CONTENT OF POLYPHENOLS AND ANTIRADICAL ACTIVITY OF BEE POLLEN

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INTRODUCTION

Recently, many investigations have been concerned with antioxidant properties of different nutritional products.

Honeybee-collected pollen is recognized as a well balanced food (Gonzáles-Güerca et al., 2001). Bee gathered pollen is regarded as valuable special food and is used also in apitherapy (Bogdanov, 2004). Bee-collected pollen ("bee pollen") is promoted as a health food with a wide range of nutritional and therapeutic properties (Campos et al., 2003).

This beehive product also has several useful pharmacological properties, such as antibiotic, antineoplastic, antidiarrhoeatic and as an antioxidant agent (Campos, 1997). The antioxidant activity of honeybee-collected pollen has been recognized as a free radical scavenger and as a lipid peroxidation inhibitor (Campos et al., 1994, Campos, 1997). This activity has been associated with the phenolic pollen content (Campos, 1997). Usually, honeybee-collected pollen is a mixture of pollen pellets from different botanical origins, each one being an important source of flavonol glycosides (Wiermann and Vieth, 1983) and, in some species, of hydroxycinnamic acids (Campos, 1997). These compounds are found in a species-specific profile (Campos, 1997), which suggests that honeybee-collected pollen from different areas or seasons could have different antioxidant activities. In spite of the relevance of honey-bee collected pollen as an antioxidant activity levels associated to the flavonol content and profile of honeybee-collected pollen from different botanical origins (Almaraz-Abarca et al., 2004).

Bee-collected pollen is apicultural product which is composed of nutritionally valuable substances and contain considerable amounts of polyphenol substrates which may act as potent antioxidants. It was concluded that pollen and propolis extracts inhibit respiratory burst within cancer cell lines probably by their antioxidant potentials (Aliyazicioglu et al., 2005).

The aim of the study was to measure content of polyphenols and the antiradical activity of dried and lyophilized bee pollen.

MATERIALS AND METHODS

MATERIAL

The pollen loads were collected by 20 honey bee colonies (*Apis mellifera*) settled in hives with bottom-fitted pollen traps, from different areas of Slovakia, during the season 2007.

The fresh bee pollen was stored at -18 °C for approximately half of year, with moisture 20 %, until analysed. The dried pollen samples were dried (moisture 9–11 %). The moisture was tested by thermo-gravimetric analyzer. The pollen loads for analysis were taken from the following plant species: *Papaver somniferum* L., *Brassica napus* subsp. *napus* L., *Helianthus annuus* L.

The pollen samples (10 g) were milled, homogenized and diluted in 100 ml 90 % ethanol. The ethanol extracts of pollen were stored at 5 °C for further analysis.

ANTIRADICAL ACTIVITY DETERMINATION

The modified method by Brand-Williams was used (Brand-Williams et al., 1995, Sánchez-Moreno et al., 1998). Antiradical activity of various bee pollen samples was determined using the free DPPH· radical. Absorbance at 515.6 nm was measured at different time intervals using Shimadzu 1601 UV/VIS spectrophotometer (UV-1601, Shimadzu, Tokyo, Japan) until the reaction reached a plateau. The absorbance of the 2,2-diphenyl-1-pikrylhydrazyle radical (DPPH·) without an antioxidant (i.e. the control), was measured first. The percent of inhibition of the DPPH· radical by the sample was then calculated according to the formula:

$$\% \text{ inhib} = [(A_{C0} - A_{At})/A_{C0}] \times 100,$$

where A_{C0} is the absorbance of the control at t = 0 minute, A_{At} is the absorbance of the antioxidant at time t minutes, % inhib equals percentage of free DPPH· radicals.

POLYPHENOLS CONTENT DETERMINATION

Total polyphenols content was quantified according to the Folin-Ciocalteau spectrophotometric method using tanin as reference standard (Singleton et al., 1999). In each sample of 0,5 ml, 0,5 ml of Folin- Ciocalteau s reagent and 5 ml of Na_2CO_3 (20% w/v) were added to 50 ml flask and , storing this mixture in dark at room temperature for 30 min. The absorbance of all samples was measured at 700 nm using a UV-VIS spectrophotometer. Results were expressed as milligrams of tanins equivalent per kilogram of pollen (mg.kg^{-1}). All values of antioxidant and antiradical activity are expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

DRIED BEE POLLEN

The antiradical activity was in the particular samples in range from 48.83 to 86.12 % (average 71.39 ± 16.45 %). Antiradical activity as determined by the DPPH radical scavenging method decreased in the order: *Brassica napus* > *Papaver somniferum* > *Helianthus annuus*. In samples of bee pollen was the polyphenol content in the range from 763.67 to 1377.67 mg.kg^{-1} (average 1026.67 ± 258.31 mg.kg^{-1}). Antiradical activity increased in the same order than content of polyphenols (Table 1). In present investigations, great variability regarding content of polyphenols as well as antiradical activity in 3 pollens was found.

