Antioxidant properties of pollen

Damir Aličić[1*], Drago Šubarić[2], Midhat Jašić[3], Hatidža Pašalić[3], Đurđica Ačkar[2]

[1]*High Secondary School "Čelić", 75246 Čelić, Bosnia and Herzegovina*
[2]*Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhača 20, HR-31000 Osijek, Croatia*
[3]*Faculty of Pharmacy, University of Tuzla, Univerzitetska 8, 75000 Tuzla, Bosnia and Herzegovina*

review

Summary

Today, bee pollen is commonly used in folk medicine and its pharmacy effects have not yet been scientifically proven. The composition and chemistry of bee pollen are not yet standardized nor defined in pharmacopoeia, and may vary due to its botanical and geographical origin, the plant species, environmental conditions, age and status of plants. Because of this, the type of bee pollen depends on the available bee pasture and types of plant species visited by bees. Bee pollen contains nutritional and essential substances and also significant amounts of polyphenolic substances, mainly flavonoids, that are considered as the main ingredients of pollen and its antioxidant properties. Researches show that pollen has significant antioxidant activity and mostly depends on phenol compounds. Large deviations of the this antioxidant activity are considerable, as well as content of phenolic compounds between pollen grains taken from different plant species and different geographical regions. The pollen antioxidant activity is usually expressed as the antioxidant capacity, and primarily depends on its botanical and geographical origin that is the subject of many scientific and research papers. This article gives an overview of bee pollen, its chemical composition and botanical origin, antioxidant properties and its capacity.

Keywords: honeybee pollen, antioxidant capacity, polyphenols, flavonoids, botanical origin

Introduction

Honey bee-derived apicultural products such as pollen have been applied for centuries in traditional medicine as well as in food diets and supplementary nutrition due to their nutritional and physiological properties, above all in regard to their health effects on the human organism (Kroyer & Hegedus, 2001). Pharmacological effects of pollen are not yet scientifically based and its chemical composition depends on the available bees pasture and species of plants visited by bees. Pollen is a considerable source of compounds with health protective potential, certain concentration of phytosterols, and is also rich in phytochemicals such as phenolic compounds, which are considered beneficial to human health. Researches show that pollen has significant antioxidant activity that mostly depends on phenolic compounds. However, large deviations of the antioxidant activity are considerable and content of phenolic compounds between pollen grains taken from different plant species and different geographical regions are remarkable. The pollen antioxidant activity is usually expressed as the antioxidant capacity, and primarily depends on its botanical and geographical origin that is the subject of many scientific and research papers. The aim of the present work was an overview of various pollen properties such as the chemical composition, the botanical origin, its collecting, phenolic compounds and an antioxidant activity.

Pollen

Pollen grains are microscopic structures found in the anthers of stamens in angiosperms (de Arruda et al., 2013), they constitute the male reproductive cells in plants (Basim et al., 2006), and their purpose is to transmit their gametes to the female sex organ of the flower (Arruda et al., 2013). Bees, other insects, wind and water pollinate plants by transferring pollen from the stamen to the stigma of another plant (LeBlanc et al., 2009). According to the regulations of honey and other bee products (Službeni glasnik BiH, br. 37/09), pollen is a product that worker bees collected in nature adding to its own specific matter which forms the pellets, and place them in the honeycomb cells. Special group of worker bees that collect pollen is called pollen-bees (Dolovac, 1997).
Pollen is very important in apiculture as a source of proteins, fats and minerals to bees and as an excess produced from the apiary (de Arruda et al., 2013). The quantity and quality of pollen collected by honeybees affects reproduction, brood rearing and

*Coressponding author: damir_alicic@yahoo.com

longevity, thus ultimately the productivity of the colony (Human & Nicolson, 2006).
The significance of pollen for bee's community is priceless and is associated with its survival. Bees use it as food for all the developmental stages in the hive (Almeida-Muradian et al., 2005; Morais et al., 2011). Apart from small quantities in nectar honeybees obtain all the proteins, lipids, minerals and vitamins they need for brood rearing and adult growth and development from pollen (Human & Nicolson, 2006). The bees place the pollen in honeycombs with their legs and cover this pollen with honey. This pollen reserve is referred to by beekeepers as ‘‘bee bread”. It was determined that an average value of 145 mg of pollen is required to rear just one worker bee (LeBlanc et al., 2009).

