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Melissopalynology analysis, determination of physicochemical parameters, sugars and phenolics in Maltese honey collected in different seasons

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Abstract: Malta, a country renowned for its honey, has not been extensively mentioned in studies based on honey. In addition to many parameters, the collection period affects honey quality, precisely due to the different floral composition that exists during a certain season. Therefore, the significance of this study refers to the provision of data on honey from Malta collected during the autumn, spring, and summer seasons. Melissopalynological analysis, determination of physicochemical parameters, and the use of analytical chromatographic methods enabled detailed analysis of this honey. Principal component analysis (PCA) provided the differentiation of Maltese honey depending on the harvest season. *Lotus* pollen, followed by *Eucalyptus*, predominated in all honey samples. Characteristic compounds for summer honey were pinocembrin, galangin, kaempferol, chrysin, *p*-hydroxybenzoic acid, vanillic acid and maltotriose, while quercetin 3-*O*-galactoside, ferulic acid, ellagic acid, protocatechuic acid, luteolin 7-*O*-glucoside and melibiose were specific for autumn honey. A higher amount of *p*-coumaric acid, genistein, catechin, as well as the content of many sugars were found in spring samples. To the best of our

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knowledge, this is the first scientific work dealing with a detailed chemical analysis of Maltese honey.

Keywords: melissopalynology; physicochemical parameters; chromatography; PCA; *Eucalyptus*; *Lotus*.

INTRODUCTION

The Maltese Islands are renowned for the production of genuine honey from different floral sources depending on the season and the location of the apiary. The endemic subspecies of the honeybee (*Apis mellifera ruttneri*) is specific to the Maltese Islands.¹ Beekeeping in the Maltese Islands is an ancient trade with the tradition being introduced by the Phoenicians thousands of years ago. This is evident by the presence of a number of ancient apiaries (locally called *Imgiebah*), scattered around the islands and by various place around the Island being named after beekeeping connections. The connection of Malta with honey is most evident from the ancient name of the island, *Melita*; which originates from the Greek language, meaning the land of honey.²

The Maltese Islands, situated in the middle of the Mediterranean, possess a mild climate with the honeybees being active almost all year round. In fact, in the Maltese Islands, beekeepers can harvest honey three times, in spring, summer and autumn. These honeys are locally known as spring honey, summer honey and autumn honey. The honey from each season has its own particular organoleptic taste, since they originate from different flora. The dependence of honey on the season from the aspect of mineral content,³ physicochemical parameters^{4,5} and content of some phenolic compounds⁶ has been published. For Maltese honey, only a few studies have dealt with seasonal effects on the physicochemical parameters of honey.^{7,8} There were other studies on Maltese honey with an emphasis on geographical origin confirmation^{2,9} based on chemometric analysis. In general, the composition of honey depends on environmental conditions, season, and floral sources. It is known that the latter causes changes in the composition of honey.¹⁰ The vegetation of Malta provides a great variety of food source for bees, such as *Lotus ornithopodioides* L. and *Lotus edulis* L., which are common species that flowers from February to May and from March to May, respectively. Further, species of Myrtaceae family are present on the Maltese Islands, such as *Eucalyptus gomphocephala* DC. Other common species were found in the Resedaceae, Rosaceae and Lamiaceae families, amongst others. Although there is extensive knowledge on the melissopalynological character of Mediterranean honeys,¹¹ only a few studies analysed the pollen spectrum of honey coming from the islands of Malta, Gozo and Comino.¹²

In this study, melissopalynological analysis,¹³ the physicochemical analysis and determination of the sugar profile, as well as correlation testing of some parameters could ensure compliance with honey quality requirements.¹⁴ In order

to determine the parameters that can potentially confirm the botanical origin of Maltese honey, or at least the presence of specific compounds that correlate with plant species, quantification of the phenolic compounds was performed. PCA was performed on the parameters that could ensure a more obvious classification of Maltese honey collected at the different seasons. A more detailed chemical analysis of Maltese honey has not been conducted so far, so the aim of this paper was to present the basic chemical parameters and floral origin of this specific honey. The extensive knowledge on the variability of honey collected from Malta (both spatial and temporal variations) is a prerequisite for differentiating this product within the protection of geographical origin. Bearing all this in mind, samples of Maltese honeys harvested in different seasons were evaluated by its pollen characteristics and combining PCA with the analysis of physicochemical parameters, sugars and phenolic compounds.

