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REVIEW ARTICLE

Comprehensive analysis of bee honey: from chemical composition to sensory characterization

Análisis integral de la miel de abeja: desde la composición química hasta la caracterización sensorial

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Abstract   Bee honey is a natural product with a complex chemical composition that varies according to its botanical and geographical origin. This article provides a comprehensive analysis of honey, addressing its chemical composition, which includes sugars, proteins, vitamins, minerals, and bioactive compounds, as well as its impact on organoleptic properties. Analytical techniques used to determine the chemical composition, such as chromatography and spectroscopy, are examined, highlighting their importance for the authenticity and quality of honey. Additionally, sensory evaluation methods are explored, which allow for the characterization of the flavor, aroma, and color of honey, and how these attributes are influenced by factors such as floral origin and processing conditions. Finally, the relevance of a multidimensional approach to honey evaluation is discussed, integrating both chemical composition and sensory characteristics, thereby contributing to a better understanding of its quality and potential as a functional product in food and health.

Keywords   bee honey, chemical composition, organoleptic properties, sensory analysis, honey authenticity.

Resumen   La miel de abeja es un producto natural con una compleja composición química que varía según su origen botánico y geográfico. Este artículo ofrece un análisis integral de la miel, abordando su composición química, que incluye azúcares, proteínas, vitaminas, minerales, y compuestos bioactivos, así como su impacto en las propiedades organolépticas. Se examinan las técnicas analíticas utilizadas para determinar la composición química, como la cromatografía y la espectroscopia, destacando su importancia para la autenticidad y calidad de la miel. Además, se exploran los métodos de evaluación sensorial, que permiten caracterizar el sabor, aroma y color de la miel, y cómo estos atributos son influenciados por factores como el origen floral y las condiciones de procesamiento. Finalmente, se discute la relevancia de un enfoque multidimensional para la evaluación de la miel, que integre tanto la composición química como las características sensoriales, contribuyendo así a un mejor entendimiento de su calidad y su potencial como producto funcional en la alimentación y la salud.

Palabras clave   miel de abeja, composición química, propiedades organolépticas, análisis sensorial, autenticidad de la miel.

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Introduction

There are approximately 20,000 species of bees worldwide, which vary in size, shape, and behavior but all share the habit of visiting flowers to collect food (Orr et al., 2022). The most well-known and widely distributed species is *Apis mellifera*, whose use in crop pollination and the production of honey, wax, and pollen dates back centuries (Papa et al., 2022).

The primary natural product of *Apis mellifera* bees is honey, which has stimulating, nutritional, and therapeutic properties, making it a highly sought-after product in the international market (Świąder & Marczewska, 2021). Currently, the work of the International Honey Commission focuses on the composition criteria of unifloral honey, which includes moisture content, sugars, water-insoluble solids, minerals, acidity, diastase activity, and hydroxymethylfurfural content (Zhang et al., 2023).

These documents also propose new international standards and other quality factors that should be considered when certifying the quality of honey specific to its geobotanical origin. These include the determination of electrical conductivity, specific monosaccharide content, invertase activity, proline content, and specific rotation angle. However, sensory property evaluation is primarily referenced in long-term studies (Świąder et al., 2021).

Honey can be floral in origin when derived from flower nectar or from honeydew, which comes from the excretions of plants or insects. Floral honeys can be monofloral (derived from a single flower species) or polyfloral (derived from multiple flower species). Honey is considered specific or unifloral if the nectar of a single floral species predominates, thus attributing the therapeutic properties of the plant from which the nectar originates (Becerril-Sánchez et al., 2021). The commercialization of unifloral honey has increased significantly due to rising international demand, benefiting exporting countries by granting them greater competitiveness in the global market (Zhang et al., 2023).

The objective of this review article was to describe the chemical composition of bee honey and its influence on physicochemical, nutritional, medicinal, and sensory properties, considering the effects of key components and factors such as botanical origin and processing on its quality, acceptance, and market consumption potential.

