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STUDY OF CHEMICAL AND BIOLOGICAL PROPERTIES OF EXTRACTS ON THE BASIS OF BEE POLLEN AND BEE BREAD

Introduction. Studies in recent years have shown that the food, especially that of plant origin contains a number of valuable substances, which can be important in prevention and treatment of various diseases, including cancer. The consumption of natural bioactive compounds included in food is good method of a prevention because the formation of malignant tumor is a multi-step process and most long-lasting. Biologically active food components may prevent exogenous (xenobiotics, pathogenic bacteria and viruses) and endogenous (oxygen free radicals, lipid peroxidation products, metal ions) genotoxic agents and affect all stages of carcinogenesis: initiation, promotion and progression (DE FLORA 1998, BEAR DUBOWSKA 2003). Examples of foods that are very good and concentrated source of bioactive substances are bee products, especially a pollen in the form of bee bread and bee pollen loads. Bee pollen apart from honey is the primary food of bees. Honey is an energetic product, while the pollen is necessary food for the proper functioning and growth of the body of bees, because it contains protein, fats, minerals, vitamins, organic acids, hormones. The bees sitting on flowers collect the pollen, mix it with a little honey, nectar or saliva, and transport molded balls (called pollen loads) to the hive in special baskets located on its hind legs. Pollen in the form of loads is called bee pollen. Pollen profited to the hive is used for food current needs for feeding bees, queen and brood, and for stocks for the period when no pollen will be in the environment. Fresh pollen is congregated by worker bees to the honeycomb cells, compacted and covered with honey. Then lactic acid fermentation occurs and as result of it propolis originates. In this form the pollen is consumed by the bees. Bee bread has a higher nutritional value, better absorption and richer chemical composition than pollen. Bee pollen is partially captured by beekeepers using pollen traps. Then it is dried and stored in a dry and cool place. It is a valuable nutritional and medicinal recommended in convalescence, after diseases as a preparation to assist regeneration. It is also recommended for the prophylaxis of various diseases such as prostatic hypertrophy, diabetes, atherosclerosis.

More than 250 substances was found in the bee pollen grains originating from different plant species. There are proteins and amino acids, lipids and fatty acids, sugars, phenolic compounds, vitamins and micronutrients. Bee pollen has a different chemical composition. For example, pollen loads collected in the spring have quite different amino acid content in compared to those collected in the summer. The amount of carotenoids and vitamin C is clearly different in pollen loads from various plants collected by bees in the same time of the year even from 30 to 400-fold. In addition, different chemical composition of pollen derived from the same plant species in different climatic zones is observed (TICHONOW et al. 2006). A characteristic feature of pollen is the content of the flavonoid compounds in combination with sugars in the form of so-called glucoside therein. Routine (3-rutinoside of quercetin) is common component in the pollen (KĘDZIA 1999 CAMPOS 2003). From 0.06 to 0.80% of phenolic acids (average 0.19%) were found in the pollen derived from 25 plants, mainly chlorogenic acid (including isomers iso- and neo-) and p-coumaric and ferulic acids. The least of phenolic acids was determined in the pollen of knotweed *Ophiuchus*, lucerne and cornflower (<0.08%), but the most of the pollen from common cherry, willow *Shiva* and brittle willow (> 0.4%) (ALMEIDA-MURADIAN 2005). Kędzia et al. also found chlorogenic, izochlorogenic and neochlorogenic acids in the ethanol extracts of pollen (KĘDZIA 1999 KĘDZIA 2000). However Kołoczek et al. determined ferulic acid in it (KŁOCZEK et al. 2005). In the *Ranunculus petiolaris* Mexican plant pollen (ARRAEZ-ROMAN 2007) the presence of gallic acid derivatives was shown, and in Brazilian pollen three derivatives of p-coumaric acid (OHTA et al. 2007) found. Phenolic compounds present in pollen exhibit a broad spectrum of biological effects on the human body, including strengthening capillaries, anti-inflammatory, anti-atherosclerotic, protecting against ionizing radiation, antioxidant, choleric, diuretic, anti-cancer and others (ROSS et al. 2005). The pollen contains from 1.40 to 1.65% of phospholipids. Phosphatidyl choline (lecithin), phosphatidylethanolamine (cephalin) and phosphatidylinositol (myo-inositol) are among them (TIKHONOV et al. 2006). According to Kołoczek et al. pollen contains an average of 0.50% of phospholipids. These compounds are part of cell membranes and selectively regulate the passage of substances into cells, thus they play a very important role in the metabolism. These substances as a lipotropic factors inhibit lipid accumulation in hepatocytes, protecting the body from hepatoza (functional disorder of the liver), and hence against the development of atherosclerosis (KŁOCZEK et al. 2005). During fermentation of pollen the bee bread is formed under the influence of microorganisms (with the participation of *Lactobacillus acidophilus* — present in bee pollen loads formed by bee pollen), followed by the distribution of molecular substances (eg, polyphenols) and creates simpler compounds (eg, phenolic acids, saccharides), easily digestible by bees in various stages of their development.

