

Pollen and chemical-physical analysis of unifloral honey from different regions of Bulgaria

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Abstract: The paper discusses the results of the pollen and chemical-physical analysis of 16 honey samples from different floristic regions of Bulgaria identified by beekeepers as unifloral honey. Unifloral honey of high quality was established in the surroundings of Turgovishte (*Tilia* and *Anethum graveolens* honey), Silistra (*Robinia pseudoacacia*), Bailovo (*Ailanthus*), Yambol (*Coriandrum sativum*), and Velingrad/Petrich (*Castanea sativa*). *Apiaceae* pollen dominates in the honey from Pleven (Danubian Plain) and Selanovtsi (Sofia region). Unifloral *Tilia*, *Coriandrum sativum* and *Castanea sativa* honey showed higher diastase activity in comparison with the results of Ivanov (1973).

Key words: diastase activity, honey, melysopalynology, nectariferous plants

Introduction

Identifying and quantifying the pollen grains in honey samples is the best way to determine the floral nectar source used by bees to produce honey, and therefore the best way to label it correctly (Lieux 1975; Louveaux & al. 1978; Moar 1985; van der Ham & al. 1999). Besides melissopalynological analysis, organoleptic and physical-chemical analysis are also necessary, because honey requires certain types of verification before being marketed (Bulgarian State Standard for Bee Honey 1990). The leading honey-producer countries in Europe have strict labelling regulations for honey products (EEU 2001).

Many melissopalynologists have tried to solve the problems of accurate unifloral honey classification on the basis of pollen content (Demianowicz 1961, 1964; Sawyer 1988; Ricciardelli D'Albore 1998; van der Ham & al. 1999). This information has important commercial value, because honey

made from some plants commands a higher price than honey produced from other plants. In order to make the results more accurate, it is necessary to take into consideration the over- or underrepresentation of certain pollen types in honey.

According to Louveaux & al. (1978), 45 % of a single pollen type is the "universal" minimal amount needed for honey to be classified as unifloral, but a correction must be made for the underrepresented or overrepresented pollen types to the level of 45 %.

Palynological investigations of Bulgarian honeys are rare (Bozilova & Anchev 1968; Bozilova & Chan 1976; Petkova 1984; Lazarova & Bozilova 2001, 2002).

The aim of this study is to increase knowledge of the Bulgarian unifloral honey. Pollen analysis together with the study of diastase activity and hydroxymethylfurfural (HMF) have immediate practical purpose.

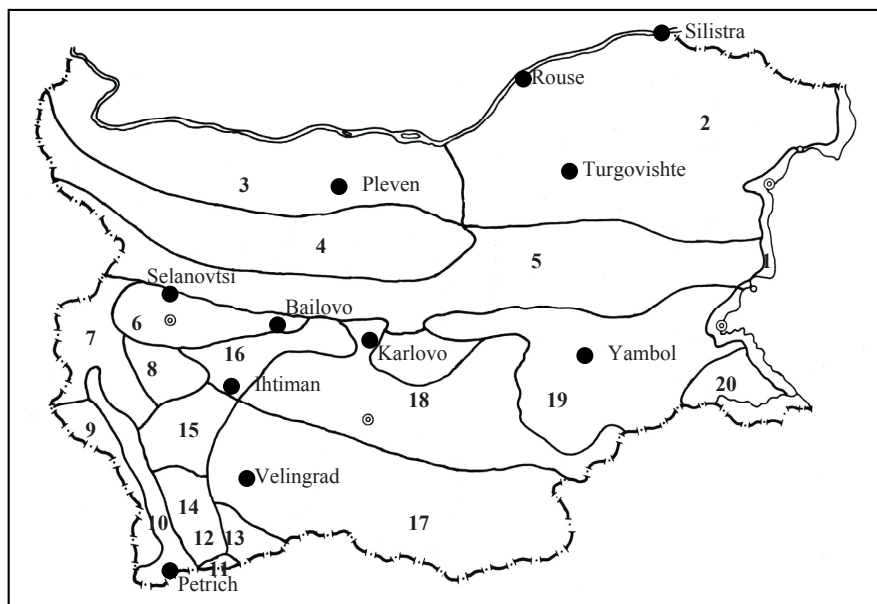


Fig 1. Map of the studied sites (the floristic regions are according to Kozhuharov 1995)