Generalities of bee honey

Honey is a food product obtained from bees *Apis mellifera*, derived from the nectar of flowers or secretions from living parts of plants. Bees collect, transform, and combine this nectar with their substances, storing it and allowing it to mature in the hive's combs.

Bee honey originates from nectar secreted by melliferous plants as a sweet, aqueous solution. When bees collect nectar from flowers, they transform it into a highly concentrated sugar solution, enriched with traces of vitamins, minerals, and other bioactive components.

Numerous varieties of bee honey can be distinguished according to various traits, including floral origin, collection regions, and production methods. Honey can be classified as floral (when derived from flower nectar) or animal origin (when derived from insect secretions). Floral honeys can be monofloral (from a single flower species) or polyfloral (from multiple flower species). There is no strict unifloral honey, as the presence of small amounts of nectar from other melliferous plants does not significantly affect the aroma, color, and flavor of the honey predominantly derived from a single floral species (Vîjan et al., 2023).

Polyfloral honey is named according to the collection location, such as meadow honey, steppe honey, forest honey, orchard honey, mountain honey, and taiga honey, among others (Fernández, 2023). However, many kind of honey marketed as polyfloral do not correspond to this classification but are mixtures of different unifloral kinds of honey.

Honey has an average energy value of 13.84 kJ/kg (3.307 kcal/kg). It is hygroscopic, easily absorbing moisture from the air, making it a dehydrating agent in the tobacco industry and baking. The glucose in honey tends to crystallize at room temperature, leaving a layer of dissolved fructose. For commercialization, honey is heated to approximately 66 °C to dissolve the crystals and then packaged in airtight containers to prevent crystallization (Amariei et al., 2020).

Chemical composition of bee honey

Bee honey primarily consists of various sugars, with fructose and glucose being the most abundant. It also contains proteins, amino acids, enzymes, organic acids, minerals, pollen, and other substances. Additionally, it may contain traces of sucrose, turanose, maltose, isomaltose, and certain oligosaccharides, as well as vestiges of fungi, yeasts, and other solid particles resulting from the honey production process.

However, presenting a summary of the chemical composition of honey as a generic value is risky due to the multiple intrinsic and extrinsic factors that influence the composition of this complex product (Al-Kafaween et al., 2023).

The chemical composition of bee honey is extremely variable, so any attempt to describe its chemical properties should be done in terms of ranges. The sugar content should not be limited to just the major sugars; it should also include other minor monosaccharides, minerals, protein-derived elements, and pigments, among others (Shapla et al., 2018).

Quality indicators

The quality of bee honey is influenced by various factors. These include aspects of bee activity, such as the sugar ratio, diastatic activity, and acidity. Environmental factors, such as the predominant flowering, the mineral content of the soil, and the composition of the nectar, are also relevant. Additionally, handling practices during the collection and preservation process, such as moisture content and hydroxymethylfurfural content, are determinants of the final product quality (Shapla et al., 2018).

The wide range of intrinsic and extrinsic factors affecting honey properties has led to hundreds of different types of unifloral honeys, each with unique chemical composition characteristics showing great variability.

Moisture content

The moisture content of honey depends on various factors, one of the most important being the time honey remains in the comb, which should be approximately three months. Additionally, moisture can be affected by technological failures, lack of precautions during the extraction process, and the use of inadequate packaging (Singh & Singh, 2018).

Moisture is the primary quality indicator of honey, as fermentation requires the presence of water. Honey extracted from partially capped combs has a higher moisture content than honey from filled and capped combs, as honey can absorb and release moisture depending on environmental conditions. Therefore, it is recommended to avoid honey extraction in humid climates until at least two-thirds of the combs are capped (Singh & Singh, 2018).

Sugar content

From a quantitative perspective, sugars constitute the major component of honey, accounting for approximately 80% of its total composition. This high content significantly contributes to the physical and energetic properties of honey (Al-Kafaween et al., 2023).

The predominant sugars in honey are the monosaccharides fructose and glucose, which are found in proportions ranging from 33 to 42% and from 27 to 36%, respectively. The presence of these monosaccharides is due to the action of the enzyme invertase (α-glucosidase) on sucrose derived from flower nectar. For honey to be considered of good quality, the content of reducing sugars should not be less than 65% (Salvador et al., 2019).

