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MIRJANA SABO

MIRJANA POTOCNJAK

INES BANJARI

DANIJELA PETROVIC

TÜBİTAK

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Pollen analysis of honeys from Varaždin County, Croatia

Mirjana SABO[1,*], Mirjana POTOČNJAK[1], Ines BANJARI[1], Danijela PETROVIĆ[2]

[1]University of Osijek, Faculty of Food Technology, F. Kuhača 20, 31000 Osijek - CROATIA

[2]University of Mostar, Agronomy and Faculty of Food Technology, BOSNIA and HERZEGOVINA

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Abstract: The palynological properties of 8 honey samples commercially produced in 1 region in Croatia (Varaždin County) were determined. Each sample was examined to determine the pollen percentage and pollen spectrum. On the basis of honey pollen analysis, in these 8 samples different botanical origin was determined. In total, 20 different types of pollen grains were identified. The dominant group of pollen grains consisted of *Castanea sativa* Mill. in samples 2 and 8, *Brassica napus* L. in samples 4 and 5, and *Trifolium pratense* L. in samples 6 and 7. The pollen analysis revealed 6 unifloral and 2 multifloral honeys. Analysis was performed using methods in accordance with national and international legislation, in an accredited laboratory.

Key words: Pollen, honey, unifloral honey, multifloral honey

Introduction

Melissopalynological analysis is still the prescribed method for botanical origin denomination and therefore it is one of the greatest discriminatory powers of honeys (Ruoff & Bogdanov, 2004). According to some authors, not only are acidity and humidity especially important parameters, but in some cases pollen analysis is also of great meaning for the geographical origin and classification of honeys (Persano Oddo & Piro, 2004a; Kaya et al., 2005; Silici & Gökçeoğlu, 2007), particularly when an individual floral species is growing in specific areas (Anklam, 1998). As emphasised by Mandić et al. (2006) there are more than 100 unifloral honeys in Europe, but most of them are produced occasionally and have a local significance. Geographic and botanical properties are important for the quality of honeys (Romas et al., 1999; Valencia et al., 2000). The taste, smell, and colour of honey changes according to the nectar of the flowers. Pollen analyses of floral honeys reveal the plant taxa of the honey's source. Bees collect the nectar and pollen from flowers at the same time. The pollen that is mixed in the honey is important for the honey's quality (Kaya et al., 2005). Nectar-containing flowering plants have been identified through pollen analysis in honey samples from various countries, including 54 samples from Louisiana (Lieux, 1972), 119 samples from New Zealand (Mear, 1985), 25 samples from the Canary Islands (Romas et al., 1999), 74 samples from various regions in Turkey (Doğan & Sorkum, 2001), and 13 other samples from various region in Turkey (Kaya et al., 2005). With the same aim, the International Honey Commission of Apimondia (IHC) recently

* E-mail: mirjana.sabo@ptfos.hr

collected data and published a descriptive sheet of 15 European unifloral honey types (Persano Oddo & Piro, 2004b). The Codex Alimentarius Commission (2001) allows specific designations for honey from particular sources (such as unifloral honeys), but does not specify the characteristics of various honey types. Most of the 15 honeys described by Persano Oddo & Piro (2004b) are widely spread in Croatia. According to Croatian legislation (Ministry of Agriculture, Forestry & Water Management, 2009), unifloral honey is honey in which the honey's insoluble sediment has at least 45% of its pollen grains deriving from the same plant species. Honey can be declared as unifloral with the name of a specific plant species if the content of the pollen grains in the honey's sediment is as follows: *Castanea sativa* Mill., 85%; *Brassica napus* L., 60%; *Phacelia tanacetifolia* Benth., 60%; *Tilia* spp., 25% (10%*); *Robinia pseudoacacia* L., 20%; *Mentha* spp., 20%; *Calluna vulgaris* L., 20%; *Satureja montana* L., 20%; *Taraxacum officinale* Weber, 20%; *Rosmarinus officinalis* L., 20%; *Salvia officinalis* L., 15% (10%*); *Arbutus unedo* L., 10%; *Citrus* spp., 10% (5%*); and *Lavandula* spp., 10% (5%*) (*: with typical sensory honey characteristics that are attached to a special plant type, i.e. smell, taste, and colour). Multifloral honey is a mixture of unifloral honeys of different species (Ministry of Agriculture, Forestry & Water Management, 2009). The aim of this study was to analyse 8 honey samples from Varaždin County, characteristic of the local nature of the flora and representing very diverse nectar and pollen sources of bees in commercially produced honeys.