Material and methods

Sixteen honey samples from different floristic regions of Bulgaria (Fig. 1) collected in the spring and summer of 2001 were analyzed palynologically at the Palynological Laboratory of the Department of Botany, University of Sofia. Most were identified by the beekeepers as unifloral honey on the basis of organoleptic features. The samples for pollen analysis were prepared following the method for non-acetylated honey described by Louveaux & al (1978) and the Bulgarian State Standard (BSS) for Bee Honey (1990). The frequency of each pollen type in the honey samples is expressed as percentage of the total pollen sum (P), which includes pollen grains only from entomophilous plants (Louveaux & al 1978). The frequency of pollen grains of the anemophilous plants is calculated on the basis of P. More than 1000 pollen grains were counted in each sample. Tablets of *Lycopodium* spores were used to calculate the absolute pollen concentration (APC) in honey and APC of the dominant pollen types. Diastase activity was analyzed for all samples, and HMF content for 7 samples; reducing sugars, total sugars, sucrose, and water content for 5 samples were studied at the Laboratory for Testing and Diagnosis of Bee Honey and Bee Products (Sofia Inspectorate for Veterinary and Sanitary Control) (Table 1). Diastase activity of all samples was analysed in September 2001, followed by a second analysis of six of them in February 2002. Diastase activity and HMF content are major parameters in the evaluation of freshness, the temperature and storage history of the honey (Ivanov

1973; Karabourniotti & Zorvalaki 2001). The honey samples were stored in the dark at 16–20 °C, and their crystallization was also recorded.

Results and discussion

Samples N 9320, 9321, 9443, 9021, 1, and 14306 are from the surroundings of Turgovishte (Northeast Bulgaria) (Fig. 1). Four of them (N 9320, 9321, 9443, and 9021) are of thick liquid honey, yellowish, clear, with delicious aroma. The crystallization was slow, setting in about six months after extraction. The honey was identified by the beekeeper as unifloral *Tilia* honey. Pollen analysis showed that the pollen spectra of the four samples were similar in taxonomic composition (Table 2). *Tilia* was the dominant pollen taxon (with 76.8 %, 77.5 %, 77.0 %, and 71.8 %). The APC of *Tilia* (Table 3) varied from 987 to 1346 pollen grains in 1 cc of honey. According to Louveaux & al. (1978), Sawyer (1988), van der Ham & al. (1999), *Tilia* pollen in honey is underrepresented, but in our case percentage values as well as APC of *Tilia* were relatively high. According to BSS (1990) and Louveaux (1978), 30 % of *Tilia* pollen is sufficient to identify the honey as unifloral. Diastase activity of the samples was also high (ranging from 16.7 to 22.4) in comparison to the results of Ivanov (1973) for *Tilia* honey in Bulgaria (Table 1).

Sample N 1. The honey was yellowish, with delicious spicy aroma and quick crystallization. It was identified by the beekeeper as *Tilia* honey. Pollen anal-

Table 1. Chemical-physical features of 16 honey samples from different regions of Bulgaria

dominant pollen type	N	Diastase activity (units after Goete)		Diastase activity (Ivanov 1973)		HMF (mg %)	reducing sugars %	total sugars %	sucrose %	water %
		09.2001	04.2002	max	min					
<i>Tilia</i>	9320	18.6	15.6							
<i>Tilia</i>	9321	22.4		21.2	8.8					
<i>Tilia</i>	9443	21.6								
<i>Tilia</i>	9021	16.7	14.6							
<i>Falcaria/Anethum</i> type	1	28.7	26.2							
<i>Trifolium</i> type	14306	11.0				2.4	72.9	76.4	4.1	15.6
<i>Tilia, Brassicaceae</i>	9248	24.6	24.0							
<i>Robinia</i>	8942	13.0	12.0	14.8	5.2	2.4	73.4	77.7	4.1	15.6
<i>Brassica</i> type	14307	20.3					74.8			
<i>Apiaceae</i>	16283	28.1				0.8				
<i>Coriandrum</i> type	13233	16.8		16.4	9.7	0.3	77.4	79.0	1.5	15.5
<i>Castanea sativa</i>	13234	22.0		18.4	13.0	0.3	70.6	78.7	7.7	15.5
<i>Asteraceae</i>	13235	29.0				0.6	75.4	77.5	2.1	15.9
<i>Apiaceae</i>	22	16.5	14.2							
<i>Ailanthus</i>	16542	28.2				0.4				
<i>Achillea</i> type	2	15.2								

