

## Physico-chemical, melissopalynological and sensory characteristics of Satsuma mandarin honey (*Citrus unshiu* Marc.)

### Fizikalno-kemijska, melisopalinološka i senzorska svojstva meda od unšijske mandarine (*Citrus unshiu* Marc.)

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#### ABSTRACT

The honey bee forage resources in the Neretva Valley enable production of unifloral Satsuma mandarin (*Citrus unshiu* Marc.) honey. Given the insufficient knowledge about this type of honey, the aim of this study was to establish its botanical origin (pollen spectrum), as well as physico-chemical and sensory properties. In total nine samples of Satsuma mandarin unifloral honey collected from Neretva Valley in the period from 2014 to 2015 were analysed. Floristic observations have recorded 78 plant species and the results of qualitative melissopalynological analysis revealed pollen originating from 50 plant species in analysed honey samples. The number of determined types of pollen in individual honey samples varied from 8 to 19 with an average of 13 pollen types. Proportion of Satsuma mandarin pollen ranged from 3 to 35% with average of 10.88%. The average water content in honey samples range from 17.1% to 17.3%, electrical conductivity from 0.22 mS/cm to 0.27 mS/cm, pH value from 3.82 to 4.02 and diastase activity from 7.78 to 9.36. The main sensory attributes of Satsuma mandarin were determined by sensory analysis: colour ranging from light to dark yellow, orange blossom odour with medium intensity, weak to medium taste persistence characterised by medium sweetness and poorly expressed acidity, and floral and fruity aroma. Due to almost monocultural orchards of Satsuma mandarin in the Neretva Valley, the possibility of producing this rare type of honey in this region should certainly be utilized.

**Keywords:** honey, *Citrus unshiu* Marc., botanical origin, physico-chemical analyses, sensory analysis

#### SAŽETAK

Na osnovu pašnih resursa u dolini Neretve značajnu proizvodnju uniflornog meda od unšijske mandarine (*Citrus unshiu* Marc.) omogućavaju površine pod navedenim agrumom. Međutim, zbog nedovoljnih saznanja o ovoj vrsti meda cilj je ovog istraživanja bio utvrditi njegovo botaničko podrijetlo te fizikalno-kemijska i senzorska svojstva. S navedenog je područja analizirano ukupno 9 uzoraka meda od unšijske mandarine prikupljenih u razdoblju od 2014. do 2015. godine. Florističkim je opažanjima zabilježeno 78 biljnih vrsta, a kvalitativnom je melisopalinološkom analizom u uzorcima meda utvrđena pelud 50 biljnih vrsta. Broj se utvrđene peludi po uzorku meda kretao od 8 do 19, a prosjek je bio 13. Udio peludnih zrnaca unšijske mandarine u istraživanim se uzorcima kretao od 3 do 35% s prosjekom od 10.88 %. Prosječni se udio vode u uzorcima meda kretao od 17.1 do 17.3%, električne provodnosti od 0.22 mS/cm do 0.27 mS/cm, pH vrijednosti od 3.82 do 4.02, dok se aktivnost dijastaze kretala od 7.78 do 9.36. Senzorskom je analizom utvrđeno da boja

meda varira od svijetlo do tamno žute, miris meda je po parfemu narančina cvijeta i srednjeg je intenziteta, okus je slabo do srednje postojan sa srednje izraženom slatkoćom i slabo izraženom kiselošću, dok je aroma srednje postojana, cvjetna, odnosno voćna. Na osnovu gotovo monokulturnih nasada unšijske mandarine u dolini Neretve trebalo bi iskoristiti mogućnost proizvodnje ove raritetne vrste meda.

