PALYNOLOGICAL AND PHYSICOCHEMICAL CHARACTERISATION OF CROATIAN HONEYS - CHRIST'S THORN (PALIURUS SPINA CHRISTI MILL.) HONEY

PALINOLOŠKA I FIZIKALNO-KEMIJSKA KARAKTERIZACIJA HRVATSKOG MEDA – DRAČIN (PALIURUS SPINA CHRISTI MILL.) MED

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ABSTRACT

The aim of this research was to determine physicochemical parameters and pollen spectrum of Christ's thorn (Paliurus spina christi Mill.) honey. 15 samples of honey from three production seasons were analysed. Pollen spectrum indicated that all honey samples were unifloral, with Paliurus spina Christi pollen content of 46 – 92 %, while number of other botanical species varied in range from 5 to 16. Moisture, electrical conductivity, pH, free, lactone and total acidity, invertase and diastase activity, hydroxymethylfurfural and proline content and specific rotation were determined, as well as content of sugars. Physicochemical parameters showed that Chist's thorn honey is characterized by high activity of enzymes diastase (25.39-50.51, mean 42.08) and especially invertase (164.9-299.7 U/kg, mean 245.5 U/kg), high electrical conductivity (0.45-0.89 mS/cm, mean 0.68 mS/cm) and high proline content (346.4-667.1 mg/kg, mean 518.6 mg/kg).

Keywords: Christ's thorn honey, melissopalynological analysis, physicochemical properties

SAŽETAK

Cilj rada bio je odrediti fizikalno-kemijska svojstva i sastav peludi meda drače (Paliurus spina Christi Mill.). Analizirano je 15 uzoraka meda proizvedenih u tri sezone. Peludna analiza je potvrdila da su svi uzorci monoflorni sa sadržajem peludi drače od 46 % do 92 %, dok je broj biljnih vrsta u uzorcima varirao od 5 do 16. Također, određen je udio vode, električna vodljivost, pH, slobodna, laktonska i ukupna kiselost, aktivnost invertaze i dijastaze, sadržaj hidroksimetilfurfurala i prolina, specifična rotacija, te sadržaj šećera. Fizikalno-kemijski parametri su ukazali da je dračin med karakterističan po visokoj aktivnosti enzima dijastaze (25.39-50.51, srednja vrijednost 42.08), te posebice invertaze (164.9-299.7 U/kg, srednja vrijednost 245.5 U/kg), visokoj električnoj vodljivosti (0.45-0.89 mS/cm, srednja vrijednost 0.68 mS/cm) i visokom sadržaju prolina (346.4-667.1 mg/kg, srednja vrijednost 518.6 mg/kg).

Ključne riječi: dračin med, melisopalinološka analiza, fizikalno-kemijska svojstva

DETALJNI SAŽETAK

Određena su fizikalno-kemijska svojstva i sastav peludi meda drače (Paliurus spina Christi Mill.). Analizirano je 15 uzoraka meda drače sakupljenih na mediteranskom dijelu Hrvatske tijekom tri sezone (2004., 2005. i 2007.). Peludna analiza je potvrdila da su svi prikupljeni uzorci meda monoflorni sa sadržajem peludi drače u netopivom sedimentu od 46 % do 92 %. Ukupno je u uzorcima utvrđena prisutnost 43 različite biljne vrste, dok je broj biljnih vrsta u pojedinačnim uzorcima varirao od 5 do 16. Uzorcima je određen udio vode, električna vodljivost, pH, slobodna, laktonska i ukupna kiselost, aktivnost invertaze i dijastaze, sadržaj hidroksimetilfurfurala (HMF) i prolina, specifična rotacija, te sadržaj šećera glukoze, fruktoze, saharoze, maltoze, melecitoze, rafinoze i ksiloze. Iz sadržaja pojedinačnih šećera izračunat je ukupni sadržaj šećera, omjer fruktoza/glukoza, te omjer glukoza/voda.