Table 2. Pollen content (in %) in 16 honey samples from different regions of Bulgaria

Pollen type	Sample N	Sample N							
		9320 %	9321 %	9443 %	9021 %	1 %	14306 %	9248 %	8942 %
<i>Tilia</i>	76.8	77.5	77.0	71.8		3.1	20.9		
<i>Helianthus annuus</i>	6.6	5.0	1.5	4.8			19.8		
<i>Cirsium/Carduus</i> type	1.4	0.7	0.6	2.0		1.5	3.2		
<i>Achillea</i> type	0.8	0.8	0.2	0.4	0.3	2.7			
<i>Centaurea cyanus</i>						0.9		1.1	
<i>Taraxacum</i> type		0.5	0.3	2.0			1.1		
<i>Apiaceae</i>	2.7	4.1	11.0	6.0	4.9	20.9	5.0	1.1	
<i>Falcaria/Anethum</i> type					78.0				
<i>Brassicaceae</i>	7.2	2.8	4.8	4.6	9.6	3.7	20.3	25.0	
<i>Trifolium repens</i> type	0.8	2.0	1.5		0.8	19.1			
<i>T. pratense</i> type		0.8				5.4			
<i>Trifolium</i> type	2.9			3.6	0.4	3.2	3.3	0.8	
<i>Vicia</i> type		1.7	0.9	0.8	1.4	20.6	0.6		
<i>Lathyrus</i> type						4.2	0.1		
<i>Lotus</i> type		2.8	0.3	1.8	0.5	3.7	6.7	7.1	
<i>Robinia</i>	0.6		1.3				1.7	45.0	
<i>Fabaceae</i>		0.2	0.3	0.1		2.5			
<i>Lamiaceae</i>		0.2			0.5	3.5	4.4	7.5	
<i>Mentha/Lavandula</i> type	0.1	0.2	0.5	0.5	0.5	1.4		0.2	
<i>Rosaceae</i>		0.1		0.4	0.5	0.9	3.3	2.4	
<i>Fragaria</i> type			0.1		0.1	1.4	0.2	0.9	
<i>Prunus</i> type			0.7		0.4		0.5		
<i>Potentilla</i> type					0.1		0.6		
<i>Scrophulariaceae</i>					0.1	0.9	0.6	1.0	
<i>Verbascum</i> type		0.1							
<i>Boraginaceae</i>			0.2						
<i>Vitis</i>		0.5		0.2	3.0		3.0	3.7	
<i>Viburnum</i>							0.8		
<i>Galium</i>				0.9					
<i>Ranunculaceae</i>		0.1				0.1	0.1	2.3	
<i>Epilobium</i>							0.1		
<i>Ailanthus</i>					2.4		2.2		
Pollen sum (P)	1533	1371	2030	1792	1156	1027	1028	1003	
<i>Corylus</i>						5.0		0.1	
<i>Betula</i>			0.2	2.3				0.4	
<i>Plantago</i>		3.1	1.2	2.0		0.2	4.8	0.2	
<i>Poaceae</i>			1.1				0.3	1.3	