The most abundant disaccharide in honey is sucrose, the predominant sugar in flower nectar. Additionally, other disaccharides such as trehalose, turanose, maltose, isomaltose, and gentibiose may be found. Among the oligosaccharides, trisaccharides such as erlose, maltotriose, and isomaltotriose are prominent (Al-Kafaween et al., 2023).

There are other hydrocarbon structures in honey, grouped under the term "dextrins," which are generally less complex than true dextrins from starch. Examples include raffinose and melecitose (Al-Kafaween et al., 2023).

Mineral content

Honey contains small amounts of minerals such as calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and silicon. These minerals come from the soil where the plants, from which honey is obtained, grow; therefore, honey from the same floral species but from different soils may present variations in mineral content (Kędzierska-Matysek et al., 2018).

Dark honey tends to be richer in mineral substances (Tlak et al., 2024). Generally, potassium is the predominant mineral, accounting for approximately one-quarter of the total minerals.

Research has shown a relationship between the color of honey and its mineral content, indicating that the darker the honey, the higher the percentage of mineral salts and, consequently, its nutritional value.

Acidity and pH

The concentration of organic acids in honey is generally low, around 0.57%. Several acids have been identified, including gluconic, citric, malic, succinic, formic, acetic, butyric, lactic, oxalic, and tartaric acids. Among these, gluconic acid is the most abundant, resulting from the action of glucose oxidase on glucose, making it the predominant acid in honey (Tischer et al., 2019).

Lactic acidity, considered as the reserve acidity when honey begins to alkalinize, always has a value lower than free acidity. The pH of honey ranges between 3 and 4, contributing to its stability against various microorganisms (Abdi et al., 2024).

Diastase activity

Diastase (α-amylase) is the enzyme responsible for the hydrolysis of starch into dextrins and simpler sugars. The diastase index, along with the content of hydroxymethylfurfural (HMF), are indicators that provide a measure of the freshness of newly harvested honey (Sajtos et al., 2024).

Invertase activity (α-glucosidase) is also sensitive to overheating and prolonged storage, making it an indicator of processing quality and honey freshness. A minimum invertase value of over 10 has been proposed, although this standard is less strict for honey with low enzymatic activity, where an invertase index greater than 4 is required.

However, interpreting results in terms of freshness based on invertase activity is complicated, as the initial value is

unknown, preventing the establishment of a reference point to assess possible deterioration due to overheating or aging of the honey. This same problem exists for diastase.

Hydroxymethylfurfural content

The content of 5-hydroxymethyl-2-furfural (HMF) is a parameter that provides information about the freshness of honey. Freshly harvested honey contains practically minimal amounts of HMF, which begins to form from the degradation of fructose under acidic conditions and at elevated temperatures. The rate of HMF formation is directly related to the moisture percentage, the initial HMF content in freshly harvested honey, and the medium acidity (Sajtos et al., 2024).

Other indicators of bee honey composition

There are useful criteria for determining the quality of honey that however are not included in international legislation. Invertase activity, proline content, and specific rotation of honey are three of these criteria (Zhang et al., 2023). Additionally, certain indices of chemical composition, besides serving as quality criteria, are useful tools for differentiating between various types of honey.

Like other components of honey, nitrogen exhibits variable concentrations influenced by multiple factors. It is estimated that approximately 40 to 80% of the nitrogen in most honey is protein nitrogen. Small amounts of proteins or other colloidal substances are sufficient to accentuate the tendency of honey to foam or retain air bubbles, which may be interpreted as a sign of fermentation, although it is not necessarily so (Martínez et al., 2024). The determination of nitrogen, among other components, has been used to assess adulteration in honey (Fakhlaei et al., 2020).

Although proteins are a minor component in honey, they are used as an internal standard in the evaluation of adulteration through the carbon isotope stability index. Since 1978, average values of 169 mg/100 g of honey have been published, with a confidence interval between 58 and 786 mg/100 g (Adams et al., 2009). This variability suggests that the variation is due not only to floral origin but also to possible errors introduced by the analytical methods used.