EXPERIMENTAL

Reagents and standards

Ultra-pure water (ThermoFisher TKA MicroPure water purification system, 0.055 mS cm⁻¹) was used to prepare the standard solutions and blanks. Syringe filters (25 mm, nylon membrane 0.45 µm) were purchased from Supelco (Bellefonte, PA). Acetonitrile and methanol (analytical grade) were purchased from Merck (Darmstadt, Germany). The Strata C18-E (500 mg/ 3 mL) solid phase extraction (SPE) cartridges used for the extraction and concentration of honey samples were obtained from Phenomenex (Torrance, CA). The standards of phenolic compounds were purchased from Sigma Aldrich (Steinheim, Germany), as well as the chemicals for melissopalynology analysis. Sugar standards were purchased from Tokyo Chemical Industry (TCI, Zwijndrecht, Belgium). Sodium hydroxide solution (0.1 mol L⁻¹) was purchased from Scharlab (Barcelona, Spain) while formic acid, 2-methoxyethanol, propanol and L-proline (all analytical grade) were purchased from Sigma Aldrich (Steinheim, Germany). Ninhydrin was purchased from Carl Roth (Karlsruhe, Germany). Tablets of *Lycopodium* tracer spores were purchased from the Department of Geology, Lund University, Sweden.

Sample collection

The honey samples were provided by individual Maltese beekeepers with a long family tradition of beekeeping and honey extraction. A total of 14 honey samples were gathered, including three samples from autumn harvest season (AU1–AU3), five samples from spring harvest season (SP1–SP5) and six samples from summer harvest season (SU1–SU6), Table I and Fig. S-1 of the Supplementary material to this paper.

Methods

Melissopalynological analysis. Pollen suspended in honey samples was extracted for analysis following the Harmonized Methods of Melissopalynology.¹³ Qualitative pollen analysis has been performed by scanning slides under Olympus BX51 light microscope at 400× magnification until a minimum of 500 pollen grains have been counted and identified. The identification was done using atlases.^{11,15-17} Where needed, 600× magnification was used for the confirmation of specific morphological characteristics. The resulting frequency of recorded pollen

grains is expressed as percentages. More detailed information of melissopalynological analysis are given in Supplementary material.

TABLE I. The location and time of collecting the honey samples from Malta

Period of sampling	Sample No	Location of apiary	Location No.
Autumn 2014	AU1	Zebbug Area, Island of Gozo	L1
	AU2	Nadur Area, Island of Gozo	L2
	AU3	Victoria Area, Island of Gozo	L3
Spring 2015	SP1	Fawwara Area, Malta	L4
	SP2	Birzebbugia, Malta	L5
	SP3	Gharb Area, Island of Gozo	L6
	SP4	Rabat Area, Malta	L7
	SP5	Wied Ghollieq, Malta	L8
Summer 2015	SU1	Naxxar Area, Malta	L9
	SU2	Gharghur Area, Malta	L10
	SU3	Mellicha Area, Malta	L11
	SU4	Wardija Area, Malta	L12
	SU5	Fawwara Area, Malta	L13
	SU6	St. Paul's Area, Malta	L14

Physicochemical parameters. The physicochemical parameters that were analysed (moisture and Brix content, electrical conductivity, pH, free acidity, diastase activity, proline and hydroxymethylfurfal (HMF) concentration) were determined by the procedures and methods described in the International Honey Commission.¹⁸ The moisture and Brix content were determined by the refractive index principle through a digital refractometer (RX900, Atago). The electrical conductivity of each honey sample was obtained by the use of a conductivity meter (Orion Star A215, Thermo Scientific). The pH and free acidity of each honey sample were obtained using an automatic titrator (TitroLine Easy, SCHOTT Instruments). Proline concentration was determined by the adapted method of Ough *et al.*¹⁹ Diastase activity of each honey sample was determined by using the Amylazyme tablets from Megazyme, Ltd. (T-AMHZY-200T) and following the manufacture description. HMF concentration of each honey sample was determined by the adapted method of White *et al.*²⁰

Sugar analysis. The honey samples were homogenized, weighed (between 0.2 and 0.3 g) and diluted 1000-fold with ultrapure water. The solutions were filtered and transferred to vials. The sugar contents were determined by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC/PAD, Dionex). The conditions were previously described in Gašić *et al.*²¹ and the details were given in Supplementary material.