Pollen type	Sample N	Sample N									
		14307 %	16283 %	16542 %	13233 %	13234 %	13235 %	22 %	2 %		
<i>Tilia</i>	2.3	0.2	1.2							0.5	
<i>Helianthus annuus</i>		2.6		0.1		2.7	33.4				
<i>Cirsium/Carduus</i> type		0.7				19.0	0.5				
<i>Achillea</i> type				0.1		17.9		65.2			
<i>Centaurea cyanus</i>						4.9		0.3			
<i>C. jacea</i> type						7.4					
<i>Taraxacum</i> type						6.0		0.9			
<i>Apiaceae</i>	13.9	84.1	0.5	2.0	3.6	9.2	49.6	1.4			
<i>Coriandrum</i> type				95.0							
<i>Brassicaceae</i>	4.9	3.8		1.0	1.2	5.3	9.2	1.9			
<i>Brassica</i> type	73.4										
<i>Trifolium</i> type	2.3	0.2	5.0	0.4		3.3	3.6				
<i>T. pratense</i> type.			5.2		0.4			0.5			
<i>Vicia</i> type		0.2	3.8			0.7	0.6	1.1			
<i>Lathyrus</i> type							1.0				
<i>Lotus</i> type		0.3	0.3			8.6	0.9				
<i>Robinia</i>	1.0										
<i>Fabaceae</i>				0.1		0.8	0.2				
<i>Lamiaceae</i>	0.4	2.0				6.7	0.6	0.9			
<i>Mentha/Lavandula</i> type		5.2			0.6	2.7	0.3				
<i>Rosaceae</i>			12.6	0.3		0.2		3.6			
<i>Fragaria</i> type		0.4				0.3		0.8			
<i>Prunus</i> type		0.1	4.8		1.1	1.2		0.8			
<i>Rosa</i> type						0.6					
<i>Scrophulariaceae</i>				0.1		0.1		1.1			
<i>Ranunculaceae</i>	0.9		0.7			0.5		5.9			
<i>Galium</i>						0.3					
<i>Sambucus</i>						0.3					
<i>Vitis</i>	0.8			0.2	1.0						
<i>Castanea sativa</i>					92.4						
<i>Ailanthus</i>			66.2					0.9			
<i>Acer</i>								10.0			
<i>Geranium</i>								1.8			
<i>Liliaceae</i>								0.1			
<i>Linum</i>							0.3				
<i>Syringa/Ligustrum</i> type							0.1				
<i>Vaccinium</i> type					0.1						
Pollen sum (P)	1137	1236	1091	2642	1602	1010	1383	1046			
<i>Betula</i>								0.3			
<i>Fagus</i>		0.2					0.1				
<i>Corylus</i>		0.1									
<i>Quercus</i>			3.4				0.2				
<i>Plantago</i>			1.1	0.5		1.8	3.7	0.1			
<i>Poaceae</i>						1.5		0.4			

Table 3. Absolute pollen concentration (APC) and APC of the dominant pollen type in 16 honey samples from different regions of Bulgaria

Sample N	APC total	APC of the dominant pollen type
9320 (Turgovishte)	1618	<i>Tilia</i> 1243
9321 (Turgovishte)	1274	<i>Tilia</i> 987
9443 (Turgovishte)	1426	<i>Tilia</i> 1116
9021 (Turgovishte)	1873	<i>Tilia</i> 1346
1 (Turgovishte)	4085	<i>Falcaria/Anethum</i> type 2982
14306 (Turgovishte)	1315	<i>Trifolium</i> type 359
9248 (Rouse)	1177	<i>Tilia</i> 246 <i>Brassicaceae</i> 220 <i>Helianthus annuus</i> 246
8942 (Silistra)	936	<i>Robinia pseudoacacia</i> 425
14307 (Pleven)	1324	<i>Brassicaceae</i> 973
16283 (Pleven)	1514	<i>Apiaceae</i> 1253
13233 (Yambol)	13807	<i>Apiaceae (Coriandrum)</i> type 13187
13234 (Velingrad/Petrich)	6822	<i>Castanea sativa</i> 6307
13235 (Karlovo)	874	<i>Cirsium/Carduus</i> type 166 <i>Mentha/Lavandula</i> type 85
16542 (Bailovo)	5145	<i>Ailanthus</i> 3408
22 (Selanovtsi)	1664	<i>Apiaceae</i> 825
2 (Ihtiman)	1157	<i>Achillea</i> type 755

ysis has shown dominance of the *Apiaceae* – *Falcaria/Anethum* type in the pollen spectrum. According to Punt & Clarke (1984), *Falcaria* type includes also *Anethum graveolens* pollen. Stojanov & Kitanov (1966) pointed out that *Anethum graveolens* is a plant with high nectar production and we considered that honey as unifloral *Anethum graveolens* honey. *Tilia* pollen was almost absent. The pollen concentration of *Falcaria/Anethum* type was high (Table 3), as was the diastase activity (Table 1).