**Ključne riječi:** med, *Citrus unshiu* Marc., botaničko podrijetlo, fizikalno-kemijske analize, senzorska analiza

## INTRODUCTION

The estuary of the Neretva River presents the northeast area in Europe (Gugić and Cukrov, 2011) where 90% of Citrus trees are Satsuma mandarin (*Citrus unshiu* Marc.) thus presenting an interesting honey bee pasture potential (Popović and Vego, 2010). Satsuma mandarin honey is not sufficiently recognized and belongs to a category of rare honey types primarily due to limited area where it can be produced, as well as insufficient knowledge about its melissopalynological properties. Also in the available scientific literature, there are only two articles discussing on physico-chemical properties of Satsuma mandarin honey, Svečnjak et al. (2015) and Svečnjak et al. (2017). Although the uniflorality of honey is generally confirmed if at least 45% of the corresponding pollen form, unfortunately the pollen to nectar ratio varies considerably between different plant species (Bryant and Jones, 2001). Therefore, the presence of pollen in honey may be influenced by numerous factors. One of them is the influence of plant morphology and physiology, where some plant species produce small amount of pollen (*Medicago sativa*, *Oxydendron* sp., *Eriogonum* sp., *Salvia* sp., *Robinia pseudoacacia*, etc.) or have sterile anthers and do not produce pollen at all, as is the case with some types of *Citrus cultivars*, like *C. unshiu*. Previous floristic studies conducted in the Neretva Valley area (Glasnović et al., 2015) were mainly based on the general representation of plant cover. In this study, one of the goals was to emphasize the representation of plant species that appear at the same time of the Satsuma mandarin flowering and as such are represented in the mandarin honey. Sensory analysis applied to honey is an important complementary part of the traditional physico-chemical and pollen analyses. It is used as an analytical tool for the quality control of honey in relation

to the evaluation of botanic origin. It also confirms the recognition of defects like fermentation, presence of impurities, off odours of smoke, metallic taste, and other characteristics that common laboratory routine analyses do not access (Marcazzan et al., 2018). Colour, odour and taste are the most important sensory characteristics of honey, depending on botanical and geographical origin, quantity and relationship of individual components of honey (colour, sugars, organic acids, minerals, proteins, amino acids), weather conditions, honey flow, beekeeping practice, including honey handling procedures during storage, processing technology and storage. It is known that sensory properties are characteristic of a certain type of honey defined by botanical origin. Some of these properties can be determined by laboratory analysis, but there are typical sensory properties without which the honey type cannot be declared and for which there is no alternative analytical method (Piana et al., 2004). Bruneau et al. (2000) developed a term for describing the odour and aroma of European uniflorous honey types. Sensory evaluation of honey uniformity is used in standard routine sensory properties estimates conducted by specially trained sensory analysts with preparation of samples and implementation of estimates according to ISO standards (Piana et al., 2004).

## MATERIALS AND METHODS

### *Honey bee colonies*

During two years of study (2014 - 2015) in Neretva Valley (43°01'11.10" N; 17°31'14.56" E), two experimental honey bee colonies of Carniolan honeybee (*Apis mellifera carnica*, Pollmann, 1879) were placed in orchards at the time of full Satsuma mandarin flowering. They were located in the central part of the study area where there are no other nectariferous plant species at the time of

Satsuma mandarin flowering. The colonies were formed from 2 kg of packaged bees which were directly set on wax foundations in a Langstroth-Root (LR) hives.

### **Honey samples**

In total nine samples of Satsuma mandarin honey were analysed; four samples were obtained from experimental honey bee colonies and five samples were collected directly from local professional beekeepers whose hives were placed also in the investigated area. To obtain as good honey samples as possible from the Satsuma mandarin, the frames from the experimental hives were individually placed in plastic bags to prevent contamination with pollen from other samples. The samples were stored in glass jars at room temperature ( $\pm 20^{\circ}\text{C}$ ) in the dark until analyses. Decrystallization of two samples was done before diastase activity analysis.

### **Physico-chemical analysis**

Water content, electrical conductivity, pH value and diastase activity (Schade units/g) were determined in accordance with the Ordinance on the quality of unifloral honey by Croatian Ministry of Agriculture, Fisheries and Rural Development (2009), European legislation (Codex Standards 12-1981, 2001; Council Directive 2001/110 / EC , 2001) and standardized methods prescribed by the International Honey Commission (2009).

### **Floristic observations**

In selected locations floristic observations included plant species that were blooming at the same time as Satsuma mandarin. Plant species were photographed by a digital camera, while the herbarium was made for the purpose of forming an internal pollen grain reference library. Determination of plant species was performed based on standard botanical keys (Horvatić and Trinajstić, 1967-1981; Domac, 1994; Dubravec and Dubravec, 2001).

### **Melissopalynological analysis**

Honey samples were analysed by qualitative melissopalynological analysis according to the method of von der Ohe et al. (2004). Microscopic examination and counting of pollen grains was carried out on a Hund h 500 (Wetzlar, Germany) light microscope attached to a digital camera (Dino-Eye Model AM423U, Dino-Lite, Taiwan) and coupled to an image analysis system (Dino Capture 2.0 Version 1.4.9, Dino-Lite, Taiwan) for morphometry of pollen grains.

### **Sensory analysis**

Sensory analysis (visual and olfactory-gustatory characteristics) of collected honey samples ( $n=9$ ) was formed by panel of three educated and trained assessors. Each honey sample was individually evaluated by descriptive grades (1-5). Sensory analysis was performed according to Piana et al. (2004).

