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Antioxidative properties of bee pollen in selected plant species

M. Leja [a,*], A. Mareczek [a], G. Wyżgolik [a], J. Klepacz-Baniak [b], K. Czekońska [b]

[a] Department of Plant Physiology, Faculty of Horticulture, Agricultural University, 29 Listopada 54, 31-425 Kraków, Poland
[b] Department of Pomology and Apiculture, Faculty of Horticulture, Agricultural University, 29 Listopada 54, 31-425 Kraków, Poland

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Abstract

Phenolic constituents (total phenols, phenylpropanoids, flavonols and anthocyanins) and antioxidant ability were determined in bee pollen of 12 plant species. Antioxidant ability was measured as total antioxidant activity, radical-scavenging activity and activity against free hydroxyl radical. Great variability of phenolic contents was observed in the pollen of investigated species. Total antioxidant activity differed considerably (0.8–86.4% inhibition of lipid peroxidation), however, in most of the examined pollens, it was high and corresponded with the phenylpropanoid level.

Great differences in the radical-scavenging activity (8.6–91.5% of DPPH neutralization) and in the hydroxyl radical-scavenging activity (10.5–98% inhibition of deoxyribose degradation) were observed and were not correlated with the content of phenolic compounds.

In most of the investigated plant species, antioxidative capacity of bee pollen was very high.

Keywords: Pollen; Antioxidant activity; Phenolics

1. Introduction

Recently, many investigations have been concerned with antioxidant properties of different nutritional products. Antioxidant ability has usually been attributed to the activity of antioxidant enzymes (mainly superoxide dismutase, peroxidase, catalase) as well as to the content of low-molecular antioxidants such as carotenoids, tocopherols, ascorbic acid, phenolic substances (Bartosz, 1997; Larson, 1988). The best source of antioxidative compounds are, undoubtedly, fruits and vegetables, recommended as health promoting components of the human diet. Their antioxidative capacity, in many cases, results from phenolic compounds (Larson, 1988). 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 (Foti, Piattelli, Baratta, & Ruberto, 1996; Natella, Nardini, Di Felice, & Saccini, 1999; Silva et al., 2000). Interdependence between the level of different phenolics and antioxidant ability of fruits, vegetables and medical plants has been studied for many years (Pietta, Simonetti, & Mauri, 1998; Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999; Velioglu, Mazza, Gao, & Oomah, 1998; Vinson, Hao, Su, & Zubik, 1998). Less often the honey bee products, particularly rich in flavonoids, have been the subject of research (Nagai, Sakai, Inoue, & Suzuki, 2001). Among them, special attention should be paid to the floral pollen used for many years as a beneficial dietary supplement (http://www.ccpollen.com/ORAC.shtml).

The purpose of the present studies was to determine the antioxidant activity of pollen loads of various origin in comparison with the level of phenolic substances (total, phenylpropanoids, flavonols and anthocyanins). The

Abbreviations: DPPH, 1,1-diphenyl-2-picrylhydrazyl; RSA, radical-scavenging activity; HRSA, hydroxyl radical-scavenging activity; TAA, total antioxidant activity.

* Corresponding author. Tel.: +48 12 662 52 07; fax: +48 12 662 52 66.
E-mail address: mleja@bratek.ogr.ar.krakow.pl (M. Leja).

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antioxidative properties of pollen were measured as the radical-scavenging activity, as the inhibition of lipid peroxidation and as the antiradical activity against free hydroxyl radical. The simultaneous application of these three methods allows a more detailed description of the antioxidative system of pollen.

2. Material and methods

The pollen loads were collected in 2004, in the Kraków area, from the beginning of May to the end of July by 20 honey bee colonies (*Apis mellifera*) settled in hives with bottom-fitted pollen traps. The fresh bee pollen was stored at −18 °C until analysed.

