*Czech Journal of Food Sciences, 37, 2019 (1): 1–8*

https://doi.org/10.17221/139/2018-CJFS

# Morphology, physicochemical properties and antioxidant capacity of bee pollens

*Roman Bleha[1]*, Tetiana Shevtsova[1], Vojtěch Kružík[2], Ján Brindza[3], Andrej Sinica[1]*

[1]*Department of Carbohydrates and Cereals, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Prague, Czech Republic*

[2]*Department of Food Preservation, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Prague, Czech Republic*

[3]*Institute of Biodiversity Conservation and Biosafety, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture, Nitra, Slovak Republic*

**Corresponding author: blehar@vscht.cz*

**Citation**: Bleha R., Shevtsova T., Kružík V., Brindza J., Sinica A. (2019): Morphology, physicochemical properties and antioxidant capacity of bee pollens. Czech J. Food Sci., 37: 1–8.

**Abstract**: Six supposedly unifloral bee pollens of various botanical origins were characterised by morphometry, SEM, CIE *L*a*b** colour parameters and FTIR spectroscopy. Botanical origin and homogeneity of bee pollens were verified by colour and morphology of pollen grains. Water activity, moisture and antioxidant capacity of bee pollens were also evaluated. The results were discussed in terms of connection between botanical origin, composition and antioxidant properties of pollen materials.

**Keywords**: beekeeping products; colour; FTIR; SEM

Bee pollen is an apicultural product often used as food supplement. Its botanical origin is important as the main factor determining the presence of nutritional compounds and antioxidants (Almeida-Muradian *et al*. 2005; Human & Nicolson 2006; Silva *et al*. 2006) responsible for various biological activities (Pascoal *et al*. 2014; Komosinska-Vassev *et al*. 2015; Denisow & Denisow-Pietrzyk 2016). This beekeeping product is interesting as natural food supplement that may supply human organism with a wide spectrum of health promoting nutrients (Kroyer & Hegedus 2001). However, it can be dangerous if consumed by some allergic people (Martin-Munoz *et al*. 2010). Chemical composition of bee pollen, including the presence of healthy and hazardous substances, depends on botanical origin of pollen grains, geographical locality, season and storage conditions (Gasparotto Sattler *et al*. 2015). Therefore, bee pollen authenticity is important for quality estimation. Bee pollen amino acids (Szczęsna 2006), fatty acids (Manning 2001; Markowicz Bastos *et al*. 2004; You *et al*. 2007), flavonoids (Tomás-Barberán *et al*. 1989; Campos *et al*. 1998), triterpene alcohols (Xu *et al*. 2012) and carbohydrates (Qian *et al*. 2008) can be used as biochemical markers of botanical origin. Otherwise, morphological signs of pollen grains, characteristic IR bands or colour parameters could be applied for bee pollen characterisation. Partially, FTIR spectroscopy has been used for estimation of bee pollen's composition (Anjos *et al*. 2017).

This article is devoted to characterisation of six supposedly unifloral bee pollens of various botanical origins by morphometry, scanning electron micros-

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project No. 21-SVV/2018.

https://doi.org/10.17221/139/2018-CJFS

copy (SEM), colour estimation and Fourier transform infrared (FTIR) spectroscopy. Water activity, moisture and antioxidant capacity of bee pollens were also evaluated.

## MATERIAL AND METHODS

***Bee pollens***. Six supposedly unifloral bee pollen samples (Table 1) were collected at different parts of Slovak Republic. Bee pollen catcher was used for this purpose under control of beekeepers. Homogenates of bee pollen were prepared for FTIR and colour measurements; intact loads were used for morphometry and SEM image analysis.

***Morphology of bee pollen loads***. Bee pollen loads were weighted individually on the Bosch SAE200 balance. Images of bee pollen loads were obtained by automatic macro magnifying glass Zeiss Discovery V12 (Carl Zeiss AG, Germany) using Zeiss AxioVision 4.7.1 software (module Automatic Measurement). The linear parameters, i.e. height (*h*) (mm) and width (*w*) (mm), were calculated from images of 100 randomly chosen loads of each bee pollen by the same software.

***SEM images***. Digital images (resolution up to 3072 × 2304 px) of bee pollen loads were obtained by scanning electron microscope Zeiss Evo LS 15 with variable pressure capability at 20 kV using a variable pressure secondary electron (VPSE) detector and Zeiss Axio Vision 4.7.1/LE 4.8.2 software (Carl Zeiss AG, Germany). Magnifications of ~200×, ~500× and ~2000× were used to obtain images of load surface, ensembles of pollen grains and individual pollen grain, respectively. Polar (*P*) and equatorial (*E*) axes (μm) of individual pollen grains in the SEM images were measured using the same software (module Heasure).