Materials and methods

Pollen analysis was done on 8 honey samples from Varaždin County, northern Croatia, which has an altitude of 180-260 m. The phytocenosis for localities from Varaždin County is a connection of mesophyll forests of *Carpinus betulus* L. and *Quercus petraea* Liebl., *Querco-Carpinetum illyricum*, and forests of *Quercus petreae* Liebl. and *Castanea sativa* Mill., *Querco-Castanetum illyricum* (*croaticum*), and *Carpino betuli-Quercetum roboris* (Rauš, 1987). Samples 1, 6, and 7 were from the meadow. Samples 4 and 5 were from cultivated fields. Samples 2, 3, and 8 were from forests. In the forests of Varaždin County, the dominant plants within the tree layers are *Quercus petraea*, *Carpinus betulus*, *Prunus avium*, *Acer campestre*, *Acer pseudoplatanus*, *Ulmus campestris*, *Ulmus montana*, *Tilia platyphyllos*, *Sorbus torminalis*, *Robinia pseudoacacia*, *Quercus robur*, *Quercus cerris*, *Acer tataricum*, and *Fraxinus excelsior*. The following plants can be found within the bush layer: *Corylus avellana*, *Euonymus europaeus*, *Rosa arvensis*, *Daphne mezereum*, *Lonicera caprifolium*, etc. The following plants can be noticed at the ground layer: *Lamium orvala*, *Helleborus atrorubens*, *Epimedium alpinum*, *Anemone nemorosa*, *Rhamnus cathartica*, *Carex digitata*, *Vicia oroboides*, *Salvia glutinosa*, etc. From the range of the joint plant species of the oak-hornbeam forests, the following plant species are also noticed: *Carex pilosa*, *Hepatica nobilis*, *Knautia arvensis*, and *Crocus albiflorus* (Rauš, 1987).

Pollen analysis was done using the methods defined by Louveaux et al. (1978). The pollen count was based on a minimum of 500 grains and the identification and observations were made with an Olympus light microscope (400× or 1000×, as appropriate). Pollen types were identified by personal reference and based on the relevant literature. Pollen grains were counted on 2 slides for each honey sample and each pollen type was presented as a percentage with respect to the total counted pollen grains numbers (von der Ohe & von der Ohe, 2003; von der Ohe et al., 2004; Ministry of Agriculture, Forestry & Water Management, 2009). The terms used for the frequency classes were: predominant pollen (more than 45% of the pollen grains counted), secondary pollen (16%-45%), important minor pollen (3%-15%), and minor pollen (less than 3%) (Louveaux et al., 1978).

Results

All of the 8 honey samples analysed were studied for pollen content in terms of quantity and diversity. Their detailed pollen load compositions were observed separately, as follows.

Honey sample 1, from the locality Sveti Ilija (northern Croatia), was selected as a meadow specimen. Honey sample 1 contained a high percentage of pollen grains from *Prunus* sp. (36%), *Castanea sativa* Mill. (21%), and *Brassica napus* L. (21%) (Table 1). The pollen of other accompanying

Table 1. Percentage of pollen grains in honey samples 1, 2, and 3 from Varaždin County.

Honey sample 1 (Sveti Ilija)		Honey sample 2 (Strmec Podravski)		Honey sample 3 (Maruševac)	
Plant species	Pollen (%)	Plant species	Pollen (%)	Plant species	Pollen (%)
Prunus sp.	36	*Castanea sativa* Mill.	97	*Castanea sativa* Mill.	40
Castanea sativa Mill.	21	*Brassica napus* L.	2	*Brassica napus* L.	27
Brassica napus L.	21	*Fraxinus ornus* L.	0.5	*Robinia pseudoacacia* L.	20
Robinia pseudoacacia L.	11	*Taraxacum officinale* Weber	0.5	*Trifolium pratense* L.	7
Zea mays L.	3			*Lotus corniculatus* L.	2
Trifolium pratense L.	3			*Centaurea montana* L.	2
Taraxacum officinale Weber	3			*Zea mays* L.	1
Centaurea montana L.	1			*Tilia platyphyllos* Scop.	1
Helianthus annuus L.	1				

plant species, such as *Robinia pseudoacacia* L. (11%), *Zea mays* L. (3%), *Trifolium pratense* L. (3%), *Taraxacum officinale* Weber (3%), *Centaurea montana* L. (1%), and *Helianthus annuus* L. (1%), were present in the total content in smaller percentages (Table 1). According to Croatian legislation, sample 1 is a multifloral honey (Ministry of Agriculture, Forestry & Water Management, 2009).