The colour of the pollen was estimated according to the tables elaborated by Hodges (1984) and Kirk (1994). Additionally, pollen loads were identified by colour and microscope observations of pollen grains (Warakomska, 1962) and compared with the pollen grains from the previously collected flowers. To describe the proper taxon, the special key for pollen grains estimation was used (Faegri & Iversen, 1978). The pollen loads of the 12 following plant species were taken for analysis:

Aesculus hippocastanum, *Chamerion angustifolium*, *Lamium purpureum*, *Lupinus polyphyllus*, *Malus domestica*, *Phacelia tanacetifolia*, *Pyrus communis*, *Robinia pseudoacacia*, *Sinapis alba*, *Taraxacum officinale*, *Trifolium* sp. and *Zea mays*.

For the estimation of phenolic constituents, extracts in 80% methanol were prepared. Total phenols, phenylpropanoids, flavonols and anthocyanins were determined by the spectrophotometric method given by Fukumoto and Mazza (2000). Reaction mixture consisted of 0.25 cm^3 of pollen extract with 0.25 cm^3 of 0.1% HCl in 96% ethanol and 4.55 cm^3 of 2% HCl. The absorbance was measured at 280 nm (chlorogenic acid as standard), 320 nm (caffeic acid as standard) and 360 nm (quercetin as standard) for total phenols, phenylpropanoids and flavonols, respectively. Absorbance of anthocyanin content was read at 520 nm and expressed as cyanidin, according to its molar extinction. Radical-scavenging activity (RSA) was determined using DPPH as the stable radical and was expressed as the percentage of its neutralization after 30 min (Pekkarinen, Stoeckmann, Schwarz, Heinonen, & Hopia, 1999). The ethanolic solution of DPPH (0.1 mM) was used as a point of reference for monitoring the decrease of its absorbance at 516 nm after addition of pollen extract. The control was prepared with 80% methanol instead of pollen extract. Pollen concentration in the reaction mixture was 0.083%. Total antioxidant activity (TAA) was determined by measurements of the inhibition of linoleic acid (LA) peroxidation, as described by Toivonen and Sweeney (1998). The products of peroxidation of exogenous LA, initiated by ferrous-EDTA, were measured spectrophotometrically at 232 nm in the solution of pollen and control. A control consisted of the above reaction mixture with 80% methanol in place of the pollen extract. Pollen concentration in the reaction mixture was 0.167%. TAA was expressed as percentage inhibition of LA peroxidation by pollen extract in comparison to the oxidation level in the control. Hydroxyl radical-scavenging activity (HRSA) of pollen extract, based on the inhibition of the degradation of deoxyribose caused by the attack of hydroxyl radicals, was evaluated using the method described by Racchi et al. (2002). The absorbance of the reaction mixture was read in a spectrophotometer at 532 nm against a solution prepared without ascorbic acid. The activity was expressed as the percentage of reaction inhibition. Pollen concentration in the reaction mixture was 0.1%.

All analyses were done in four replications (four extracts) and the results were statistically evaluated using Duncan's test for significance $P < 0.05$.

3. Results

3.1. Antioxidant activity

Total antioxidant activity, expressed as the percentage of inhibition of lipid peroxidation, differed considerably in respect to the pollen species; however, most of them (*P. communis*, *M. domestica*, *T. officinale*, *A. hippocastanum*, *R. pseudoacacia*, *P. tanacetifolia* and *S. alba*) exceeded 60% (60–90%). The medium ability of inhibition of lipid peroxidation was associated with four species: *C. angustifolium*, *L. polyphyllus*, *L. purpureum* and *Trifolium* sp. (27–55%), while low ability was found in the *Z. mays* pollen (7%) (Table 1).

Great discrepancies between radical-scavenging activity in different pollen species were observed. According to the results, presented in Table 1, the pollen species can be divided into three groups: those of high ability of DPPH neutralization (61–91.3%, *L. polyphyllus*, *P. tanacetifolia*, *Trifolium* sp., *S. alba*, *R. pseudoacacia* and *A. hippocastanum*), those of medium RSA (23.5–29.6%, *Z. mays*, *C. angustifolium* and *P. communis*), and those of low RSA (8.6–16%, *L. purpureum*, *T. officinale* and *M. domestica*).

