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CONFIRMING THE BOTANICAL ORIGIN OF THE CROATIAN BLACK LOCUST HONEY (ISTRIA REGION) USING PHYSICOCHEMICAL PARAMETERS DURING TWO SEASONS

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Abstract

Honey is a sweet, thick, viscous, liquid or crystallized product produced by honeybees (*Apis mellifera*) from the nectar of honey plants or secretions of living parts of plants or excretions of insects. Its chemical composition makes it a complex mixture of over 70 ingredients. The most common among them are carbohydrates (fructose and glucose) and water (they make 99% of honey). Other substances (which make only < 1%) are proteins (including enzymes), minerals, vitamins, organic acids, phenolic compounds, aroma compounds (volatile compounds) and various chlorophyll derivatives, which are also responsible for sensory and nutritional properties of honey.

In Croatia, the largest production pertains to the production of black locust honey. The aim of this paper is to present the share of physicochemical parameters (water, free acids, electrical conductivity, reducing sugars, sucrose, diastasis, and hidroksimetilfurfurala HMF) as an indicator of whether the samples meet the general requirements of the Honey Regulations to confirm the botanical origin of black locust honey (as defined by the manufacturer).

The research was conducted through two seasons. Forty samples of honey collected by beekeepers in the region of Istria were included.

The methods that were used to determine the physicochemical parameters are various. The determination of water was based on refractometry. Titration using sodium hydroxide was used to determine the free acid, while the electrical conductivity is defined as the conductivity of 20% aqueous solution of honey at 20 ^{0}C. Reducing sugars and sucrose were identified by titration using Fehling's solution, and diastasis was proven using the hydrolysis method. HMF was defined using the Winkler method.

All the samples tested met the requirement on the quality of black locust honey (water content, free acids, electrical conductivity, reducing sugars, sucrose, diastase, and HMF) thus confirming the botanical origin of honey, i.e. all the samples are black locust honey.

***Key words**: Black locust honey, Physicochemical analysis, Botanical origin.*

1. Introduction

Variability and non-existence of the same two samples of honey are the characteristics that describe the chemical composition of honey in the best possible way (Uršulin-Trstenjak [12]). According to its chemical composition, honey is a complex mixture of over 70 ingredients, which reach honey in a variety of ways: added by bees; originating from plants; or occur by ripening of honey in the comb (Krell [5]). The most common ingredients that make up 99% of honey are carbohydrates (mostly fructose and glucose) and water. Other substances (which make only < 1%) are proteins (including enzymes), minerals, vitamins, organic acids, phenolic compounds, aroma compounds (volatile compounds) and various chlorophyll derivatives, which are also responsible for sensory and nutritional properties of honey (Vahčić & Matković [14]).

In Croatia, the largest production pertains to the production of black locust honey (Uršulin-Trstenjak [13]). The aim of this paper is to present the share of physicochemical parameters (water, free acids, electrical conductivity, reducing sugars, sucrose, diastasis, and hydroxymethylfurfural HMF) as an indicator of whether the samples meet the general requirements of the

Honey Regulations to confirm the botanical origin of black locust honey (as defined by the manufacturer) (MPRRR [7] and [8]).

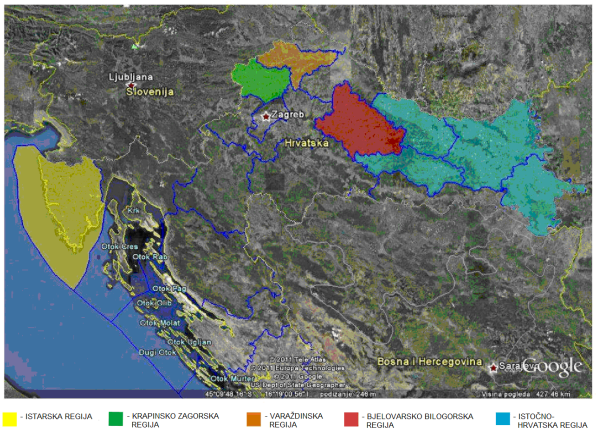

Figure 1. The map of geographical origin of honey

The research was conducted through two seasons. Forty samples of honey collected by beekeepers in the region of Istria were included (marked in yellow) (Figure 1).

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HMF was defined using the Winkler method.

All the samples tested met the requirement on the quality of black locust honey (water share, free acids, electrical conductivity, reducing sugars, sucrose, diastase, and HMF) thus confirming the botanical origin of honey, i.e. all the samples are black locust honey (MPRRR, [7]).

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.

In order to determine the physicochemical parameters – water, free acids, electrical conductivity, reducing sugars, sucrose, diastasis, and HMF – specific methods have been used:

- The share of water in honey is determined using the method which is based on refractometry and is prescribed by the International Honey Commission (IHC [4]).

