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DETERMINATION OF BOTANIC ORIGIN OF THE CROATIAN BLACK LOCUST HONEY (Istria region) USING MELISSOPALYNOLOGICAL ANALYSIS

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Abstract

In Croatia, the largest honey production is related to the production of black locust honey. For the purpose of proving botanical and geographical origin of honey melissopalynological analysis is used - it includes quantitative analysis of pollen in honey, i.e. the determination of the relative content of pollen in honey.

The aim of this paper is to present the results of melissopalynological analysis of forty samples of black locust honey collected by the Istria region beekeepers during two seasons. The analyses of honey samples confirmed the botanical origin of honey defined by the manufacturer.

All of the collected samples conform to the criteria of uniflorality according to the requirements of the Croatian regulations, which prescribe the minimum of 20% of the pollen grains of a plant *Robinia pseudacacia* [1]. Conducting pollen analysis on samples of black locust honey over two seasons has led to identifying pollen grains of 36 plant species. The most common ones are pollen grains of genus *Robinia pseudacacia* (family Fabaceae), rose family (Rosaceae), cabbage family (Brassicaceae) and bean family (Fabaceae).

***Key words**: Black locust honey, Botanic origin of honey, Melissopalynological analysis.*

1. Introduction

In accordance with the EU regulations, the directive on honey quality (*The European Union Council Directive Relating to Honey*), and the current legislation in the Republic of Croatia, the condition for identifying botanical origin of nectar honey is the content of dominant floral pollen (it is determined by pollen analysis), either the unifloral or multifloral pollen [1, 2 and 3]. The requirements for conducting pollen analysis, that is melissopalynological analysis, are good knowledge of honey pollen morphology, expertise and specialized analysts.

Melissopalynologists have developed a list of corrective values for particular pollen sorts, and they have called it the *pollen coefficient values* or PC for compensating pollen sorts in honey which contains the dominant pollen grains with trace grains (e.g. *Asclepias*, *Epilobium*, *Oxydendronarboreum*, *Salvia*, *Robinia pseudoacacia*, *Nyssa*, *Mysotis*, etc.) [4]. Not all the experts agree with this way of interpreting the results, since there are no uniquely disclosed PC values. This is because very different variables have been used which have influence on PC values calibration [5].

Black locust honey is one of the most valuable types of honey on the European market, and its characteristics are very much appraised by the consumers - it is liquid; it does not tend to crystallize; its colour is very light; it has a floral scent or that of a fresh fruit which is mildly expressed; and, the taste is also light [4]. In Croatia, the largest honey production is directly related to the black locust honey production [6].

In order to prove the botanical origin of honey of the Croatian black locust honey in the Istria region (marked in yellow) (Figure 1) that is to check whether the samples meet the requirements determined by the Croatian legislation, melissopalynological analysis was used in this paper [1].

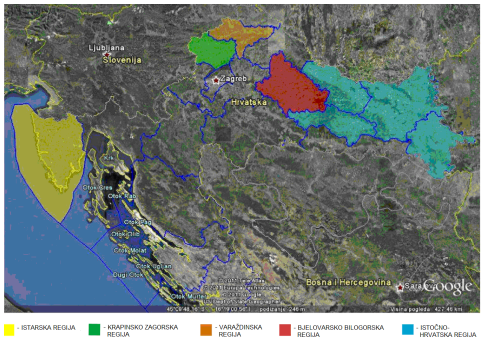

Figure 1. The map of geographical origin of honey

2. Materials and Methods

This research has included in total 40 honey samples extracted during two seasons, and collected by the beekeepers on different locations of honey pastures within the Istria region (Figure 2); they are marked from I-1 to I-20 (Table 1). One sort of unifloral honey was chosen: black locust honey.

Table 1. Black locust honey samples of the Istria region and the method of their marking in the further text

Istria region mark			
I-1	I-6	I-11	I-16
I-2	I-7	I-12	I-17
I-3	I-8	I-13	I-18
I-4	I-9	I-14	I-19
I-5	I-10	I-15	I-20