Honey sample 2, from the locality Strmec Podravski (northern Croatia), was selected as a forest specimen. Sample 2 contained pollen grains from *Castanea sativa* Mill. at a percentage of nearly 97%; thus, the pollen of this species is dominant, suggesting that this plant is the chief source of pollen and nectar in bee foraging. Pollen grains of *Brassica napus* L. (2%) were present in a smaller percentage, while *Fraxinus ornus* L. (0.5%) and *Taraxacum officinale* Weber (0.5%) pollen grains were present in trace amounts (Table 1). In sample 2, pollen grains of *Castanea sativa* Mill. were predominant and, according to Croatian legislation, with 85% of *Castanea sativa* Mill. pollen grains in the total honey sediment, this honey sample is a unifloral chestnut honey (Ministry of Agriculture, Forestry and Water Management, 2009).

Honey sample 3, from the locality Maruševac (northern Croatia), was also selected as a forest specimen. This honey sample contained pollen grains of *Castanea sativa* Mill. at 40%, *Brassica napus*. L. at 27%, *Robinia pseudoacacia* L. at 20%, and smaller percentages of pollen of other accompanying plant species, such as *Trifolium pratense* L. (7%), *Lotus corniculatus* L. (2%), *Centaurea montana* L. (2%), *Zea mays* L. (1%), and *Tilia platyphyllos* Scop. (1%) (Table 1). Although *Castanea sativa* Mill. pollen grains were highly represented in comparison to other pollen types, according to Croatian legislation (Ministry of Agriculture, Forestry & Water Management, 2009) this honey could only be declared as a black locust (*Robinia pseudoacacia* L.) unifloral honey, because pollen grains of *Robinia pseudoacacia* L. were 20% of the honey's total insoluble sediment (Table 1).

Sample 4, from the locality Ivanec (northern Croatia), was selected as a honey from a cultivated field of rapeseed (*Brassica napus* L.). Sample 4 contained pollen grains of *Brassica napus* L. in a quantity of nearly 90%, suggesting thereby that this plant is the chief source of pollen and nectar in bee foraging. The honey production occurred in the late spring as *Brassica napus* L., a common oil seed

plant of the region, flowers profusely during this period. On the basis of the honey pollen analysis, 5 other botanical species were identified: *Robinia pseudoacacia* L. (3%), *Castanea sativa* Mill. (3%), *Trifolium pratense* L. (2%), *Taraxacum officinale* Weber (1%), and *Lathyrus sylvestris* L. (1%), all present in smaller percentages (Table 2). On the basis of these results, honey sample 4 is a unifloral rapeseed (*Brassica napus* L.) honey (Ministry of Agriculture, Forestry & Water Management, 2009).

Sample 5, from the locality Vinica (northern Croatia), was also selected as an example of honey from a cultivated field of rapeseed (*Brassica napus* L.). *Brassica napus* L. (59%) was the dominant pollen, with relatively much higher frequencies then the others retrieved from the sample. *Fraxinus ornus* L. (15%), followed by *Castanea sativa* Mill. (9%), *Trifolium pratense* L. (5%), *Prunus* sp. (5%), *Robinia pseudoacacia* L. (4%), and *Taraxacum officinale* Weber (2%), were present in smaller percentages. *Zea mays* L. (0.5%) and *Viola tricolor* L. (0.5%) were present in trace amounts (Table 2). According to Croatian legislation, based on the pollen analysis, sample 5 should be declared as a unifloral rapeseed (*Brassica napus* L.) honey (Ministry of Agriculture, Forestry & Water Management, 2009).