The National Honey Board of the U.S. reports that honey has an average protein content of 168.6 mg/100 g, varying from 57.7 to 567 mg/100 g (S = 70.9) (Zavala et al., 2024). This range, although lower than that reported by White and Rudyl, is still wide for comparisons between honeys of different botanical origins. Additionally, this organization indicates that the protein content in honey is 0.266%, equivalent to 266 mg/100 g, while nitrogen is found at 43 mg/100 g and amino acids range from 50 to 100 mg/100 g, leading to inconsistencies with previous data (Erban et al., 2019).

The proteins in honey generally come from bees, although there are also contributions from pollen and nectar from plants. Most publications on honey proteins focus on the enzymes that bees incorporate into honey. The main enzymes found are invertase (α-glucosidase), α-amylase (diastase), and catalase (Erban et al., 2019).

Honey contains, although in small quantities, around 18 free amino acids, which represent between 0.05% and 0.1%, among which histidine, proline, lysine, serine, phenylalanine, asparagine, and glutamine stand out (Martínez et al., 2024). Proline is the most abundant amino acid, representing between 39.6% and 46.9% of the total in honey from Korea, or levels between 133 and 1,245 mg/kg in honey from Brazil (Chang et al., 2022). The amount of proline has been used as an indicator of adulteration, as there is a negative correlation between proline and the typical sugars in honey, and it has been suggested that genuine honey should contain a minimum of 180 mg/kg of proline.

Although honey is not a significant food in terms of protein contribution, knowing the total protein and nitrogen content is useful, as the low protein content is one of the reasons why honey has low microbiological contamination and contributes to its antimicrobial power. This is also explained by the high C ratio, which does not meet the needs of most microorganisms. The presence of certain proteins also influences the non-Newtonian (thixotropic) behavior of some honey and is responsible for foaming and the retention of air bubbles, undesirable characteristics, as consumers associate them with fermentation.

Some authors have linked variations in honey color with the presence of certain proteins, and it has been shown that these also participate in variations in honey's thermal properties, as the thermal capacity decreases with increasing total solids, including proteins (Singh & Singh, 2018).

On the other hand, the honey vitamin content is low and does not represent a significant nutritional contribution. During storage, these decrease due to oxidation and thermal degradation. The vitamins include thiamine, riboflavin, ascorbic acid, pantothenic acid, and nicotinic acid.

Lipids in honey are scarce and likely come from the wax generated during extraction. Plant pigments, such as carotenoids, xanthophylls, and anthocyanins, are found in small amounts and have been little studied. On the other hand, flavonoids, which are also responsible for honey color, have been studied more for their therapeutic properties than their relationship with botanical origin (Becerril-Sánchez et al., 2021).

Furthermore, variations in honey color are considered to be due to the presence of pigments (carotenoids, chlorophylls, and xanthophylls) that differentiate between light and dark honey. Likewise, very old or dark combs enhance the natural color of honey, as pigments retained in the cells dissolve into it.

Physical properties of honey
Color

Color is one of the most relevant physical parameters for defining the quality of honey and is an important factor to consider in commercial presentation, as it influences consumer preferences (Haidamus et al., 2019). The nature of color as an indicator of the botanical and geographical origin of honey from *Apis mellifera* is a complex and debated topic.

Although these aspects have generally been studied independently, the trend in the scientific community is to consider minerals as the main contributors to honey coloration. However, the characteristic color of honey is a multifactorial phenomenon that must be approached comprehensively. The color of honeys varies according to the predominant floral species during a given period, as well as by external factors such as aging, beekeeper management, and storage conditions (Haidamus et al., 2019).

To classify color, the visual colorimetric comparator "Pfund" is used, which classifies honey from water white to dark amber, is the internationally recognized standard for the buying and selling of honey. According to this method, honey is considered to have a certain color when at least 5% of the analyzed containers contain honey of different colors, provided that no sample has a reading below the immediate following color (Bodor et al., 2021).

