

Because of its nutritional and medicinal properties, honey is known as a functional food product that guarantees biological functions are balanced [6]. Clinical studies have confirmed the health benefits of honey resulting from its bioactive compounds [7].

Natural honey has been used as a therapeutic agent in treating human diseases since ancient times [8]. The earliest references emphasizing the use of honey include clay tablets from Sumerian (6200 BC), Egyptian papyri (1900–1250 BC), Veda documents (5000 years ago), the Holy Quran, the Talmud, and the Bible as well as other sacred texts from different countries [9]. Honey has also been used as a remedial agent for infections and wounds for over 5000 years. Hippocrates (460–357 BC) and Aristotle (384–322 BC) both used natural honey as an effective remedy for treating wounds. Dioscorides, known as the father of pharmacognosy, used natural honey to treat sunburn, ulcers, inflammation of the throat and tonsils, and coughs. He introduced the yellow honey of Attica (Greece) as the best honey. In his medical manuscripts, the famous Iranian physician Abu Ali Sina mentioned honey being used medicinally for wound repair, to strengthen the stomach and cardiovascular system, to treat skin diseases, as an antidote, and for relief from insect bites [8].

Medicinal honey is organic, free of contaminants or toxic substances, gamma sterilized under standardized conditions, and free of dangerous microorganisms. It can be used safely in medical therapies, must adhere to strict production and storage standards as well as legal and safety regulations, and must comply with the physicochemical criteria that are important for the use of honey as a wound-care product [10–12].

Prominent papers have reported MGH being applied for wound management as well antibacterial effects [10, 13–17].

Providing medicinal honey involves production ecology, harvesting and storage, authentication, grading, and clinical trials. Accordingly, from production to supply is a unique process based on scientific regulations and guidelines that will be discussed in this manuscript. Little attention has been paid to the integrative and comprehensive criteria for MGH production and evaluation. Because of the high importance of this valuable natural product and its use as a medicinal supplement, treatment aid, and even a therapeutic agent, it is vital that the guidelines and criteria for identifying and authenticating MGH be reviewed and analyzed. The current study aims to provide an overview of production criteria and authentication as well as the determination of plant origin and health and classification of medicinal-grade honey.

2. Methods

Searches of the online databases of Wiley, Oxford, Springer, PubMed, Google Scholar, and Science Direct were conducted using diverse mixtures of the terms: medical grade, authenticity, botanical origin, honey ecology, and therapeutic effects. Abstracts of identified articles were carefully assessed, key data were identified, and analyses were extracted from the main texts. Our review firstly provides an introduction to authenticity and grading standards and guidelines; then we analyze medical grade honey using an integrated and multidisciplinary approach.

3. Authentication and grading

CODEX Alimentarius [18] states that honey is a natural sweet substance produced by honey bees from the nectar of plants, secretions of living parts of plants, or the excretions of plant-sucking insects on the living parts of plants which bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store, and leave in the honeycomb to ripen and mature. Natural honey must strictly adhere to the mentioned definition; any violation constitutes fraud in honey and causes its nutritional and medicinal properties to be reduced or destroyed.

Honey for consumption must comply with international food standards. Higher quality standards are necessary for MGH [19]. Accordingly, determining the medical grade of honey is a complex procedure that includes authenticity assessment, determination of the botanical origin, and confirmation of the health factors. One of the most important achievements of honey quality control is grading, which effects fair pricing, increases consumer confidence, and improves community health while also increasing demand and reducing or removing fake honey from the economic cycle.

Identifying and authenticating natural honey require the integrity of diverse methods, including physico-chemistry, phyto-chemistry, melissopalynology, microbiology, and organoleptic analyses. The first criterion for determining MGH is the evaluation of the honey's authenticity. The United States composed the first standards for honey which include extracted honey [20] and comb honey grading [21]. CODEX [18] and ISO [22] have published international standards for the quality control of honey. The Republic of China (Taiwan), China, Europe, Japan, Korea, ANZ, and Iran [23] have developed honey standards at the national level.

MGH must meet a wide range of physicochemical, phytochemical, melissopalynological, and microbiological (INSO.org) component standards to ensure the level of quality established by the European Union [24].

4. Physicochemical factors

Physicochemical factors mainly comprise sugar content (sucrose and fructose/glucose), pH, proline, moisture percentage, diastase activity, free acidity, hydroxymethylfurfural (HMF) content, ash content, and electrical conductivity (EC) [25, 26]. The first step in evaluating MGH is to determine whether the honey complies with the basic physicochemical standards (primary authenticity). Honeys with better physicochemical characteristics are more suitable choices for further screening. On the basis of Codex Standard [18], the main quality factors include diastase activity, $\geq$8 Schade unit, hydroxylmethylfurfural (HMF) content, $\leq$40 mg/kg (or $\leq$80 mg/kg in honey from tropical climates), ≤20 g/100 g free acidity, ≤50 mequiv/kg), and a pH ranging from 3.4 to 6.1 (averaging about 3.9). The honey's freshness and quality are also strongly affected by storage conditions, and the quantity of hydroxymethylfurfural (HMF) and diastase activity are strangely correlated with the freshness and quality of honey [27].

The enzyme activity of honey is mainly connected to the concentration of the nectar flow as well as the composition of the nectar. Enzyme content can be significantly reduced by processing and warming; elongated storage times also affect the authenticity and quality [28]. Some natural honeys, however, originate from rich nectar (e.g., citrus and acacia) that have low levels of natural enzyme activity [29]. The α- and β-amylase (diastase) number (diastase activity) indicate honey's freshness. Natural honey also contains the active enzymes α-glucosidase (invertase), glucose oxidase, catalase, acid phosphatases, proteases, and esterases [30].