Few data indicate that polyphenols act not only as anti but also as pro-oxidants (in high concentrations and under certain conditions). These compounds are treated by the body as xenobiotics, they are metabolized, so their properties are partially modified, and their concentration in the body is often too small to could say that their

direct antioxidant effect is only one protective effect on the cells of the body. Probably polyphenol interactions with cellular signaling pathways and the effect on gene expression, including those responsible for the synthesis of antioxidant enzymes are of importance. It has been shown that the polyphenols in green tea, absorbed in higher concentrations (e.g. during chewing tea leaves) cause to produce large amounts of hydrogen peroxide. It is usually associated with the process of oxidation of polyphenols in the presence of transition metal ions, resulting in the formation of cytotoxic products, such as superoxide anion, H₂O₂ and a mixture of quinones. It is known that the polyphenols are oxidized at high temperatures, this process was also observed in the culture media.

Methodology and results. The total content of phenolic compounds in beebread, bee pollen and propolis was tested by the Folin–Ciocalteu method. A typical protocol using the Folin-Ciocalteu method was outlined by Singleton (SINGLETON 1999). Each sample is extracted with 70 % methanol and filtered. This solution is then mixed with a 0.2 M Folin–Ciocalteu reagent for 5 min and a sodium carbonate solution (Na₂CO₃) is then added. After incubation in dark at 25 °C for 2 h, the absorbance of the reaction mixture at 760 nm is determined against the blank (the same mixture without the sample). Gallic acid is used as standard to produce the calibration curve. The total phenolic content is expressed in mg of gallic acid equivalents/100 g of sampl.

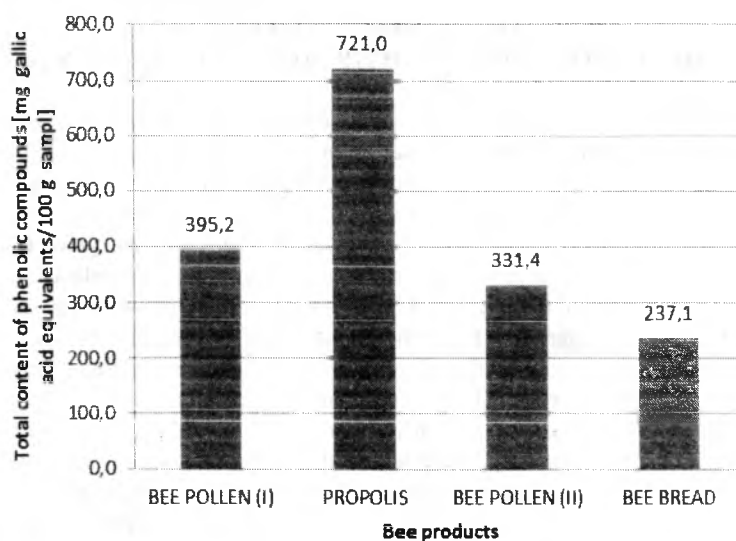


Fig.1. The total content of phenolic compounds in beebread, bee pollen and propolis with the Folin-Ciocalteu method

Conclusion. Carried studies out by our research team have shown, that selected phenolic acids have antimicrobial activity when they used separately. Whereas plant extracts containing a large variety of phenolic compounds can stimulate the growth of analyzed microorganisms strains. This suggests that the effect of isolated phenolic compounds is different from that of the same substances in biological systems. The subject of research in this project were extracts with unpopular bee products: bee pollen and beebread. It is significant that these products do not undergo any treatment technology (except for drying and during packing) they are produced by "nature", i.e. by bees. A few scientific reports on bee pollen loads among researchers who are interested in the widely understood apitherapy. Since about 15 years great interest of research teams all over the world is directed to a study of the properties and bioavailability of a bioactive polyphenolic compounds which exhibit the strongest antioxidant activity in humans.

