

New Advances in the Antioxidant Properties of Bee Products

Claudia Pașca *  and Daniel Severus Dezmirean 

Department of Apiculture and Sericulture, Faculty of Animal Science and Biotechnology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 400372 Cluj-Napoca, Romania; ddezmiorean@usamvcluj.ro

* Correspondence: claudia.pasca@usamvcluj.ro; Tel.: +40-733-696-413

1. Introduction

Bee products—including honey, pollen, propolis, beeswax, royal jelly, bee bread, bee venom, and apilarnil (a Romanian bee-derived product)—have been utilized since antiquity for their nutritional value and diverse therapeutic applications. They are increasingly recognized as rich sources of natural antioxidants, capable of mitigating oxidative stress, a key factor implicated in the pathogenesis of numerous diseases. Recent advances in analytical techniques and extraction methods have significantly improved the characterization and valorization of these compounds, enabling a deeper understanding of their functional properties and therapeutic relevance.

Honey is a natural product rich in antioxidant compounds, produced by honeybees (*Apis mellifera*) and composed primarily of a complex mixture of carbohydrates [1,2]. This bee product has more than 300 different chemical compounds belonging to various chemical classes. The main constituents are carbohydrates and water, while minor components include fatty acids, proteins, minerals, pigments, aroma compounds, enzymes, essential oils, sterols, phospholipids, and organic acids [3]. The phenolic composition of different honey varieties is generally similar and includes phenolic acids such as caffeic, ellagic, ferulic, and p-coumaric acids; flavonoids including apigenin, chrysin, galangin, hesperetin, kaempferol, pinocembrin, and quercetin; as well as antioxidant-related compounds and enzymatic biomarkers, such as tocopherols, ascorbic acid, superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) [4]. These compounds and enzymatic activities were evaluated to characterize the antioxidant properties of the different types of honey. Nevertheless, certain honey varieties may also contain specific compounds that can serve as markers of their botanical origin.

Accumulating evidence suggests that honey exerts a wide range of beneficial biological effects, including gastroprotective, hepatoprotective, reproductive, hypoglycemic, antioxidant, antihypertensive, antibacterial, antifungal, and anti-inflammatory activities.

Bee pollen is a natural mixture of floral pollen combined with honeybee secretions and nectar. It is typically collected at the hive entrance using specialized traps. Due to its nutritional and functional properties, bee pollen is widely used as a dietary supplement and is associated with potential beneficial effects in the prevention and management of various human diseases. It represents a valuable source of essential nutrients, including proteins, lipids, carbohydrates, vitamins, minerals, and trace elements, as well as significant amounts of polyphenolic compounds, particularly flavonoids [5]. Bee pollen is widely recognized as a functional food with diverse biological properties, including antimicrobial, antifungal, antioxidant, anti-radiation, hepatoprotective, chemoprotective, and anti-inflammatory activities [6]. It has also been associated with beneficial effects in the prevention of metabolic and chronic disorders, including prostate diseases, arteriosclerosis, and gastrointestinal and



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respiratory conditions, as well as in allergy desensitization. In addition, bee pollen supports cardiovascular and digestive health, enhances immune function, and may contribute to delaying aging processes.

Propolis is characterized by a highly complex and variable chemical composition, which is influenced by multiple factors, including botanical origin, harvesting season, geographical location, local flora, climatic conditions, and honeybee species at the collection site [7,8]. To date, more than 300 chemically distinct compounds have been isolated and identified from this natural product. Propolis possess several health properties including antiallergic, antimicrobial, antioxidant, cardioprotective effects, anti-cancer, anti-inflammatory, antiulcer, antitumor, hepatoprotective, neuroprotective and antidiabetic effects. The efficacy of neuroprotective flavonoids primarily depends on their high antioxidant, immunomodulatory and anti-inflammatory functions [8–10].

Beeswax is secreted by worker bees through wax glands in their abdomens. Beeswax is produced mostly in late spring, during the colony expansion period, and is used to make combs. The crystalline form of beeswax, which is produced from honey sugars, makes it perfect for beehive construction. Its chemical makeup varies depending on the species of bee and the area in which it is found. It is composed of hydrocarbons, free fatty acids, free fatty alcohols, linear wax monoesters, hydroxymonoesters made from palmitic, 15-hydroxypalmitic, and oleic acids, and complex wax esters that contain diols and 15-hydroxypalmitic acid [11].