Sample 6, from the locality Cerje Tužno (northern Croatia), was selected as a meadow specimen. It contained pollen grains of *Trifolium pratense* L. in a quantity of nearly 48%, and pollen grains of *Robinia pseudoacacia* L. in a share of 37%. In the sediment of honey sample 6, pollen grains of other plants species were identified: *Taraxacum officinale* Weber (4%), *Castanea sativa* Mill. (3%), *Brassica napus* L. (3%), *Centaurea montana* L. (2%), *Cichorium intybus* L. (1%), and *Knautia arvensis* L. (1%) in very small percentages, and *Sambucus nigra* L. (0.5%) and *Loranthus europaeus* Jacq. (0.5%) in trace amounts (Table 2). According to Croatian legislation, based on the pollen analysis, this honey sample could be declared as a unifloral red clover (*Trifolium pratense* L.) honey or a unifloral black locust (*Robinia pseudoacacia* L.) honey; the final decision should be made using physicochemical parameters, as well (Ministry of Agriculture, Forestry & Water Management, 2009).

Table 2. Percentage of pollen grains in honey samples 4, 5, and 6 from Varaždin County.

Honey sample 4 (Ivanec)		Honey sample 5 (Vinica)		Honey sample 6 (Cerje Tužno)	
Plant species	Pollen (%)	Plant species	Pollen (%)	Plant species	Pollen (%)
Brassica napus L.	90	*Brassica napus* L.	59	*Trifolium pratense* L.	48
Robinia pseudoacacia L.	3	*Fraxinus ornus* L.	15	*Robinia pseudoacacia* L.	37
Castanea sativa Mill.	3	*Castanea sativa* Mill.	9	*Taraxacum officinale* Weber	4
Trifolium pratense L.	2	*Trifolium pratense* L.	5	*Castanea sativa* Mill.	3
Taraxacum officinale Weber	1	*Prunus* sp.	5	*Brassica napus* L.	3
Lathyrus sylvestris L.	1	*Robinia pseudoacacia* L.	4	*Centaurea montana* L.	2
		Taraxacum officinale Weber	2	*Cichorium intybus* L.	1
		Zea mays L.	0.5	*Knautia arvensis* L.	1
		Viola tricolor L.	0.5	*Sambucus nigra* L.	0.5
				Loranthus europaeus Jacq.	0.5

From the melissopalynological analysis of honey sample 7, selected as a meadow specimen from the locality Margečan (northern Croatia), pollen grains of *Trifolium pratense* L. (70%) were seen to be the dominant component with relatively much higher frequencies than other pollens retrieved from the sample. *Robinia pseudoacacia* L. (10%), followed by *Zea mays* L. (6%), *Centaurea montana* L. (6%), *Brassica napus* subsp. *oleracea* DC. (4%), and *Taraxacum officinale* Weber (4%) were present in smaller percentages (Table 3). After melissopalynological analysis, it was clear that honey sample 7 from Margečan should be declared as a unifloral red clover (*Trifolium pratense* L.) honey (Ministry of Agriculture, Forestry & Water Management, 2009).

Honey sample 8 was selected as a forest specimen from the locality Donja Voća (northern Croatia); it contained pollen grains of *Castanea sativa* Mill. at 59% and pollen grains of *Trifolium pratense* L. at 35%, while pollen grains of *Fraxinus ornus* L. (3%) and *Centaurea montana* L. (2%) were present in smaller amounts. *Taraxacum officinale* Weber (0.6%), *Centaurea jacea* L. (0.2%), and *Raphanus raphanistrum* L. (0.2%) were present in trace amounts (Table 3). Although pollen grains of *Castanea sativa* Mill. dominated in sample 8, this honey should be declared and considered as a multifloral honey (Ministry of Agriculture, Forestry & Water Management, 2009).

Discussion

Based on the number of pollen species and the share of each species in the total pollen count, 6 out of 8 analysed samples were identified as unifloral honeys and 2 samples were identified as multifloral honeys. The pollen analyses of the honeys collected from Varaždin County in Croatia generated significant information pertaining to geographic and botanical origins of honeys, whether unifloral or multifloral, and documentation of bee foraging of plants, as well. The investigation depicts the characteristic local nature of the flora, which serves as a very diverse nectar and pollen source for bees for commercially produced honeys. The quantification of the pollen types in relation to their overall distribution in the local flora brings knowledge of the principles and importance of the forage plants for each honey sample. Microscopic analysis revealed that plant species variability is greatest in the minor pollen group (less than 3%), followed by the important minor pollen, secondary, and dominant groups. This seems to confirm the view that variability is always small among pollen species in the dominant groups, while greater among minor pollen (less than 3%), important minor pollen, and secondary pollen groups. According to Kaya et al. (2005), pollen grains in the dominant and secondary groups supply the nectar source, which plays a crucial role in the formation of honey, while the taste, smell, and colour

Table 3. Percentage of pollen grains in honey samples 7 and 8 from Varaždin County.