Niske vrijednosti sadržaja HMF-a (0-6 mg/kg, prosječno 1.2 mg/kg) potvrdile su da su uzorci svježi i termički ne procesirani, te da su kao takvi reprezentativni za karakterizaciju meda. Fizikalno-kemijski parametri su ukazali da dračin med ima karakteristične visoke vrijednosti aktivnosti enzima dijastaze (25.39-50.51, srednja vrijednost 42.08), posebice aktivnosti invertaze (164.9-299.7 U/kg, srednja vrijednost 245.5 U/kg), visoku električnu vodljivosti (0.45-0.89 mS/cm, srednja vrijednost 0.68 mS/cm), te visok sadržaj aminokiseline prolina (346.4-667.1 mg/kg, srednja vrijednost 518.6 mg/kg).

INTRODUCTION

Round the Globe, many types of monofloral honey are produced, and yet, only some 20 types of unifloral honeys, multifloral honey, honeydew honey and blend honey are well known in most of the countries and described in the literature.

Only in Europe over 100 botanical species are known to produce unifloral honey, but most of them are produced occasionally or are only of local interest [12]. Fifteen the most abundantly produced and marketed unifloral honey types were selected by the International Honey Commission of Apimondia (IHC) and described through their mellisopalynological, physicochemical and sensory parameters [12]. Some specific, but not so widespread monofloral honey types, were studied and described in the literature by different authors [6,9,15,16], while data for others are mostly unavailable.

Christ's thorn (Paliurus spina-christi Mill.) is deciduous scrub which can be rarely found across the Mediterranean (Italy, Greece, South-East Europe) [11], while its occurrence in Croatian Mediterranean region is high enough to enable unifloral honey production. Christ's thorn flourishes from May till July (depending on the weather conditions), giving honey which is yellow coloured, of medium sweetness and light bitterness, and which crystallises very fast with big crystals. Complete patterns of headspace, volatile and semi-volatile compounds present in unifloral Christ's thorn honey from sub Mediterranean part of Croatia, were recently published [5]. Precise data on other physico-chemical properties of this type of unifloral honey are, to the best authors knowledge, not available in the scientific literature.

The aim of this paper was to characterise Christ's thorn honey by its pollen spectrum and physicochemical properties.

MATERIALS AND METHODS

Samples

Honey samples were purchased directly from the beekeepers whose hives were located in the Mediterranean region of the Republic of Croatia. According to their knowledge (position of hives and blooming of flowers) purchased samples were denominated as Christ's thorn honey. Altogether 15 samples of Christ's thorn honey from 3 production seasons (2004., 2005. and 2007.) were gathered.

Pollen spectrum determination

The pollen analysis was performed according the method of Louveaux, Maurizio and Vorwohl [8] and Croatian regulations [10] i.e. by pollen grains identification and counting. On five samples just orienting analysis was performed with the aim of confirming that Paliurus spina christi pollen grains are the most frequently occurring i.e. that their share is high enough to declare the sample as Christ's thorn honey. For that purpose only share of Paliurus spina christy pollen grains in total number of pollen grains was expressed. Other ten samples were completely analysed and frequency classes for all present pollen types were determined. Identification of present pollen grains was made by reference to the literature data [19] and/or personal comparative preparation.

Determination of physicochemical parameters

All physicochemical parameters were determined by the methods officially prescribed methods [1,4].

Moisture (water content) was determined from the refractive index of the honey read on Abbé refractometer by reference to a standard table [4]. Electrical conductivity was measured in a solution of 20 g honey dry matter in demineralised low conductivity water at 20°C [4]. pH of solution of 10 g of honey in 75 mL of distilled water

Table 1: Average values of physicochemical parameters, shares of *Paliurus spina christi* pollen grains and number of pollen types represented in analysed Christ's thorn honey samples
Tablica 1: Srednje vrijednosti fizikalno-kemijskih parametara, udjeli *Paliurus spina christi* peludi i broj zastupljenih biljnih vrsta u analiziranim uzorcima meda drače