Analysis of phenolic compounds. The method described by Gašić *et al.*²¹ was used for the extraction and isolation of phenolic compounds from the honey samples. Prior to the analysis of phenolic compounds, the extracts were filtered through a 0.45 µm nylon membrane filter. The separation, determination, and quantification of the components in the honey samples were performed using a Dionex Ultimate 3000 ultra-high-performance chromatography (UHPLC) system equipped with a diode array detector (DAD) that was connected to TSQ Quantum Access Max triple–quadrupole mass spectrometer (Thermo Fisher Scientific) (UHPLC-DAD MS/MS). Details of the analysis of phenolic compounds were given in Supplementary material.

Statistical analysis. Statistical analyses were performed using the Analysis ToolPak from Microsoft Office Excel 2010 Professional. PCA was realized using the PLS Tool Box software package for MATLAB 7.12.0 (Eigenvector Research, Inc., Wenatchee, WA). All data

were pre-treated (mean-centred and scaled to the unit standard deviation) prior to PCA. The singular value decomposition algorithm (SVD) and a 0.95 confidence level for Q and Hotelling T^2 limits for outliers were chosen.

RESULTS AND DISCUSSION

Melissopalynology analysis of Maltese honey samples

The melissopalynology analysis of 14 honey samples from Malta provided 30 pollen types classified in 21 botanical families. The presence of different types of pollen and their maximum frequency were shown in Fig. S-2 of the Supplementary material. The most represented pollen was *Lotus*, exhibited in all analysed honey samples from Malta, mainly as predominant (>45 %). The next abundant pollen was *Eucalyptus*, which was present in eight honey samples. The detailed melissopalynology assessment of the honey samples with the following terms for frequency classes²² were given in the Table S-I.

The first season of harvesting was autumn. *Eucalyptus* and *Reseda*-type pollen prevailed in these samples, as well as *Rubus* pollen in sample AU2 (Table S-I). In the second harvest season, spring, pollen types were diverse. *Lotus* pollen was predominant (>45 %) in sample SP1, SP3, SP4 or as secondary pollen (43 % in sample SP2 and 35 % in sample SP5) with additional *Reseda*-type pollen presented in sample SP6 (36 %, Table S-I). The third season considered summer honey samples, in which *Lotus* pollen predominated with 49-72 %, and *Reseda*-type and *Thymus* as secondary pollen (Table S-I).

As it appears from the melissopalynological analysis (Table S-I), different sorts of pollen occurred in the various period of sampling, and they that did not always correlate with a flowering period of the plant. This could be a consequence of contamination of honeycombs with honey from previous season. This may be either due to honey retained in the comb after previous harvest, or there are shifts in the flowering season for earlier flowering plants. When the bees are searching for food, they also collect pollen from various plants, as well as honeydew. It is important to mention that some of the plants whose pollen has been found in samples, can also provide honeydew secretion. The occurrence of aphids on carob (*Ceratonia siliqua*) and *Citrus* during the autumn season was mentioned by Mifsud *et al.*²³ The potential content of honeydew secretion may be the reason for the later higher electrical conductivity of several honey samples in this study. Nevertheless, the International Honey Commission has established that honey with a percentage of pollen higher than (or equal to) 45 % is considered as monofloral.¹³ Therefore, the honeydew content in honey samples remains an assumption.

Determination of physicochemical parameters of Maltese honey samples

The moisture content of all honey samples was in range from 15.58 to 19.76 % (Table S-II of the Supplementary material), following the International