Honey's freshness and quality are closely related to its effective medicinal compounds. Polyphenols, minerals, vitamins, and fragrance are all significantly higher in medical grade honey than in food grade honeys. Both pH and EC are chemical indicators of the mineral and acid contents of honey. They are also the most valuable markers that differentiate between blossom and honeydew honeys which have diverse therapeutic effects [31]. EC value depends on the acid and ash content of honey; higher values represent higher conductivity [32]. The free acidity (acid content) of honey is useful for evaluating fermentation in honey. Microbial modifications affect total acidity over the permissible range. EC and pH are also valuable markers for differentiating between nectar and honeydew honey. Higher levels of minerals in honey indicate greater EC values [33].

The mineral components of honey are correlated with its botanical origin, color [34], geographical origin, environmental factors [35], honey comb age [36], and length of storage [37]. Lighter honeys have lower levels of minerals than darker ones.

Component	General requirement	Exceptions
Fructose and glucose content	Blossom honey: _60 g/100 g Honeydew honey: _45 g/100 g	
Sucrose content	5 g/100 g	False acacia, Alfalfa, Firewood Banksia, French honeysuckle, Red gum, Leatherwood, citrus spp.: _10 g/100 g, Lavender, Borage: _15 g/100 g
Moisture content	18%	Calluna, Erica (_23%)
Water-insoluble content	_0.1 g/100 g	Pressed honey: _0.5 g/100 g
Electrical conductivity	Blossom honey: _0.8 mS/cm Honeydew Honey: _0.8 mS/cm	Strawberry tree, Bell heather, Eucalyptus, Lime tree, Tea tree, Ling heather, Manuka or jelly bush
Free acid	50 milli-equivalents acid/1000 g	
Diastase activity	8 (Schade scale)	Honey with natural low enzyme content (e.g., citrus honey) and HMF content _15 mg/kg: _3 (Schade scale)
HMF	40 mg/kg	Fresh and unheated honey: _15 mg/kg Honeys of tropical origin: _80 mg/kg

Table 1.
Criteria for guaranteeing honey quality [41].

A significant correlation has also been reported between metal content and both total phenolic and antioxidant activities of honey [38]. Moreover, the Maillard reaction (MR), a non-enzymic reaction between free amino acids and sugars, occurs during prolonged heating and storage. In addition, decreased diastasis activity indicates an increased level of HMF; however, increased temperatures have been reported in honey stored for lengthy periods [39].

HMF is another bio-indicator of honey safety which occurs through the dehydration of hexose in honey under acidic conditions. It depends mainly on the chemical structure of honey (e.g., pH, sugar, total acidity processing, and storage temperature). Accordingly, the maximum acceptable level of HMF is 40 mg/kg in both blossom and honeydew honey [40]. The amount of proline decreases with increases in the artificial sugar compounds added to honey. Moreover, it decreases when honey is heat-treated (**Table 1**).

5. Palynological factors

Pollen assessment (melissopalynology) is significant in the grading, authenticating, and quality control of honey [42]. Melissopalynology was first explained and proposed by the International Commission for Bee Botany (ICBB), then updated by Louveaux [43]. Analysis efficiently aids in determining the geographical and botanical origin of honey [44]. Melissopalynology also provides valuable data on honey extraction and filtration, fermentation [45], some types of adulteration [46], and contamination with mineral dust, soot, or starch grains [43]. This method is also key to determining monofloral honey [47]. Honeys derived from different botanical and geographical origins are remarkably distinct in phytochemical and biological characters that significantly affect their phytochemical components as well as their medical and therapeutic features. The International Commission for Bee Research (ICBR) recommends a pollen count higher than 1200 grains per honey sample to achieve percentages of plant taxa with an accuracy of about 1%. However, it recommends a count of 500–1000 pollen grains per honey sample. The Louveaux method [43] is the most well-known method in melissopalynology and requires a lot of expertise and experience. This method divides pollen frequency into the following four classes: Predominant pollen (more than 45% of total pollen count), secondary pollen (between 16 and 45%), important minor pollen (between 3 and 15%), and minor pollen (below 3%). Some exceptions (e.g., Medicago, Citrus and Ziziphus) should be considered in evaluations [48].

Melissopalynology is a vital tool for developing both regulatory standards and certification for honey. It also provides key data for determining poisonous pollen or other adulterations [49] as well as allergenic pollens. Some pollen grains transferred into the honeycomb by the honey bee can cause allergic responses in humans. Honey pollen proteins are one of the main causes of allergic reactions to honey [50]. Honey bees collect nectar from diverse flowers, some of which, such as *Rhododendron* spp. (Ericaceae), *Lasiosiphon* sp. (Thymelaceae), and *Serjania letbalis* (Sapinaceae), are very toxic. Notably, the pollen of *Euphorbia geniculata* is highly poisonous for bees, yet honey contaminated with this pollen does not affect human health [48].

DNA metabarcoding is an efficient method for tracking honey bees and determining their geographical and botanical origins. It also has some advantages over melissopalynology, as it does not require knowledge or systematic experience and, therefore, can easily be used to analyze a honey's botanical and geographical origin [51].

6. Phytochemical and biochemical factors (functional components)

To date, about 300 different types of honey have been documented and connected mainly to botanical origins. These various types show variations in biological activity, phytochemical constituents (e.g., volatile compounds and carbohydrates), and organoleptic properties (color, taste, and smell), and thus induce diverse therapeutic effects [52]. The chemical and biological features of honey depend mainly on the botanical and geographical origins of nectar. Seasonal and environmental features have a great impact on honey properties [53]. According to CODEX [18], natural honey is produced by honey bees from the nectar of plants and honeydew. The main chemical constituents (e.g., polyphenols, carbohydrates, and amino acids) originate from plant species. These phytochemical components are affected by ecological factors and geographical origin [54]. The high diversity of the mentioned constituents makes botanical origin the most variable marker for the authentication and quality control of honey. The main phytochemical markers include carbohydrates, amino acids, polyphenols, and aromatics. Minerals and vitamins, however, are classified as subsidiary evidence to authentication [55].