	Unity Jedinica	Mean Srednja vrijednost	SD	Min	Max	Number of samples analysed Broj analiziranih uzoraka
Melissopalynological parameters Melisopalinološki parametri						
Specific pollen Specifična pelud	%	69	15	46	92	15
Number of plant species represented in each sample Broj biljnih vrsta zastupljenih u pojedinom uzorku		12	3	5	16	10
Physicochemical parameters Fizikalno-kemijski parametri						
Water / Voda	%	16.6	0.7	15.2	18.0	15
Electrical Conductivity Električna vodljivost	mS/cm	0.68	0.13	0.45	0.89	15
pH		4.80	0.39	4.21	5.55	15
Free acidity / Slobodna kiselost	mmol/kg	17.1	4.6	8.5	24.6	15
Lactones / Laktonska kiselost	mmol/kg	7.5	3.4	2.7	14.1	10
Total acidity / Ukupna kiselost	mmol/kg	24.0	7.9	12.3	38.7	10
Diastase / Dijastaza		42.08	6.38	25.39	50.51	15
Invertase / Invertaza	U/kg	245.5	42.6	164.9	299.7	9
HMF	mg/kg	1.3	1.6	0.0	6.0	15
Proline / Prolin	mg/kg	518.6	96.6	346.4	667.1	9
Specific Rotation Specifična rotacija	$[\alpha]_D^{20}$	-6.71	4.00	-12.59	-1.45	10
Fructose / Fruktoza	g/100 g	38.1	3.6	33.5	45.0	15
Glucose / Glukoza	g/100 g	33.8	3.9	26.9	38.8	15
Sucrose / Saharoza	g/100 g	2.2	0.6	0.7	3.3	15
Maltose / Maltoza	g/100 g	2.4	1.5	0.7	5.1	15
Melezitose / Melecitoza	g/100 g	0.6	1.1	0.0	2.9	15
Raffinose / Rafinoza	g/100 g	0.1	0.1	0.0	0.4	15
Ksilose / Ksiloza	g/100 g	0.1	0.1	0.0	0.2	15
Total sugars / Ukupni šećeri	g/100 g	77.3	4.3	64.7	83	15
Fructose + Glucose Fruktoza + glukoza	g/100 g	71.9	4.2	61.8	76.7	15
Fructose/Glucose Fruktoza/glukoza		1.1	0.2	1.0	1.6	15
Glucose/Water Glukoza/voda		2.0	0.3	1.6	2.5	15

was measured for all samples, but afterwards for some of them only free acidity was determined by titration with 0.1 mol/L NaOH to pH=8.3 [4], while for others lactones and total acidity were determined as well [1].

For sugar (carbohydrates) content determination samples were analysed by HPLC method [4]. For that purpose liquid chromatographic system consisting of Varian ProStar 230 Solvent Delivery Module, Varian ProStar 500 Column Valve Module and Varian ProStar 350 Refractive Index Detector coupled to a computer with ProStar Chromatography Workstation was used. Separation of sugars was performed on Zorbax NH_2 analytical column (Agilent Technologies, 4.6 mm ID x 250 mm), using mixture of acetonitrile and water (70:30) as a mobile phase at a flow rate of 1ml/min. Identification of separated sugars was performed on the basis of the retention times, and quantities of present sugars through external calibration.

Optical activity i.e. specific rotation of clear, filtered aqueous honey solution was determined by the polarimetric method [4]. Diastase activity (Gothe or Schade units) was determined according the method after Schade [4]. Invertase activity (U/kg) was determined through the reaction in which p-nitrophenyl-α-D-glucopyranoside is split into glucose and p-nitrophenol by invertase, and from the p-nitrophenol resulting nitrophenolate anion is determined photometricaly [4]. Proline content was determined by ninhydrin-based photometric method [1] and hydroxymethylfurfural (HMF) content according the method after White by determination of UV absorbance of HMF [4].