According to the results obtained, hydroxyl radical-scavenging activity was above 60% (61–98%) in eight cases (*S. alba*, *Trifolium* sp., *C. angustifolium*, *P. tanacetifolia*, *Z. mays*, *L. purpureum*, *M. domestica* and *P. communis*), medium (15.8–24.7%) in two species (*R. pseudoacacia* and *L. polyphyllus*), and relatively low (10.5%) in the pollen of *A. hippocastanum* (Table 1).

3.2. Phenolic constituents

Great variability of phenolic content was observed in the investigated species of pollen (Table 1). The highest and the lowest levels of total phenols were found in pollens from *P. communis* and *Z. mays*, respectively. Similarly, the highest content of phenylpropanoids was observed in *P. communis* pollen, followed by *P. tanacetifolia*, while the lowest level was determined in *Z. mays*. The participation of phenylpropanoids in total phenolics seems to be similar

Table 1
Antioxidative properties of selected bee pollens

Pollen species	TAA (%)	RSA (%)	HRSA (%)	Total phenols (mg 100 g^{-1})	Phenylpropanoids (mg 100 g^{-1})	Flavonols (mg 100 g^{-1})	Anthocyanins (mg 100 g^{-1})
Sinapis alba	86.4 g[a]	90.0 h	61.0 d	3924 e	1503 e	914 e	236 ef
Phacelia tanacetifolia	85.9 g	66.3 e	73.5 ef	8025 i	2243 g	815 e	327 g
Robinia pseudoacacia	84.4 g	91.0 h	15.8 ab	6178 g	1875 f	1068 f	251 f
Aesculus hippocastanum	81.9 fg	91.3 h	10.5 a	3375 d	1159 d	624 cd	183 c
Taraxacum officinale	77.3 f	15.2 b	50.7 c	6307 g	1496 e	503 bc	233 ef
Malus domestica	76.5 f	16.0 b	92.7 g	7288 h	1825 f	1070 f	206 cd
Pyrus communis	66.4 e	29.6 d	98.0 g	8243 i	2307 g	1349 g	253 f
Trifolium sp.	55.1 d	82.2 g	65.1 de	1515 ab	432 ab	195 a	91.7 a
Lamium purpureum	51.1 d	8.6 a	76.1 f	3570 de	825 c	171 a	123 b
Lupinus polyphyllus	38.5 c	61.7 e	24.7 b	2836 c	741 c	595 cd	217 de
Chamerion angustifolium	27.2 b	23.7 c	67.5 def	1829b	506b	683d	147 b
Zea mays	6.8 a	23.5 c	75.8 f	1293a	308 a	378 b	92.4 a

[a] Means followed by the same letters are not significantly different.

in most of the examined pollen samples (25% on average) excepting *S. alba* (38.3%).

Flavonol content showed discrepancies in the examined pollen samples and ranged between 170 (*L. purpureum*) and 1349 mg 100 g^{-1} (*P. communis* pollen). The participation of flavonols in total phenolics differed considerably, depending on the pollen species, from 4.78% (*L. purpureum*) to 37.3% (*C. angustifolium*).

The content of anthocyanins was relatively low in comparison with the other phenolic compounds and ranged between 92 (*Trifolium* sp. and *Z. mays*) and 327 mg 100 g^{-1} (*P. tanacetifolia*). The percentage of anthocyanins in total phenols varied in the species from 3% (*L. purpureum* and *P. communis*) to 13% (*Trifolium* sp.).

Variability of total antioxidant activity in the investigated species seems to correspond to their phenylpropanoid contents (Fig. 1), being manifested by the significant positive correlation coefficient ($R^2 = 0.6132$).

4. Discussion

The honey bee products are considered to be abundant sources of antioxidants. In honey, royal jelly and propolis high antioxidant activity, expressed as the inhibition of lipid peroxidation was found (Nagai et al., 2001). In propolis water extracts high radical-scavenging activity, activity against superoxide anion and hydroxyl radical-scavenging activity were reported (Nagai, Inoue, Inoue, & Suzuki, 2003). Studies of Campos et al. (2000), and of Campos, Webby, and Markham (2002), concerning antioxidant properties of bee pollen as well as the recent reports (http://www.ccpollen.com/ORAC.shtml), confirm a very high antiradical activity of this product. Antioxidative ability of pollen seems to be due to phenolic compounds. In floral pollen mostly flavonoids, their glycosides and derivatives of cinnamic acid are present (Markham & Campos, 1996). Apart from common flavonoids (quercetin, kaempferol, luteolin and their derivatives), specific flavonoid glycosides, characteristic of some floral pollen, such as 7-α-8-*O*-methylherbacetin-3-*O*-sophorosides (Markham & Campos, 1996) or found in the Myrtaceae family aglycone triacin (Campos et al., 2002) were determined.