## **RESULTS AND DISCUSSION**

The physico-chemical composition of honey depends primarily on the botanical origin of nectar (Kaškonienė and Venskutonis, 2010), water and sugar content. According to Makhloufi et al. (2010) the moisture content determines the capability of the product to remain stable and to resist spoilage by yeast fermentation. Persano Oddo and Piro (2004) reported that average content of sugars in *Citrus* honey amounts 38.7% fructose, 31.4% glucose and 1.2% sucrose. Data in this study were compared with previous investigations on Satsuma mandarin honey (Svečnjak et al., 2015; Svečnjak et al., 2017) and on other *Citrus* honey types. Based on the results of physico-chemical analyses (Table 1), the average water content in Satsuma mandarin honey samples ranged from 17.1% to 17.3% which is lower than the value obtained in the study by Svečnjak et al. (2015). Similar values were found in other types of *Citrus* honey (Serra-Bonvehi and Ventura Coll, 1995; Persano Oddo and Piro, 2004; Makhloufi et al., 2010; Rodriguez et al., 2010; Kadar et al., 2011; Karabagias et

al., 2017), while a slightly higher average value of 20% was found in *Citrus* honey from the Morocco (Aazza et al., 2014; Chakir et al., 2016). Water content is a parameter that have significant effect on the quality of all types of honey (Persano Oddo and Piro, 2004) and it is strongly depended on the beekeeping technology and honey extraction time. The increased water content negatively affects the physical (viscosity and crystallization) and sensory properties (colour, taste, odour) of honey and increases fermentation potential (Escuredo et al., 2013). The production area, i.e., weather conditions, such as rainfall and relative humidity, may also affect the water content in the honey (Karabagias et al., 2017).

Since Vorwohl (1964) has introduced a measure of electrical conductivity in honey analytics, it is considered to be one of the most useful parameters for classifying unifloral honey types. Electrical conductivity in honey is dependent on the proportion of mineral substances and organic acids (da Silva et al., 2016). On the basis of electrical conductivity, the honey is classified by source from which the bees collected the nectar and / or the honeydew (Bogdanov et al., 2004). Electrical conductivity value varies significantly, depending on the botanical origin of honey, ranging from 0.06 to 2.17 mS/cm (Persano Oddo and Piro, 2004). As presented in Table 1, the average values of electrical conductivity in the investigated honey samples ranged from 0.22 mS/cm to 0.27 mS/cm, which is in agreement with Svečnjak et al. (2015) and Svečnjak et al. (2017). The values reported in

some other studies investigating different *Citrus* honeys (Terrab et al., 2003; Serrano et al., 2004; Kadar et al., 2011; Rodriguez et al., 2010) were also in the obtained range. It is important to mention that a sample with only 3% Satsuma mandarin pollen grains has a lower electrical conductivity value (0.23 mS/cm) compare a sample with 35% of Satsuma mandarin pollen grains (0.25 mS/cm).

However, Makhloufi et al. (2010) have reported that the average value of electrical conductivity in Algerian samples was 0.40 mS/cm, while the values determined in *Citrus* samples from Egypt (Alqarni et al., 2014) were 0.80 mS/cm, which is significantly higher than the values obtained in this research. Given that Persano Oddo and Piro (2004) established the average values of electrical conductivity on 299 samples of *Citrus* honey ranging from 0.11 to 0.31 mS/cm, the values obtained for Algerian and Egyptian honey samples should be taken with the reserve. It can be assumed that higher values reported for these samples were probably due to the presence of nectar from other plant species, notably in Egyptian samples, especially given that a value of 0.80 mS/cm is the limit for discrimination between nectar honey and honeydew honey.

The determined pH values of honey samples ranged from 3.81 to 4.13, while a slightly lower minimum (3.72) and higher maximum (4.17) values were observed in previous Satsuma mandarin honey study from Croatia (Svečnjak et al., 2015; Svečnjak et al., 2017). Serrano et al. (2004) reported an average pH value of 4.02, Azeredo

**Table 1.** Descriptive statistics for selected physico-chemical parameters of analysed Satsuma mandarin honey samples (n=9)

Parameter	Year	N	$\bar{x}$	Sd	Min	Max
Water content (%)	2014	4	17.1	0.02	16.2	17.8
	2015	5	17.3	0.54	16.6	17.8
El. conductivity (mS/cm)	2014	4	0.27	0.02	0.24	0.29
	2015	5	0.22	0.04	0.18	0.29
pH value	2014	4	3.82	0.04	3.81	3.87
	2015	5	4.02	0.07	3.93	4.13
Diastase activity (Schade units/g)	2014	4	7.78	2.11	4.90	9.40
	2015	5	9.36	2.23	7.30	12.70

et al. (2003) 4.00, as well as Makhloufi et al. (2010). Furthermore, Aazza et al. (2014) reported 3.68, while Persano Oddo and Piro (2004) averaged 3.80. The lowest pH values ranging from 3.22 to 3.67 were recorded in the study of Karabagias et al. (2017). It can be concluded that all reported pH values do not actually vary significantly, regardless of *Citrus* species.