Data analysis

After the analytical work was carried out, for each parameter the average value and standard deviations were calculated.

RESULTS AND DISCUSSION

On the basis of the values obtained for analysed parameters of particular samples, characteristic values for the honey type are calculated and presented on Figure 1, and in Table 1.

Christ's thorn honey is not specifically listed in Croatian legislation as unifloral if some specific content of Paliurus spina christi pollen grains is found in it, and therefore according to the general rule this type of honey can be declared as unifloral in case if Paliurus spina christi pollen grains are represented with 45 % or more in insoluble honey sediment [10]. Taking this into consideration, all analysed samples of Christ's thorn honey complied with prescribed legal values. European Directive [18] does not give any specific values for honey's quality control by pollen grains representation, and pollen analysis results are expressed as frequency classes.

Due to obtained values, Paliurus spina christi pollen grains are in all analysed samples represented as predominant pollen (>45 % in total pollen).

Specific share of Paliurus spina christi pollen grains in the insoluble honey sediment varied from 46 % up to 92 % giving the average of 69 % (Table 1). Various ranges of specific pollen share in unifloral honeys are reported earlier for many types of honey, and while for some honey types this range is rather narrow (chestnut 85.6-100 %), or extremely wide (sunflower 12.0-92.0 %), for most of unifloral honeys variability in the presence of specific pollen has similar difference between maximal and minimal shares [6,12,17].

Wide range of specific pollen shares in avocado (Persea americana Mill) honey (5-60 %) reported by Terrab and Herredia [17] was explained by the avocado flowering behaviour and the impact of the climatic conditions on this flourishing. In the case of here presented Christ´s thorn honey differences in the P. spina christi pollen grains shares are probably the result of the fact that Christ's thorn is a wild growing plant which is unequally represented in different areas and flourishes at the same time as many other Mediterranean plants.

Besides Paliurus spina Christi pollen grains, in indissoluble sediment of analysed honey samples, pollen grains of other 43 different plant species were represented. Presence of different types of pollen, even in cases when they are represented as important minor pollen (IMP) or even minor pollen (MP), provides useful information for geographical characterisation of honey. Namely, since pollen spectrum of honey depends on botanical species available in some area, geographical origin of honey can be established either by the presence of characteristic pollens limited to a certain region, or by the presence of pollen combinations [8]. From all pollen species present in analysed samples, only Trifolium spp in one sample was represented as secondary pollen (SP) (16 - 45 % of total pollen), while 24 plant species were represented as important minor pollen (IMP) (3 - 15 % of total pollen), and 36 of them as minor pollen (MP) (< 3 % of total pollen). Sage (Salvia officinalis L.) was present in the most samples (8 out of 10 analysed), followed by nectarless olive (Olea europea L.) and Poaceae family pollen grains (in 7 out of 10 analysed samples). 17 plant species were present only in one analysed honey sample each, 5 out of them as important minor pollen, and 12 as minor pollen (Figure 1). Number of plant species present in each particular honey sample varied from 5 up to 16, giving the average of 12 plant species per sample. Number of present plant species

in Portuguese honeys reported by Andrade et al. [2] is much lower (28) but number of plants represented in each particular sample varied from 4 to 18, what is similar to our results. On the other hand, Sanz et al. [13] reported much higher variability of plant species in Spanish honeys. Data on variability of plant species in other monofloral honeys are, to the best authors knowledge, not available, mostly due to the fact that Directive [18] does not prescribe pollen analysis as obligatory in honey quality control.