requirement¹⁴ (below 20 %). By measuring the Brix, the amount of solids in honey is determined. The Brix is connected to the moisture content, as they were completely negatively correlated (-1.0 , Table S-III of the Supplementary material), and their sum were more than 98 % of honey content for all analysed samples. The pH range in analysed samples was from 3.58–4.00 (Table S-II), which is in accordance with EU Directive¹⁴ and descriptive sheets.²⁴ Higher values of moisture content and pH values were found for autumn honey samples. A similar observation was already noted for Maltese honey samples in different periods of harvesting published by Attard *et al.*⁷ As we noted a positively good correlation between the mean values obtained for pH and electrical conductivity (0.96, Table S-III), it was expected that some of the highest pH values were also related to the highest conductivity. There were minimal differences between the moisture content, Brix and pH values within each particular seasons of sampling. This was in the line with results by other authors.^{5,7} Electrical conductivity of honey depends on the minerals, salts, ash, organic acid and protein content, and it could be useful for the determination of the botanical origin of honey.^{25,26} International Honey Commission¹⁴ states that honeydew honey has typical electrical conductivity higher than 0.8 mS cm^{-1} . There are some exceptions established for several types of honey such as strawberry honey or *Eucalyptus* honey, which are allowed to possess higher values than the recommended values for floral types of honey¹⁴. The range of electrical conductivity in the analysed samples was 0.87 – 1.52 mS cm^{-1} for autumn, 0.49 to 0.98 mS cm^{-1} for spring and 0.46 – 0.71 mS cm^{-1} for summer honeys (Table S-II). Autumn honey samples (AU1–AU3) and one spring honey (SP5) possessed higher values than 0.8 mS cm^{-1} . Additionally, many reports published the electrical conductivity $>0.8 \text{ mS cm}^{-1}$ for several different type of honey such as *Lotus* or *Eucalyptus* honey (0.81 and 0.89 mS cm^{-1})²⁷ or heather and chestnut honey.²⁶ Furthermore, higher electrical conductivity was already reported by other authors for autumn Maltese honey (0.96 – 1.89 mS cm^{-1}),⁷ as well as for Gozitan honey (0.88 mS cm^{-1}).^{28,29} These authors stated high soil and atmospheric salinity on Maltese islands as a reason for higher electrical conductivity of their analysed samples. Apart from that, do Nascimento *et al.*⁶ reported the correlation of electrical conductivity with acidity, but for our data the average values of these parameters did not correlate (Table S-III). The parameter of acidity content refers to the free amino acid that occurs in honey, as well as to the existing fermentation of sugars into organic acid.³⁰ The obtained values of acidity for honey samples from three seasons (Table S-II) were in the line with requirement¹⁴ of $\leq 50 \text{ meq kg}^{-1}$, except for two summer samples (SU1 and SU4), with the values exceeded the recommended value of acidity (57.40 and $54.77 \text{ mmol kg}^{-1}$, respectively). The correlation between acidity and proline content was 0.97 (Table S-III), which suggests the contribution of proline to the honey acidity. The content of proline varies a lot between different types of

honey¹⁸ due to its protein dependence of which it mainly consists. The obtained values for proline were in the range from 373.0 to 928.5 mg kg⁻¹ and they differ through the seasons (Table S-II). The lowest value was noted for spring sample (373.0 mg kg⁻¹), but still, this value was higher than those other authors reported for Maltese honey⁷. Higher average value and the range of proline content were found in summer samples (Table S-II). Comparing to the results of proline content published by other authors, for *Eucalyptus* honey was noted similar^{10,26} or even higher amount.¹⁰ Determined values for HMF were in accordance with permitted levels¹⁴ of ≤ 40 mg kg⁻¹, indicated the appropriate storage of samples. Nevertheless, our values were notably lower, ranging from 0.89 to 15.61 mg kg⁻¹, which differed the most in the spring samples. It was noticeable that HMF content positively correlated with the moisture content (0.99) and contrariwise, negatively with the Brix content (-0.99, Table S-III). The obtained values were similar to those reported for *Eucalyptus* honey²⁶ and smaller than other reported for Maltese honey.^{7,28} Diastase activity indicates the catalysis of sugars by an enzyme. The obtained range for diastase activity was from 4.04 to 12.62 Schade units, and the lowest values exhibited in spring honey samples (Table S-II). The recommended values are ≥ 8 Schade units. Moreover, the obtained lower values of diastase activity that we noted for one autumn sample, four spring samples and one summer honey (AU2, SP2-SP5 and SU2, Table S-II) followed the EU Directive's statement for honey with low natural enzyme content such as Citrus honey.¹⁴ In addition, the diastase values of ≥ 3 Schade units are also permitted¹⁴ when the honey samples have HMF values lower than 15 mg kg⁻¹. In accordance with that, Serrano *et al.*²⁶ reported low values of diastase activity for some *Eucalyptus* and *Citrus* honey (1.47 and 5.94 Schade units, respectively) and low HMF content (0.96 and 1.10 mg kg⁻¹, respectively). Similar amount of diastase activity in some Maltese honey was reported by Attard *et al.*⁷ Do Nascimento *et al.*⁶ also reported differences between harvesting seasons, as they obtained lower values of diastase activity with HMF content between 3-15 mg kg⁻¹ for some polyfloral and Quitoco (*Pluchea Sagittali*) honey samples. In our study, there was an exception of these observations, noted for summer honey SU2 (Table S-II). In this sample, the low value of diastase activity (6.33 Schade units) was found, but HMF was above 15 mg kg⁻¹, which was not high enough to be considered as significant.