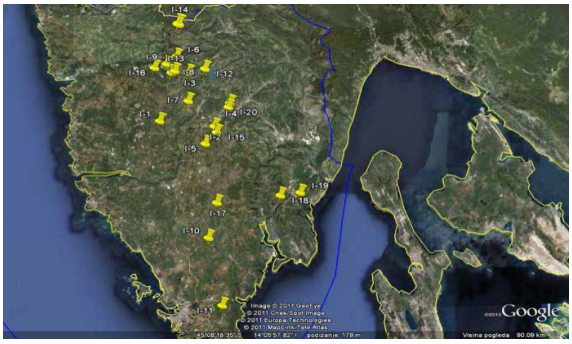

Figure 2. The map of honey pastures locations in the Istria region

For the purpose of proving botanical origin of honey, melissopalynological analysis was used in this research. It was conducted in a way that the pollen grains were identified and quantified using the microscope (400x magnification) on the honey sample preparation. At least 500 pollen grains were counted cumulatively, in steps of 100 in a row, in total 5 horizontal rows. The interspace between each horizontal row is one field of sight. The entire preparation was examined through these 5 horizontal rows. The content of specific pollen sort is calculated as percentage of the total number of pollen. The collection of reference pollen preparations was used for pollen grains determination from the samples by the principle of comparison (DIN 10760:2002-05 [7]).

3. Results and Discussion

The results represent an overview of pollen analyses with percentage content of pollen grains of each and every plant species of black locust honey in the Istria region for both seasons (Tables 2 and 3).

Confirmation of botanical origin of honey, defined by the manufacturer, was conducted by melissopalynological analysis that is pollen analysis. It conforms to the values defined by the Regulations on the Unifloral Honey Quality with the minimum of 20% content of *Robinia pseudacacia* pollen grains (black locust family) [1].

Considering the complexity of pollen analysis as a method that demands time and exceptional analysts' expertise, and the fact that, until today, this method has been defined through the law, and as essential technique in detecting botanical origin of honey, the application of other more simple and faster techniques is being tested, such as spectroscopic techniques - infrared and fluorescence spectroscopy [8]. Fluorescence spectroscopy was applied by Golob *et al.* [9] in defining botanical classification achieved through pollen spectrum which contained > 40% (> 80% for chestnut honey) of adequate dominant pollen in different sorts of honey from four Slovenian regions: Alpine, Dinaric, Mediterranean and coastal.

Table 2. Pollen analysis of the Istria region black locust honey– first season

	SAMPLE																			
POLLEN SORT (%)	I-1	I-2	I-3	I-4	I-5	I-6	I-7	I-8	I-9	I-10	I-11	I-12	I-13	I-14	I-15	I-16	I-17	I-18	I-19	I-20
Robiniasp	68	51	42	38	63	47	55	64	71	47	61	71	62	60	48	61	50	49	62	64
Rosaceae	5	8	12	22	4	21	7	24	7	16	16	10	9	15	22	8	15	13	10	11
Brassicaceae																				
Fabaceae	6	8	4	6	2	5	5	2	2	6	11	2	*p*	3	6	9	5	5	1	3
Asteraceae	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*p*		*p*	*p*	*p*	*p*	*p*	*p*	*p*	*p*	
Poaceae	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*p*		*p*	*p*		*p*	*p*	*p*	*p*	*p*	
Amorphafrutcosae	3	14	17	16		10	10	4	8	2	3	2	14	*p*	2	4	5	17	8	2
Castaneasativia	*p*		*p*	3			*p*	*p*				*p*				*p*				7
Rhamnaceae	*p*		*p*	*p*	*p*	*p*			*p*		*p*	*p*	*p*	*p*	*p*	*p*		*p*		
Apiaceae	*p*	*p*	*p*	*p*	*p*		*p*	*p*	*p*						*p*	*p*	*p*			
Plantago	*p*															p	p	p		
Quercussp	2		*p*	*p*	*p*		*p*		*p*	*p*	4	2	*p*	1	*p*	*p*	*p*	2	1	2
Salixsp	*p*									*p*	*p*		*p*	*p*	*p*		*p*		*p*	*p*
Fraxinussp		*p*				*p*				4		2	*p*	1	*p*	3	1	*p*	*p*	
Polygonaceae																				
Sambucussp																				
Lamiaceae					*p*	*p*	*p*		*p*						11		*p*	*p*	2	
Comussp										8		5	7	4	1	3	5		1	6
Tiliasp																				
Loranthussp	*p*	*p*	*p*	*p*	*p*		*p*	*p*	*p*											p
Cistaceae	*p*	2	4	2	5	1	2	*p*	3											
Taxus	*p*		*p*		*p*		*p*			*p*										
Pinussp	*p*	*p*			*p*	*p*														
Oleaceae	9	13	14	5	21	9	15	2	5	11		3	2	9	4	6	11	10	10	
Rumixsp			*p*	*p*	*p*	*p*				*p*						*p*	*p*		*p*	
Impatiens																				
Boraginaceae																				
Vitaceae																				
Moraceae																				
Ericaceae											*p*	*p*	*p*							
Fagussp.																				
Aarsp										*p*				*p*						*p*
Almus																				
Clusiaceae											1									
Caryophyllaceae																				
Convolvulaceae																				
INDET	3	1	3	4	1	4	2	1	2	3	2	1	3	4	2	2	3	2	2	3
Others	4	3	4	4	4	3	4	3	2	3	2	2	3	3	4	4	5	2	3	2