***Colour estimation***. The CIE *L*a*b** parameters of bee pollen homogenates were calculated from diffuse reflectance visible (Vis) spectra. Ten Vis spectra of each sample (380–780 nm, step 10 nm) were recorded on UV-Vis spectrophotometer UV4 (UNICAM, UK) using Labsphere holder (Labshere, USA) and Chroma 2.0 (ThermoSpectronic, USA) software.

***FTIR spectroscopy***. FTIR spectra (4000–400 $cm^{-1}$, 64 scans and spectral resolution 2 $cm^{-1}$) of bee pollens were recorded in KBr tablets on Nicolet 6700 FT-IR spectrometer using Omnic 7.0 (Thermo Scientific, USA) software. The smoothed and baseline corrected spectra were exported in the CSV format into Origin 6.0 (Microcal Origin, USA) software for graphical outputs.

***Moisture and water activity***. Karl Fischer titration method was done on Volumetric Karl Fischer titrator AF8 (Thermo Orion, USA) for determination of moisture in thawed fresh-frosted bee pollen tempered at 25°C. Water activity of bee pollens was measured using a LabMaster-aw neo (Novasina AG, Switzerland) and represented as the ratio of partial pressure of water vapour above the surface of the product to pressure of saturated water vapour at 25°C.

***Antioxidant capacity***. Two methods were used for evaluation of bee pollen antioxidant activity: 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) and 2-2´-azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS) photometric assays. The former one is based on decolouration of stable free radical DPPH$^{\bullet}$ having a strong purple colour (Brand-Williams *et al*. 1995); the latter one quantifies decolouration of blue-green coloured radical-cation $ABTS^{\bullet+}$ generating from ABTS by metmyoglobin/ $H_2O_2$ (Miller & Rice-Evans 1996). ABTS assay was applied for the methanolic extracts from bee pollens; DPPH assay for both methanolic and aqueous extracts. Bee pollens (0.1 g) were mixed with 25 ml of water or methanol, exposed to ultrasound during 1 h and then filtrated using syringe filters. Commercial set Randox (Randox Laboratories, USA) was used for

Table 1. Specification of bee pollen samples

| Sample | Specie | Collecting place[a] |
|---|---|---|
| 1 | oilseed rape (*Brassica napus* L. var. *napus*) | Slepčany |
| 2 | sunflower (*Helianthus annuus* L.) | Šahy |
| 3 | opium poppy (*Papaver somniferum* L.) | Dvory nad Žitavou |
| 4 | blue tansy (*Phacelia tanacetifolia* L.) | Zemianske Podhradie |
| 5 | black locust (*Robinia pseudoacacia* L.) | Mošovce |
| 6 | white clover (*Trifolium repens* L.) | Tesárske Mlyňany |

[a]Slovak Republic

https://doi.org/10.17221/139/2018-CJFS

ABTS assay. The absorbance was measured at 517 nm ($DPPH^{\bullet}$) and at 600 nm ($ABTS^{\bullet+}$). The resulting antioxidant capacity was expressed as an equivalent of TROLOX concentration (mmol/l) for trapping of the same amount of ABTS or as % of DPPH bleaching.

***Statistical evaluation***. For the morphological and CIE $L^*a^*b^*$ parameters, moisture, water activity and antioxidant capacity, the results were compared among the samples using the ANOVA technique with Statistica 10 (StatSoft, USA). ANOVA indicated differences among the means, and Tukey (HSD) analysis of the differences was used for the comparisons between individual bee pollens. All data are reported as mean values with confidence intervals ($P < 0.05$).

## RESULTS AND DISCUSSION

***Morphology of bee pollen loads***. Typical bee pollen loads of samples 1–6 are shown in Figure 1. There are evident differences in size, shape, surface micro-texture and colour of these loads. Variability of the mass, height and width for bee pollen loads is represented in Table 2. The average masses of loads were in the range of ~8.4–15.3 g; variability coefficients confirmed moderate to very high variability in mass. The average heights and widths of loads were in the range of ~2.7–3.5 mm and ~3.3–3.8 mm, respectively; the corresponding variability coefficients confirmed moderate to high variability in these parameters. Bee pollen samples 3 and 4 consisted of loads with maximal and minimal values of all morphometric parameters, respectively. Surface texture of bee pollen loads is informative about palinological composition of bee pollen (Nôžková *et al*. 2010). The differences in the surface texture of bee pollen loads were caused by specific structure of exine typical for mentioned botanical species.


