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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

IDENTIFICATION OF THE BOTANICAL ORIGIN AND DETERMINATION OF PHYSICOCHEMICAL PARAMETERS OF HONEY SAMPLES COLLECTED FROM THE SARY-CHELEK BIOSPHERE RESERVE OF KYRGYZSTAN

Kırgızistan'ın Sarı-Çelek Biyosfer Rezervinden Toplanan Bal Örneklerinin Botanik Kökeninin ve Fizikokimyasal Parametrelerinin Belirlenmesi

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ABSTRACT

Sary-Chelek Biosphere Reserve is one of the richest rare flora and animal reserves in Kyrgyzstan. The natural environment of the Sary-Chelek Biosphere Reserve is exceptionally favourable to high-quality honey production. Botanical authenticity is the main factor influencing honey prices, as it has a direct impact on the quality of honey. Producing monofloral honey in a natural landscape is challenging because, throughout the beekeeping season, multiple melliferous plants bloom at the same time. The article describes for the first time the botanical origin and physicochemical characteristics of honey collected in this biosphere reserve and also determines the influence of the dominant honey plant on organoleptic, physicochemical, and food safety indicators. Pollen grains of 50 taxa of nectar sources and about 10 taxa of pollen sources were identified. There were unique plant pollens in the samples; nine of the samples were monofloral honey—three of sage (*Salvia officinalis* L.), four of thyme (*Thymus vulgaris* L.), and two of eremurus (*Eremurus fuscus* O.Fedtsch), and eight samples were polyfloral honey. High diastase activity was found in samples of thyme honey (41.1 ± 2.9 Gothe) and in samples of sage honey (31.3 ± 2.2 Gothe). The physicochemical and food safety parameters of the honey samples comply with the established norms and international standards.

Keywords: Melliferous plants, Honey, Nectar, Pollen analysis, Quality

ÖZ

Sary-Chelek Biyosfer Rezervi, Kırgızistan'daki en zengin nadir bitki örtüsü ve hayvan rezervlerinden biridir. Sary-Chelek Biyosfer Rezervi'nin doğal ortamı, yüksek kaliteli bal üretimi için son derece elverişlidir. Botanik özgünlük, balın kalitesi üzerinde doğrudan bir etkiye sahip olduğu için bal fiyatlarını etkileyen ana faktördür. Doğal bir peyzajda tek çiçekli bal üretmek zordur çünkü arıcılık sezonu boyunca birden fazla melez bitki aynı anda çiçek açar. Bu makale, bu biyosfer rezervinde toplanan balın botanik kökenini ve fizikokimyasal özelliklerini ilk kez tanımlamakta ve ayrıca baskın

bal bitkisinin organoleptik, fizikokimyasal ve gıda güvenliği göstergeleri üzerindeki etkisini belirlemektedir. Nektar kaynaklarının 50 taksonuna ait polen taneleri ve polen kaynaklarının yaklaşık 10 taksonu tanımlanmıştır. Örneklerde benzersiz bitki polenleri vardı; örneklerin dokuzu monofloral baldı - üçü adaçayı (Salvia officinalis L.), dördü kekik (Thymus vulgaris L.) ve ikisi eremurus (Eremurus fuscus O. Fedtsch) ve sekiz örnek polifloral baldı. Yüksek diastaz aktivitesi kekik balı (41.1 ± 2.9 Gothe) ve adaçayı balı (31.3 ± 2.2 Gothe) örneklerinde bulunmuştur. Bal örneklerinin fizikokimyasal ve gıda güvenliği parametreleri belirlenmiş normlara ve uluslararası standartlara uygundur.

Anahtar kelimeler: Melez bitkiler, Bal, Nektar, Polen analizi, Kalite

GENİŞLETİLMİŞ ÖZET

Amaç: Toktogul, Sary-Chelek, Kara-Shoro ve At-Bashy, Kırgızistan'da bal üretimi için en önemli dağlık bölgelerdir. Sarı-Çelek Biyosfer Rezervi, Kırgızistan'daki en zengin nadir flora ve hayvan rezervlerinden biridir. Sarı-Çelek biyosfer rezervinin doğal ortamı, yüksek kaliteli bal üretimi için son derece elverişlidir. Doğal bir manzarada tek çiçekli bal üretmek zordur çünkü arıcılık sezonu boyunca birden fazla melez bitki aynı anda çiçek açar. Botanik özgünlük, balın kalitesi üzerinde doğrudan bir etkiye sahip olduğu için bal fiyatlarını etkileyen ana faktördür. Bu bölgeden gelen balın botanik kökeni şimdiye kadar hiç araştırılmamıştır. Kırgızistan'daki Sary-Chelek Biyosfer Rezervi'nden elde edilen dağ balının fizikokimyasal özelliklerini ve botanik kökenini detaylandıran bilimsel çalışmaların eksik olduğu göz önüne alındığında, bu çalışma "SaryChelek" Biyosfer Rezervi'nden toplanan balın botanik kökenini belirlemeyi ve fizikokimyasal parametrelerini belgelemeyi amaçlamaktadır.