The chemical composition of pollen

Flower pollens' composition can vary due to their botanical and geographic origin (Almaraz-Abarca et al., 2004). The major components of bee pollen are carbohydrates, crude fibers, proteins and lipids at proportions ranging between 13 % and 55 %, 0.3 % and 20 %, 10 % and 40 %, 1 % and 10 %, respectively (Villanueva et al., 2002). Other minorcomponents are minerals and trace elements, vitamins and carotenoids, phenolic compounds, flavonoids, sterols and terpenes (Bogdanov, 2011). Proline, aspartic acid, phenylalanine and glutamic acid are the primary amino acids in pollen (Roldán et al., 2011). However, the composition of bee pollen depends strongly on plant source, together with other factors such as climatic conditions, soil type and beekeeper activities (Morais et al., 2011).
Few studies on the active enzymes in bee pollen have been published (Xue et al., 2012). According de Arruda et al. (2013), bee pollen is rich in B complex vitamins (thiamine, niacin, riboflavin, pyridoxine, pantothenic acid, folic acid and biotin) and carotenoids, which can be provitamin A. However, according to the same author, there is no significant amount of vitamin C or lipid soluble vitamins.
There are numerous reports of bioactive substances in the pollen such as phenols, flavonoids, anthocyans, phospholipids and proteins. The main bioactive flavonoids are naringenin, isorhamnetin3-*O*-rutinoside, rhamnetin3-*O*-neohesperidoside, isorhamnetin, quercetin3-*O*-rutinoside, quercetin3-*O*-neohesperidoside, kaempferol and quercetin and their total amounts are in the range 0.3-1.1 % w/w (Han et al., 2012).
Phenolic compounds are one of the most critical ingredients related to antioxidant activity in pollen. Usually, it contains vanillic acid, protocatechuic acid, gallic acid, *p*-coumaric acid, hesperidin, rutin, kaempferol, apigenin, luteolin, quercetin, and isorhamnetin (Bonvehi et al., 2001).

Pollen collection

The collection of this natural product is a relatively recent development, dependent primarily on the basic concept of scraping pollen off of the bees’ legs as they enter the hive (Feás et al., 2012). Honey bees collect pollen by adding sugars from nectar and their own secretions to bind the grains together (Cheng et al., 2013) and then transfer them back to the colony by packing them into hairs on the corbiculae (hind legs) of bees (LeBlanc et al., 2009).
For the commercial bee pollen collection, indoor or outdoor pollen collectors can be used. There are different versions of these collectors depending on the type of the hive and the principle of the pollen subtraction is the same. Bee with pollen must scrape through small openings in the pollen collector where it passes and the balls of pollen fall into the prepared drawer. The advantage of outdoor pollen collectors is cleaner pollen but its deficiency is smaller amount in comparison with indoor pollen collectors.
The collected raw pollen with about 20 % moisture content is subject to microbial spoilage and kept in a frozen state at - 18 °C up to a certain analysis or dried to 7-8 % moisture content and kept in a cool, dark place. For pollen analyzing, its extracts in different solvents and their mixtures are prepared, and most commonly used solvents are methanol, ethanol and water.
Kroyer & Hegedus (2001) used ethanol, methanol/water and water as pollen solvents, and reported that the content of polyphenolic compounds in pollen extract was significantly increased in absolute ethanol.

Botanical origin of pollen

Botanical origin of pollen is determined by palynological (microscopic) analysis respectively by the microscopic identification and counting of pollen grains. Each plant species has its own characteristic pollen grain that can be used to determine its botanical origin i.e. determining the plants that bees visited by gathering the pollen.
Pollen grains vary in terms of their morphological characteristics such as form, size, openings/apertures and ornamentation, as well as in terms of color and appearance. Color and other characteristics of pollen grains can be used to identify the genus of plants and, sometimes, the plant species (Bačić, 1995; de Arruda et al., 2013).

Pollen analysis allows the identification of the major pollen sources used by the bees, as well as the periods of pollen production in the field and possible times of shortage (de Arruda et al., 2013). Microscopic examination showed that each pellet of honeybee-collected pollen was largely homogeneous, confirming the observations of Almaraz-Abarca et al. (2004) who observed that pollen pellets predominantly consist of pollen grains from one species.