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

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

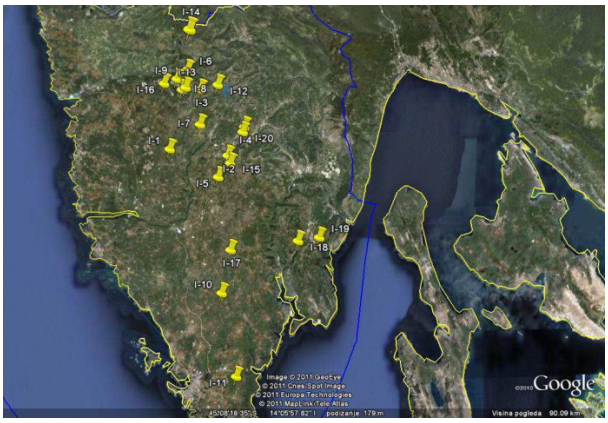

Figure 2. The map of honey pastures locations in Istria region

- Determination of free acids is carried out so that the prepared sample is titrated using the solution of 0.1M of sodium hydroxide to a pH of 8.30, according to the method prescribed by the International Honey Commission (IHC [4]).
- Electrical conductivity of honey is defined as the conductivity of a 20% aqueous solution of honey at 20 ^{0}C, where 20% refers to the dry weight of honey. The results are expressed in mS/cm (IHC [4]).
- Reducing sugars and sucrose in honey were determined using the method based on the reduction of Fehling's solution by titration with a solution of sugar from the honey, with the use of the methylene blue dye as an indicator. The share of sucrose is obtained from the difference between the results obtained before and after the inversion (IHC [4]).
- The method for the determination of diastase activity in honey is based on the hydrolysis of 1% solution of starch by the enzyme from 1g of honey during one hour at a temperature of 40 ^{0}C (IHC [4]; SLMB [10]; Bogdanov *et al*. [1]; DIN 10750:1990 [2]).
- Hydroxymethylfurfural (HMF) in honey was defined using the Winkler method and is based on the reaction of the hydroxymethylfurfural with barbituric acid and p-toluidine, which gives it a pink color, the intensity of which is measured at a wavelength of 550 nm (IHC, [4]; SLMB, [10]).

Statistical data analysis

The mean value of the physicochemical parameters in samples of black locust honey has been calculated.

3. Results and Discussion

The results of the analysis of the physicochemical parameters of black locust honey are presented for each of the samples respectively for both the first and the second season and their mean values have been calculated (Tables 2 and 3).

Table 2. Physicochemical parameters of black locust honey in the Istria region – first season

	ANALYSIS	water (%)	free acids (mEq/ 1000g)	electrical conductivity (mS/cm)	reducing sugars (g/100g)	sucrose (g/100g)	diastase (DN)	HMF (mg/kg)
SAMPLES	I-1	14.48	9.4	0.11	70.86	0.00	9.67	4.60
	I-2	16.64	9.3	0.16	70.86	0.00	11.07	5.90
	I-3	15.53	8.1	0.13	70.86	0.00	13.51	3.90
	I-4	17.28	9.2	0.14	70.86	0.19	13.25	4.50
	I-5	17.36	9.9	0.14	70.86	0.00	13.67	3.20
	I-6	15.40	8.1	0.13	70.86	0.00	14.06	0.90
	I-7	16.00	9.5	0.16	71.37	0.38	11.77	2.80
	I-8	14.84	8.4	0.11	70.86	0.00	12.32	3.30
	I-9	15.92	8.0	0.14	71.47	0.34	12.27	7.10
	I-10	16.40	9.2	0.15	70.86	0.00	12.80	2.20
	I-11	16.04	9.2	0.16	70.66	0.19	12.45	5.40
	I-12	18.20	8.4	0.12	70.06	0.26	12.56	2.90
	I-13	15.68	9.1	0.14	71.26	0.29	11.58	5.60
	I-14	15.84	10.3	0.20	70.86	0.19	15.55	4.20
	I-15	15.40	8.2	0.13	70.86	0.00	9.77	5.60
	I-16	16.24	8.3	0.13	70.86	0.38	10.79	5.40
	I-17	16.04	11.9	0.19	70.86	0.00	14.97	4.50
	I-18	14.84	8.2	0.14	69.86	0.36	12.87	1.00
	I-19	16.20	9.5	0.17	70.06	0.37	12.40	6.00
	I-20	15.53	9.1	0.13	68.51	0.29	9.89	4.80
	$\bar{x}$	15.99	9.06	0.14	70.68	0.16	12.36	4.19

$\bar{x}$ – mean value

Table 3. Physicochemical parameters of black locust honey in the Istria region – second season