Sample N 14306 was a thick liquid honey, light-yellow, clear, transparent, with delicious flavour. There was no sign of crystallization 10 months after extraction. The honey was identified by the beekeeper as unifloral *Trifolium* honey. Pollen analysis has shown predominance of pollen grains of different *Fabaceae* species (Table 2). *Trifolium* type accounted for 26.6 % (including *Trifolium repens* type and *T. pratense* type). The APC was 1315 pollen grains in 1 cc of honey, but for *Trifolium* type only 358 pollen grains in 1 cc. Diastase activity registered the lowest values (11 units after Goete) (Table 1).

Sample N 9248 from Rouse (Northeast Bulgaria). The honey was thick, liquid, dark-yellow, clear, and with delicious flavour. No crystallization after 9 months. It was identified by the beekeeper as unifloral *Tilia* honey. Pollen analysis has shown dominance of *Tilia* (20.9 %) and *Brassicaceae* (20.3 %). High percentage of *Helianthus annuus* was also present (19.8 %).

Diastase activity was high (24.6). The APC of *Tilia* was low (204 pollen grains in 1 cc of honey).

Sample N 8942 from Silistra (Northeast Bulgaria). The honey was liquid, light-yellow, clear, transparent, and with delicious aroma. No crystallization 10 months after the extraction. The honey was identified by the beekeeper as unifloral *Robinia pseudoacacia* honey. Pollen analysis has shown predominance of *Robinia* (45 %). *Robinia* pollen in honey is under-represented (Louveaux & al. 1978; Sawyer 1988) and 30 % of it is sufficient to identify the honey as unifloral *Robinia pseudoacacia* honey (BSS 1990). *Robinia pseudoacacia* is a fine nectariferous plant but a low pollen producer (Ricciardelli D'Albore 1998), which is the main reason for the low APC of *Robinia* (Table 3). Slow crystallization is characteristic of *Robinia* honey. Diastase activity was 13 units after Goete.

Unifloral *Robinia* honey was established also by Lazarova & Bozilova (2001) in Oryahovo and Pleven (Danubian Plain), with high percentage in the pollen spectrum but low diastase activity (5.4) for Oryahovo. According to Ivanov (1973), the values of diastase activity for *Robinia* honey in Bulgaria range from 5.2 to 14.8.

Samples N 14307 and 16283 are from the Pleven region (Danubian Plain). Sample N 14307 came from the beekeeper in crystallized form. The honey was whitish, finely crystallized, with delicious aroma, and was identified by the beekeeper as *Carduus* honey. *Brassica* type (73.4 %) predominated in the pollen spectrum, and *Apiaceae* was the accompanying pollen type. The APC of *Brassica* type was 973 pollen grains in 1 cc of honey. Diastase activity was 20.3 units after Goete (Table 1). Quick crystallization is characteristic of honey produced by different *Brassicaceae* species (Lazarov & al. 1971).

Sample N 16283 was of thick liquid honey, dark-orange, clear, with delicious aroma. No sign of crystallization after 10 months. The honey was identified by the beekeeper as polyfloral honey. Pollen analysis has shown predominance of *Apiaceae* pollen (*Chaerophyllum* type): 84.1 %. High diastase activity was also established (Table 1).

Sample N 16542 from Bailovo (Sofia region). The sample for pollen analysis came in fine crystallized form from the beekeeper. The honey was whitish, with strong delicious flavour, identified by the beekeeper as *Lavandula* honey. *Ailanthus* pollen (66.2 %) predominated in the pollen spectrum. *Ailanthus altissima* was introduced in Europe in the 18th century (Vetvička

1998) and has been naturalised also in Bulgaria. Its great adaptability and quick growth make it a common tree species in the parks and cities of Bulgaria. For Italy, *Ailanthus* is one of the most frequently occurring pollen types in bee honey (Ricciardelli D'Albore 1998). One of the highest diastase numbers was established for this honey: 28.2 (Table 1).

