COMPARISON OF THE CROATIAN AND MACEDONIAN HONEYDEW HONEY
USPOREDBA HRVATSKOG I MAKEDONSKOG MEDLJIKOVCA

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ABSTRACT

The aim of this research was to compare physicochemical characteristics of honeydew honeys originating from Croatia and Macedonia. 11 samples of Macedonian and 17 samples of Croatian honeydew honey were collected during the harvest season 2005 and 2006, respectively. All collected samples were subjected to the melissopalynological analysis, and afterwards physicochemical characteristics were determined.

In comparison with Croatian samples, the samples of Macedonian honeydew honey show statistically significant higher electrical conductivity, prolin content, free and total acidity, glucose and sucrose, as well as fructose and glucose content (F + G), and lower specific rotation, almost negative values. Croatian samples have higher content of maltose (with celobiose and trehalose) and raffinose, until melezitose (with erlose) content in both sample groups is low.

KEY WORDS: honeydew honey, physicochemical characteristics, melissopalynological analysis

SAŽETAK

Zadatak ovog istraživanja bio je usporedba fizikalno-kemijskih parametara medljikovca podrijetlom iz Hrvatske i Makedonije. Prikupljeno je 11 uzoraka proizvedenih u Makedoniji 2005. i 17 uzoraka proizvedenih u Hrvatskoj 2006. godine. Po provedenoj melisopalinološkoj analizi, određeni su fizikalno-kemijski parametri uzoraka.

U usporedbi s hrvatskim uzorcima, makedonski uzorci pokazuju statistički značajno višu električnu provodnost, udio prolina, slobodnu i ukupnu kiselost, udio glukoze i saharoze, udio fruktoze i glukoze (F + G), te nižu specifičnu rotaciju, uglavnom negativnu. Hrvatske uzorke karakterizira viši udio maltoze (sa celobizom i trehalozom) i rafinoze, dok je udio melecitoze (sa erlozom) u obje skupine uzoraka nizak.

KLJUČNE RIJEČI: medljikovac, fizikalno-kemijske karakteristike, melisopalinološka analiza

DETALJNI SAŽETAK

Medljikovac je vrsta meda koji pčele proizvode od medljike ili medne rose, slatkog soka koji luče biljke ili insekti koji žive na biljkama, bjelogoričnom i crnogoričnom drveću [9,10, 22]. I dok je medljikovac kod nas slabije poznat i manje zastupljen u proizvodnji, u mnogim je zemljama posebno cijenjen (Njemačka, Švicarska), i karakteriziran (Švicarska, Grčka, Italija) [4,7,22,26]. Zahvaljujući velikim područjima hrastovih šuma u Slavoniji, te jelovih i smrekovih šuma u Gorskom Kotaru i Lici, Hrvatska ima velike mogućnosti za proizvodnju medljikovca [2,25]. U Makedoniji, s kojom smo bili partneri u projektu, posebno u njenom zapadnom dijelu, rasprostranjene su šume sa specifičnom dendroflorom (Pinus peuce, Quercus macedonicus)), koje su glavni izvor meda.