Figure 1. Typical bee pollen loads of samples 1–6

***Morphology of pollen grains***. SEM images of pollen loads of 1–6 at three levels of magnification are shown in Figure 2. The samples 1, 2, 4 and 5 were pure unifloral bee pollens of botanic species mentioned above, whereas samples 3 and 6 were evidently contaminated by sunflower pollen grains. This admixture could appear during pollen collection by bee visiting flowers of more than one species. Otherwise, the surface scratches between bee pollen loads of different palinological origin may occur during bee pollen storage. Variability of pollen grains in bee pollen samples is summarised in Table 2. The grains of studied botanical species demonstrated significant difference in linear parameters, i.e. lengths at polar ($P$) and equatorial ($E$) axes. The shape index (SI, $P/E$ ratio), which is a measure of elongation or roundness of pollen grains, also showed significant variability. Blue tansy pollen 4 is oblong (SI = 2.03 ± 0.20), while sunflower pollen 2 is about roundish (SI = 1.17 ± 0.08).

***Colour***. The CIE $L^*a^*b^*$ parameters of bee pollen samples are summarised in Table 3. Among all bee

Table 2. Variability of bee pollen loads and grains in samples 1–6

| Sample | Bee pollen loads ($n$ = 100) | | | Pollen grains | | | |
|---|---|---|---|---|---|---|---|
| | $m$ (mg) | $h$ (mm) | $w$ (mm) | N | $P$ (µm) | $E$ (µm) | SI |
| 1 | 11.47 ± 0.18$^{d}$ | 3.31 ± 0.018$^{cd}$ | 3.77 ± 0.016$^{b}$ | 40 | 39.99 ± 0.03$^{d}$ | 25.22 ± 0.07$^{c}$ | 1.59 ± 0.006 |
| 2 | 10.70 ± 0.19$^{c}$ | 3.38 ± 0.008$^{d}$ | 3.72 ± 0.008$^{b}$ | 54 | 45.27 ± 0.01$^{f}$ | 38.81 ± 0.08$^{e}$ | 1.17 ± 0.003 |
| 3 | 15.32 ± 0.23$^{f}$ | 3.45 ± 0.004$^{d}$ | 3.77 ± 0.006$^{b}$ | 72 | 37.67 ± 0.01$^{c}$ | 25.90 ± 0.03$^{cd}$ | 1.46 ± 0.002 |
| 4 | 8.40 ± 0.14$^{a}$ | 2.66 ± 0.006$^{a}$ | 3.34 ± 0.006$^{a}$ | 105 | 32.53 ± 0.01$^{a}$ | 16.13 ± 0.02$^{a}$ | 2.03 ± 0.004 |
| 5 | 9.21 ± 0.22$^{b}$ | 2.91 ± 0.008$^{b}$ | 3.42 ± 0.006$^{a}$ | 87 | 41.20 ± 0.01$^{e}$ | 21.56 ± 0.03$^{b}$ | 1.92 ± 0.002 |
| 6 | 12.26 ± 0.20$^{e}$ | 3.36 ± 0.006$^{d}$ | 3.74 ± 0.006$^{b}$ | 90 | 34.62 ± 0.01$^{b}$ | 27.23 ± 0.03$^{d}$ | 1.27 ± 0.002 |

$m$ – mass; $h$ – height; $w$ – width; $P$ – polar axes; $E$ – equatorial axes; SI – shape index ($P/E$); different letters indicate significant differences between bee pollens ($P < 0.05$)