Sample N 13233 from Yambol (Toundzha Hilly Country). The sample for pollen analysis came in crystallized form from the beekeeper. Rapid and very fine crystallization was characteristic. The honey was beige in colour, with strong delicious aroma, identified by the beekeeper as *Thymus* honey. No pollen grains of *Thymus* were identified in this sample. Pollen analysis has shown predominance of *Apiaceae* (*Bifora/Coriandrum* type – 95%), with the highest pollen concentration of all investigated samples (13807 pollen grains in 1 cc of honey and 13187 for the dominant pollen type). According to Punt & Clarke (1984), *Bifora radians* pollen type includes *Coriandrum sativum* pollen. Bearing in mind also the organoleptic features, we consider that honey as unifloral *Coriandrum*. Stojanov & Kitanov (1960) and Petkov (1973) had pointed out that *Coriandrum* is a very good and important for the bees nectariferous plant, predominantly planted in South Bulgaria.

Sample N 13234. This sample was combined from two sites: Petrich (Valley of Strouma River) and Velingrad (Western Rhodopes). The honey was thick liquid, dark-orange to brown, with strange but not unpleasant flavour. The beekeeper identified it as *Castanea sativa* honey. *Castanea* pollen predominates in the pollen spectrum (92.4%) and pollen concentration is also high, typical for small-sized pollen (Louveaux & al. 1978; Ricciardelli D'Albore 1998). According to van der Ham & al. (1999), 90% of *Castanea* pollen is necessary to identify the honey as unifloral *Castanea* honey. After 10 months no sign of crystallization was established.

Sample N 13235 from Karlovo (Thracian Lowland). The honey showed quick crystallization (soon after extraction). It was yellowish, with delicious aroma, identified by the beekeeper as *Lavandula* honey. Pollen analysis has shown only 2.7% presence of *Mentha/Lavandula* pollen type. Pollen of *Asteraceae* species predominated (Table 2). For this sample was established the highest diastase activity (29 units after Goete).

Sample N 22 from Selanovtsi (Sofia region). The honey was a thick liquid mass, transparent, with pleas-

ant flavour, identified by the beekeeper as polyfloral honey. Major crystallization began after 3–4 months. *Apiaceae* (49.6%) predominated in the pollen spectrum, accompanied by *Helianthus annuus* (33.4%).

Sample N 2 from Ihtiman (Mt Sredna Gora). The honey was identified by the beekeeper as *Cirsium* honey. It was very finely crystallized, dark-yellow, with fine delicious aroma. *Asteraceae* pollen (*Achillea* type) predominated (65.2%), but *Cirsium/Carduus* type was absent. Diastase activity was high: 28.2.

Conclusions

Each of the studied honey samples has a characteristic pollen composition, depending on the floristic composition of the region. Human activity (farming, agriculture and plant introduction) is an important factor to be considered in understanding the presence or absence of some taxa in the pollen spectra of honey. The dominant pollen types in the pollen spectra for different floristic regions are different. Unifloral *Tilia* honey of high quality was found in the surroundings of Turgovishte and *Robinia pseudoacacia* honey for Silistra (Northeast Bulgaria), unifloral *Ailanthus* honey was established for Bailovo (Sofia region), *Castanea sativa* honey for Velingrad/Petrich (the Valley of Strouma River and Western Rhodopes). As compare to the previous investigations of honey in Bulgaria (Bozilova & Chan 1976, Lazarova & Bozilova 2001, 2002), *Apiaceae* species manifests a greater importance as source of nectar for the bees in different regions of the country. Unifloral *Anethum graveolens* honey was established for Turgovishte (Northeast Bulgaria) and *Coriandrum sativum* honey for Yambol (Toundzha Hilly Country). *Apiaceae* pollen dominates in the honey from Pleven (Danubian Plain) and Selanovtsi (Sofia region). *Apiaceae* pollen is represented with relative high percentages in almost all pollen spectra.

More melissopalynological investigations are necessary to draw more precise conclusions about the unifloral honey specific for different floral regions in Bulgaria.

The chemical-physical analysis of the honey has also shown interesting results (Table 1).

Diastase activity ranges from 11 to 29 units after Goete, with a minimum of 9 for all types of honey according the BSS (1990), and 8 for *Robinia* honey. Higher diastase activity is established for unifloral

Tilia, *Coriandrum*, and *Castanea sativa* honey, as compared to the results of Ivanov (1973) (Table 1). The investigations of diastase activity in six of the samples within an interval of five months have shown decrease in the diastase number of one unit or more.

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