Zadatak ovog rada bio je odrediti fizikalno-kemijske parametre kvalitete, te usporediti svojstva hrvatskog i makedonskog medljikovca. Analizirano je 17 uzoraka deklariranih kao medljikovac iz Hrvatske, te 11 iz Makedonije, koji su dobiveni direktno od pčelara. Po provedenoj melisopalinološkoj analizi [19] prema kojoj su na bazi dominacije elemenata medljike uzorci klasificirani kao medljikovci, određeni su fizikalno-kemijski parametri: udio vode, električna provodnost, aktivnost dijastaze prema Shade-u i invertaze po Siegentheler-u, udio hidroksimetilfurfurala (HMF-a) po White-u, udio prolina, pH i kiselost titrimetrijski, specifična rotacija, te sastav šećera visokodjelotvornom tekućinskom kromatografijom [1,3]. Postojanje razlika između uzoraka testirano je Mann-Whitney U testom. Boja hrvatskih (H) uzoraka kretala se od jantarne, preko jantarne sa crvenom ili zelenom nijansom, pa do crvenkasto-smeđe, dok je boja makedonskih (M) uzoraka varirala od svijetlo jantarno-smeđe, preko smeđe do tamno smeđe sa crvenkastim odsjajem. U odnosu na hrvatske, analizirani makedonski uzorci imali su statistički značajno višu provodnost (M : H = 1.14 : 1.00 mS/cm), udio prolina (M : H = 754.7 : 410.4 mg/kg) i slobodnu kiselost (M : H = 44.2 : 27.7 mmol/kg), a nižu specifičnu rotaciju, pretežno negativnu (M : H = -3.6 : 6.6 $[\alpha]^{20}_{D}$). U pogledu sastava šećera, hrvatski medljikovci su imali značajno viši udio maltoze (H : M = 9.7 : 2.3 %) i rafinoze (H : M = 0.2 : 0.1 %), niži udio glukoze (H : M = 31.0 : 36.8 %) i saharoze (H : M = 1.4 : 2.1%), te zajedno fruktoze i glukoze (H : M = 63.4 : 70.4 %). Udio melecitoze u obje je skupine uzoraka bio nizak. Sastav šećera direktno se odražava na specifičnu rotaciju, te su tako hrvatski uzorci s izuzetkom dva uzorka bili desnoskretni, dok su makedonski uzorci uz dva izuzetka bili lijevoskretni. Negativna specifična rotacija medljikovaca rijetko se sreće u literaturi, te istraživanja na karakterizaciji makedonskog, ali i hrvatskog medljikovca svakako treba nastaviti.

INTRODUCTION

Honeydew honey is made by honey bees from secretions of living parts of plant or excretions of plant-sucking insects (Hemiptera) on the living part of plants [9,10] from the latifoliae trees as oak (Quercus spp.) and lime (Tilia spp.) or coniferous trees as fir (Abies alba), spruce (Picea abies) and etc [2,22,28]. With the exception of Metcalfa honeydew honey produced by Metcalfa pruinosa, the main physicochemical parameters of other honeydew honey types are considered quite uniform [22]. Common characteristics are high electrical conductivity and pH, positive values of specific rotation, low values of fructose, glucose, F+G and higher shares of oligosaccharides [22,24], while organoleptic characteristics are more variable. Croatia has wide areas of oak (Slavonia), fir and spruce (Gorski kotar, Lika) forests and accordingly high possibility of honeydew production [2,28]. Many studies on physicochemical properties of honeydew honey are reported, but studies on Croatian honeydew honey are still sporadic. Recently, Lušić et al. have published volatile profiles of fir (Abies alba) honeydew [16]. Research and published data on Macedonian honey, where our project partners are from, are even less, though their production of honeydew honey is relatively high. Namely, in Macedonia, especially in the west part of country (around mountains Baba, Nidze, Kozuf, Galicica, Bistra, Strogovo, Karaorman, Lopusnic, Ilinska, Bigla and other), forest with specific dendroflora (for example endemic five-needle pine Molika (Pinus peuce) and Macedonian oak (Quercus macedonicus)), are the main source of honey.

Therefore, the aim of this study was to determine physicochemical characteristics of honeydew honey from Croatia and FR Macedonia, and to compare them.

MATERIALS AND METHODS

Samples

11 honeydew honey samples from different parts of Macedonia and 17 samples from different parts of Republic Croatia were provided by beekeepers during the harvest season 2005 and 2006, respectively.

Melissopalynological analysis

Though beekeepers themselves declared honey as honeydew honey, samples were subjected to qualitative melissopalynological analysis. Analysis was performed by counting of 200-300 honeydew elements or pollen grains, according the method of Lauveaux et al [15] and

Croatian regulations [19].

Physicochemical analysis

Moisture, electrical conductivity, HMF (after White), specific rotation, diastase activity (after Schade), invertase activity and sugar content (HPLC) were analysed according to the Harmonised methods of the European Honey Commission (2002) [3], while proline, pH, total, free and lactone acidity were determined according to AOAC Official Methods (2002) [1].

Data analysis

Average values, medians, minimal and maximal values were calculated. Significance of differences of investigated parameters between samples of Croatian and Macedonian honeydew honey were examined by Mann – Whitney U test, and an association between parameters was evaluated using the Pearson correlation coefficient. Statistica 7.0 software was used.