Carbohydrates comprise the main chemical constituent of nectar and include sucrose, glucose, and fructose, which range from 7% to 70% w/w and are a result of adaptation to pollinators [56] and phylogenetic structure [57]. Additionally, other monosaccharides (e.g., mannose, arabinose, and xylose), disaccharides (maltose and melibiose) or, more rarely, oligosaccharides (raffinose, melezitose, stachyose) show less frequency in nectar. Nectar sugars show significant variations, both within and between species, which severely affect the sugar profiles and medicinal properties of honey. It has been reported that carbohydrate profiles can be effective markers for differentiation in some monofloral honeys [58]. Some authors, however, believe that the sugar profile alone is not enough to identify the botanical and geographical origins of honeys. Accordingly, the quantitative spectra of sugars can assist the quality control and grading of honey [59]. The total or ratio of sugars (glucose and fructose) show higher efficiency than other evaluated sugars of honey. The main disaccharides in blossom honeys are sucrose, maltose, trehalose, and turanose. Honeydew honeys show higher levels of oligosaccharides than blossom honeys, especially trisaccharides (e.g., melezitose and raffinose) which have not been reported in blossom honey. Higher levels of erlose and isomaltose have also been found in honeydew honeys. Moreover, blossom honey contains higher levels of glucose and sucrose [60].

Prebiotics are known as non-digestible food elements that usefully improve the activity of some bacteria in the colon of the host. They are usually polysaccharides or oligosaccharides. Other known prebiotics comprise malto-oligosaccharides, especially isomaltose, cellobiose, panose, maltotriose, melezitose, raffinose, maltose, turanose, and maltotriose. Additionally, prebiotic agents of honey include nulobiose, kestose, nystose, isomaltose, and faffinose [61]. Kestose, inulobiose, and nystose have been reported in Malaysian [62], raffinose in Italian [63], and isomaltose and melezitose in New Zealand [61] honeys. Moreover, erlose and raffinose have been reported in honeydew honey [64].

Low levels of proteins have also been found in honey samples, but they have displayed low efficiency in authenticating honey. Honey proteins are derived mostly from the pollen and nectar of plants [65] but originate mainly from the enzymatic process honey bees use to break down pollen and nectar [66]. Short or bioactive peptides in foods are composed of a small number of amino acids and are mostly produced by the enzymatic hydrolysis of large proteins from animal and plant origins. *Acacia* and *sidr* honey have the mentioned peptides [67]. Potent antioxidant peptides originating from honey can improve the tolerance of eukaryotic cells against oxidative stress [68]. The results of a new study on honey [69] affirmed the role of the peptides created by honey bees as antibacterial agents in honey.

The enzyme activity of honey, related mainly to α-glucosidase (invertase), α- and β-amylase (diastase), glucose oxidase, catalase, acid phosphatase, proteases, and esterases [70], depend on the strength of the nectar flow. Moreover, enzyme content in honey, considered as a bio-indicator of processing, depends on heating as well as the storage properties [28].

Diastase shows great variations among the studied honey based on the floral and geographical origin. Nectar flow, foraging patterns of the honey bee, and pH are other factors affecting diastase activity, which decreases in old and heated honey. Similarly, invertase activity depends mainly on floral origin. Glucose oxidase is inactive under the low pH of honey. Gluconic acid and H_2O_2 is generally made through honey ripening. Nevertheless, it represents a very slow action in ripe honey. Diluting honey improves glucose oxidase activity. The proteases derived from pollen, nectar, and cephalic gland secretions of the honey bee are recognized as protolithic agents in honey. They mainly improve the bee's immune system reaction against parasites [71].

The H_2O_2 produced from glucose oxidase activity motivates photolytic activity in honey enzymes, which leads to the digestion of dead tissues and improves the development of blood vessels to promote the delivery of oxygen, nutrients, and fibroblasts for tissue regeneration. A total of 26 free amino acids have been reported in honey [72] derived from nectar, pollen, and bees [73]. In addition, storage changes and reduces honey's amino acids. Pollen is a main source of amino acids in honey, so different honeys can be distinguished by their botanical and/or geographical origin [74]. Moreover, the variability of the amino acid content in honey depends mainly on the floral sources and production time [75]. The existence of some amino acids (e.g., cysteine, tryptophan, and arginine) is recognized as a diagnostic character of certain honey types [76]. Proline is the main amino acid of honey originating from the honey bee and is a main factor in the authentication and ripening of honey [44]; however, it varies according to the botanical and geographical origin of the honey [47]. In addition, the total profile of amino acids is effective in distinguishing between various types of honey. Nevertheless, neither a solitary amino acid nor a collection of them plays any prominent role in differentiating some kinds of honey. Additionally, the quantitative profile of some amino acids can be effective in determining honeys originating from different geographical regions. The enantiomeric ratio of amino acids is reported to be an indicator of processing methods, storage conditions, and age [54]. Thermal treatment or heating (e.g., up to 90°C) reduces the protein and amino acid contents of some honeys (e.g., Tualang, Gelam, and Acacia) [77].

Some glycoproteins (e.g., the MRJP family) of honey display antibacterial properties [78]. Some glycoproteins and glycopeptides are considered immunomodulatory agents in some natural honeys (e.g., Sidr and Acacia) [33]. Moreover, some honey peptides display antigiardial activity [79], and arabinogalactan proteins in honey help regulate the inflammatory process. Honey enzymes are main agents in metabolic processes, freshness, and some antimicrobial features of natural honey. The invertase content is significantly higher in honeydew honey than blossom honey. Protease is known to improve immune reactions as well as biological defenses against pathogens [71]. Acid phosphatase is a bio-indicator of fermentation in honey [80].

Low fatty acids contents (e.g., palmitic acid, oleic acid, lignoceric, linoleic acid, stearic acid) in honey have been reported to have no significant clinical effects on consumers [81].