https://doi.org/10.17221/139/2018-CJFS

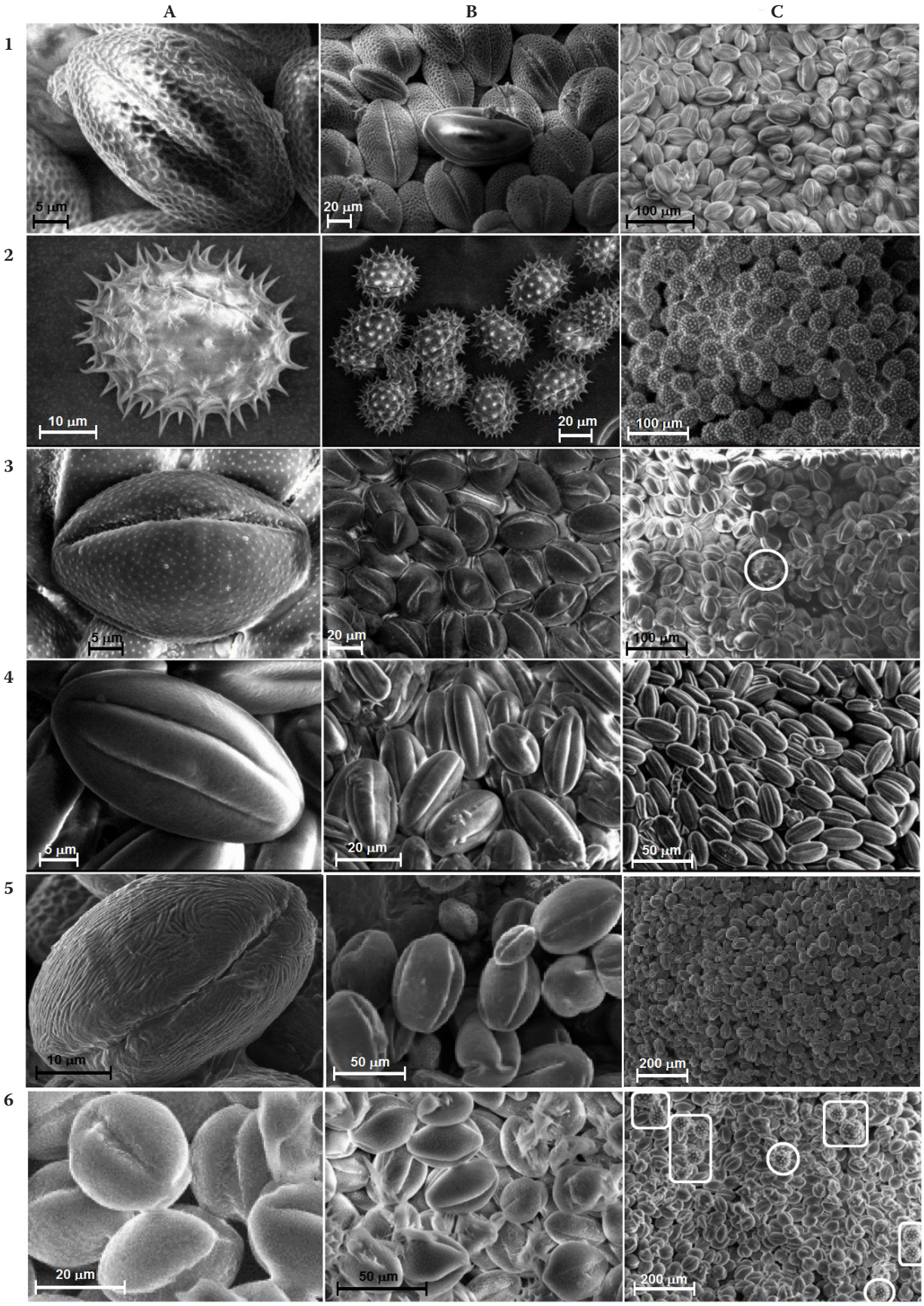


Figure 2. SEM images of typical bee pollen loads of samples 1–6

https://doi.org/10.17221/139/2018-CJFS

Table 3. CIE $L^*a^*b^*$ parameters of bee pollens ($n$ = 10)

| Sample | $L^*$ | $a^*$ | $b^*$ | $C_{ab}^*$ | $H_{ab}^*$ |
|---|---|---|---|---|---|
| 1 | 75.19 ± 0.09$^{d}$ | 7.85 ± 0.19$^{b}$ | 29.86 ± 0.57$^{d}$ | 30.88 ± 0.54$^{d}$ | 1.313 ± 0.007$^{e}$ |
| 2 | 57.95 ± 0.38$^{b}$ | 19.99 ± 0.41$^{e}$ | 25.84 ± 1.50$^{c}$ | 32.69 ± 1.26$^{d}$ | 0.911 ± 0.027$^{c}$ |
| 3 | 51.40 ± 0.18$^{a}$ | 14.79 ± 0.58$^{d}$ | 8.99 ± 1.35$^{b}$ | 17.42 ± 0.53$^{b}$ | 0.543 ± 0.074$^{b}$ |
| 4 | 51.75 ± 0.27$^{a}$ | 1.11 ± 0.22$^{a}$ | −10.34 ± 0.87$^{a}$ | 10.42 ± 0.88$^{a}$ | −1.467 ± 0.018$^{a}$ |
| 5 | 75.23 ± 0.17$^{d}$ | 9.54 ± 0.20$^{c}$ | 38.60 ± 0.71$^{e}$ | 39.76 ± 0.66$^{e}$ | 1.328 ± 0.008$^{e}$ |
| 6 | 64.70 ± 0.19$^{c}$ | 10.00 ± 0.26$^{c}$ | 25.40 ± 0.73$^{c}$ | 27.31 ± 0.71$^{c}$ | 1.195 ± 0.012$^{d}$ |

Different letters indicate significant differences between bee pollens ($P$ < 0.05)

pollens, samples 1 and 5 were the lightest ($L^*$ ~ 75) and samples 3 and 4 were the darkest ($L^*$ ~ 51–52). The colour parameter $a^*$ (red–green axes) was positive for all bee pollens, and the colour parameters $b^*$ (yellow–blue axes) and $H_{ab}^*$ (hue) were also positive for all the samples except blue coloured bee pollen 4. For studied bee pollens the colour parameter $C_{ab}^*$ (saturation) varied from ~10 (sample 4) to ~40 (sample 5). Differences in the CIE $L^*a^*b^*$ parameters mentioned above are useful for distinguishing of bee pollens 1–6 according to their botanical origin.

