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Bee Collected Pollen – General Aspects and Chemical Composition

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Abstract. Pollen represents the male reproductive cells collected by honeybees from flower stamens. The nutritional and medicinal value of bee pollen is known for centuries. With orange to red pigments, it provides nutrition through its considerable amounts of proteins, sterols, vitamins, fatty acids, phenol compounds and other physiologically active compounds. As a healthy bee product, pollen is considered to be the first-rate reservoir of antioxidants. Worldwide interest in bee pollen has recently developed as more information on their chemical composition and physiological effects has become highlighted. Bee collected pollen is regarded as valuable special food, having different health enhancing effects, also used in apitherapy. Its value is given by the content in amino acids, lipids, carbohydrates, mineral salts, vitamins, polyphenols, carotenoids, which depends on the botanical source available to bees.

Keywords: bee pollen, carotenoids, chemical composition, therapeutically effects, vitamins

INTRODUCTION

Bee pollen is a beehive product obtained by *Apis mellifera* honeybees by gathering millions of floral pollen grains and mixing it with plant nectar and bee saliva rich in enzymes, thus transforming its composition and improving its therapeutically potential. Floral pollen grains are therefore forming a pollen pellet carried by bees on their hind legs until inside the hive where it is stored separately from honey cells. During a pollen collecting trip, one honeybee can only carry two of these pollen pellets (Cocan, 2005).

The bees find pollen as a very fine powder inside flowers anthers. It is made out of grains of different shapes and colors, specific to each plant. Pollen grains are different from one another by the shape of the exterior surface, nutritive substances content, vitamins, amino acids and also phenolic acids and flavonoids. (http://www.proapicultura.ro).

The pollen is collected from the hive by special pollen traps. Fresh, bee collected pollen contains about 20-30g water/100g. This high humidity content is an ideal culture medium for microorganisms like bacteria and yeast. For prevention of spoilage and preservation of a maximum quality, the pollen has to bee harvested daily and immediately placed in a freezer. Two days later, the pest insects in pollen will be killed. After thawing, can be kept only of few hours and should be further processed as soon as possible (Campos, 2008).

DRYING, PURIFICATION AND STORAGE

Pollen is best dried in an electric oven, where humidity can continuously escape. The maximum recommended temperature is 40^{o}C and the drying time should be as short as possible in order to avoid losses of volatile compounds until the humidity is 6% or lower.

Such pollen remains stable during storage for 15 months. Pollen containing more than 6% of water will easily ferment upon storage. Storage one year or longer will reduce the free radical scavenging capacity of pollen (Campos, 2008).

Mostly the collected pollen pellets contain impurities, which should be removed, most efficiently by air specially constructed purifiers. The air should be free of dust and bacteria. In order to have minor looses of nutrients, pollen should be stored in a cool, dry place in well closed glass or plastic recipients (Bogdanov, 2004). Nutrient content of pollen also changes due to storage.

Literature date reveals different ways of storage for pollen such as freezing, drying at 40°C and liophilization (Campos, 2008). Freezing does not cause modification on chemical composition level and it is recommended as storage method when pollen is used as food or for its therapeutically effects. Liophilization has caused the loss of vitamin C and provitamin A. Drying pollen at 40°C has significantly caused a diminutions of vitamin C content, sugars, total proteins and provitamin A. Freezing followed by storage in liquid nitrogen at -20°C is the most recommended method for pollen to be kept for 6 months. For a longer period, liophilization followed by storage in liquid nitrogen is the most adequate (Campos, 2008).

CHEMICAL COMPOSITION OF BEE POLLEN

The high biological value of pollen is due to the presence of essential amino acids, vitamin complex, flavonoids and carotenoids. Beside the mentioned compounds, honeybee collected pollen contains also mineral salts, carbon hydrates, water and vegetal fibers.

Differences in chemical composition are due to botanical origin and harvesting mode. Pollen collected directly from flowers has a much lower content in sugars, compared with honeybee-collected pollen, because here the bee intercedes with the flower pollen, adding their own saliva and some nectar (Marghitas, 2005).

Out of the 83% sugars, the following classes of carbohydrates have been identified: monosaccharide (46% fructose, 37% glucose) and disaccharides (8% sucrose, 7% maltose and 1% trehalose and turanose) (Szczęsna, 2007).

The total amount of carbohydrates ranges between 13 and 55 g/100g dry matter after the loss of proteins and lipids.