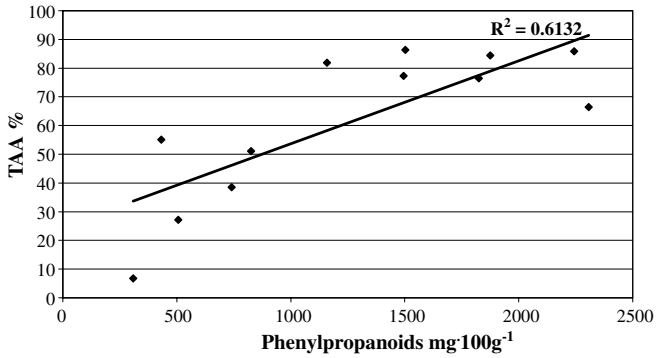

Fig. 1. Variability of total antioxidant activity.

High levels of phenolic constituents are often accompanied by high antioxidative capacity of pollen; however, according to reports of Campos, Webby, Markham, Mitchall, and Cunha (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).

In the present investigations, great variability regarding content of total phenols, phenylpropanoids, flavonols and antioxidant capacity in 12 examined pollens was found. 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.

In pollen of *R. pseudoacacia* and *A. hippocastanum*, high and medium levels, respectively, of phenolic compounds (total, phenylpropanoids and flavonols) were manifested by high TAA (84.4% and 82%) and high RSA (91% and 91.3%), while the HRSA was low (15.8% and 10.5%). In

the case of *M. domestica* and *P. communis*, high inhibition of lipid peroxidation and very high hydroxyl radical-scavenging activity agreed with the high level of total phenols and phenylpropanoids; however, radical-scavenging activity was low and medium, respectively (16.0% and 29.6%). Special attention should be paid to *L. purpureum* pollen: the relatively high (51%) TAA and high (76%) HRSA did not reflect a poor (8.6%) RSA and low flavonol content.

The most distinct interdependence was found between phenylpropanoids and total antioxidant activity, resulting in a significant positive correlation coefficient ($R^2 = 0.6132$). According to these results, phenylpropanoids might be treated as the main phenolic compounds responsible for the protection against lipid peroxidation in bee pollen tissue. Cinnamic acid derivatives are considered as efficient antioxidants due to the substitutions on the aromatic ring and the structure of the side chain, and better antioxidants than their benzoic acid counterparts (Natella et al., 1999). Very strong antioxidative properties of caffeic acid and its derivatives were described by Silva et al. (2000). However, according to Foti et al. (1996), the best protective action against linoleic acid peroxidation was attributed to flavonoids, followed by coumarins and cinnamic acids.

In general, the direct correlation between phenolic constituents and antioxidant capacity of bee pollen was questionable in some examined samples. The above results are partially in agreement with the Campos et al. (2003, 2000) reports. However, these authors determined only RSA activity by the DPPH method. The simultaneous estimation of RSA, TAA and HRSA allowed antioxidative properties of pollen to be characterized more precisely. Results showed that 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.

References

Bartosz, G. (1997). Oxidative stress in plants. *Acta Physiologiae Plantarum, 19*, 47–64.

Campos, M. G., Webby, R. F., & Markham, K. R. (2002). The unique occurrence of the flavone aglycone tricetin in Myrtaceae pollen. *Zeitschrift fur Naturforschung, 57c*, 1–3.

Campos, M. G., Webby, R. F., Markham, K. R., Mitchall, K. A., & Cunha, A. P. (2003). Age-induced diminution of free radical scavenging capacity in bee pollens and the contribution of constituent flavonoids. *Journal of Agricultural and Food Chemistry, 51*(3), 742–745.