***Moisture and water activity***. Pollen is known as highly hygroscopic material and thus susceptible for microbial or fungal growth (Carpes *et al*. 2009). Moisture is tightly connected with quality of bee pollen because wet conditions support grows of microorganisms, fungi and enzyme activities that may decrease safety and sensory properties of this product. The water activity indicates resistance of bee pollen to microbial contamination because if this value is too high, undesirable microorganisms may occur (Morgano *et al*. 2011). To avoid this, for better and longer storage, bee pollen is usually frozen or dried.

The fresh-frozen bee pollens 1–6 contained 11.5–19.5% of water. Water activity of these samples was in the range of 0.57–0.67 (Table 4). These values were similar to those measured for fresh-frozen bee pollens and intermediate between those of freshly harvested and dried bee pollens, i.e. 0.52–0.59 (Sagona *et al*. 2017), 0.62–0.82 (De-Melo *et al*. 2015) and 0.21–0.55 (Carpes *et al*. 2009; Estevinho *et al*. 2012; Feás *et al*. 2012; Nogueira *et al*. 2012; Deveza *et al*. 2015), respectively. All values obtained for thawed bee pollens were below 0.7 and thus low enough to avoid bacterial growth, but yeast growth is still possible (Sagona *et al*. 2017).

Table 4. Moisture and water activity of bee pollens ($n$ = 5)

| Sample | Moisture (% m/m) | Water activity |
|---|---|---|
| 1 | 14.784 ± 0.195$^{b}$ | 0.665 ± 0.036$^{b}$ |
| 2 | 15.524 ± 0.808$^{b}$ | 0.594 ± 0.056$^{ab}$ |
| 3 | 19.539 ± 2.238$^{c}$ | 0.661 ± 0.070$^{ab}$ |
| 4 | 15.741 ± 2.211$^{b}$ | 0.574 ± 0.032$^{a}$ |
| 5 | 11.534 ± 0.743$^{a}$ | 0.595 ± 0.047$^{ab}$ |
| 6 | 15.933 ± 1.048$^{b}$ | 0.650 ± 0.043$^{ab}$ |

Different letters indicate significant differences between bee pollens ($P$ < 0.05)

***FTIR spectra***. Spectra of bee pollens 1–6 are shown in Figure 3. Broad band around 3300–3400 cm$^{-1}$ originated from OH stretching vibrations of water and hydroxyls. Two narrow bands near 2923 and 2852 cm$^{-1}$ were assigned to antisymmetric and symmetric $CH_2$ stretching vibrations in lipids, respectively (Vlachos *et al*. 2006). Weak band or shoulder near 3012 cm$^{-1}$ arose from =CH stretching vibrations of unsaturated compounds. Several overlapped bands in the region of 1500–1800 cm$^{-1}$ mainly indicated the presence of carboxylic compounds, i.e. carbonic acids, esters and amides. Two intense bands near 1656 cm$^{-1}$ (amide I) and 1545 cm$^{-1}$ (amide II) confirmed the presence of proteins (Barth 2007). Less pronounced bands near 1740 and 1710 cm$^{-1}$ were assigned to

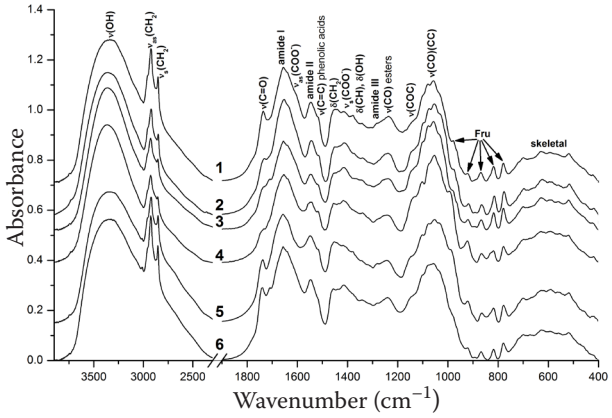


Figure 3. FTIR spectra of bee pollens 1–6

https://doi.org/10.17221/139/2018-CJFS

C=O stretching vibrations of ester and carboxylic groups, respectively. Weak band near 1518 $cm^{-1}$ arose from C=C stretching vibrations of phenolic acids (Kačuráková *et al.* 1999). The bands at 1451–1458 and 1378 $cm^{-1}$ were assigned to $CH_2$ and $CH_3$ bending vibrations in aliphatic groups. Band at 1414 $cm^{-1}$ arose from symmetric $COO^-$ stretching. The band or shoulder near 1280 $cm^{-1}$ was assigned to amide III vibrations in proteins. Broad band near 1240 $cm^{-1}$ originated from CO stretching vibrations in carboxyls and esters. Highly overlapped bands in the region of 900–1200 $cm^{-1}$ were assigned mainly to CO, CN a CC stretching vibrations of sugars and proteins. Bands at 777, 817, 865, 922, and 978 $cm^{-1}$ are typical for amorphous fructose (Ibrahim *et al.* 2006). The bands below 700 $cm^{-1}$ could be assigned to various skeletal vibrations.