Protein content is reported in literature being 10-40 g/100g dry matter (Campos, 2008). Certain amino acids are derived from bees and are common to many honeys, while others originate from nectar and honeydew. Proline is the major free amino acid both for honey and for pollen, with amounts that may surpass widely half of the total free amino acid content. In the case of honey it is mainly contributed by the bees and is originated in the pollen they consume early in life.

Interest in the knowledge of amino acid profile of honey and pollen has centered on three fields: first, as a potential tool for the botanical or even the geographical differentiation of honeys, secondly, from a nutritional point of view, in the case of pollen, as a source of proteins or essential amino acids and third, for quality control as indicator of freshness and adequacy of the drying process and storage of pollen, based on the content of a few free amino acids (Paramas *et al.*, 2005).

A special attention is placed on essential amino acids found in the chemical composition of pollen, such as: lysine, leucine, isoleucine, phenylalanine, valine, tryptophan, methionine and threonine. Amino acid content, however may define the nutritional value of pollen more accurately than protein content, since the nutritional value is reduced when inadequate amounts of the essential amino acids are present (Paramas, 2005).

The pollen contains all essential amino acids (Szczęsna *et al.*, 2006). Lipid content in pollen is evaluated to 1-13 g/100 g dry matter (Campos, 2008). Esters of different fatty acids are the main representative compounds of lipids. Most pollen types have sterols in their composition, which are necessary for the cholesterol metabolism by the honeybees. Lecithin and fatty oils were also found in pollen. Mineral content is dependent upon the botanical origin and is components of amino acids, phospholipids and enzymes present in honeybee-collected pollen (Marghitas, 2005). A general composition regarding the content in minerals and vitamins is presented in Tab. 1.

Tab. 1

Minerals and vitamins content (in mg/kg) in bee collected pollen

Compounds	Quantity
Minerals	
potassium	4000-20000
magnesium	200-3000
calcium	200-3000
phosphorus	800-6000
iron	11-170
zinc	30-250
copper	2-16
manganese	20-110
Vitamins	
β-Carotene	10-200
B1; Thiamin	6-13
B2; Riboflavin	6-20
B3; Niacin	40-110
B5; Pantothenic acid	5-20
B6; Pyridoxin	2-7
C; Ascorbic acid	70-560

Bee pollen has been used as a food supplement because of its importance as a source of essential nutrients, among them, vitamins. Vitamin B_2 (riboflavin) has an important role in cell respiration, metabolism of proteins, fats and carbohydrates and has participation in vitamins B_6, B_9 and B_{12} metabolism. Riboflavin is stable during food processing and storage and is very sensitive to light (Santos Pereira, 2010).

In addition, pollen contains significant amounts of polyphenolic substances, mainly flavonoids (Almeida-Muradian, 2005). These phenolic compounds are essential for the plants physiology due to its contribution in the morphology (form and structure). The polyphenols are involved in plants growth and reproduction; they also supply resistance against pathogens by the action of the phytoalexins and protection against plagues increasing the astringency of the plant as a food, becoming indigestible to predators. The polyphenols constitute one of the most numerous metabolic groups of plants and are integral part of people and animals diet.

The interest in phenolic compounds has increased due to the antioxidant and free radical scavenging activities. Several researchers found out that polyphenols are antioxidants with redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. The polyphenols also have metal chelating properties. Epidemiologic studies have shown a correlation between an increased consumption of phenolics antioxidants and a reduced risk of cardiovascular disease and certain types of cancer (Carpes, 2007).

Another aspect of pollen pellets is the presence of pigments. The color of pollen loads, and therefore of pollen pellets is determined by the presence of pigments such as flavonoids

and/or carotenoids. Flavonoids determine colors such as red, pink, purple and blue. The carotenoids comprise a large family consisting of approximately 600 compounds of lipid nature derived from isoprenoids, which determine colors from pale yellow to dark red and pass through red-orange and yellow-orange (Montenegro, 1997).

Most recent studies regarding some parameters of honeybee collected pollen are presented in Tab. 2.