Research by de Arruda et al. (2013) indicates that bees use a variety of flora for the production of bee pollen and other bee products. When collecting pollen, bees generally visit the same type of plants to make pollen grains, and that pollen is mainly monofloral origin, with minor additions of pollen grains of other species of plants. According to de Arruda et al. (2013), pollen samples that have amounts exceeding 45 % of a botanical taxon in their composition can be considered as unifloral pollen. Morais et al. (2011) proved that pollens with same color belong to the same family. According to Luz et al. (2010) the pollen types observed in the pollen pellets can vary according to the region where they are offered, a factor which depends on the available surrounding bee pasture in the apiary vegetation, as well as on the climate conditions for flowering. Therefore, the composition of the pollen may vary due to its botanical and geographical origin (Almaraz-Abaraca et al., 2004) and according to Szczesna et al. (2002), the chemical composition of bee pollen varies according to the plant species, environmental conditions (different locations, seasons and years), age and status of the plant (when the pollen is developing).

For microscopic analysis, homogeneous pollen sample is taken in the amount of 2 g, which corresponds to the number of 300 pollen grains (Almeida-Muradian et al., 2005), which are classified into groups with grains of the same color (Mărghitaş et al., 2009), determining their percentage participation in the main sample. The colour of the pollen can be estimated according to the tables elaborated by Hodges (1984) and Kirk (1994) and identified by colour and microscope observations of pollen grains (Warakomska, 1962). For the determination by the palynological analysis also some others standardized taxons for the specific area or country may be used.

Antioxidant properties of pollen

In the literature, the term "antioxidant" is defined in many ways. The word antioxidant, as the same name indicates, means "something that is opposite to oxidation." Antioxidant opposes oxidation or inhibits reactions induced by oxygen or peroxides. Thus, the presence of antioxidants in the pollen reduces the harmful effects of the free radicals in the cell and can slow oxidation reactions in food.

Antioxidant ability has usually been attributed to the activity of antioxidant enzymes (mainly superoxide dismutase, peroxidase and catalase) as well as to the content of low-molecular antioxidants such as carotenoids, tocopherols, ascorbic acid, phenolic substances (Leja et al., 2007). Antioxidants are considered as possible protection agents reducing oxidative damage to important biomolecules, including lipoprotein and DNA (deoxyribonucleic acid) from ROS (reactive oxygen species). Oxidative stress, the consequence of an imbalance between ROS generation and antioxidants in the organism, initiates a series of harmful biochemical events which are associated with diverse pathological processes which can lead to various cellular damages and diseases (Mărghitaş et al., 2009).

It is believed that the bee products are large sources of antioxidants. According to Nagai et al. (2001) there is significant antioxidant activity in pollen and other bee products.

Bee pollen, like other bee products (honey, propolis), is due to the abundant and qualitatively and quantitatively different contents of phenolic and flavonoid antioxidants related to botanical species and origin, valuable sources of these healthy beneficial constituents characterized by high antioxidant activity (de Arruda et al., 2013). This various mechanisms of antioxidant activity permit a wide range of free radicals scavenging and lipo-peroxidation assays in order to evaluate the complete antioxidant potential (Mărghitaş et al., 2009).

Many of the present studies are concerned with determining the antioxidant activity of pollen samples of different geographical origin and establishing a correlation with the content of phenolic and other compounds.

Antioxidant capacity

The measure of antioxidant activity can be expressed by antioxidant capacity. Many factors may affect accurate determination of antioxidant activity (Kukrić at al., 2013). A number of methods based on different mechanisms of antioxidant defense system, are developed to determine antioxidant capacity, such as the removal or inhibition of free radicals or chelating metal ions, which otherwise may lead to the formation of free radicals (Greblo, 2009). The antioxidant properties of the pollen extracts cannot be evaluated by just one method due to the complex

nature of their constituents. Recent investigations show differences between the test systems in determining antioxidant capacity. Use of at least two methods is recommended to assess and compare the antioxidant capacity of a sample (Sakanaka & Ishihara, 2008). There are various methods available in the assessment of the antioxidant capacity of samples. They provide useful data, however, they are not sufficient to estimate a general antioxidant ability of the sample (Filipiak, 2001). These methods differ in terms of their assay principles and experimental conditions. Consequently, in different methods, particular antioxidants have varying contributions to total antioxidant potential (Mărghitaş et al., 2009).

The enzymatic and non-enzymatic methods are used to determine the antioxidant capacity. Of the non-enzymatic method, indirect methods (DPPH, $ABTS^{+}$, FRAP) and direct methods (ORAC method) (Kukrić at al., 2013) are used mostly.