Honey sample 7 (Margečan)		Honey sample 8 (Donja Voća)	
Plant species	Pollen (%)	Plant species	Pollen (%)
Trifolium pratense L.	70	*Castanea sativa* Mill.	59
Robinia pseudoacacia L.	10	*Trifolium pratense* L.	35
Zea mays L.	6	*Fraxinus ornus* L.	3
Centaurea montana L.	6	*Centaurea montana* L.	2
Brassica napus L.	4	*Taraxacum officinale* Weber	0.6
Taraxacum officinale Weber	4	*Centaurea jacea* L.	0.2
		Raphanus raphanistrum L.	0.2

of honey change according to the flower nectar, as in our investigations.

According to the results, pollen grains of family Fagaceae (*Castanea sativa* Mill.) were dominant in honey samples 2 and 8 (Tables 1 and 3). Of family Brassicaceae, *Brassica napus* L. pollen grains were predominant in samples 4 and 5 (Table 2), while secondary in samples 1 and 3 (Table 1). In honey samples 4 and 5, *Brassica napus* L. was the chief source of nectar and pollen as manifested by much higher percentages, 90% and 59%, respectively, of total honey insoluble sediment. According to Pınar et al. (2009a), pollen and seed morphology of the Turkish *Hesperis* L. (*Brassicaceae*) taxa are taxonomically significant characters and the main pollen and seed morphological differences have been found at the section level, especially in pollen and seed types.

Of the family Fabaceae, *Trifolium pratense* L. pollen grains were dominant in sample 6 (Table 2) and in sample 7 (Table 3). According to Pınar et al. (2009b), investigations of the pollen morphology of *Trifolium pratense* L. (Fabaceae) showed that the main values for diploid, triploid, and tetraploid species are very similar, but greater variations in the maximum and minimum values occur in the tetraploid. *Robinia pseudoacacia* L. pollen grains were secondary in sample 2 (Table 1) and in sample 6 (Table 2). Of the family Oleaceae, *Fraxinus ornus* L. pollen grains were an important minor group in honey sample 5 (Table 2) and a minor group pollen (less than 3%) in samples 2 (Table 2) and 8 (Table 3). Pollen grains of family Asteraceae were found in all analysed samples, but in small percentages and in minor pollen (less than 3%) spectrum amounts (Tables 1-3). Akyalçın et al. (2011) reported that the size of pollen grains of genus *Achillea* (Asteraceae) show wide variations. In the analysed honey samples, 20 plant species were identified, of which 4 types of pollen grains, *Castanea sativa* Mill., *Brassica napus* L., *Trifolium pratense* L., and *Robinia pseudoacacia* L. were significant in the identification of the analysed honey samples. However, all of the other identified pollen grains that are mixed in the honey still significantly influence the quality of the honey. According to Lieux (1979), many of the pollen grains of this group have been mixed into the honey in a random fashion. The highest percentage of pollen grains in the analysed honey samples was of the species *Castanea sativa* Mill., *Brassica napus* L., and *Trifolium pratense* L., followed by *Robinia pseudoacacia* L. and *Prunus* sp. The remaining species of pollen grains were defined as important minor pollen and minor pollen (less than 3%) (Tables 1-3). Pollen grains from families Fagaceae, Brassicaceae, and Fabaceae were abundant in the highest amounts, while pollen grains of Asteraceae, Rosaceae, Oleaceae, Poaceae, Tiliaceae, Caprifoliaceae, Loranthaceae, Dipsacaceae, and Violaceae were abundant in smaller percentages. Out of 8 analysed honey samples from Varaždin County, 6 were identified as unifloral (samples 2, 3, 4, 5, 6, and 7) and 2 as multifloral honeys (samples 1 and 8).

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