Average values, standard deviation, minimal and maximal values of all analysed physicochemical parameters and from them derived values for Christ's thorn unifloral honey are presented in Table 1. Results are compared

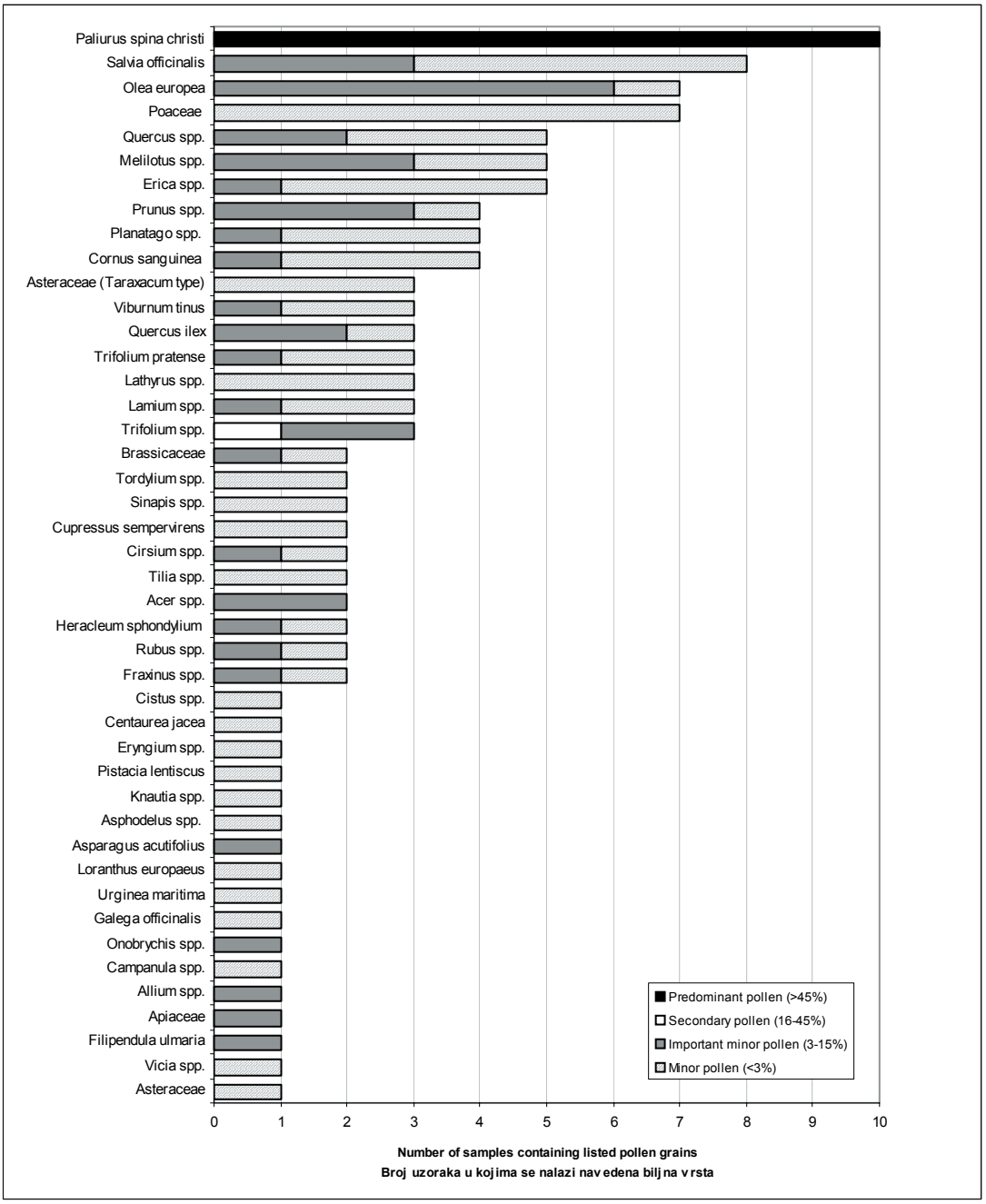

Figure 1 Number of samples (out of 10 analysed) in which listed plant species are represented
Slika 1 Broj uzoraka (od 10 analiziranih) u kojima su navedene biljne vrste zastupljene

to legal requirements and with the values reported for other Mediterranean types of honey, which are produced under the same climatic conditions as Christ's thorn honey (citrus, orange honey, lavender, rosemary, sage, eucalyptus and avocado unifloral honey) and were available to authors.