	ANALYSIS	water (%)	free acids (mEq/ 1000g)	electrical conductivity (mS/cm)	reducing sugars (g/100g)	sucrose (g/100g)	diastase (DN)	HMF (mg/kg)
SAMPLES	I -1	18.04	10.1	0.14	70.57	0.00	19.17	2.28
	I -2	18.00	13.0	0.27	71.26	2.90	15.25	1.17
	I -3	16.60	14.2	0.19	70.57	0.00	21.00	1.72
	I -4	18.68	13.1	0.21	65.78	2.50	15.62	1.02
	I -5	20.28	13.1	0.19	70.57	0.00	14.92	1.63
	I -6	18.32	13.9	0.27	70.57	1.09	17.73	1.69
	I -7	17.32	12.1	0.16	70.57	1.06	15.91	1.22
	I -8	18.44	14.2	0.17	71.26	1.09	17.67	1.81
	I -9	17.24	15.2	0.30	70.66	0.00	19.86	1.41
	I -10	19.08	13.0	0.25	70.57	1.20	15.61	1.12
	I -11	18.16	15.0	0.25	70.57	0.00	13.97	3.31
	I -12	19.84	12.1	0.19	70.57	1.20	14.46	1.69
	I -13	17.36	13.0	0.22	71.26	1.60	16.44	1.34
	I -14	17.08	11.9	0.26	70.57	0.00	16.15	1.08
	I -15	17.08	11.3	0.19	71.26	1.09	10.72	18.99
	I -16	17.24	13.0	0.21	70.57	0.87	10.54	1.34
	I -17	15.92	15.1	0.22	71.47	0.93	10.84	10.02
	I -18	17.32	12.0	0.27	70.57	0.00	11.22	1.68
	I -19	17.92	11.1	0.20	71.26	1.29	11.78	1.54
	I-20	20.64	13.1	0.21	70.57	1.70	13.84	1.55
	$\overline{x}$	18.03	12.97	0.22	70.55	0.93	15.14	2.88

$\overline{x}$ – mean value

Permitted water share in black locust honey is a maximum of 20% (MPRRR [7]). This is visible from the mean obtained values from the analyzed samples, ranging from 15.99 to 18.03%, and thus follows the results obtained by other researchers on the samples of black locust honey from Croatia (from 15.40 to 16.30%) and Romania (17.90%) (Šarić *et al*. [11]; Marghitas *et al*. [6]).

Permitted value of free acid in honey is < 50 mEq of acid per 1000g of honey (MPRRR [7]), which accordingly shows the average values of the study from 9.06 to 12.97 mEq/1000g - they are lower or equal to the results obtained by Golob and Plestenjak [3] (24 mEq/1000g).

Mean values of the electric conductivity in the samples used in this research range from 0.14 to 0.22 mS/cm, and thus satisfy the general requirements of the maximum of 0.8 mS/cm (MPRRR [7]).

The sum of glucose and fructose shares, i.e. of reducing sugars in honey, should be at least 60g/100g (MPRRR [7]). At the level of the European research on 454 samples of black locust honey the mean value obtained was 69.2 g/100g (Persano Oddo & Piro, [9]), the relations of which are the values obtained in this study, from 70.55 to 70.68 g/100g.

All the samples tested met the requirement of sucrose share (up to 10 g/100g of the sample); both for the first and the second season (MPRRR [7]). The sucrose share ranges from 0.16 to 0.93 g/100g and is equal to the obtained range in samples of black locust honey tested by other researchers from Romania (Marghitas *et al*. [6]).

General requirements for the value of the diastase activity equal 8DN, except for species with low enzyme activity (citrus, black locust), where DN can be < 8, but the share of HMF then needs to be ≤ 15 mg/kg (MPRRR [7]). The resulting averages are from 12.36 to 15.14 DN and follow the European results (10.5 DN) (Persano Oddo & Piro [9]).

The share of HMFis low – a very large percentage of honey samples had less than 10 mg/kg (calculated as the upper limit for a first-class honey), and are in the range of 2.88 to 4.19 mg/kg.

4. Conclusions

- All the tested samples (Tables 2 and 3) satisfy the prescribed criteria listed in the Honey Regulations regarding the physicochemical parameters (MPRRR [7]).

- the share of water ranges on average from 15.99 to 18.03%,
- the mean values of free acid in this research range from 9.06 to 12.97 mEq/1000g,
- the mean values of electrical conductivity in the samples used in this research range from 0.14 to 0.22 mS/cm,
- the sum of glucose and fructose shares, i.e. of reducing sugars and the values obtained in this research range from 70.55 to 70.68 g/100g,
- the sucrose share ranges from 0.16 to 0.93 g/100g,
- the obtained averages of the diastase activity range from 12.36 to 15.14 DN,
- the HMF share ranges from 2.88 to 4.19 mg/kg.

- As regards the botanical origin of honey, it can be concluded that all the tested samples of honey are of black locust honey, as defined by the manufacturer.

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