It has been proved that the antioxidant activity of honey is mainly dependent upon its botanical origin [82]. Polyphenol compounds, flavonoids, carotenoid derivatives, catalase, peroxides, glucose oxidase enzymes, ascorbic acid, organic acids, Maillard reaction products, amino acids, and proteins show antioxidant activity in honey [83].

The phenolic acids (chlorogenic, coumaric, ellagic, caffeic, and ferulic acid), flavonoids (pinosembrine myceticine, quercetin, galangin, hesperetin chrysin, and kaempferol), carotenoids, ascorbic acid, catalase, peroxidase, and Maillard reaction products are the main biological and phytochemical constituents responsible for the antioxidant activity of honey [84]. Polyphenols have high potential for use in key formulations of nutrition- and health-oriented bee products. The antioxidant, anti-inflammatory, antimicrobial, pro-oxidant, antihypertensive, anticancer, and antiatherosclerotic effects of honey are related mainly to honey polyphenols (e.g., quercetin, apigenin, myricetin, and luteolin).

The antibacterial features of honey result from its high osmolarity, hydrogen peroxide, low pH, glucose oxidase secreted by the hypopharyngeal of the honey bee as well as the catalase activity resulting from flower pollen and nectar, and propolis and its phenolic derivatives. The antioxidant activity in honey is known as an indicator of the strength of the antibacterial, anti-inflammatory, anti-allergenic, anticoagulant, and anticancer features [85], especially those effective on breast, cervical, and prostate cancers and osteosarcoma [86]. The direct and indirect therapeutic activities of honey against COVID-19 are related mainly to its phenolic component contents [87]. The polyphenols have a significant relationship with the color of the honey; darker honey has a higher polyphenol content, and as a result, more antioxidant power. Blossom and honeydew honeys vary significantly in chemical and biological characteristics, which causes prominent variations in their antimicrobial, anti-inflammatory, and antioxidant properties. Therefore, it is necessary to distinguish between these honeys for medical uses.

Honey also has volatile components that belong to the following seven classes: aldehydes, ketones, acids, alcohols, esters, hydrocarbons, and cyclic compounds. These volatile compounds reflect the botanical and geographical origins of honey, as confirmed by several studies [88]. They display anti-inflammatory, wound healing, antioxidant, pain relieving, antitumor, antibacterial, anticancer, antihyperglycemic, and hypotensive properties; however, some of them, such as furan derivatives and acetone, exhibit low toxic effects. In addition, a wide range of volatile compounds has been used as phyto-markers for differentiating between honeydew and blossom honey [89].

There are some aromatic and non-aromatic organic acids in honey resulting from aerobic and anaerobic fermentation [90]. Organic acids exhibit variations based on the botanical and geographical origins of the honey [91]. Non-aromatic acids are responsible for the antibacterial and antioxidant activities of honey (They accelerate the action of other antioxidants) [92], the phyto-indicator of honey fermentation, the treatment of Varroa infestation [93], and honey authenticity. They also aid in the determination of the botanical and geographical origins of honey [94].

Honey contains fat-soluble and water-soluble vitamins that are useful for the physiological health of the body. These, too, vary on the basis of the botanical and geographical origins of the honey [95] and are present in minute volumes, and thus have low biological effects (e.g., antioxidant and metabolic) on human health [27]. Although consuming more honey than the usual dosage can compensate for this deficiency, this, itself, can create complications in the metabolism of the body. Furthermore, honey vitamins are subsidiary markers of honey authenticity. Heating and storage reduce the nutritional value of honey vitamins [59].

7. Safety and health factors

Honey is an important bio-indicator of ecological conditions, such as environmental pollution (heavy metals, toxins). In modern beekeeping, contamination can occur directly because of veterinary actions or indirectly because of the bee itself through collecting nectar, pollen, or consuming contaminated water [96]. Honey must be free of measurable levels of pesticides, herbicides, antibiotics, and heavy metals that show toxicity even at low levels (arsenic, lead, and cadmium). In addition, the amounts of iron and zinc should not exceed permissible levels for foods. Many chemical contaminations, including residual toxins, heavy metals, antibiotics, and radioactive elements, have been reported in honey [97]. Accordingly, medical grade honey must be free of these polluting chemicals. CODEX [18] has described the prominent criteria for evaluating residue, pesticides, veterinary drugs, and heavy metals.

Gathering honey under organic conditions is not sufficient to guarantee the absence of all possible contaminants. A wide range of bacteria, yeasts, and molds have been reported in honey [98] which may affect its safety as well as other features [99]. Scientific evidence has shown the microorganism contamination of honey originating from three sources: One, pollen, digestive tracts of honey bees, air, soil, dust and nectar; two, animals including insects, rodents, etc. that penetrate the hives during honey maturation; and three, human activities (e.g., harvesting and equipment) [100].

Clostridium botulinum is a known bacterium in the environment whose endospore content in honey varies from 5% to 64% [101]. Clostridium spores cause botulism and can cause fatal poisoning in infants [102]. In addition, several documents have reported cases of anaphylactic shock in humans, especially infants, after the consumption of raw honey caused by these contaminants.