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STUDY OF ANTIRADICAL ACTIVITY OF FLAVONOIDS AND THEIR METAL COMPLEXES

Introduction. In recent years there has been an increased interest in free radicals, which are responsible for many lifestyle diseases such as cardiovascular diseases, tumors and atherosclerosis [1]. In the development of atherosclerosis, for example, an important role is attributed to free radicals, which by initiating the process of lipid peroxidation present in lipoproteins and lipids of biological membranes cause the upsetting of prooxidative and antioxidant body balance as well as a decrease in an activity of antioxidant enzymes [2]. It was shown that important role in the process of “scavenging” of free radicals play antioxidant substances. These substances are widely distributed in natural products of plant origin. Flavonoids are a large group of natural phenolic compounds contained in high concentrations in vegetables, fruits and plants. Many flavonoids are shown to have antioxidative activity, coronary heart disease prevention, and anticancer activity, while some flavonoids exhibit potential for anti-human immunodeficiency virus functions. The mechanism of antioxidant action of these compounds is related to their ability to capture and scavenge free radicals, the termination of free radical reactions, conversion of radicals into compounds with lower reactivity, the binding of heavy metal ions, stimulation of certain enzymes involved in the destruction of toxic substances [3]. Many studies have shown that flavonoids can directly scavenge reactive oxygen species: superoxide, hydrogen peroxide, hydroxyl radical, singlet oxygen or peroxy radicals. Their antioxidant action relies mainly on their ability to donate electrons or hydrogen atoms [4]. Some studies have shown that the presence of metal ions can cause modification of the antioxidant properties of these compounds [5].

Antiradicals properties of flavonoids. Many works are devoted to the mechanism of reactions occurring between certain flavonoids and free radicals. Radical scavenging reactions of flavonoids are associated typically with the formation of less reactive (stabilized by the mesomeric effect) phenoxyl radical, which then is rearranged into more stable forms. Tamba and Torreggiani studied the place of radical attack on the basis of the reaction of quercetin and catechin in free radicals ($\bullet\text{OH}$, $\text{N}_3\bullet$, $\text{SO}_4^{2-\bullet}$) generated by pulse radiolysis. They found a significant effect of pH on the course of the reaction of quercetin, resulting from various forms of occurrence of this flavonoid in the solution at different pH. The $\bullet\text{OH}$ radical produced different products (O-)querc(OH)₄O \bullet , or (O-)₂querc(OH)₂O \bullet , probably through the reaction of deprotonation. During the attack of radical $\bullet\text{OH}$, the $\bullet\text{OH}$ — connections are mainly formed, together with the derivatives of phenoxyl radical, which for a moment absorbed at different wavelengths. In catechin the radical attack occurs mainly in ortho - dihydroxy B ring with the creation of semi-quinone radical ($\lambda = 310\text{nm}$), while in quercetin the significant relocation of electrons contributes to the formation of fenoxyl radical characterised by many mezomeric structures, which causes the shifts of the characteristic bands toward longer wavenumbers. Spectral properties of both compounds as well as the kind of radical depend on the pH. The acid-base and redox properties of phenoxyl radical are presented as an important indicator influencing the assessment of the antioxidative capacities of natural flavonoids as electron donors [6].

Jovanovic et al. showed that there is no difference in the course of the reaction of flavones and flavonols with two and three double bonds. The authors found a linear correlation between the number of hydroxyl groups in ortho position and the rate of quenching free radicals [7]. Antioxidant activity of flavonoids mainly results from the presence and proper position of hydroxyl and methyl groups as well as glycoside connections among themselves [8]. Antioxidant capacities of compounds endowed with a greater number of hydroxyl groups are stronger. Acker et al. indicated that myricetyne (having six hydroxyl groups) showed higher antioxidant capacity than for example kaempferol — with four hydroxyl groups [9]. Pannala et al. studied the antioxidant activity of flavonoids on the basis of the reaction with the stable radical ABTS⁺. They found the relationship