Diastase is one of the enzymes naturally occurring in the honey and lighter honey types have a lower diastase activity. Quantity of diastase in honey depends on various factors, but the most important are botanical and geographical origins and freshness of honey. Namely, long-term storage or heating of the honey increases diastase activity (Fallico et al., 2006). In this context, diastase activity along with HMF (hydroxymethylfurfural) is commonly used to detect excessive heating of honey (Tosi et al., 2008). In Codex Alimentarius (2001), minimal values of 3 Schade units/g of diastase activity were determined for honey types that have a naturally low level of that enzyme. Due to the known specificity of Satsuma mandarin honey compared to other types of honey, which is reflected through low diastase activity, the mentioned parameter is also monitored. Its value ranged from 4.90 to 12.70 Schade units/g. Persano Oddo and Piro (2004) state that the average value of diastase activity for *Citrus* honey is 9.6, which is in agreement with the values obtained in this study. Serrano et al. (2004) state that the average diastase activity in their study was 13.96 with a range of 5.94 to even 35.35. Furthermore, Serra Bonvehi and Ventura Coll (1995) in Spanish *Citrus* honey samples found an average of 17.60, while the lowest values of 7.0 to 9.0 were recorded by Makhloufi et al. (2010) for Algerian samples. Svečnjak et al. (2017) reported values range from 7.00 to 17.80 with the average of 12.02 Schade units/g.

Based on floristic observations, 78 plant taxa were determined on investigated location. They are presented in Table 2 together with the relative amount of nectar and pollen produced.

Of the total number of determined plant species (Table 2), nine of them were the source of nectar, 45 nectar and

pollen, while 24 honey bees attended solely for pollen.

Dimensions of Satsuma pollen grains ranged from 21.79 to 23.75  $\mu\text{m}$  with an average of 22.68  $\mu\text{m}$  in polar view. In the equatorial view, values ranged from 20.28 to 24.16  $\mu\text{m}$  with an average of 22.57  $\mu\text{m}$ . According to aperture type, the pollen grains of Satsuma mandarin belong to the tetracolporate and the exine sculpturing type belongs to the reticulate/foveolate (Figure 1).

Based on qualitative melissopalynological analysis, a total of 50 plant species were identified (Figure 2). The number of determined pollen types in honey sample varied from 8 to 19 and the average was 13. In 33% of the samples, the pollen of Satsuma mandarin (*C. unshiu*) was classified as secondary and in 47% as the group of rare pollen. Pollen originating from other nectariferous plant species were also present in analysed honey samples, i.e., *Capsela bursa pastoris*, Asteraceae *Taraxacum* form, *Rhamnus alaternus* and *C. sinensis*. In addition to the nectariferous plant species, a large number of pollen of non-nectariferous species was determined: *Fraxinus ornus*, *Quercus ilex*, *Dactylis Glomerata*, *Olea europea*, *Cistus* spp., *Cupressus sempervirens* and *Pistacia lentiscus*.

Although there are studies related to the botanical origin of *Citrus* honey, according to available scientific literature, there are only two studies (Svečnjak et al., 2015; Svečnjak et al., 2017) that deal exclusively with Satsuma mandarin honey. However, they do not have information on the botanical origin. In countries with traditional *Citrus* breeding, orchards are mostly planted with different types of *Citrus* trees (orange, lemon, mandarin and grapefruit), but not in monocultural orchards, as in the Neretva Valley with the Satsuma mandarin. Based on melissopalynological analysis, the share of pollen of the Satsuma mandarin including nectarless plant species ranged from 1 to 5% with average of 2.44%. When the percentage of pollen of nectarless plant species was excluded from the calculation, the proportion of Satsuma pollen ranged from 3 to 35% with average of 10.88% (Table 3).