Diverse fungi varieties are reported to grow in honey, despite the unsuitable conditions for mycotoxin making, and they can cause different infections. The prominent microorganisms reported in honey comprise the following yeasts (e.g., *Debaryomyces hansenii*, *Zygosaccharomyces rouxii*, *Zygosaccharomyces mellis*, *Aureobasidium pullulans*, and *Cryptococcus uzbekistanensis*) and bacteria (e.g., *Bacillus cereus*, *Clostridium perfringes*, *Bacillus*, *Bacteridium*, *Streptococcus*, *Achromobacter*, *Citrobacter*, *Enterobacter*, *Erwinia*, *Escherichia coli*, *Flavobacterium*, *Klebsiella*, *Proteus*, and *Pseudomonas*, *Enterobacteriaceae*, *Penicillium* spp., *Torulopsis* spp., *Aspergillus* spp., *Actinomyces*, *Bacteroides*, *Clostridium*, *Enterobacter*, *Enterococcus*, *Escherichia*, *Klebsiella*, *Lactobacillus*, *Proteus*, *Pseudomonas*, *Staphylococcus*, and *Streptococcus*) [103]. *Gluconobacter oxydans*, *Lactobacillus kunkeei, Pseudomonas* spp., and *Bacillus* spp. have been reported in honey and can act as probiotics. *Saccharomyces, Rhodotorula*, *Debaryomyces*, *Hansenula*, *Lipomyces*, *Oosporidium*, *Pichiu*, *Torulopsis*, *Trichosporon*, *Nematospora*, and *Schizosaccharomyces* are the main extracted yeasts that can be used in foods. These biological contaminations are inactive in honey; however, they represent new side effects when moved into a living host through consumption [11].

The same applies to antibiotic treatment of bee colonies, as in the long run, trace amounts can contribute to the global burden of antibiotic resistance. To produce MGH, raw honey should at least meet organic food standards and be free of detectable amounts of pollutants. Preferably, it should be certified as organic. A worldwide collection of standards, guidelines, and codes of practice has been collated by the Revised Codex Alimentarius Commission (CAC) to create uniform international food standards. Residual pollutions are known as an important factor in genetic mutations as well as cellular degradation [104]. The chemical pollutant of honey is related mainly to soil formation and floral origin that penetrate plants, are passed to the nectar, and finally enter the honey through the honey bees [105]. The negative results of chemical pollutants cover a wide range of both acute and chronic disease, leading to coma or even death. A wide range of pesticides (e.g., insecticides, bactericides, herbicides, organic acids, and fungicides) used in agriculture lead to the contamination of bee products [106]. Several studies have reported all macro-elements (Fe, Cu, and Zn) and, to a lesser extent, micro-elements (Cd, Pb, Ag, Si, Br, and Co) as present in honey [107]. Heavy metals as well as toxic trace elements have been reported in honey found in close proximity to industrial areas. Additionally, the water, soil, and air of contaminated urban and agricultural zones are agents that aggregate these toxic elements in honey [106]. Accordingly, the amount of these compounds in honey should always be monitored so as to identify products with these compounds in minimum amounts or amounts within the standard range.

Studies have shown that HMF and its derivatives have organotoxic, enzyme-inhibitory, mutagenic, genotoxic, carcinogenic, and DNA-damaging effects [108]. HMF is known as the main intermediate product resulting from two reactions: the acid-catalyzed degradation of hexose and the 3-deoxyosone in the Maillard reaction. The occurrence of simple carbohydrates (glucose and fructose), several acids, and minerals can increase HMF production in honey. Additionally, storing honey in metal containers enhances HMF levels. Moreover, HMF is produced from oligosaccharides and polysaccharides that can produce hexoses (e.g., fructose, sucrose, and glucose) in the hydrolysis reaction. Long-term storage [109], heating, and certain physicochemical properties of honey (e.g., pH, free acids, total acidity, lactones, and mineral content) are critical to increasing HMF levels. Additionally, increasing the humidity, length of heating [110], and density of metallic ions in honey (e.g., Mn, Mg, Ze, and Fe(II)) exhibit a high correlation with HMF formation. In low pH or acidic conditions, HMF can form at low temperatures and in high water content. A high fructose-to-glucose ratio can also accelerate HMF production. Additionally, the concentrations of metallic ions (e.g., Mg, Zn, and Fe(II)) affect HMF production positively during storage.

CODEX [18], European Commission (EC) regulations [24], DIN [111], and ISO [112] have presented the most important guidelines and standards for honey health.

8. Clinical trials

Clinical trials produce valuable data on the safety, dosage, and efficacy of drugs. They aim to guarantee the scientific validity of research results. Pharmacological studies on bee products have improved in recent years, with both *in vitro* and *in vivo* studies proving the therapeutic effects in humans [113]. Accordingly, multiple drugs and supplements have been developed from bee products throughout the world. Several studies have recommended that the use of MGH be evaluated in clinical studies [114]. To date, several clinical studies have investigated MGH [115]. Honey's greatest potential lies in its antimicrobial effects, as it prevents a wide spectra of bacterial taxa (anaerobic, aerobic, gram-negative bacteria, and gram-positive). MGH is an alternative to antibiotics for wound treatment. Honey has also been successfully used to cure a wide range of mucositis [116], herpes simplex labialis [117], and surgical and chronic wounds [118]. It also inhibits a wide range of yeasts and fungi as well as some viruses. Based on concentration, honey exhibits bacteriostatic or bactericidal effects [119]. The antibacterial features of honey are related to its physical and chemical factors, namely high-pressure osmosis, the presence of hydrogen peroxide (H_2O_2), high acidity (low pH), and antioxidants, all of which reduce the growth of the mentioned microorganisms. The presence of diverse phytochemical components in honey, such as polyphenols, also prevents bacterial activity [120]. Some specific phytochemical components (e.g., MGO in Manauka honey) exhibit specific activities against microbial organisms. The acidity of honey is a well-known trait of its antibacterial effectiveness resulting from the existence of certain key organic acids (e.g., gluconic acid). Nevertheless, this factor is not effective against bacteria alone, especially when diluted in foods or biological fluids of the body. H_2O_2 is produced enzymatically, and enzyme activity increases when honey is diluted. Additionally, a linear connection exists between the H_2O_2 content and the antibacterial potency of honey [121]; however, H_2O_2 concentrations vary among honey from different botanical and geographical origins. Nevertheless, some honey samples present high antibacterial activity while producing low amounts of H_2O_2 and vice versa [122].