Obtained values of all analysed parameters were within the limit prescribed by the Croatian national [10] and international legislation [18]. Furthermore, low HMF content (0-6 mg/kg, average 1.3 mg/kg) purports the fact that all samples were fresh and unprocessed, and as such could give representative data for honey type characterisation.

Relatively high proline content in analysed samples (364.4 – 667.1 mg/kg) confirms that samples were genuine. Namely, minimum proline content in genuine honey (not adulterated with sugar) should be above 180 mg /kg of honey, though considerable variations in its content are related to the honey type [3].

Water content provides information on the resistance of honey to spoilage by fermentation. The lower the water content, the lower the probability that honey will ferment during storage. Maximal water content of 20% prescribed by legislation [10,18] is for most types of honey needed to keep it stabile during the shelf life, and there are some tendencies to lower that limit to 19 % [4]. Water content of analysed Christ's thorn honey varied from 15.2 % up to 18.0 %, what confirms stability of honey during the storage. Also, obtained values are in agreement with values obtained for other Mediterranean types of honey [7,12,14], and are the result of the climate in which they are produced.

Electrical conductivity values varied from 0.45 mS/cm to 0.89 mS/cm (Table 1) giving the average value of 0.68 mS/cm. These values are considerably higher from values reported for other Mediterranean honeys like sage, lavender, citrus and rosemary (0.304 mS/cm, 0.21 mS/cm, 0.19 mS/cm and 0.15 mS/cm, respectively) [6,12].

Christ's thorn honey is characterised by high enzymatic activity (diastase, invertase) in comparison with other types of Mediterranean honeys. Activity is especially high for the enzyme invertase (164.9 U/kg – 299.7 U/kg, mean 245.5 U/kg), for which values are, to the best authors knowledge, higher from all in the literature reported values. For example, invertase activity of honey types produced in Mediterranean areas are much lower (citrus 40.0 U/kg, lavender 106.5 U/kg, rosemary 56.4 U/kg) and even the highest maximal value of all 15 described European honey types, which is for honeydew honey 244.4 U/kg, is lower than average of here presented Christ's thorn honey [12]. High enzymatic activity (especially activity of the enzyme invertase which is much more sensitive on the temperature and prolonged storage) indicates that all samples were fresh and unprocessed, but at the same time they are a characteristic of the honey type [12].

Acidity of honey, besides that gives valuable information on the presence of fermentation process if raised above prescribed limit [10,18], also presents one of the parameters for characterisation. Christ's thorn honey studied in this research, like many other honey types [12] can be categorised into the group of honeys with average acidity (pH 4.21- 5.55, free acidity 8.5-24.6 mmol/kg, lactones 2.7-14.1 mmol/kg, total acidity 12.3-38.7 mmol/kg).

Considering sugar profile, Christ's thorn honey is similar to other Mediterranean unifloral honey types [6,12], and negative values of specific rotation, which are the characteristic confirmation of the nectar honey type, are also the result of sugar content.

Sucrose content (0.7-3.3 g/100 g; mean 2.2 g/100 g) is higher than in all of other Mediterranean unifloral honey types (sage 1.7 g/100 g; citrus 1.2 g/100 g; rosemary 1.3 g/100 g) except lavender (5.7 g/100 g) honey [6,12].

Due to relatively high glucose/water ratio, Christ's thorn honey chrysalises rather fast after the extraction, and crystallisation is furthermore purported by relatively low fructose/glucose ratio.

CONCLUSION

Spectrum of pollen grains represented in this unifloral honey type is rather wide, but domination of Paliurus spina christi pollen grains is always marked, while other plant species are represented mostly as minor pollen.

On the basis of the obtained values of physicochemical parameters Christ's thorn honey can be described as honey with high enzymatic activity, especially invertase activity, and high proline content, while all the other characteristics are similar to those of other Mediterranean honeys.

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