**Table 2.** The list of vascular plant taxa (n=78) identified in the Neretva Valley 2015 during Satsuma mandarin flowering

No	Latin name	Family name	Nectar*	Pollen*
1	<i>Allium subhirsutum</i> L.	Amaryllidaceae	xx	x
2	<i>Alyssum sinuatum</i> L.	Brassicaceae	x	x
3	<i>Anacamptis pyramidalis</i> (L.) Rich.	Orchidaceae		x
4	<i>Anthyllis vulneraria</i> L. ssp. <i>praepropera</i>	Fabaceae	xx	x
5	<i>Antirrhinum majus</i> L.	Scrophulariaceae		xx
6	<i>Avena sterilis</i> L.	Poaceae		x
7	<i>Blackstonia perfoliata</i> (L.) Huds.	Gentianaceae	x	x
8	<i>Callistemon</i> spp	Myrtaceae	x	x
9	<i>Calystegia sepium</i> (L.) R. Br.	Convolvulaceae	x	x
10	<i>Capsella bursa-pastoris</i> (L.) Medik.	Brassicaceae	x	x
11	<i>Carduus micropterus</i> (Borbás) Teyber	Asteraceae	xx	xx
12	<i>Carduus pycnocephalus</i> L.	Asteraceae	xxx	xxx
13	<i>Centaurium erythraea</i> Rafn	Gentianaceae	x	
14	<i>Cerastium brachypetalum</i> Pers.	Caryophyllaceae	x	xx
15	<i>Colutea arborescens</i> L.	Fabaceae	xx	x
16	<i>Convolvulus althaeoides</i> L. ssp. <i>tenuissimus</i> (Sibth. et Sm.) Stac	Convolvulaceae		x
17	<i>Coronilla cretica</i> L.	Fabaceae	x	xx
18	<i>Coronilla emerus</i> L. ssp. <i>emeroides</i> Boiss. et Spruner	Fabaceae	xx	xx
19	<i>Dactylis glomerata</i> L. ssp. <i>hispanica</i> (Roth)	Poaceae		x
20	<i>Fumaria capreolata</i> L.	Fumariaceae		x
21	<i>Geranium purpureum</i> Vill.	Geraniaceae	x	x
22	<i>Gladiolus illyricus</i> W.D.J.Koch	Iridaceae	x	x
23	<i>Gleditsia triacanthos</i> L.	Fabaceae	xx	x
24	<i>Helichrysum italicum</i> (Roth) G.Don	Asteraceae	xx	x
25	<i>Hypericum perforatum</i> L.	Clusiaceae		xx
26	<i>Inula candida</i> Ten.	Asteraceae		x
27	<i>Iris pseudacorus</i> L.	Iridaceae	x	x
28	<i>Leontodon crispus</i> Vill.	Cichoriaceae	x	x
29	<i>Lithospermum purpureocaeruleum</i> L.	Boraginaceae	x	x
30	<i>Lonicera etrusca</i> Santi	Caprifoliaceae		x
31	<i>Malva sylvestris</i> L.	Malvaceae	xx	x
32	<i>Marrubium incanum</i> Desr.	Lamiaceae	x	x

Table 2. Continued

No	Latin name	Family name	Nectar*	Pollen*
33	<i>Medicago minima</i> (L.) Bartal.	Fabaceae	x	x
34	<i>Medicago sativa</i> L.	Fabaceae	xxx	x
35	<i>Micromeria juliana</i> (L.) Benth. ex Rchb.	Lamiaceae	x	
36	<i>Muscari comosum</i> (L.) Mill.	Asparagaceae		x
37	<i>Nigella damascena</i> L.	Ranunculaceae	xxx	x
38	<i>Orlaya grandiflora</i> (L.) Hoffm.	Apiaceae		x
39	<i>Ornithogalum pyramidale</i> L.	Asparagaceae		x
40	<i>Osyris alba</i> L.	Santalaceae	x	
41	<i>Paliurus spina-christi</i> Mill.	Rhamnaceae	xxx	xx
42	<i>Pallenis spinosa</i> (L.) Cass.	Asteraceae		x
43	<i>Papaver rhoeas</i> L. ssp. <i>strigosum</i> (Boenningh.)	Papaveraceae		xxx
44	<i>Parietaria judaica</i> L.	Urticaceae		x
45	<i>Pittosporum tobira</i> (Thunb.) Aiton f.	Pittosporaceae	xxx	x
46	<i>Plantago coronopus</i> L.	Plantaginaceae		x
47	<i>Plantago lanceolata</i> L.	Plantaginaceae		x
48	<i>Poa bulbosa</i> L.	Poaceae		x
49	<i>Polygala nicaeensis</i> Risso ex Koch	Polygalaceae	x	
50	<i>Potentilla pedata</i> Nestl.	Rosaceae	x	x
51	<i>Prasium majus</i> L.	Lamiaceae	xx	x
52	<i>Psoralea bituminosa</i> L.	Fabaceae	x	x
53	<i>Punica granatum</i> L.	Punicaceae	x	x
54	<i>Quercus ilex</i> L.	Fagaceae		xx
55	<i>Rosa agrestis</i> Savi	Rosaceae	x	xx
56	<i>Rosa marginata</i> Walln.	Rosaceae	x	xx
57	<i>Salvia officinalis</i> L.	Lamiaceae	xxx	
58	<i>Salvia verbenacea</i> L.	Lamiaceae	xxx	x
59	<i>Scorpiurus muricatus</i> L.	Fabaceae		x
60	<i>Sedum acre</i> L.	Crassulaceae	x	xx
61	<i>Sedum ochroleucum</i> Chaix	Crassulaceae	x	xx
62	<i>Senecio vulgaris</i> L.	Asteraceae	xx	xx
63	<i>Sideritis romana</i> L.	Lamiaceae	xx	
64	<i>Sisymbrium officinale</i> (L.) Scop.	Brassicaceae		x