RESULTS AND DISCUSSION

According to the results of qualitative melissopalynological analysis, all honey samples have been classified as honeydew honey (based on the domination of honeydew elements such as mold hyphae and spores, unicellular algae). Afterwards, samples were subjected to the analysis of physicochemical parameters and sugars content. Only samples which complied with international regulation for electrical conductivity (≥ 0.8 mS/cm) [9,10] and HMF content (≤ 10 mg/kg), as to ensure the honey freshness, were considered for honey characterization (16 samples from Croatia and 10 samples from Macedonia).

Colour, as significant organoleptic characteristic, is useful criterion for the classification of unifloral honeys [14]. Visually, colour of Croatian samples varies from amber, through amber with reddish and greenish shades, to reddish-brown. Colour of Macedonian samples varies from bright amber-brown, to dark brown with reddish shades.

The results of the physicochemical parameters (Table 1) show that although there are statistically significant differences between some of evaluated parameters, all samples have low water content, while electrical conductivity, pH value and acidity are inherent for honeydew honey [8, 22]. Free acidity of Macedonian samples is however very high, 44.2 mmol/kg compared to 27.7 mmol/kg in Croatian samples.

Enzymatic activity, as honey freshness parameter, is generally higher in honeydew honey then blossom honey, which is in consistence with the fact that enzymes present in honeydew honey originate from bees as well as row honeydew [2,8]. With exception of a few samples, Croatian and Macedonian samples have high enzymatic

Table 1. Physicochemical characteristics of honeydew honeys
Tablica 1. Fizikalno-kemijske karakteristike medljikovca

Parameter Parametar	Unity Jedinica	Croatian honeydew honey Hrvatski medljikovac				Macedonian honeydew honey Makedonski medljikovac			
		N = 16				N = 10			
		Median	$\overline{x}$	SD	min-max	Median	$\overline{x}$	SD	min-max
Water Voda	%	15.9	16.1	0.9	14.9 - 17.5	16.00	16.1	0.6	15.2 - 17.3
Electrical Conductivity * Električna provodnost *	mS/cm	0.98	1.00	0.10	0.81 - 1.16	1.13	1.14	0.14	0.80 - 1.30
Diastase Dijastaza	DN	25.4	25.9	7.1	16.6 - 40.2	29.0	28.5	3.0	23.7 - 32.9
Invertase Invertaza	U/kg	208.7	208.6	57.0	108.7 - 321.1	280.5	246.9	132.6	31.3 - 376.7
HMF HMF	mg/kg	0.2	0.8	1.3	0.0 - 3.9	0.1	0.2	0.3	0.0 - 0.8
Proline ** Prolin **	mg/kg	411.1	410.4	127.5	261.7 - 749.7	779.3	754.7	123.5	512.9 - 877.5
pH pH		4.8	4.8	0.3	4.1 - 5.4	4.8	4.7	0.2	4.3 - 4.9
Free Acidity ** Slobodna kiselost **	mmol/kg	28.2	27.7	9.8	14.2 - 45.7	42.6	44.2	5.4	38.1 - 52.5
Lactonic Acidity Laktonska kiselost	mmol/kg	3.9	3.8	2.0	0.3 - 8.3	4.5	5.3	1.9	3.7 - 9.7
Total Acidity ** Ukupna kiselost **	mmol/kg	32.3	31.5	11.4	17.1 - 50.6	48.5	49.5	6.3	42.4 - 59.4
Specific Rotation ** Specifična rotacija **	$[\alpha]_D^{20}$	5.7	6.6	7.4	(-7.7) - 18.2	-5.0	(-3.6)	4.7	(-7.8) - 7.5

Statistically significant difference between samples of Croatian and Macedonian honeydew honey *(p ≤ 0.01); **(p ≤ 0.001)/
Statistički značajna razlika između uzoraka hrvatskih i makedonskih medljikovaca *(p ≤ 0.01); **(p ≤ 0.001)

Table 2. Sugar profiles of honeydew honeys
Tablica 2. Sastav šećera u medljikovcu