Royal jelly is produced by worker bees from the hypopharyngeal and mandibular glands through partial digestion of honeydew, and it is crucial for the development and caste differentiation of honeybee larvae [12]. RJ is an acidic secretion with a pH of 3.5–4.2. It mostly consists of water (60–70%), sugars (7–18%), proteins (9–18%), lipids (3–8%), minerals, and trace amounts of vitamins. Its major lipids consist of 10-hydroxy-2-decanoic acid and sebatic acid [13]. 10-H2DA is known for its anti-cancerous and anti-angiogenic activity, whereas sebatic acid has anti-aging effects. Major RJ proteins (MRJPs) present in the RJ increase the lifespan of the queen bee. They consist of peptides such as royalisin, jelleines, and royalactina. The various pharmacological aspects of RJ are attributed to its unique and rich composition of proteins, carbohydrates, vitamins, lipids, minerals, flavonoids, and polyphenols, along with various biologically active substances [12].

Bee bread is an apitherapeutic product obtained through the lactic fermentation of pollen collected by honeybees. It is recognized as a natural source of antioxidants, including phenolic compounds and coenzyme Q10 [14], and as a bioactive-rich product with a nutritional composition strongly influenced by botanical origin and geographical conditions. Flavonoids and carotenoids are among its key constituents, contributing to a range of biological activities, including antioxidant, anti-inflammatory, anticancer, neuroprotective, and antidiabetic effects [15,16]. Bee bread also contains fat- and water-soluble vitamins (A, D, E, K, C, and B complex), with vitamin E playing a major role in its antioxidant potential. Overall, bee bread exhibits multiple reported bioactivities, including antimicrobial, antifungal, gastroprotective, neuroprotective, and anti-aging effects [14,17].

Bee venom is secreted by the venom gland located in the abdominal cavity of female honeybees [18]. This gland is connected to a reservoir sac that stores the venom prior to its release. In honeybees of the genus *Apis*, the venom apparatus represents a key defensive mechanism essential for colony protection. Bee venom contains bioactive compounds with marked antioxidant activity [19], including phospholipase A2, apamin, and melittin. Its antioxidant effects are mediated through multiple mechanisms, such as free radical scavenging, hydrogen donation, metal ion chelation, and quenching of reactive oxygen species. These activities are further supported by the inhibition of lipid peroxidation and the enhancement of superoxide dismutase activity, a key enzymatic defense against

oxidative stress. In addition, other constituents, such as vitellogenin, contribute to cellular protection by mitigating reactive oxygen species and oxidative damage.

Apilarnil is obtained by harvesting drone larvae 3 to 10 days after hatching. This bee-derived product was first identified by the Romanian researcher Nicolae V. Iliesiu, and its name derives from “api” (bee), “lar” (larva), and “nil” (an abbreviation of the discoverer’s name). *Apilarnil* is a nutrient-dense bee-derived product rich in proteins and amino acids, with high levels of glutamic acid, valine, aspartic acid, lysine, and leucine. It also contains low concentrations of sugars, predominantly glucose, along with minor amounts of fructose and other disaccharides. Its lipid fraction includes fatty acids; mono-, di-, and triacylglycerols; sterols; and phospholipids, in addition to vitamins and essential minerals [20].

Produced by honeybee larvae, *apilarnil* contains hormonally active compounds, including testosterone, estradiol, progesterone, and prolactin. Experimental studies have reported multiple biological activities, such as antioxidant, androgenic, and estrogenic effects, neuroprotection, immunomodulation, anti-atherosclerotic activity, and protective effects against testicular and hepatic toxicity, as well as sexual dysfunction.

In the context of the growing interest in bioactive compounds derived from natural sources for health promotion and disease prevention, this Special Issue entitled “New Advances in the Antioxidant Properties of Bee Products” represents a continuation of the previous Special Issue dedicated to the quality control and functional characterization of bee products. It reflects the growing need for integrated research approaches that connect plant sources, bee biology, environmental factors, and technological processes in order to better assess and enhance antioxidant potential.

2. Contributions to This Special Issue

This Special Issue comprises eight original research articles and one review, encompassing multiple subfields, including bioactive compounds from bee products, functional food components, and analytical validation methods for functional constituents. Collectively, these contributions highlight cutting-edge methodologies and emphasize the applied significance of bee products research.

2.1. LC–MS/MS Analysis of Phenolic Compounds

LC–MS/MS analyses were conducted using a validated targeted analytical protocol routinely employed for phenolic profiling in complex food matrices. Chromatographic separation was carried out on an Agilent 1260 Infinity HPLC system (Agilent Technologies, Palo Alto, CA, USA) coupled with an AB SCIEX Triple Quad 3500 mass spectrometer (AB Sciex, Foster City, CA, USA) equipped with an electrospray ionization (ESI) source. Separation was achieved using a Phenomenex Luna C18 column (150 mm × 2.0 mm, 3 µm particle size) (Phenomenex, Torrance, CA, USA). Phenolic compounds were identified based on retention time matching and confirmed using two multiple reaction monitoring (MRM) transitions per analyte, in comparison with authentic standards. The limit of detection (LOD) was defined as the lowest concentration corresponding to a signal-to-noise ratio ≥ 3 and was established at 0.01 mg/kg [21].