Finally, the physicochemical analysis indicates higher moisture content, pH values and conductivity for autumn samples, higher proline content for summer samples, and low values of proline and distase activity for spring samples. However, physicochemical parameters also differ within the seasons, so seasonal differences for these honey samples can be defined in more detail with PCA.

Sugar profile of Maltese honey samples

With HPAEC/PAD 15 sugars were quantified (three monosaccharides, seven disaccharides and five trisaccharides) in analysed Maltese honey samples (Table S-II). According to the following criteria of sugar compositions, the obtained results for fructose and glucose, as well as sucrose content in analysed honey samples were appropriate.¹⁴ The sugars analysis showed that the total amount of the quantified sugars was in the range 64.16 to 80.53 % (Table S-II). The total amount of disaccharides, trisaccharides as well as many other individual sugars such as sucrose, maltose, isomaltose, turanose, gentobiose, melezitose, raffinose were higher in the spring than in the summer samples. An exception of these observations were values for isomaltotriose, which were the highest in the autumn samples AU2 and AU3 (0.98 and 0.90 g kg⁻¹, respectively), Table S-II. The sucrose concentration differs with the season, and it was in correlation with the obtained Brix values for different seasons. Contrary to these results, Attard *et al.*⁷ found lower sucrose content for Maltese spring honey (1.69) than we noted in this present study. Mahmoudi *et al.*⁵ obtained different results from ours, as their summer honey showed higher values for sucrose content (5.51 %) than those obtained for autumn and spring samples (3.78 and 3.4 %, respectively). Pita-Calvo *et al.*³¹ reported that trisaccharides could be useful to differentiate honeydew honey from floral honey. In our study, the highest concentration of melezitose was found in sample SP2 (3.84 g kg⁻¹, Table S-II), which exhibited the predominant *Lotus* pollen. The next high amount of melezitose was found in sample SP5 (3.75 g kg⁻¹) that contained dominant *Lotus* and *Reseda* pollen (Table S-II). Attard *et al.*⁷ obtained different results, as they found melezitose only in some autumn samples, but not in spring and summer honey. However, the content of other trisaccharide, such as raffinose and maltotriose, was higher in summer samples (Table S-II). In general, the content of trisaccharides does not indicate a higher amount of honeydew honey in autumn samples, as might be expected due to the tree-related nectars.⁷ Similarly, notably higher content of oligosaccharides was observed in study that summarized results for *Eucalyptus* honey.¹⁰

Quantification of phenolic compounds in Maltese honey samples

UHPLC–DAD MS/MS technique was performed for the quantification of 31 phenolic compounds, of which 11 phenolic acids and 20 flavonoids with their derivatives (Table S-IV of the Supplementary material). Phenolic acids such as *p*-hydroxybenzoic acid, caffeic acid, vanillic acid, syringic acid, *p*-coumaric acid and cinnamic acid, have been found in all studied samples. Considering flavonoids, all samples contained luteolin, quercetin, naringenin, kaempferol, and chrysin. The highest content, among all quantified phenolic acids, has been found for *p*-coumaric acid, 6.04 mg kg⁻¹, in one of spring honey samples (SP3). In