Type of sugar Vrsta šećera	Unity Jedinica	Croatian honeydew honey Hrvatski medljikovac				Macedonian honeydew honey Makedonski medljikovac			
		N = 16				N = 10			
		Median	$\bar{x}$	SD	min-max	Median	$\bar{x}$	SD	min-max
Xyilose **Ksiloza**	%	0.0	0.1	0.2	0.0 - 0.4	0.0	0.0	0.0	0.0 - 0.1
Fructose **Fruktoza**	%	32.7	32.4	2.7	27.7 - 37.0	33.6	33.6	1.4	31.5 - 35.7
Glucose ** **Glukoza ****	%	30.7	31.0	3.1	25.7 - 35.2	37.4	36.8	4.0	29.5 - 40.7
Sucrose * **Saharoza ***	%	1.4	1.4	0.7	0.4 - 2.8	2.0	2.1	0.3	1.8 - 2.7
Maltose [a]*** **Maltoza [a]*****	%	10.6	9.7	5.1	1.0 - 17.8	1.7	2.3	1.3	1.3 - 5.1
Melezitose [b] **Melecitoza [b]**	%	0.0	0.2	0.3	0.0 - 0.9	0.0	0.1	0.1	0.0 - 0.4
Raffinose * **Rafinoza ***	%	0.0	0.2	0.5	0.0 - 2.1	0.0	0.1	0.2	0.0 - 0.7
Fructose/Glucose *** **Fruktoza/Glukoza *****		1.1	1.1	0.1	1.0 - 1.2	0.9	0.9	0.1	0.8 - 1.1
Fructose + Glucose ** **Fruktosa/Glukoza ****	%	63.6	63.4	5.5	53.4 - 72.2	71.5	70.4	4.8	62.2 - 76.4
Total sugars **Ukupni šećer**	%	75.0	75.0	2.3	71.4 - 80.7	75.5	75.0	3.5	68.2 - 79.5
Glucose/Water **Glukoza/Voda**		2.0	1.9	0.2	1.6 - 2.3	2.3	2.3	0.3	1.8 - 2.6

Statistically significant difference between samples of Croatian and Macedonian honeydew honey
*($p \leq 0.05$); **($p \leq 0.01$); *** ($p \leq 0.001$) /
Statistički značajna razlika između uzoraka hrvatskih i makedonskih medljikovaca
*($p \leq 0.05$); **($p \leq 0.01$); *** ($p \leq 0.001$)
[a] Includes the contribution of celobiose and trehalose / [a] uključen sadržaj celobioze i trehaloze
[b] Includes the contribution of erlose / [b] uključen sadržaj erloze

activity (diastase and invertase). Mean diastase activity is 25.9 for Croatian and 28.5 for Macedonian honeydew honey samples. Lower values of diastase activity were reported by Golob and Plastenjak [13] and Terrab et al. [27] while higher diastase activity characterises Spanish honeydew honey [20]. Furthermore, regarding to literature data for Italian (175.5 U/kg) [21] and another European honeydew honey (139.0 U/kg) [22], Croatian (208.6 U/kg) and Macedonian (246.9 U/kg) samples also have high mean invertase activity.

Prolin is criterion of ripeness. Analyzed Macedonian samples have significantly higher prolin content (512.9 – 877.5 mg/kg) than Croatian samples (261.7 – 749.7 mg/kg), $p<0.001$. Similar results as Croatian honeydew honey samples were found in European samples (240 – 718 mg/kg) [22], and extremely high prolin content was reported by Terrab et al. [27] for Moroccan honeydew honey (142 - 301 mg/100 g).

Concerning sugar profile, significant differences (Table 2, U test) between Croatian and Macedonian samples are also notable. Croatian honeydew honeys have slightly higher fructose content than glucose (32.4 : 31.0 %), while Macedonian honeydew honeys show opposite results, 36.8% glucose and 33.6% fructose. According to Oddo et al. [22] and Ruoff [24], fructose content was higher than glucose content, and therefore fructose/glucose ratio is between 1.01 and 1.48 [22], namely between 1.07 - 1.53 for fir honeydew honey (Abies spp. i Picea spp.) and between 1.03 - 1.31 for oak honeydew honey (Quercus spp.) [24]. Range of glucose and fructose sum for Croatian and Macedonian are in agreement with those published earlier for honeydew honey, while some literature data show also wider ranges than in presented study (45.1 - 71.8 %) [6].