p - below 1%

Table 3. Pollen analysis of the Istria region black locust honey – second season

	SAMPLE																			
POLLEN SORT (%)	I-1	I-2	I-3	I-4	I-5	I-6	I-7	I-8	I-9	I-10	I-11	I-12	I-13	I-14	I-15	I-16	I-17	I-18	I-19	I-20
Robiniasp	65	33	44	41	61	63	59	62	61	44	62	66	43	53	54	53	49	43	57	61
Rosaceae	12	20	17	24	6	8	7	22	12	18	5	8	8		18	14	16	2	14	13
Brassicaceae		1				4			p		*p*	4					p			
Fabaceae	5	18		3	*p*	8	6	4	5	8	11		16	18	8	6	9	19	*p*	*p*
Asteraceae	*p*	*p*	*p*	1	2	1	*p*	1	1	1		4	*p*	2	*p*	3	7		2	
Poaceae	*p*		*p*	*p*	*p*		*p*	1	*p*	3	p	*p*		1	*p*		*p*		2	
Amorphafrutcosae	2		19	11			4	4	8	*p*		*p*			4	*p*			*p*	*p*
Castaneasativia	*p*	3	1	6		1	1	*p*			8	*p*		9		1	1	18		14
Rhamnaceae	*p*		1	*p*								2			*p*					
Apiaceae	*p*		*p*	*p*	*p*	1	1	*p*	*p*						*p*	1	*p*			
Plantago	*p*	*p*									1					*p*		*p*		
Quercussp	2	*p*	*p*	*p*	*p*		*p*		*p*	1		*p*				3	*p*	1	*p*	2
Salixsp	*p*	1				2				*p*	*p*		1	3	*p*		*p*	1	*p*	*p*
Fraxinussp						*1*				*p*		8			*p*	*p*			*p*	
Polygonaceae									*p*											
Sambucussp																		*p*		
Lamiaceae		1			*p*		*p*		*p*		4			*p*	1		*p*	*1*	2	
Comussp						6			5	10	*p*	3	13	2	3	1	*p*	*p*	3	2
Tiliasp																				
Loranthussp	*p*		*p*	*p*	*p*	*p*	*p*	*p*	*2*		*p*									*p*
Cistaceae	*p*	2	*p*	*p*	4		*p*	1									4	2		
Taxus	*p*		*p*		*p*		*p*			1							*p*			
Pinussp	*p*	*p*			*p*												*p*			
Oleaceae	9	13	13	7	21		15	*p*	*p*	8	4	*p*	11	2	5	11	5	8	15	3
Rumixsp			*p*	*p*						*p*						*p*			*p*	
Impatiens																				
Boraginaceae		1												2			*p*	1		
Vitaceae																				
Moraceae																				
Ericaceae											*p*	*p*	*p*	*p*		*p*	3			
Fagussp.						1			*p*				2							
Acersp						1				1							*p*	*p*		*p*
Rubiaceae														4						
Cucurbitaceae																				
Convolvulaceae																				
Ligustrum		*p*																		
INDET	3	3	2	3	2	1	3	2	1	2	2	2	2	2	3	3	2	2	2	3
Others	2	4	3	4	4	2	4	3	4	3	3	3	4	2	4	4	4	2	3	2

p - below 1%

4. Conclusions

- In conducting pollen analysis of the black locust honey samples from both seasons in the Istria region, pollen grains of 36 plant species were detected. The most common are the ones from *Robinia pseudacacia* family (black locust family), *Rosaceae* (rose family), *Brassicaceae* (cabbage family) and *Fabaceae* (bean family) (Tables 2 and 3).
- The resulting values of pollen grains of *Robinia pseudacacia*-black locust meet the criteria of the minimum 20% content, and therefore this is the confirmation of botanical origin of honey. It also means that the honey conforms to the sort defined by the manufacturer.
- Pollen grains values of *Robinia pseudacacia*-black locust range from 38% to 71% in the first season and from 33% to 66% in the second season.

5. References

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