Table 1. Antiradical activity and content of polyphenols in pollen

Pollen	DPPH [% of inhibition]	Polyphenols [mg.kg^{-1}]
H. annuus	48.43 ± 0.29	763.67 ± 5.56
P. somniferum	79.61 ± 0.45	938.67 ± 3.09
B. napus	86.12 ± 0.48	1377.67 ± 3.68

LYOPHILIZED BEE POLLEN

The antiradical activity was in the particular samples in range from 49.87 to 86.43 % (average 72.46 ± 15.71 %). Antiradical activity as determined by the DPPH radical scavenging method decreased in the order: *Brassica napus* var. *oleifera* (Moench) Delile > *Papaver somniferum* L > *Helianthus annuus* L. In samples of bee pollen was the polyphenol content in the range from 799 to 1550 mg.kg^{-1} (average 1090.11 ± 325.51 mg.kg^{-1}). Antiradical activity increase in the same order than content of polyphenols (Table 2).

Table 2. Antiradical activity and content of polyphenols in pollen

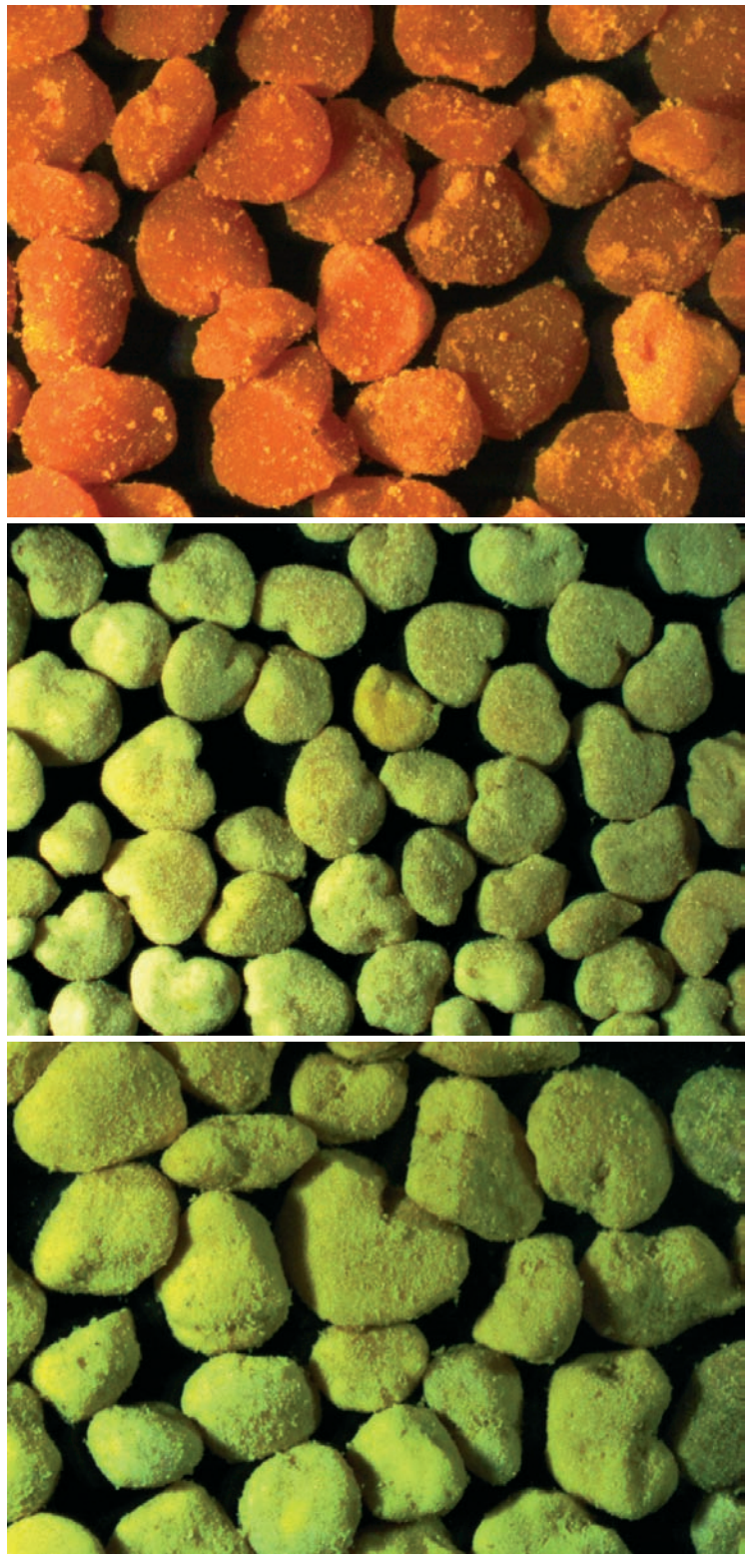
Pollen	DPPH [% of inhibition]	Polyphenols [mg.kg^{-1}]
H. annuus	48.43 ± 0.29	763.67 ± 5.56
P. somniferum	79.61 ± 0.45	938.67 ± 3.09
B. napus	86.12 ± 0.48	1377.67 ± 3.68