Regarding disaccharides, Croatian honeydew honey samples show high level of maltose with celobiose and trehalose (because of peaks overlap) (1.0 - 17.8 %), but values are much lower in Macedonian samples (1.3 - 5.1 %). Literature data for maltose are between 0 to 4.9 % for fir honeydew honey (Abies spp. i Picea spp.) [24] and 3.43 - 6.22 % for Spain honeydew honey [18], which under maltose peak contains nigerose and turanose. Content of maltose in floral honeys is usually 0 to 8.6%, with median of 1.43 % [24].

Content of melezitose and raffinose, oligosaccharides identified as characteristic for honeydew honey [8], is low. Croatian honeydew honey melezitose content with erlose is 0 - 0.9 %, and content of Macedonian samples is even lower (0 - 0.4 %, Table 2) and similar to the content of Moroccan honeydew honey (0.43 %) reported by Díez et al. [11]. Ruoff et al. [24] reported that in fir honeydew honeys (Abies spp. i Picea spp.) range of these oligosaccharides varies from 0 to 8.4 % (median 2.6), while Mateo et al. [18] reported lower values range (0.15 - 3.4 %). Clearly higher content of melezitose was found in Slovenian fir honeydew honey (5.15 - 21.46%) [13], while several times higher content was found in Greek larch honeydew honey (Larix decidua), 44.5% melezitose [17]. Croatian honeydew honeys also have low content of raffinose, in range from 0 to 2.1%, and in Macedonian barely 0 to 0.7%.

Content and sugar type are correlated to the specific rotation, which is useful for differentiation of blossom (laevorotatory, negative specific rotation) and honeydew honeys, which mostly have positive values (dextrorotary). Except that, specific rotation is useful additional parameter for botanical identification, but there are no clear bounds between different honey types [3,8]. With exception of two laevorotatory samples, analysed samples from Croatia are dextrorotary, while Macedonian samples, with exception of two dextrorotary samples, are laevorotatory (with relatively low negative values). Similar, rather low positive values (4.2 ± 1.3 $[\alpha]^{20}_{D}$) of specific rotation were earlier reported by Dinkov et al. [12] for Bulgarian honeydew honey, while Pridal et al. have reported negative values of specific rotation for honeydew honey [23].

As expected, high negative correlation (r = -0.909, $p < 0.05$) (Figure 1.) between fructose and glucose sum (F+G) and specific rotation is found. Namely, considering obtained F/G values, and the fact that fructose has stronger negative specific rotation than glucose the positive specific rotation, values resulting from only these two sugars are always negative. Maltose is highly positively correlated with specific rotation (r=0.901, $p < 0.05$) (Figure 2.), which is in accordance with the high specific rotation angle of maltose. Yet, in case of high glucose and fructose sum, lower amounts of carbohydrates that can bring toward positive rotation (like maltose) are present in samples, and the resulting values remain negative.