Table 2. Continued

No	Latin name	Family name	Nectar*	Pollen*
65	<i>Sonchus oleraceus</i> L.	Cichoriaceae		xx
66	<i>Spartium junceum</i> L.	Fabaceae	x	x
67	<i>Stachys germanica</i> L.	Lamiaceae	xx	x
68	<i>Stenactis annua</i> (L.) Nees	Asteraceae		x
69	<i>Teucrium polium</i> L.	Lamiaceae	x	x
70	<i>Thymus dalmaticus</i> (Rchb.) Freyn	Lamiaceae	xxx	
71	<i>Torilis heterophylla</i> Guss.	Apiaceae		x
72	<i>Tragopogon</i> sp.	Asteraceae	x	x
73	<i>Trifolium angustifolium</i> L.	Fabaceae	xx	x
74	<i>Trifolium arvense</i> L.	Fabaceae	xx	x
75	<i>Trifolium repens</i> L.	Fabaceae	xx	
76	<i>Trifolium tomentosum</i> L.	Fabaceae	x	x
77	<i>Verbascum phlomoides</i> L.	Scrophulariaceae	x	xx
78	<i>Vicia grandiflora</i> Scop.	Fabaceae		x

\*data from literature (nectar and pollen source)

x -low, xx -medium, xxx - high

Table 3. Share of Satsuma mandarin pollen in analysed honey samples

Share (%)	N	$\bar{x}$	SD	Range
Included nectarless plant species	9	2.44	1.33	1 - 5
Excluded nectarless plant species		10.88	13.27	3 - 35

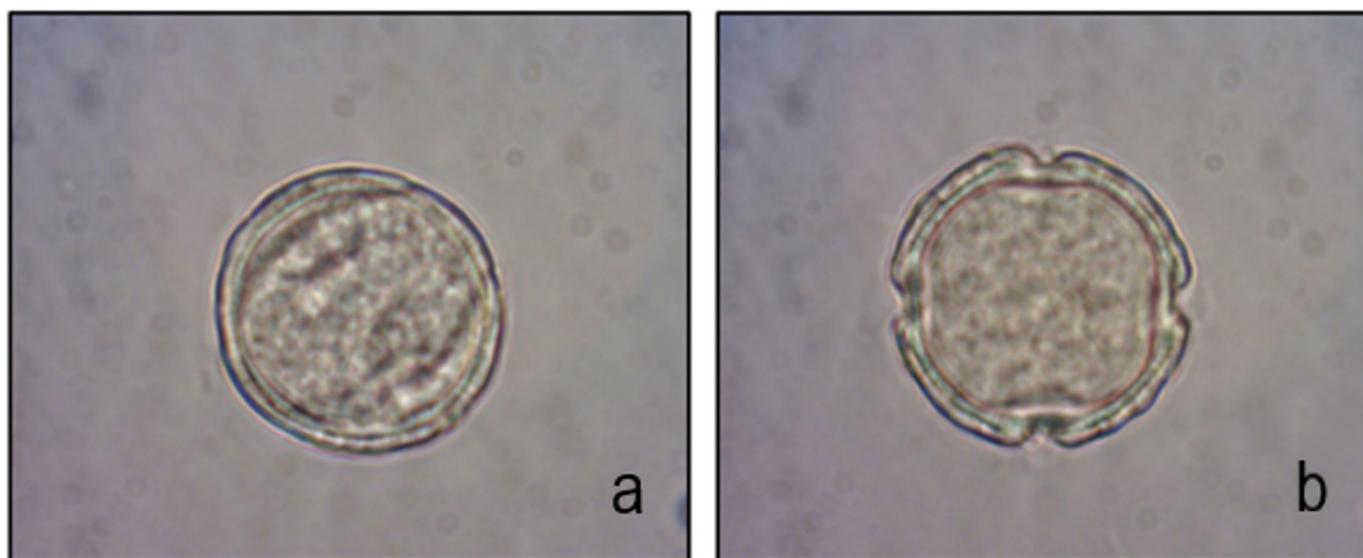


Figure 1. Pollen grain of Satsuma mandarin in equatorial a) and polar view (b)