In spite of the relevance of honey-bee collected pollen as an antioxidant substance, there is not enough systematic information about the antioxidant activity levels associated to the flavonol content and profile of honey-bee collected pollen from different botanical origins. Comparing the antiradical scavenging activity of the total extract of the mixture of honey-bee collected pollen and those of its six constituent pollens indiviadually reported by Almaraz-Abarca et al. (2004), no correlation seems to exist between the flavonol content and the antiradical activity for pollen from different botanical origins. The flavonol and phenolic acid composition, rather than the concentration, could be the determinant factor. The particular combination of flavonol glycosides and phenolic acids could define the level of antioxidant capability of pollen of different origin. Antioxidant activities were different for each species and were not clearly associated to the flavonol content in pollen. Pollen from different botanical origin had different antioxidant capacity. This beehive product can be considered as an important source of natural flavonol antioxidants (Almaraz-Abarca et al., 2004).

Phenolic constituents (total phenols, phenylpropanoids, flavonols and anthocyanins) and antioxidant ability were determined in bee pollen of 12 plant species by Leja et al. (2007). Great variability of phenolic contents was observed in the pollen of investigated species. Great differences in the radical-scavenging activity (8.6-91.5 % of DPPH neutralization) were observed and were not correlated with the content of phenolic compounds. The pollen species can be divided into three groups: those of high ability of DPPH neutralization (61-91.3 %, *Lupinus polyphyllus, Phacelia tanacetifolia, Trifolium* sp., *Sinapis alba, Robinia pseudoacatia* and *Aesculus hippocastanum*), those of medium radical-scavenging activity (RSA) (23.5-29.6 %, Zea *mays, Chamerion angustifolium* and *Pyrus communis*), and those of low RSA (8.6-16 %, *Lamium purpureum, Taraxacum officinale* and *Malus domestica*). The highest and the lowest levels of total phenols were found in pollens from *P. communis* and *Z. mays*, respectively. In some of them (*P. tanacetifolia* and *S. alba*), a very high antioxidant activity, expressed as radical scavenging activity, inhibition of lipid peroxidation and hydroxyl radical-scavenging activity, corresponded to high levels of total phenols, phenylpropanoids and flavonols (Leja et al., 2007).

In the present investigations very high and high antiradical activity in the case of *Brassica napus* and *Papaver somniferum* L., respectively, were manifested by very high and high content of polyphenols. Antioxidant activity, expressed as antiradical activity, corresponded to high radical-scavenging activity of 6 pollen with high ability of DPPH neutralization examined by Leja et al. (2007). Lower antiradical activity of *Helianthus annuus* L. pollen agreed with the lower level of polyphenols. This pollen species integrated to second group with medium RSA reported by Leja et al. (2007). *Helianthus annuus* pollen integrated to second group with medium RSA reported by Leja et al. (2007). None of investigated pollens from our research is being possible to integrate to third category with the low RSA. The above results are partially in agreement with the Leja et al. (2007) reports.

Antioxidant ability of pollen seems to be due to phenolic compounds (Markham, Campos, 1996). High levels of phenolic constituents are often accompanied by high antioxidative capacity of pollen; however, according to reports of Campos et al. (2003) and Campos et al. (2000), no direct correlation between flavonoids and radical-scavenging activity was found. The gradual decrease of RSA in the pollen stored for 4 years was not accompanied by a parallel reduction of flavonoids (Campos et al., 2003) and some pollens with high levels of phenolics did not present significant antiradical activity (Campos et al., 2000). Pollen, containing more than 6 % of water will ferment upon storage. Storage for one year or longer will reduce the free radical scavenging capacity of pollen (Campos et al., 2003). Pollen aging over 3 years is demonstrated to reduce the free radical scavenging activity



by up to 50 % in the most active floral pollens, which tend to contain the highest levels of flavonoids/phenolic acids. It is suggested that the freshness of a bee pollen may be determined from its free radical scavenging capacity relative to that of fresh bee pollen containing the same floral pollen mix (Campos et al., 2003).

Various constituents (phenolics, and probably, other compounds) are engaged in neutralization of different active oxygen species. The separation of the individual phenolics and detection of the other antioxidants will be necessary in further investigations of the pollen antiradical system (Leja et al., 2007).

CONCLUSIONS

Further studies of the antioxidant properties (include antiradical activity) and the antioxidant components of bee pollen from different botanical origins are required, especially identification and quantification of individual antioxidants contained in pollen.

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