spring samples, the presence of chlorogenic acid was found in two samples (SP2 and SP5), while there was the absence of *p*-hydroxyphenylacetic acid in them. Catechin was quantified only in one spring honey (No. 7) in the amount of 0.14 mg kg⁻¹. Protocatechuic acid was found only in autumn honey samples with the values of 0.02 and 0.05 mg kg⁻¹. Do Nascimento *et al.*⁶ noted no presence of this phenolic compounds in spring and summer *Eucalyptus* honey samples from the same site of collection, neither in polyfloral honey, but it was present in autumn *Eucalyptus* honey from other sampling sites. Our autumn samples possessed higher values of ferulic acid and ellagic acid than spring and summer honey. Similar amount for ferulic acid was found in honey obtained from Chaste tree.²⁵ Luteolin 7-*O*-glucoside was found in all three autumn honey samples (AU1–AU3) in the range from 0.69–2.08 mg kg⁻¹, and in one spring sample (SP5) in lower amount, without its presence in summer samples (Table SI-V of the Supplementary material). Conversely, apigenin 7-*O*-apioglucoside was found only in summer samples. Summer honey samples possessed higher values of vanilic acid and syringic acid, than those found in spring samples, but still being lower than what was previously found in various honey types²⁵. Furthermore, the highest values of many other phenolic compounds such as *p*-hydroxybenzoic acid, aesculetin, luteolin, baicalein, genistein, kaempferol, amentoflavone, chrysin, pinocembrin and galangin were found in summer sample SU2 (Table S-IV).

PCA performed on determined parameters

Descriptive statistics provided some general information concerning phenolic content, sugar profiles and physicochemical parameters, but independently they cannot be considered as specific parameters that would enable decisive characterization of seasonal variability of honey. In fact, PCA was applied to differentiate groups of Maltese honey collected at different seasons.

As far as the determination of botanical origin of honey, physicochemical parameters mainly gave a substantially better classification when applied alone, while all other analytical parameters should be combined to evaluate better the results.³⁰ The identification of biomarkers among sugar compounds is mainly disabled by high variability of sugar composition among honeys of the same botanical species, but from different geographical locations, and by small differences in sugar profile among various types from the same region.³² Additionally, due to the complex nature of honey that contains vast number of compounds, one class of compounds present in small quantities, such as phenolic compounds, are difficult to use for differentiation and classification of groups of particular origin.

Grouping tendency of samples produced in summer was imposed by free acidity, diastase activity and proline content, differentiation of autumn honey is determined by moisture, electrical conductivity and HMF content, while spring samples have the lowest value of all physicochemical parameters (Fig. 1A). In

the case of PCA performed on phenolic and sugar data samples were grouped according to seasonal variability, similar to that obtained for physicochemical parameters, indicating specific chemical profile of samples depending on time of production. The compounds specific for autumn honey were melibiose, quercetin 3-*O*-galactoside, ferulic acid, ellagic acid, protocatechic acid, luteolin 7-*O*-glucoside (Fig. 1B). Samples harvested in spring contained higher amount of maltose, *p*-coumaric acid, melezitose, turanose, raffinose, genistein, catechin. Summer honey is specific with pinocembrin, galangin, genistein, chrysin, *p*-hydroxybenzoic acid, vanillic acid, maltotriose, kaempferol and caffeic acid.