Although the Pfund method allows for visual comparison, it includes honey with a wide range of values within the same category. Therefore, it would be beneficial to use spectrophotometric evaluations for a more precise characterization of the chromatic properties of honey. Since 1925, relative characteristics of the color of various types of honey have been established (Bodor et al., 2021).

Density and viscosity

Density is a physical property of honey that is closely related to its moisture content and determines its consistency or viscosity. Honey generally has a relatively high density, and honey with low density tends to have a high moisture content, making them less viscous and more prone to fermentation. Currently, it is established that the minimum acceptable relative density for honey should be 1.400 kg/L (Ciursa et al., 2021).

Viscosity is one of the most important physical properties of honey, as it significantly influences its processing and preservation. Research on the rheological behavior of honey has been fundamental in achieving a longer shelf life, as well as facilitating handling, packaging, and processing. Generally, honeys behave as Newtonian fluids. The viscosity value largely depends on the concentration of sugars at a specific temperature.

The ratio of fructose to glucose also influences viscosity; a fructose solution is less viscous than a glucose solution, which explains why tupelo honey, which have a high fructose content, are less viscous (Ciursa et al., 2021). Additionally, it has been suggested that the presence of dextrins can increase the viscosity of honey more than the sugar ratio, which may lead to a rheological behavior known as dilatancy.

Refractive index

The refractive index provides a quick, accurate, and simple measure from which the water content in honey can be inferred. This indicator is useful precisely because it is not related to the botanical origin of the honey. Honey from bees has a refractive index corresponding to its high sugar concentration, ranging from 1.47 to 1.50 at a temperature of 20 °C (Rababah et al., 2024).

Specific rotation angle

A common characteristic of all sugary solutions is their ability to rotate the plane of polarized light, either to the right or to the left, depending on the type of sugar and its concentration. In honey, the value of the rotation angle is the sum of the rotatory power of the present sugars. Most nectar honey is levorotatory, while honeydew honey tends to be dextrorotatory (Zhang et al., 2023).

Electrical conductivity

Electrical conductivity is an indirect measure of the mineral salt content in honey and has replaced the determination of ash in routine analysis, as it is directly proportional to the content of ash and metals, especially transition metal salts (Sancho et al., 1991). This measurement is widely used to identify unifloral honey, so the inclusion of electrical conductivity among international standards is urgently recommended (Addi & Bareke, 2021).

It is suggested that floral honeys and their mixtures should have electrical conductivity values below 0.8 mS/cm, while honeydew and chestnut honey should present higher values. However, there are exceptions, such as honey from Arbutus, Eucalyptus, and Tilia, which show high variability in their electrical conductivity (Piana et al., 2004).

Hygroscopicity and water activity

Hygroscopicity is the ability of certain foods and chemical products, such as honey, to absorb and retain moisture. This phenomenon has been studied in various sugars, where it has been found that fructose, the predominant sugar in honey, exhibits higher hygroscopicity compared to other common

sugars (Erejuwa et al., 2012). However, under certain conditions, honey can show a level of hygroscopicity greater than that of fructose, due to the influence of other components present in honey that enhance its ability to retain moisture (Al-Kafaween et al., 2023). The water activity (aw) in honey is one of the most stable indicators and has been observed to vary between 0.5 and 0.6 (Chen, 2019).

Sensory evaluation

In the last 25 years, it has been shown that while instrumental methods are faster and more precise for determining food quality, they do not always manage to measure all aspects of food, only specific characteristics. Therefore, sensory evaluation conducted by consumers is considered the most direct way to assess food quality, allowing the determination of its organoleptic properties (Żak et al., 2023).

Sensory evaluation tests are classified into analytical, which are the most suitable for assessing product quality (Żak et al., 2023), and affective, which focus on consumer acceptance and preference. Among analytical tests, descriptive tests are the most common.

The initial steps in the sensory analysis of unifloral honeys in Belgium, primarily focusing on aroma profiles, have been used as a tool to identify their botanical origin in future studies (Zhang et al., 2023). Despite the numerous references on sensory evaluation tests, especially of the descriptive type, the application of quantitative descriptive analysis to differentiate honey has been scarcely explored.