***Antioxidant activity***. Antioxidant activities of bee pollen samples are shown in Table 5. Both ABTS and DPPH methods confirmed that bee pollen samples had valuable antioxidant activity that is in agreement with literature (Leja *et al.* 2007; Morais *et al.* 2011; Feás *et al.* 2012). It is known that bee pollens contains significant amounts of antioxidants including hydrophilic (ascorbate, phenolic acids, flavonoids, etc.) and hydrophobic (unsaturated fatty acids, tocopherols and carotenoids) ones (Rice-Evans *et al.* 1996; Krinsky 2001; Richard *et al.* 2008; Rzepecka-Stojko *et al.* 2015). According to DPPH assay, all methanolic extracts showed significantly higher antioxidant capacity than aqueous extracts due to the prevalence of mentioned antioxidants. Methanolic extracts from samples 1 and 4 showed maximal amounts of DPPH bleaching. In earlier investigation (Fatrcová-Šramková *et al.* 2013), the antioxidant properties of unifloral bee pollens decreased in the order 1 > 3 > 2 that is in agreement with our results of DPPH assay obtained for methanolic extracts. By contrast, aqueous extracts from samples 1 and 2 demonstrated lower antioxidant capacity than those from the other samples according to both assays. Many reports (Campos *et al.* 2003; Leja *et al.* 2007; Pérez-Pérez *et al.* 2012) confirmed that high antioxidant capacity of bee pollen ethanolic or methanolic extracts correlated with high amount of polyphenols, mainly flavonoids and phenolic acids. The contribution of non-phenolic compounds to the whole antioxidant capacity of these extracts cannot be excluded. Moreover, free radical scavenging activity of bee pollen demonstrated significant decrease with aging that could be explained by instability of some antioxidants. Campos *et al.* (2003) suggested that such decline in free radical scavenging can be used as a measure of bee pollen age if compare with fresh bee pollen of the same floral origin.

Table 5. Antioxidant capacity of bee pollens 1–6

| Sample | ABTS ($n$ = 3) | DPPH ($n$ = 5) | |
|---|---|---|---|
| | TROLOX (mm/l) | DPPH bleaching (%) | |
| | | methanol | water |
| 1 | 0.83 ± 0.10$^a$ | 93.69 ± 5.80$^d$ | 22.53 ± 1.40$^a$ |
| 2 | 1.90 ± 0.20$^b$ | 25.96 ± 1.61$^a$ | 19.66 ± 1.06$^a$ |
| 3 | 2.08 ± 0.25$^b$ | 61.30 ± 3.84$^c$ | 43.95 ± 2.63$^c$ |
| 4 | 2.01 ± 0.25$^b$ | 93.06 ± 5.74$^d$ | 50.29 ± 3.05$^c$ |
| 5 | 2.00 ± 0.22$^b$ | 92.10 ± 5.65$^d$ | 47.21 ± 2.83$^c$ |
| 6 | 2.07 ± 0.27$^b$ | 43.55 ± 2.58$^b$ | 34.28 ± 2.16$^b$ |

Different letters indicate significant differences between bee pollens ($P$ < 0.05)

## CONCLUSIONS

Six samples of unifloral bee pollen were characterised. Morphology and SEM images of bee pollen loads confirmed their botanical origin and homogeneity. Bee pollen samples demonstrated valuable antioxidant activity mainly due to the presence of methanol soluble phenolics and, to a lesser extent, carotenoids.

### References

Almeida-Muradian L.B., Pamplona L.C., Coimbra S., Barth O.M. (2005): Chemical composition and botanical evaluation of dried bee pollen pellets. Food Composition and Analysis, 18: 105–111.

Anjos O., Santos A.J.A., Dias T., Estevinho L.M. (2017): Application of FTIR-ATR spectroscopy on the bee pollen characterization. Journal of Apicultural Research, 56: 210–218.

Barth A. (2007): Infrared spectroscopy of proteins. Biochimica et Biophysica Acta, 1767: 1073–1101.