2.2. Comparison Between Ozone and Drying Treatments on Phenolic Compounds and Antioxidant Activity in Bee Pollen

Five 50 g subsamples were prepared from each sample and subjected to different treatments: ozonation at 200 mg/h for 1 h (O1) and 2 h (O2) using a portable ozone generator, and drying at 40 °C for 4 h (D4) and 8 h (D8) using an airflow pollen dryer. The selected drying temperature was considered optimal for reducing moisture content to approximately 6%, ensuring adequate preservation [22,23].

Ozone treatments did not adversely affect total phenolic content or antioxidant activity in the samples. In contrast, drying led to a reduction in total phenolics, while enhancing the antioxidant activity of bee pollen [24].

2.3. Validation of Microplate Methods for Phenolics and Antioxidant Activity in Honey: Comparison with Conventional Assays

Microplate-based Folin–Ciocalteu and DPPH assays provide a validated, reliable, and environmentally friendly alternative to conventional spectrophotometric methods, offering reduced reagent consumption, shorter analysis time, and comparable accuracy, precision, and sensitivity [25].

2.4. Flavonoids and Phenolic Acids as Markers for Differentiating Honey Origin

Flavonoids and phenolic acids quantified by HPLC-DAD proved effective chemical markers for differentiating honeys according to geographical origin, with multivariate analyses (PCA and discriminant analysis) enabling clear discrimination between Dominican Republic and Spanish samples and achieving up to 80.3% correct classification, thereby demonstrating their value for honey authentication [26].

2.5. Innovative Flavoring of Rapeseed Honey with Essential Oils

Cinnamon and clove essential oils were used for the first time to flavor rapeseed honey, and the resulting products showed enhanced antioxidant properties and altered chemical profiles depending on oil origin, suggesting potential for developing novel functional honey-based products [27].

2.6. Solvent Extraction of Propolis Bioactive Compounds for Food Applications

Portuguese propolis from the Guarda region was extracted using different solvents, revealing that ethanolic extracts—particularly 70% and 96% ethanol—yielded higher phenolic and flavonoid contents, stronger antioxidant activity, and greater enzyme inhibitory potential, underscoring the critical role of solvent selection in optimizing its bioactive properties for food and related applications [28].

2.7. Effect of Altitude on Polyphenols, Antioxidant Activity, and Elemental Composition of Wildflower Honey

This study demonstrated that altitude exerts a significant influence on the phenolic profile, antioxidant activity, and mineral composition of multifloral honeys, with observed variations reflecting differences in agro-climatic conditions, soil characteristics, and floral biodiversity, thereby confirming the strong dependence of honey's physicochemical and biofunctional properties on its geographical origin [29].

2.8. Functional and Antimicrobial Properties of Romanian Propolis

This study evaluated the chemical composition and antimicrobial activity of hydroalcoholic propolis extracts from different regions of Romania, demonstrating that their bioactive properties are strongly influenced by local botanical sources, with higher flavonoid content generally associated with increased antimicrobial activity against tested bacterial and fungal strains [30].

2.9. Honey-Based Delivery Systems for Wound Healing: Current Trends and Perspectives

Honey is a multifunctional therapeutic agent in wound management due to its antimicrobial, anti-inflammatory, antioxidant, and tissue-regenerative properties; however, its direct application is limited by high viscosity and compositional variability, leading to the de-

velopment of advanced delivery systems such as nanoparticles, electrospun nanofibers, and hydrogels that improve stability, control release, and enhance wound-healing efficacy [31].

3. Challenges and Future Perspectives

The science of bee products is currently advancing rapidly due to innovations in analytical technologies, deeper insights into biological mechanisms, and expanding applications, although key scientific and technical challenges remain for sustainable and health-oriented development. Future research will focus on smart, multifunctional products with biosensing capabilities, advanced 3D/4D bioprinting for personalized and responsive designs, and the integration of computational chemistry, molecular docking, and multi-omics approaches to elucidate bioactive mechanisms and support the rational design of bee products.

4. Conclusions

Research on bee products is expected to advance toward greater precision, sustainability, functionality, and standardization, driven by interdisciplinary integration across analytical, materials, life, food, and pharmaceutical sciences, ultimately enabling the development of safe and effective bee-derived health products.

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