The functional structure of MGH in wound healing is highly correlated with the presence of hydrogen peroxide, high osmolality, acidic pH, non-peroxide elements, nitric oxide, and phenols. Honey also improves autolytic debridement, promotes regeneration of wound tissues, and stimulates anti-inflammatory activities, thus accelerating wound healing. In addition, honey reduces the occurrence of extreme scar formation [123].

MGH improves the defense of the heart system by improving lipid metabolism, weakening cell apoptosis through its antioxidant features and antiaging activities, blood pressure variation, recovery of the pulsation of the heart, and reducing heart attack risk [124]. Additionally, the anticancer mechanisms of MGH include modulation of insulin signaling and estrogenic activity, facilitation of the antitumor effects of anticancer drugs, control of cancer-related complications, free radical scavenging effects, fixing wounds and chronic ulcers, anti-proliferative activity, immunomodulatory activity, anti-inflammatory effects, antioxidant activity, antimicrobial effects, anti-mutagenic activity, the induction of apoptosis and angiogenesis, and P53 regulation [125].

Various pre-clinical and clinical studies have confirmed the protective activities of MGH against metabolic syndrome. MGH decreases blood sugar levels, thus preventing weight gain. It also increases the metabolism of lipids by decreasing total triglycerides (TG), cholesterol (TC), and low-density lipoprotein (LDL) and improving high-density lipoprotein (HDL), thereby reducing the risk factors of atherogenesis. Furthermore, it improves the sensitivity of insulin to maintain stable blood glucose levels and protect the pancreas from high motivation-caused insulin resistance. Additionally, the antioxidative activities of MGH help decrease oxidative stress. Finally, MGH protects the vasculature system from endothelial disorders and tissue rebuilding [126]. The antioxidant features of honey display hepatoprotective and cardioprotective effects [127].

MGH also exhibits hypolipidemic, anti-obesity, antihypertensive, and antidiabetic effects resulting from its low glycemic index (GI), thereby limiting overweight, improving fat storage, and enhancing insulin sensitivities. Honey also reduces glucose levels in blood, increases the metabolism of lipids, and thus prevents atherogenesis, limits oxidative stress, and defends against endothelial dysfunction. Accordingly, MGH is an effective agent against metabolic syndrome [126].

MGH exhibits neurological (antinociceptive, anticonvulsant, anxiolytic, antidepressant) effects and improves memory capacity [128]. Additionally, oral consumption of MGH is known to prevent cisplatin nephrotoxicity caused by a reduction in oxidative stress, leading to the suppression of inflammation [129].

MGH has exhibited a significant effect on non-alcoholic steatohepatitis (NASH), hepatotoxicity, liver fibrosis, cirrhosis, liver disease, and liver injury. In addition, honey displays significant effects against liver cancer cells [130]. It also plays an important role in supporting and improving sports performance, bone health, and immune function when combined with a suitable sports plan. MGH is also effective against immune disorders and human immunodeficiency virus, and it is an effective therapeutic agent for respiratory tract diseases, wound healing, gastroenteritis, and several illnesses in children and infants [131].

MGH is effective against gastrointestinal disorders, such as gastroesophageal reflux, malabsorption, dyspepsia, gastritis, gastric ulcer, gastroenteritis, IBS, constipation, hemorrhoids, anal fissures, IBD, and pancreas diseases. It is also effective against periodontal diseases, pharyngitis (sore throat), cough, and hiccups. MGH exhibits high potential in boosting the immune system, being an anti-inflammatory agent, and in healing chronic ulcers to prevent the cancer. In addition, it is effective in cancer therapy [132]. MGH is used as a supplementary treatment in the management of chemotherapy-associated oral mucositis in pediatric patients. It is also effective in preventing disease progression in cancer patients [125].

MGH is effective against several conditions in women. For example, it decreases the initial pains of dysmenorrhea, improves cesarean section and episiotomy wounds, controls the quantity/period of menstrual bleeding as well as the space between two menstrual cycles, treats headache, nausea, vomiting, and menstruation pain, and aids in labor development. It is also effective in treating candida, a vaginal disease in women [133].

MGH exhibits high antioxidant activity resulting mainly from the phenolic compounds related to free radical scavenging, hydrogen-donation, singlet oxygen quenching and/or metal ion chelation. Those honeys with a higher level of polyphenols display higher antioxidant activity; however, a wide range of compounds, including catalase, glucose oxidase, peroxidase, ascorbic acid, α-tocopherol, carotenoids, amino acids, proteins, organic acids, Maillard reaction products, and other minor components have less effect on this activity [134].

MGH has antinociceptive, anxiolytic, anticonvulsant, and antidepressant properties. It also enhances the oxidative status of the brain and exhibits neuroprotective and nootropic effects. It decreases microglia-induced neuro-inflammation resulting from ischemia-reperfusion injury or immunogenic neurotoxins, reduces neuroinflammation in the hippocampus, and improves memory [135].

Currently, the number of approved products under the name of medicinal honey and with specific trade names in the world is very limited. The US Food and Drug Administration (FDA) has approved some products originating from MGH, especially those formulated on Manuka honey, including dressings, pastes, ointments, and gels [136]. The main known MGH types are described below.

Manuka honey is made in Australia and New Zealand by bees that pollinate the native *Leptospermum scoparium* (Myrtaceae), also known as a tea tree. Methylglyoxal (MGO) and dihydroxyacetone (DHA) are the main phyto-chemical components found in the nectar of *Leptospermum scoparium*. The higher the concentration of MGO is, the higher the antibacterial effect classified by grades named the Unique Manuka Factor (UMF™) will be. Manuka is used mainly for wound and burn healing, but its other therapeutic uses include treatments for skin conditions (e.g., eczema and dermatitis), cough or sore throat, and digestive disorders.