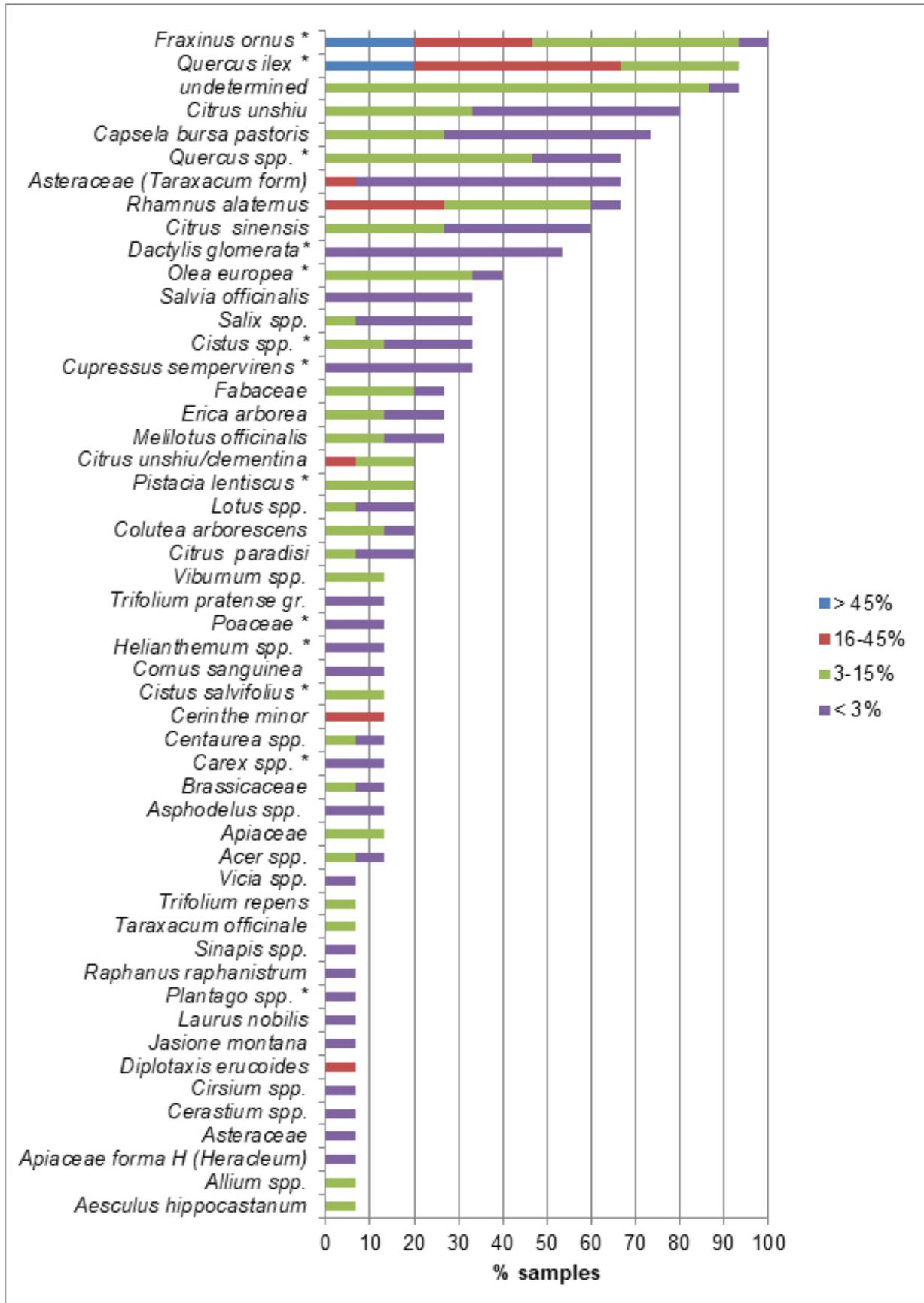


Figure 2. Pollen spectrum of analysed Satsuma mandarin honey samples (n=9)