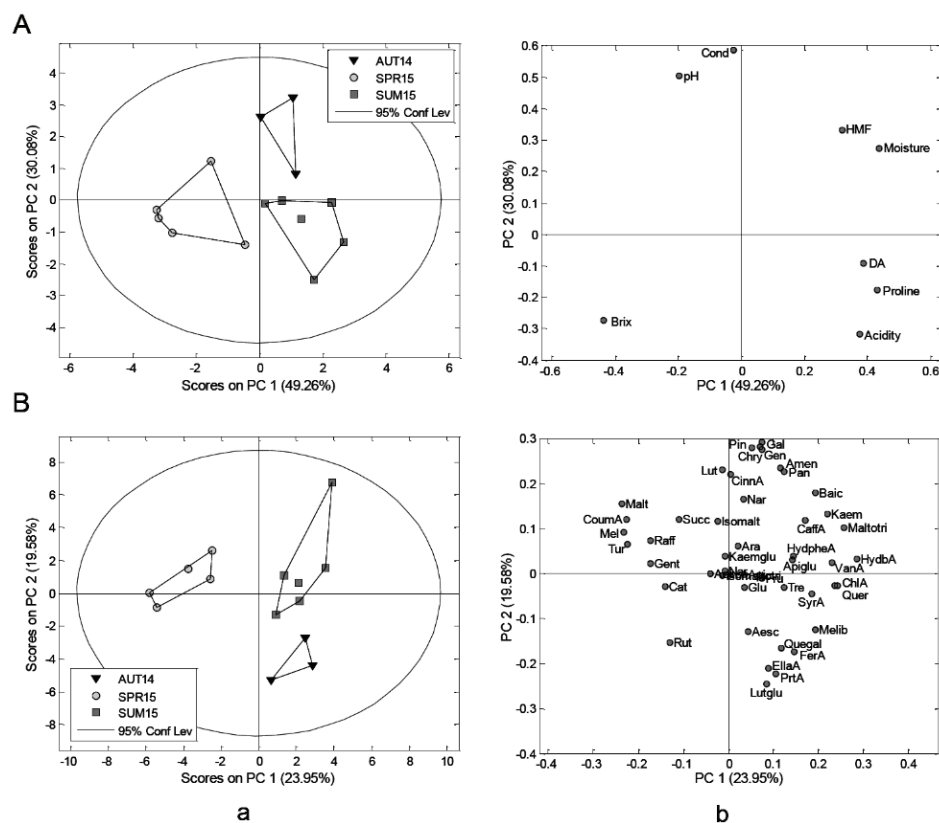


Fig. 1. PCA model based on: A) physicochemical parameters and B) phenolic and sugar content, for Maltese honey collected at different seasons, a) scores plots, b) loading plots.

CONCLUSION

The presented study provides a physicochemical analysis of Maltese honey, with emphasis on their seasonal comparison. Based on mellissopalynological analysis, *Lotus* pollen was predominant in honey samples, followed by *Eucalyptus*. The botanical origin of honey samples was diverse as it was accompanied

by other pollen types, of which most species were from Fabaceae and Rosaceae family. From the physicochemical parameters, it was noted that all samples showed a low content of diastase activity, with also a low content of HMF. In addition, four honey samples, (three samples from the autumn season and one from spring) were characterized by electrical conductivity higher than 0.8 mS cm^{-1} . The honey samples from summer season were differentiated by a higher content of diastase activity, proline, acidity, as well as monosaccharides, while spring samples were characterized by a higher content of disaccharides, trisaccharides, as well as individual sugars. There was the specificity of appearances of several phenolic compounds, such as the presence of protocatechuic acid only in autumn samples, then apigenin 7-*O*-apioglucoside only in summer samples, and catechin only in one spring sample. Furthermore, there was no occurring of *p*-hydroxyphenylacetic acid in spring samples and luteolin 7-*O*-glucoside in summer samples. All these differentiations were confirmed by PCA. We should emphasize that these are preliminary results, as we have small number of samples, particularly from autumn.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/11481>, or from the corresponding author on request.

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ИЗВОД

МЕЛИСОПАЛИНОЛОШКА АНАЛИЗА, ОДРЕЂИВАЊЕ ФИЗИЧКО-ХЕМИЈСКИХ ПАРАМЕТАРА, САДРЖАЈА ШЕЋЕРА И ФЕНОЛА У МАЛТЕШКОМ МЕДУ САКУПЉЕНОМ У РАЗЛИЧИТИМ ГОДИШЊИМ ДОБИМА

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Малта, земља позната по свом меду, није много помињана у радовима који се баве испитивањем меда. Поред многих параметара, период сакупљања меда утиче на квалитет меда, управо због различите цветне флоре која постоји током одређене сезоне. Стога се значај ове студије односи на пружање података о меду са Малте прикупљеног

током јесење, пролећне и летње сезоне. Мелисопалинолошка анализа, одређивање физикохемијских параметара и примена аналитичких хроматографских метода омогућили су детаљну анализу меда са ових простора. Анализа главних компоненти (РСА) омогућила је диференцијацију малтешког меда у зависности од сезоне прикупљања. У свим узорцима меда је доминирао *Lotus* полен, а затим *Eucalyptus* полен. Карактеристична једињења за летњи мед била су пиноцембрин, галангин, кемпферол, хрисин, *p*-хидроксибензоева киселина, ванилинска киселина и малтотриоза, док су кверцетин 3-*O*-галактозид, ферулинска киселина, елагинска киселина, протокатехуинска киселина, лутеолин 7-*O*-глукозид и мелибиоза били специфични за јесењи мед. У пролећним узорцима нађена је већа количина *p*-кумаринске киселине, генистеина, катехина, као и многих шећера. Према нашим сазнањима, ово је први научни рад који се бави детаљном хемијском анализом малтешког меда.

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