Tualang (TH) is a monofloral forest honey produced by the *Apis dorsata* (rock bee) that builds its hive among the branches of the Tualang tree (*Kompassia excelsa*), distributed mainly in tropical rain forests. Tualang honey has bactericidal and bacteriostatic activity and is rich in antioxidant components that exhibit a high potential for preventing cancer [132].

Gelam, another monofloral honey produced by wild *Apis dorsata* (rock bee), originates from *Melaleuca cajupati* Powell. (Myrtaceae), known as the "Gelam tree." Gelam honey is mainly used in therapies to treat cholera, vaginal infection, thrush, acne, verruca, warts, cold sores, nits, athlete's foot, and insect bites and to aid wound healing [137].

Kelulut is a forest honey produced in Indonesia by the wild kelulut bee (*Trigona* sp.) that contains high levels of antioxidant components. This honey has a sweet and sour taste and is used by indigenous people to treat canker sores [138].

Kanuka honey is derived from the Kanuka tree (*Kunzea ericoides* (A.Rich.) Joy Thomps) from Myrtaceae that grows in New Zealand. It exhibits significant antibacterial, anti-inflammatory, and antioxidant activity [139], which is effective in wound management.

Revamil honey (RS) is made under controlled conditions in greenhouses and exhibits high antibacterial effects [140]. RS has also shown anti-bactericidal activity against antibiotic-resistant gram-positive and gram-negative bacteria.

Ulmo honey originates from the Ulmo tree (*Ucryphia cordifolia*) and is produced in Chile. It is equal to Manuk UMF25+ in terms of its antibacterial power [141].

9. Discussion

Honey can be classified as either a medical food (MF) or medical grade (MG). It contains nutritive components (primary metabolites, proteins, lipids, carbohydrates, and micronutrients such as minerals, vitamins, and water) as well as secondary metabolites (e.g., bioactive or phytochemical components). The ratio between these two components determines the medicinal or food value of honey. Medical grade honey contains effective medicinal compounds (including pharmacological effects). The primary step to determining the medical grade of honey is to evaluate the authenticity of natural honey based on set standards. The second step is to evaluate the levels of its functional components. A supplementary step can be clinical trials to screen the honey's therapeutic effects. The evaluation of honey factors and their compliance with set standards will aid in guaranteeing the primary conditions for the quality and therapeutic potential of medicinal grade honey. The biological and phytochemical components of honey are mainly dependent on botanical origins and ecological factors. Storage and processing also affect the medical constituents of honey (e.g., polyphenols and volatiles) [92]. The Apimondia Working Group on International Organic Standards for bee products has provided guidelines on the following for the production of organic products: The usage of young and productive queens, providing honey bees suitable pollen and nectar resources and access to clean water, feeding bees in necessary situations, arrangement order in the establishment of hives, implementing measures to reduce stress to the honey bees, feeding or removing sufficient honey and pollen resources for the dearth period, protective measures against bee diseases, appropriate use of medicines, healthy management of bee products, and locating proper establishments for honey bees. Successful beekeeping is strongly dependent upon the existence of a sufficient number of good quality forage resources (e.g., nectar and pollen) [142]. One of the most vital actions of the beekeeper is selecting the number of hives to be located in a particular habitat so as to achieve the highest efficiency in foraging performance [143]. Key factors in producing the top grades of honey (such as medical grade) are the ecological conditions governing the establishment of honey bee colonies. These factors in habitats include vegetation types, density as well as frequency spectra of valuable plant taxa for the honey bee, non-fragmented habitats, distance from permanent water sources, distance from roads, distance from power lines and antennas, altitudinal variations, geomorphologic diversity, and organic crop surface [144]. Therefore, the use of chemically synthesized allopathic products in organic apiculture (the basis of medicinal honey production) is forbidden. In addition, apiaries must be at least 3 km away from sources of industrial pollution [145]. Some experts [146], however, believe that the settlement zone of the bee colony should be up to 10 km away from contaminated areas. Evaluating the ideal number of bee colonies is vital to reducing the competition and ensuring the high quality of honey [147]. Imbalances in the standard establishment of colonies can reduce the quantity and quality of production [148]. To counteract this, beekeepers move colonies from one habitat to another so as to provide food for the colony and increase the quantity and quality of the honey. Additionally, the migration rate of the colonies depends on the density and quality of vegetation in the habitats [149].

Because of the diversity in possible honey sources resulting from a wide range of plant taxa, each honey is unique. Plant nectars are the main sources for the production of honey. Quantitatively and of course to a greater extent qualitatively, nectar profiles display a lot of variety. The qualitative diversity of chemical components is often related to the species, genus, and family of the nectar-producing plants; however, quantitative diversity is often related to the ecological and geographical conditions of the honey production. Monofloral honeys (obtained from the nectar of specific plant species) are the main sources of bioactive compounds [150] and display a prominent potential for therapeutic features [84]. Thus, they are known as a higher class of medical honeys [47]. Several valid studies have confirmed that monofloral honeys have different bioactive compositions for medicinal uses [84, 151, 152]. Monofloral honeys are also main sources of antioxidant activities. They contain a wide range of phytochemical components with functional properties (e.g., phenolic acids, flavonoids, and minerals) and thus exhibit a marvelous potential for medical uses [84]. There are significant differences in the effective medicinal compounds of blossom honey and honeydew. Accordingly, differentiating between the mentioned kinds of honey is necessary to control the quality and grading of honey. The therapeutic effects of several monofloral honeys, such as Citrus [153], Ziziphus [154], Thymus [155], Teucrium [156], and Astragalus [157], have been confirmed in clinical trials.

The glycemic index (GI) indicates how rapidly different foods affect blood glucose levels as well as the relative increase in blood glucose level 2 hours after consuming a particular food. Its values range from 0 to 100 (pure glucose). Glycemic load (GL) is calculated on the basis of the highest dose of honey consumption per drink and can range between 50 and 80 g. Honey can have a glycemic index of between 32 and 87, depending on its botanical and geographical origins and fructose content [158]. Monofloral honeys exhibit diverse ranges of fructose content and fructose/glucose. Accordingly, honey containing the lowest level is the most appropriate choice for diabetic patients. A study in Turkey identified Citrus and Thyme honeys as having a low GI and Lime, Chestnut, and Pine honeys as having a medium GI [159].