Tab. 2

Parameters that characterize the honeybee collected (Stanciu, 2008)

Determined parameter	Method used for determination	References
Water	Karl Fisher titration	Gergen et al.,2004
Proteins	Kjeldahl	Almeida-Muradin et al., 2005
Total nitrogen	Kjeldahl	Gergen et al.,2004
Amino acids	HPLC with OPA	Paramas et al., 2005
Minerals	TXRF (Total Refection X-ray Fluorescence)	Kump et al.,1996
Reducing sugars	Luff Schoorl	Gergen et al.,2004
Lipids	Soxhlet extraction (petroleum ether) Soxhlet extraction (diethyl ether)	Gergen et al.,2004 Almeida-Muradin et al., 2005
Ash	Gravimetric mthod (550 ^{0}C) Matis-Steiner (1992)	Almeida-Muradin et al., 2005 Gergen et al.,2004
Saccharose Fructose Glucose	HPLC-PAD	Quian et al., 2008
Carotenoids	Open column chromatography	Almeida-Muradin et al., 2005
C Vitamin	AOAC microfluorimetry	Almeida-Muradin et al., 2005

THERAPEUTICALLY EFFECTS

Bee pollen has been used in folk medicine for centuries, to alleviate or cure conditions such as colds, flu, ulcers, premature ageing, anemia, colitis, allergic reactions, and enteritis with different pollens showing different specificities. It is currently marketed widely in Europe and Asia as a tonic primarily with appeal to the elderly to ameliorate the effects of ageing. Demand for this product is rising and consequently, pharmaceutical and health food companies are increasing the product range containing bee pollen (Campos, 1997).

Pollen is thought to have a wide range of health benefits, including anti-tumor function and immunostimulation. Several investigations have stated that the pollen had anti-tumor effects because the extracts of the pollen were good scavengers of active oxygen species. Other studies have also stated that pollen could significantly inhibit tumor growth and enhance immunity when used as anti-tumor drug or adjuvant in the course of tumor patient's clinic treatment (Yang, 2007).

The β-carotene constitutes the principal source of provitamin A is of vital importance for vision, bone growth and reproduction. Its deficiency constitutes a serious problem, particularly in children of underdeveloped countries. Furthermore, effects such as enhancing the immune response: inhibiting mutagenesis, diminishing nuclear damage, blocking reactions mediated by free radicals. Similarly, it has been observed in epidemiological studies that a diet rich in carotenoids diminishes the risk of some types of cancer (Montenegro, 1997).

There is some evidence that ingested pollen can protect animals as well as humans against the adverse effects or x-ray radiation treatments. Improve general condition by

nutritive balance equilibration, liver function improving, capillary permeability, kidney and digestive function improvement, blood cholesterol reducing, treatment of chronic prostates. (Cocan, 2005).

MICROBIOLOGY AND CONTAMINANTS

The microbiological content should correspond to the hygienic standards. The European Union standard for microbiological quality follows the A.O.A.C. methods and level.

Tab. 3

Microbiological analysis of pollen (Campos *et al*., 2008)

Salmonella	Absent / 10 g
Staphylococcus aureus	Absent / 1 g
Enterobacteriaceae	Max.100/g
Escherichia coli	Absent./ g
Total aerobic plate count	<100 000/g
Mould and yeast	< 50 000/g

The viability of pollen is affected by environmental pollution and its use as a bio-indicator is proposed. Such effects can be observed and quantified by biological tests. However, a more accurate identification of the agents affecting the viability is required in order to validate the biological assay for environmental monitoring. The chemical analysis of pollen is meant to ascertain the existence of a correlation between its reduced biological functions and the presence of pollutants. Moreover, such biological systems act as accumulators and allow the detection and quantification of species present in the environment at low concentrations (Peponi *et al.*, 2004).

Table 4 shows the maximum values of contaminants found in honeybee collected pollen.

Tab. 4

Contaminants of bee pollen (Campos *et al*., 2008)

Organochlorine pesticides	< MRL*
Organophosphate pesticides	< MRL
Pyrethroids	< MRL
Alfatoxin B1	Max. 2 Ig/kg
Alfatoxin B1+B2+G1+G2	Max. 4 Ig/kg
Cloramphenicol (CAP)	absent
Nitrofuran metabolites	absent
Sulfonamides	absent
Heavy metal Pb	max 0,5 mg/kg
Heavy metal Hg	max 0,01 mg /kg
Heavy metal Cd	max 0,03 mg/kg
Radioactivity (Cs-134 and Cs-137)	<600 Bq / kg

*should be smaller than values established for honey.

CONCLUSIONS

Bee collected pollen is regarded as valuable special food; its value is given by the content in amino acids, lipids, carbohydrates, mineral salts, vitamins, polyphenols, carotenoids, which depends upon the botanical source available to bees. Worldwide interest in bee pollen has recently developed as more information on their chemical composition and physiological effects has become highlighted.

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