The results of the qualitative melissopalynological analysis of honey samples investigated in this study were compared with those of other *Citrus* species. In the samples of orange honey from the Greek area *Citrus* pollen (*Citrus* spp.) ranged from 2.9 to 26.5% (Tsigouri et al., 2004). According to Ricciardelli D'Albore (1997), in Italian samples of *Citrus* honey (orange) pollen was usually represented with more than 5% or 10%. In the Spanish samples, Serra Bonvehi and Ventura Coll (1995) set a range of 8% to 25% of *Citrus* pollen grains, while the range of 10 to 46% was found by Rufino and Bosch-Reig (1998). Similar values, from 10 to 53%, for samples from Morocco were reported by Terrab et al. (2002 and 2003) and Zerrauk et al. (2014) for the samples from Algeria (27% - 50%). Recent research by Karabagias et al. (2017), on the samples of *Citrus* honey from the Mediterranean area based on melissopalynological analyses established certain differences. Namely, the content of the *Citrus* pollen grains ranged from 32% for samples from Egypt, followed by 19% from Spain, 11% from Morocco and 8% from Greece as confirmed by Persano Oddo and Piro (2004) who reported the pollen content range from 2% up to 42%.

Since pollen grains of *Citrus* spp. belong to the under-represented category (relatively small amount of pollen compared to the quantity of nectar), low content in this type of honey is very common (Von der Ohe et al., 2004). Such a large range of *Citrus* pollen share determined in numerous studies investigating various *Citrus* honey types, primarily depends on the plant species from which the bees collected the nectar. It can be concluded that exceptionally low proportion of *Citrus* pollen in certain honey types (such as *C. unshiu*) is the result of sterile hybrids and cultivars of *Citrus* species, while in fertile plants species the share of *Citrus* spp. pollen increases, and in some species, exceeds a share of 45% (normally represented category - equal amount of pollen compared to the amount of nectar).

It is known that sensory properties are characteristic of a certain type of honey defined by botanical origin. Some of these properties can be determined by

laboratory analysis, but for typical sensory properties without which the honey type cannot be declared, there is no alternative analytical method (Piana et al., 2004). Bruneau et al. (2000) developed a term for describing the odour and aroma of European unifloral honey types. The main sensory attributes of investigated Satsuma honey samples are presented in Table 4. Sensory analysis revealed that the colour of Satsuma honey varies from light to medium yellow. The honey is characterized by orange blossom odour with medium intensity. The taste is weak to medium, stable, with medium sweetness and poorly expressed acidity, while the aroma is moderately persistent, floral and fruity.

**Table 4.** Sensory profile of Satsuma mandarin honey

Sensory characteristics		Description
Olfactory assessment	Intensity	Medium
	Description	Floral, fresh (orange flower)
Tasting assessment	Intensity	Short to medium
	Sweetness	Medium
	Acidity	Weak
	Bitterness	Absent
Aroma	Intensity	Medium
	Persistence	Short to medium
	Description	Floral, fresh (anise), fruit

## CONCLUSIONS

A detailed description of physico-chemical, melissopalynological and sensory characteristics of unifloral Satsuma mandarin honey has been presented in this study. The results have revealed that physico-chemical characteristics (average water content from 17.1% to 17.3%, electrical conductivity from 0.22 mS/cm to 0.27 mS/cm, pH value from 3.82 to 4.02 and diastase activity from 7.78 to 9.36 Schade units/g) of Satsuma

mandarin honey were in accordance with international standards established for various *Citrus* honeys. The results of melissopalynological analysis showed that all investigated honey samples were rich in various pollen grain types (average 13; predominant ones were *Capsela bursa pastoris*, Asteraceae *Taraxacum* form, *Rhamnus alaternus* and *Citrus sinensis*), but with low percentages of Satsuma mandarin pollen, which was expected since this *Citrus* cultivar have a sterile anther. Sensory analysis revealed that the colour of Satsuma honey varies from light to medium yellow. The honey is characterized by moderate orange blossom odour, and the taste by medium sweetness and poorly expressed acidity. The aroma is moderately persistent, floral and fruity. Considering above mentioned, especially the honey identification issue related to small number of *C. unshiu* pollen grains that can be found in corresponding honey, it is suggested that Satsuma mandarin honey samples, which do not have sufficient number of pollen grains according to current IHC standards but are characterized with physico-chemical and sensory profiles typical for *Citrus* honey types, are allowed to be declared as Satsuma mandarin (*C. unshiu*) unifloral honey.

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