Campos, M.G., Webby, R.F., Mitchell, K.A., Coleta, M., Markham, K.R., Cunha, A.P., 2000. Free-radical scavenging properties of bee-pollens – the non-involvement of flavonoids? *Polyphenols Communications* 2000, September 11–15. Freising-Weihenstephan Germany.

Faegri, K., Iversen, J., 1978. Podręcznik analizy pylkowej. Warszawa

Foti, M., Piattelli, M., Baratta, M. T., & Ruberto, G. (1996). Flavonoids, coumarins, and cinnamic acids as antioxidants in micellar system. Structure-activity relationship. *Journal of Agricultural and Food Chemistry, 44*(2), 497–501.

Fukumoto, L., & Mazza, G. (2000). Assessing antioxidant and prooxidant activities of phenolic compounds. *Journal of Agricultural and Food Chemistry, 48*(8), 3597–3604.

Hodges, D. (1984). *The pollen loads of the honey bee: a guide to their identification by colour and form*. London: Bee Research Association.

Kirk, D. J. (1994). *A colour guide to pollen loads of the honey bee*. Cardiff: International Bee Research Association.

Larson, R. A. (1988). The antioxidants of higher plants. *Phytochemistry, 27*(4), 969–978.

Markham, K. R., & Campos, M. G. (1996). 7-α-8-*O*-Methylherbacetin-3-*O*-sophorosides from bee pollens and some structure/activity observations. *Phytochemistry, 43*(4), 763–767.

Nagai, T., Sakai, M., Inoue, R., & Suzuki, N. (2001). Antioxidative activities of some commercially honeys, royal jelly, and propolis. *Food Chemistry, 75*, 237–240.

Nagai, T., Inoue, R., Inoue, H., & Suzuki, N. (2003). Preparation and antioxidant properties of water extract of propolis. *Food Chemistry, 80*, 29–33.

Natella, F., Nardini, M., Di Felice, M., & Saccini, C. (1999). Benzoic and cinnamic acid derivatives as antioxidants: structure–activity relation. *Journal of Agricultural and Food Chemistry, 47*(4), 1453–1459.

Pekkarinen, S. S., Stoeckmann, H., Schwarz, K., Heinonen, I. M., & Hopia, A. I. (1999). Atioxidant activity and partitioning of phenolic acids in bulk and emulsified methyl linoleate. *Journal of Agricultural and Food Chemistry, 47*(8), 3036–3043.

Pietta, P., Simonetti, P., & Mauri, P. (1998). Antioxidant activity of selected medicinal plants. *Journal of Agricultural and Food Chemistry, 46*(11), 4487–4490.

Racchi, M., Daglia, M., Lanni, C., Pepetti, A., Giovoni, S., & Gazzani, G. (2002). Antiradical activity of water soluble components in common diet vegetables. *Journal of Agricultural and Food Chemistry, 50*(5), 1272–1277.

Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P., & Glover, W. (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry, 66*, 401–436.

Silva, F. A., Borges, F., Guimaraes, C., Lima, J. L. F. C., Matos, C., & Reis, S. (2000). Phenolic acids and derivatives: studies on the relationship among structure, radical scavenging activity and physicochemical parameters. *Journal of Agricultural and Food Chemistry, 48*(6), 2122–2126.

Toivonen, P. M. A., & Sweeney, M. (1998). Differences in chlorophyll loss at 13 °C for two broccoli *Brassica oleracea* L. cultivars associated with antioxidant enzyme activities. *Journal of Agricultural and Food Chemistry, 46*(1), 20–24.

Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *Journal of Agricultural and Food Chemistry, 46*(10), 4113–4117.

Vinson, J. A., Hao, Y., Su, X., & Zubik, L. (1998). Phenol antioxidant quantity and quality in foods: vegetables. *Journal of Agricultural and Food Chemistry, 46*(9), 3630–3634.

Warakomska, Z. (1962). An investigation into pollen collection by *Apis mellifera* L. from two different parts of Poland. *Annales Universitatis Mariae Curie-Sklodowska. Lublin, Polonia, 17*(5), 67–106.