Characterization of unifloral honey

The floral origin of honeys is an essential characteristic in assessing their quality. Unifloral honey is distinguished by aromas that primarily come from the nectar of the flowers from which they are obtained, indicating the presence of volatile compounds that can act as specific markers. However, the identification of the floral origin of honeys is generally performed using chemical, and physical indicators, pollen patterns, and organoleptic properties (Jandrić et al., 2015).

In Italy, a country notable for the production and marketing of unifloral honeys, the Ministry of Agricultural and Forestry Policies has established a characterization scheme that requires the analysis of chemical and physical parameters, as well as sensory and palynological analysis (Conti et al., 2007). To determine if a honey is indeed unifloral, it is necessary to compare these characteristics with established standards.

Chemical and physical analyses provide objective measurements but do not always guarantee adequate differentiation. Sensory and palynological analyses provide more specific data that are complemented by utilizing sufficient taxonomic variables and appropriate statistical methods (Rodríguez et al., 2015). Analytical techniques for determining chemical and physical indicators of honey are internationally validated and considered official methods of analysis (Tsagkaris et al., 2021).

The relationships between the amounts of glucose and fructose are influenced by floral nectar, allowing for the characterization of honey based on their geographical and/or botanical origin (Mongi, 2024).

Free amino acids are a characteristic component of unifloral honey, with an average content of approximately 980 mg/kg, enabling regional and botanical discrimination of honeys (Yang et al., 2024). The minerals present in honey primarily come from the raw materials collected by bees and vary according to the botanical and geographical origin (Bogdanov et al., 2007). Research in Canada has analyzed the mineral fraction in honey using neutron activation techniques and multivariate statistical methods to differentiate them based on their geographical origin (Burton et al., 2023).

The electrical conductivity of honey is related to the presence of organic and inorganic acids, as well as the dissociation of mineral salts into ions. Recent results indicate that electrical conductivity is a useful parameter for classifying honey based on its botanical origin (Majewska et al., 2019).

Multivariate statistics have been widely used to classify honey. Principal component analysis has been applied to authenticate honeys (Torres et al., 2022), while other studies have classified nectar and honeydew honey samples based on physical and chemical indicators (Fernández-Estellé et al., 2023). In Spain, statistical techniques have been applied to classify honeys from different geographical origins using quality control data (Ghidotti et al., 2021).

To identify and differentiate unifloral honey, sensory analysis has been used, where the appropriate selection of tasters and statistical methods contributes to obtaining more objective results (Piana et al., 2004). Commercial honeys were studied through chemical, physical indicators, and sensory evaluations (Anupama et al., 2003).

Melissopalynological analysis, which studies the pollen grains present in honey, is fundamental for certifying unifloral origin. This analysis involves counting the absolute number of pollen grains per unit volume, which varies according to the foraging behavior of bees and floral morphology (Selvaraju et al., 2019). Unifloral honey must contain more than 45% pollen grains from a single species (Calaça et al., 2018).

Currently, attention is being paid to the detection and identification of micro-components in honey, which are often responsible for differences in their characteristics depending on their botanical origin. These compounds are referred to as

chemical markers. Gas chromatography is primarily used to identify volatile compounds, while high-performance liquid chromatography (HPLC) is employed to quantify phenolic and flavonoid compounds, which are important chemical markers in unifloral honeys (Becerril-Sánchez et al., 2021; Ouchemoukh et al., 2017).

Conclusions

The chemical composition of honey is highly complex and varies significantly depending on factors such as bee species, available flora, environmental conditions, and processing methods. Carbohydrates, predominantly glucose and fructose, constitute the majority of its composition, while phenolic compounds and flavonoids give honey notable antioxidant and antimicrobial properties. These properties, in turn, influence the sensory profile of honey, affecting attributes like color, aroma, and flavor, which are crucial for consumer preference. Sensory characterization remains an evolving area seeking standardization and improvement, especially in the evaluation of honey from specific botanical origins. The integration of advanced analytical chemistry methods and sensory analysis techniques is projected as essential for quality and authenticity standardization of honey in the food industry.

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