The DPPH method (Brand-Williams, Cuvelier, & Berset, 1995) principle is the reaction of DPPH (2,2-diphenyl-1-picrylhydrazyl), a stable free radical, which accepts an electron or hydrogen radical to become a stable molecule, and, accordingly, is reduced in presence of an antioxidant. DPPH radical is widely used for the preliminary screening of compounds capable to scavenge activated oxygen species since they are much more stable and easier to handle than oxygen free radical (Tominaga et al., 2005). The absorbance changing is monitored at 517 nm (Parkash, 2001).

The TEAC assay is based on the inhibition of the absorbance of the radical cation of ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonate) by antioxidants. Due to its operational simplicity, the TEAC assay has been used in many research laboratories for studying antioxidant capacity, and TEAC values of many compounds and food samples are reported.

The FRAP assay measures the ferric-to-ferrous iron reduction in the presence of antioxidants and is very simple and convenient in terms of its operation (Mărghitaş et al., 2009).

The antioxidant capacity determination results of an extract depend greatly on the methodology used, that is the oxidant and the oxidisable substrate used in the measurement. Therefore, it is important to compare different analytical methods varying in their oxidation initiators and targets in order to understand the biological activity of an antioxidant and to obtain accurate data for a better comparison with other literature. On the other hand, different antioxidants respond differently in various measurement methods which involve specific reaction conditions and mechanisms of action. This may explain the various results for DPPH, FRAP and TEAC assay, in regard with the antioxidant content of bee pollen samples analysed. In conclusion, future analysis is required, not only in testing other different systems of evaluating the antioxidant activity, but also in separation and identification the specific bioactive compounds in bee pollens with different botanical origin, in order to elucidate the differences between various samples (Mărghitaş et.al., 2009).

Phenolic compounds in pollen

Bee-collected pollen contains significant amounts of polyphenol substances mainly flavonoids which furthermore are regarded as principal indicating ingredient substances of pollen and can be used for setting up quality standards in relation to their nutritional-physiological properties and for quality control of commercially distributed pollen preparations (Kroyer & Hegedus, 2001).

In addition to testing the total phenolic compounds in pollen its constituents are tested, such as flavonoids, anthocyanins, fenilpropanoids and others. There are many studies that explore the contents of phenolics and flavonoids and their common relationship to antioxidant activity. Significant and mutual dependencies between these components and antioxidant capacity, botanical and geographical origin are established.

The antioxidant activity of polyphenols is mainly due to their redox properties, which can play an important role in neutralizing free radicals, quenching oxygen, or decomposing peroxides (Nijveldt et al., 2001).

According to Carpes et al. (2007), the pollen collected by bees generally shows characteristic amounts of total polyphenols due to its botanical and geographical origin.

Antioxidant activity is not necessarily correlated with high amounts of phenolic compounds and total phenolic content, measured by the Folin–Ciocalteu procedure, and does not give a full idea of the nature of the phenolic constituents in the extracts (Mărghitaş et al., 2009).

Studies by Almaraz-Abarca et al. (2004) and Mărghitaş et.al. (2009) show that the polyphenol composition of pollen, can be a factor in its determination. Mărghitaş et al. (2009) require the detailed examination of phenolic composition in bee pollen extracts for the comprehensive assessment of individual compounds exhibiting antioxidant activity. The results in most studies show large variations and significant differences in the amount and content of phenolic compounds in pollen from different geographical destinations and different botanical origin.