Though most of their analysed physicochemical

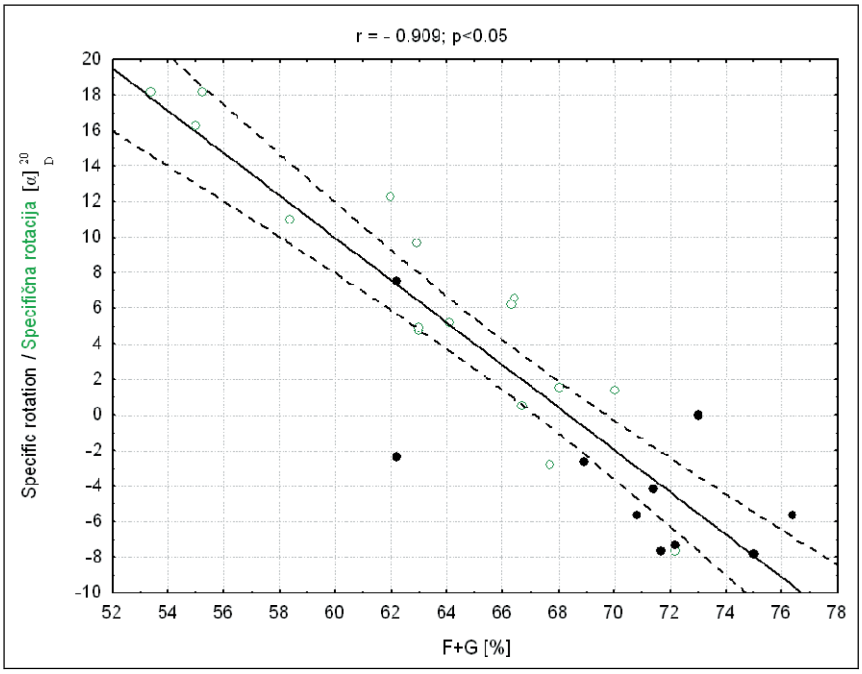

Figure 1. Correlation between fructose and glucose content (F+G) [%] and specific rotation $[\alpha]^{20}_{D}$ in samples of Croatian and Macedonian honeydew honey

Slika 1. Povezanost između udjela fruktoze i glukoze (F+G) [%] i specifične rotacije $[\alpha]^{20}_{D}$ u uzorcima hrvatskog i makedonskog medljikovca

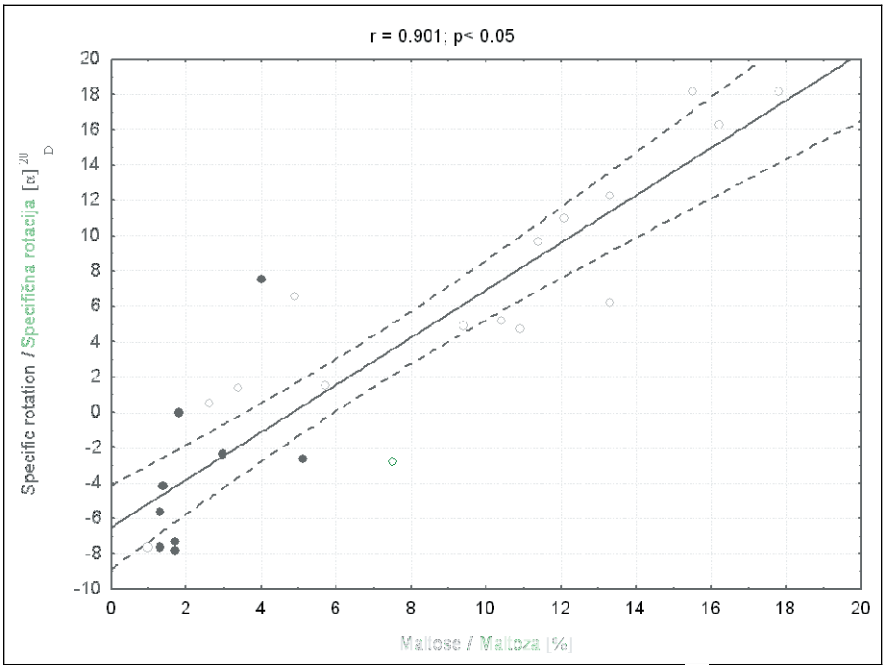

Figure 2. Correlation between maltose content [%] and specific rotation $[\alpha]^{20}_{D}$ in samples of Croatian and Macedonian honeydew honey

Slika 2. Povezanost između udjela maltoze [%] i specifične rotacije $[\alpha]^{20}_{D}$ u uzorcima hrvatskog i makedonskog medljikovca

characteristics (colour, conductivity, acidity and etc.), as well as information obtained by microscopic analysis are in accordance with values reported earlier by different authors, and prescribed by the legislation for honeydew honey, sugar profile and specific rotation which deviate from the common values imply that Macedonian honeydew honeys should be more intensively studied.

CONCLUSIONS

In comparison with Croatian samples, the samples of Macedonian honeydew honey show statistically significant higher electrical conductivity, prolin content, free and total acidity, and lower specific rotation, mostly negative values.

Croatian samples have statistically significant higher content of maltose (with celobiose and trehalose) and raffinose, until Macedonian samples show higher content of glucose and sucrose, as well as F + G content.

Croatian and Macedonian honeydew honey samples differentiate in more physicochemical parameters, especially in sugar profile. Therefore, research should be continued with a focus on botanical origin and geographical location of researched honeydew honey combined with more comprehensive melissopalynological analysis.

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