Brand-Williams W., Cuvelier M.E., Berse C. (1995): Use of a free radical method to evaluate antioxidant activity. LWT-Food Science Technology, 28: 25–30.

Campos M., Markham K.R., Mitchell K.A., da Cunha A.P. (1998): An approach to the characterization of bee pollens via their flavonoid/phenolic profiles. Phytochemical Analysis, 8: 181–185.

Campos M.G., Webby R.F., Markham K.R., Mitchell K.A., da Cunha A.P. (2003): Age-induced diminution of free radical scavenging capacity in bee pollens and the contri-

https://doi.org/10.17221/139/2018-CJFS

bution of constituent flavonoids. Journal of Agricultural and Food Chemistry, 51: 742–745.

Carpes S.T., Mourão G.B., De Alencar S.M., Masson M.L. (2009): Chemical composition and free radical scavenging activity of *Apis mellifera* bee pollen from Southern Brazil. Brazilian Journal of Food Technology, 12: 220–229.

De-Melo A.A.M., Miranda Fernandes Estevinho M.L., Gasparotto Sattler J.A., Rodrigues de Souza B., da Silva de Freitas A., Barth O.M., Almeida-Muradiana L.B. (2015): Effect of processing conditions on characteristics of dehydrated bee-pollen and correlation between quality parameters. LWT-Food Sciences and Technologies, 65: 808–815.

Denisow B., Denisow-Pietrzyk M. (2016): Biological and therapeutic properties of bee pollen: a review. Journal of the Science of Food and Agriculture, 96: 4303–4309.

Deveza M.V., Keller K.M., Lorenzon M.C.A., Nunes L.M.T., Sales E.O., Barth O.M. (2015): Mycotoxicological and palynological profiles of commercial brands of dried bee pollen. Brazilian Journal of Microbiology, 46: 1171–1176.

Estevinho L.M., Rodrigues S., Pereira A.P., Feás X. (2012): Portuguese bee pollen: Palynological study, nutritional and microbiological evaluation. International Journal of Food Science and Technology, 47: 429–435.

Fatrcová-Šramková K., Nôžková J., Kačániová M., Máriássyová M., Rovná K., Stričík M. (2013): Antioxidant and antimicrobial properties of unifloral bee pollen. Journal of Environmental Science and Health B, 48: 133–138.

Feás X., Vazquez-Tato M.P., Estevinho L., Seijas J.A., Iglesias A. (2012): Organic bee pollen: Botanical origin, nutritional value, bioactive compounds, antioxidant activity and microbiological quality. Molecules, 17: 8359–8377.

Gasparotto Sattler J., Pereira de Melo I., Granato D., Araujo E., Freitas A., Barth O., Sattler A., Almeida-Muradian L. (2015): Impact of origin on bioactive compounds and nutritional composition of bee pollen from southern Brazil: A screening study. Food Research International, 77: 82–91.

Human H., Nicolson S.W. (2006): Nutritional content of fresh, bee-collected and stored pollen of *Aloe greatheadii* var. *davyana* (Asphodelaceae). Phytochemistry, 67: 1486–1492.

Ibrahim M., Alaam M., El-Haes H., Jalbout A.F., de Leon A. (2006): Analysis of the structure and vibrational spectra of glucose and fructose, Elética, 31: 15–21.

Kačuráková M., Wellner N., Ebringerová A., Hromádková Z., Wilson R.H., Belton P.S. (1999): Characterisation of xylan-type polysaccharides and associated cell wall components by FT-IR and FT-Raman spectroscopies. Food Hydrocolloids, 13: 35–41.

Komosinska-Vassev K., Olczyk P., Kaźmierczak J., Mencner L., Olczyk K. (2015): Bee pollen: chemical composition and therapeutic application. Evidence-Based Complementary and Alternative Medicine, vol. 2015, Article ID 297425, 6 pages. doi 10.1155/2015/297425.

Krinsky N.I. (2001): Carotenoids as antioxidants. Nutrition, 17: 815–817.

Kroyer G., Hegedus N. (2001): Evaluation of bioactive properties of pollen extracts as functional dietary food supplement. Innovative Food Science & Emerging Technologies, 2: 171–174.

Leja M., Mareczeka A., Wyżgolika G., Klepacz-Baniak J., Czekońska K. (2007): Antioxidative properties of bee pollen in selected plant species. Food Chemistry, 100: 237–240.

Manning R. (2001): Fatty acids in pollen: a review of their importance for honey bees. Bee World, 82: 60–75.

Markowicz Bastos D.H., Barth O.M., Rocha C.I., da Silva Cunha I.B., de Oliveira Carvalho P., Silva Torres E.A., Michelan M. (2004): Fatty acid composition and palynological analysis of bee (Apis) pollen loads in the states of São Paulo and Minas Gerais, Brazil. Journal of Apicultural Research, 43: 35–39.