The most important and largest group polyphenols are flavonoids that appear in almost all parts of the plant and today approximately 4000-5000 various types of flavonoids are known (Kukrić et al., 2013).
Flavonoids are pigments responsible for the coloration of flowers and leaves and are important for normal growth, development and defense of plants. Each type of pollen has its own specific system of flavonoids (Crane, 1990).
Recent studies have shown that flavonoids derived from the pollen of different geographical and botanical origin containing compounds of different nutritional significance. The reactions of free radicals and scavenging capacity to reactive species of oxygen in the pollen may be due to differences in atmospheric and environmental conditions, soil or plant physiology. Flavonoids have different structural features and show several biological activities. It appears that they may strongly influence antioxidant activity, gene expression, drug-metabolizing enzymes, such as cell signaling or cytochrome P450 (CYP) enzymes, express phytoestrogenic potential, protect against toxicity of the environmental contaminant dioxin (Šarić et al., 2009).
It is known that only flavonoids of a certain structure and particularly hydroxyl position in the molecule, determine antioxidant properties. In general, these properties depend on the ability to donate hydrogen or electron to a free radical (Mărghitaş et al., 2009).
The high ability of phenolic constituents to neutralize the active oxygen species is strongly associated with their structure, such as the conjugated double bonds and the number of hydroxyl groups in the aromatic ring, mostly attributed to flavonoids and cinnamic acid derivatives (Leja et al., 2007).
In addition, the redox properties of polyphenol compounds, especially flavonoids, play an important role in absorbing and neutralising free radicals, quenching oxygen and decomposing peroxides (Damintoti et al., 2005).
The antioxidant activity of flavonoids is reflected in the inhibitory effect on lipid peroxidation and increasing the activity of antioxidant enzymes (Kukrić et al., 2013). Flavonoids have different structural features and show several biological activities (Šarić et al., 2009).
The best-described property of almost every group of flavonoids, which are the predominant
phenolic class present in honeybee-collected pollen, is their capacity to act as antioxidants (Kroyer & Hegedus, 2001). One way is the direct scavenging of free radicals. Flavonoids are oxidised by radicals, resulting in a more stable, less-reactive radical. In other words, flavonoids stabilize the reactive oxygen species by reacting with the reactive compound of the radical (Nijveldt et al., 2001).
Total phenols is usually determined spectrophotometrically (Moreira et al., 2008; Kroyer & Hegedus, 2001; Mărghitaş et al, 2009), by modified Folin-Ciocalteu method which is based on phenol coloured reaction with the Folin-Ciocalteu reagent, measuring the resulting intensity of coloration (Kukrić et al., 2013), and total flavonoids by colorimetric tests with reference standards (Kim et al., 2003).

Health effects of pollen

Recently, increasing evidence suggests its potential therapeutic benefits, including antioxidant (Leja et al., 2007), bioactive (Kroyer & Hegedus, 2001; Roldán et al., 2001), and antimicrobial properties (Basim et al., 2006; Carpes et al., 2007; Morais et al., 2011), suggesting that it could be useful in prevention of diseases in which free radicals are implicated (Pascoal et al., 2013).
It is considered to be a natural health food which constitutes a potential source of energy and functional components for human consumption (Silva et al., 2006), with a wide range of therapeutic properties, such as antimicrobial, antifungal, antioxidant, anti-radiation, hepatoprotective, chemoprotective and/or chemopreventive and anti-inflammatory activities (Pascoal et al., 2013), free radical scavenging activities (Leja et al., 2007; Silva et al., 2006), inhibition of lipid peroxidation and suppressing the cellular and humoral response (Xu et al., 2009). These therapeutic and protective effects have been related to the content of polyphenols by Almeida-Muradian et al., (2005) and flavonoids by Šarić et al., (2009).
The daily ingestion of bee pollen can regulate the intestinal functions, effectively reduce capillary fragility and has beneficial effects on the cardiovascular system, vision and skin (Pietta, 2000). In addition, it has been reported to trigger beneficial effects in the prevention of prostate problems, arteriosclerosis, gastroenteritis, respiratory diseases, allergy desensitization, improving the cardiovascular and digestive systems, body immunity and delaying aging (Estevinho et al., 2012).
Phytochemicals, such as phenolic compounds are considered beneficial for human health since they decrease the risk of degenerative diseases by reducing oxidative stress and inhibiting macromolecular oxidation. They have been shown to possess free radical-scavenging and metalchelating activity in addition to their reported anticarcinogenic properties (Morais et al., 2011).

Conclusions

Effects of pharmacological bee pollen still have not been scientifically based and is commonly used in folk medicine. The composition and chemistry of pollen are not yet standardized nor defined with pharmacopoeia, and may vary due to its botanical and geographical origin, the plant species, environmental conditions, age and status of plants. Because of this, the type of bee pollen depends on the available bee pasture, types of plant species visited by bees and the period of flowering for characteristic plant species.

Antioxidant capacity of bee pollen, as well its other physico-chemical properties primarily depend on its botanical and geographical origin of and that is the subject of many scientific and research papers. Studies have generally shown significant association between antioxidant capacity and total content of polyphenols or flavonoids.

Further studies of bee pollen for certain geographic region can be directed to the determination of its botanical origin, phenolic constituents and to determine the quality of the characteristic pollen types from the point of antioxidant capacity, and as a result, to provide recommendations of applying pollen into functional, nutritional and pharmaceutical purposes.

Pollen should be used preventively in enrichment of everyday food or as a natural dietary supplement, due to it contains all essential amino and fatty acids and all the ingredients for a healthy and normal development of the organism.

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