Additionally, Citrus and Pod Locust with low GI values and Christ Thorn, Mixed Floral, and Thistle honeys with medium GIs have been reported in Jordan. Honey can be polluted by environmental factors as well as by beekeeping activities. The main chemical contaminants of honey include pesticides, antibiotics, and heavy metals, all of which are harmful to human health. These chemical components biomagnify and bio-accumulate, leading to high concentrations in the body over time. The negative effects of these compounds in sick people are extremely dangerous. Accordingly, MGH should have the lowest amounts of these pollutants so as not to impose secondary pressures on the patient.

Because of their chemical and physical characteristics, microorganisms have a strong ability to spread in honey. Therefore, reducing or even eliminating pollutants is very important to obtaining MGH. Honey absorbs bacterial spores and keeps them for a long time. The harmful effects of these biological contaminants, especially *C. botulinum* spores that are acquired through honey consumption cannot be ignored. Numerous reports have indicated the negative effects of these bacteria. Because honey can become biologically contaminated, sterilization seems to be necessary, especially MGH to be used for wound healing [160]. Up to now, several sterilization methods have been reported, for example, pasteurization, refrigeration, freezing, and most especially gamma radiation for MGH [19, 161]. Moreover, membrane processing completely separates yeast cells from honey [162]. Ultrasound, infrared heat, and microwave processing are other unconventional ways to sterilize honey [163]. The bubbling ozone gas placed in an ozone-resistant container displays high efficacy in destroying endospores in honey. While bacteria are destroyed by sterilization, their endotoxins can penetrate the honey and eventually have negative effects on consumer health. There is a positive correlation between released endotoxins and the amount of endospores. Accordingly, screening for low levels of microorganisms in honey is a necessary step in MGH selection. The maximum limit is suggested to be 10 CFU/g honey for fungi and molds and 10 CFU/g honey for bacteria [10]. Bacteria are destroyed by sterilization; however, some endotoxins (e.g., LPS) can penetrate honey and lead to inflammatory pyrogenic reactions. Higher endospore counts release a higher number of toxins. Accordingly, a honey with fewer microorganisms (maximum limits of 10 CFU/g honey for bacteria and 10 CFU/g honey for fungi and molds) is the better choice [10]. It has been reported that low-dose gamma irradiation (e.g., 2.5 KGy and 5 KGy) is not effective in honey sterilization. Larbi et al. [100] revealed that 20 KGy gamma radiation is suitable for destroying bacteria and maintaining the nutritional-medicinal values of honey. Furthermore, gamma irradiation ranging from 20 KGy to 30 KGy at a temperature of 25°C had no significant negative effect on the physicochemical features of honey. Using gamma irradiation (25 KGy) has not been shown to have significant effects on reducing the antibacterial effects of honey [19, 120]. Gamma radiation, however, has been found to reduce all values of HMF [160] and to effect a minor reduction in pH after irradiation at 40 KGy. Gamma irradiation for the sterilization of honey has been reported to be a safe method for eliminating microorganisms and bacterial spores in honey for use in pediatric patients and in wound healing.

The medical grade of honey can be improved by several complementary natural products (e.g., medicinal plants and mineral elements). Numerous studies have shown that honey, in combination with other natural products such as medicinal plants, has stronger therapeutic effects. Some examples include honey and cinnamon for breast cancer [164] and honey and ginger against *Escherichia coli*. The mixture of honey and cider vinegar improves the detoxification of the liver to enhance overall health and aids in weight loss. Additionally, garlic, cinnamon, Zataria multiflora, cardamom, and basil [82] have been shown to improve the therapeutic properties of honey. Moreover, honey and royal jelly combined exhibit a synergic effect [165]. In addition, medicinal honey increases the effectiveness of medicines [8].

Some known MGH products have additional elements, such as hypoallergenic lanolin (CAS 8006-54-0) and PEG 4000 (CAS 25322-68-3). PEG (CAS 57-55-6), polypropylene glycol, lanolin, polyethylene, omega 3, and vitamins E, A, and D have been known to improve the quality of MGH treatment in wound healing [10].

Honey is recognized as an allergenic food that induces different levels of allergenic reactions varying from mild (e.g., cough, etc.) to severe (e.g., anaphylaxis). The allergenic factors of honey include gland secretions and wax from the honey bee, flower nectar, and pollen proteins. Sensitization to honey allergens and allergenic reactions from honey should be considered when using honey in treatments [166].

Medicinal grade honey is achieved through a continuous chain from the location of colony establishment to the production process to storage and screening. A disruption in any part of this chain will cause problems in the entire process. Furthermore, numerous geographical zones lack the ability to produce medicinal honey. Accordingly, harsh conditions are considered in the production of natural honey for medicinal use so as to guarantee the health of consumers. Medical grade honey covers a limited range of naturally produced honey in the world. It must be categorized on the basis of effective chemical components; some classes are suitable for oral consumption, while others must only be used externally, especially for wound treatment. Medical food products have become an important assistant in hospitals and nursing homes, promoting the recovery of patients.

Several produced honeys originating from various botanical and geographical regions in the world have a high potential for becoming medicinal grade honeys. This requires several steps: Sampling of all ecological regions producing each type of honey, and comprehensive analysis including physicochemistry, phytochemistry, microbiology, melissopalynology, safety, and health. Finally, clinical trials must be conducted to evaluate the therapeutic effects of selected honeys. Accordingly, such comprehensive characterization (authenticity, grading, and clinical trials) provides valid and referable documentation for the use of a honey as an MGH.

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