Martin-Munoz M.F., Bartolome B., Caminoa M., Bobolea I., Ara M.G., Quirce S. (2010): Bee pollen: a dangerous food for allergic children. Identification of responsible allergens. Allergologia et immunopathologia, 38: 263–265.

Miller N.J., Rice-Evans C.A. (1996): Spectrophotometric determination of antioxidant activity. Redox Report: Communications in Free Radical Research, 2: 161–171.

Morais M., Moreira L., Feás X., Estevinho L.M. (2011): Honeybee-collected pollen from five Portuguese Natural Parks: Palynological origin, phenolic content, antioxidant properties and antimicrobial activity. Food and Chemical Toxicology, 49: 1096–1101.

Morgano M.A., Milani R.F., Martins M.C.T., Rodriguez-Amaya D.B. (2011): Determination of water content in Brazilian honeybee-collected pollen by Karl Fischer titration. Food Control, 22: 1604–1608.

Nogueira C., Iglesias A., Feas X., Estevinho L.M. (2012): Commercial bee pollen with different geographical origins: A comprensive approach. International Journal of Molecular Sciences, 13: 11173–11187.

Nôžková J., Brindza J., Ostrovský R., Stehlíková B., Fatrcová-Šramková K. (2010): Evaluation of qualitative and quantitative traits of bee pollen and their classification according to proposed list of descriptors. Potravinarstvo, 4: 204–216.

Pascoal A., Rodrigues S., Teixeira A., Feás X., Estevinho L.M. (2014): Biological activities of commercial bee pollens: antimicrobial, antimutagenic, antioxidant and anti-inflammatory. Food and Chemical Toxicology, 63: 233–239.

Pérez-Pérez E.M., Vit P., Rivas E., Sciortino R., Sosa A., Tejada D., Rodríguez-Malaver A.J. (2012): Antioxidant activity of four color fractions of bee pollen from Mérida, Venezuela. Archivos Latinoamericanos de Nutricion, 62: 375–380.

Qian W.L., Khan Z., Watson D.G., Fearnley J. (2008): Analysis of sugars in bee pollen and propolis by ligand exchange

https://doi.org/10.17221/139/2018-CJFS

chromatography in combination with pulsed amperometric detection and mass spectrometry. Journal of Food Composition and Analysis, 21: 78–83.

Rice-Evans C.A., Miller N.J., Paganga G. (1996): Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine, 20: 933–956.

Richard D., Kefi K., Barbe U., Bausero P., Visioli F. (2008): Polyunsaturated fatty acids as antioxidants. Pharmacological Research, 57: 451–455.

Rzepecka-Stojko A., Stojko J., Kurek-Górecka A., Górecki M., Kabała-Dzik A., Kubina R., Moździerz A., Buszman E. (2015): Polyphenols from bee pollen: structure, absorption, metabolism and biological activity. Molecules, 20: 21732–21749.

Sagona S., Bozzicolonna R., Nuvoloni R., Cilia G., Torracca B., Felicioli A. (2017): Water activity of fresh bee pollen and mixtures of bee pollen-honey of different botanical origin. LWT-Food Science and Technology, 84: 595–600.

Silva T.M.S., Camara C.A., da Silva Lins A.C., Barbosa-Filho J.M., da Silva E.M.S., Freitas B.M., dos Santos F. de A.R. (2006): Chemical composition and free radical scavenging activity of pollen loads from stingless bee *Melipona subnitida* Ducke. Journal of Food and Composition Analysis, 19: 507–511.

Szczęsna T. (2006): Protein content and amino acid composition of bee-collected pollen from selected botanical origins, Journal of Apicultural Sciences, 50: 81–90.

Tomás-Barberán F.A., Tomás-Lorente F., Ferreres F., Garcia-Viguera C. (1989): Flavonoids as biochemical markers of the plant origin of bee pollen. Journal of Sciences in Food and Agriculture, 47: 337–340.

Vlachos N., Skopelitis Y., Psaroudaki M., Konstantinidou V., Chatzilazarou A., Tegou E. (2006): Applications of Fourier transform-infrared spectroscopy to edible oils. Analytica Chimica Acta, 573–574: 459–465. doi 10.1016/j.aca.2006.05.034

Xu X., Gao Y., Sun L. (2012): Free and esterified triterpene alcohol composition of bee pollen from different botanical origins. Food Research International, 48: 650–656.

You J., Zhu F., Zhao W., Zhao X., Suo Y. Liu, S. (2007): Analysis of saturated free fatty acids from pollen by HPLC with fluorescence detection. European Journal of Lipid Science and Technology, 109: 225–236.

Received: 2018–05–21
Accepted after corrections: 2019–01–30
Published online: 2019–02–18