Gereç ve Yöntemler: Sarı-Çelek Biyosfer Rezervi'nden toplanan toplam 17 bal örneği üzerinde çalışılmıştır. Polen analizi Uluslararası Arı Botanik Komisyonu tarafından belirlenen yönteme göre yapılmıştır. Tanımlayıcı niteliksel yöntem ISO standartlarına göre uygulanmıştır (ISO 5492, 2008; ISO 6658, 2005). Bu yöntem görünüş, kıvam, renk, koku, aroma ve lezzet gibi tüm tanımlayıcıları dikkate almaktadır. Nem, serbest asitlik ve diastaz aktivitesi gibi kalite parametrelerinin analizi, Avrupa Bal Komisyonu'nun Uyumlaştırılmış Yöntemlerine karşılık gelen GOST 19792-2017'ye (Bal Doğal. Teknik Koşullar) göre yapılmıştır.

Bulgular ve tartışma: Polen analizi, bu bölge için karakteristik melez bitki türlerini belirlememizi sağlamıştır. Bal örneklerinde 50 takson (nektar kaynağı) ve yaklaşık 10 takson (polen kaynağı) tespit edilmiştir. Ana polen taneleri 10 familyaya aittir: Apiaceae Lindl, Asteraceae Bercht. & J.Presl, Campanulaceae Juss., Hypericaceae Juss., Rosaceae Juss., Lamiaceae L., Liliaceae Juss., Fabaceae Lindl. Boraginaceae Juss. ve Plantaginaceae Juss. Polen analizi sonuçları, bal hasadı dönemi boyunca arılık çevresinde çiçekli bitkilerin bolluğuna rağmen arıların çoğunlukla bir (birincil) veya iki (ikincil) çiçek türünden topladığını göstermektedir. Örneklerin sekizi monofloral baldır - üçü adaçayı (Salvia officinalis L.), dördü kekik (Thymus vulgaris L.) ve ikisi eremurus (Eremurus fuscus O. Fedtsch) - ve geri kalanı rezervin farklı yerlerinden toplanan Rosaceae ve Apiaceae familyalarının polifloral polenleridir. Fizikokimyasal parametreler tüm uluslararası ve ulusal normları ve gereklilikleri karşılamaktadır. Bal örneklerinde pestisit tespit edilmemiştir. Elde edilen sonuçlar, Kırgızistan'da üretilen balın botanik ve coğrafi kökeninin belirlenmesine önemli bir katkı sağlamaktadır.

Sonuç: Sarı-Çelek rezervinden farklı bölgelerinden toplanan 17 bal örneğinin polen analizi ve bu bölgeler özgü bitki türlerinin berlilenmesi sağlanmıştır. Nektar toplama döneminin tamamı boyunca arılık çevresinde çiçekli bitkilerin bol olması polifloral balların toplanmasını olanak sağladığı gibi adaçayı, kekik, eremurus gibi bazı değerli ve nadir monofloral balların toplanması mümkün olmaktadır. Eremurus balının organoleptik ve fizikokimyasal paremetreleri ilk kez açıklanmıştır. Öte yandan araştırma, korunan alanlarda arıcılığın avantajlarını ortaya koymaktadır. Elde edilen sonuçlar Kırgizistan'da üretilen balların botanik ve coğrafik kökeninin belirlenmesine önemli katkı sağlayacak ve adaçayı, kekik, ve eremurus gibi monofloral ballara yönelik standartların oluşturulmasında kullanılabilir.

INTRODUCTION

Honey varieties that are most renowned and widely consumed in Kyrgyzstan are sourced from mountainous regions, including Toktogul, Sary-Chelek, Kara-Shoro, and At-Bashy. The Sary-

Chelek Biosphere Reserve is one of five natural reserves located in the western Tien Shan mountains in Kyrgyzstan (Fig.1). Lake Sary-Chelek (1873 m a.s.l.) and the surrounding 238.33 km^2 area were established as a protected area in 1959 and granted Biosphere Reserve status by UNESCO in 1979. The Sary-Chelek Biosphere Reserve is one of the richest rare flora and animal reserves in Kyrgyzstan, habitats in the reserve include forest, meadow, steppe, rock escarpments, and some aquatic systems (Lake Sary-Chelek, Iiri-Köl, Kyla-Köl) (Cantarello et al. 2014). The reserve occupies a mountain basin in the range of altitudes from 1200 to 4247 m a.s.l. (Umurakov et al. 1987, Duisenov et al. 2016). The territory of the reserve includes landscapes of the following altitudinal belts: low-altitude forest (1200–1800 m), middle-altitude forest-meadow (from 1800 to 2600–2700 m), and high-altitude meadow (2700–4000 m) (Umurakov et al. 1987). The reserve's area successfully integrates the distinctive and typical elements of southern Kyrgyzstan's natural landscape, including rare flora and animals, multi-age landscapes and plant communities. The uniqueness of the landscapes is due to the combination of walnut and coniferous forests with subalpine and alpine meadows, shrub formations and steppes. The seasons are quite distinct; precipitation falls mainly in the form of snow and is accompanied by a noticeable drop in temperature. In the second half of April, frosts stop, and vegetation begins to grow actively. The best time for plant development is during the first half of summer, from late May to mid-July, when the optimal temperature and moisture levels are present (Ionov and Lebedeva 2002, Umurakov et al. 1987).

The natural environment of the Sary-Chelek biosphere reserve is exceptionally conducive to honey production. The flora is composed of plants belonging to 63 families and 378 genera (Ionov and Lebedeva 2002). In terms of species richness, the families of *Asteraceae* (115 species), *Poaceae* (105) and *Fabaceae* (89) are distinguished. Diversely represented plants belong to the families *Lamiaceae* (69), *Rosaceae* (68) and *Cruciferae* (61 species), and contain many fodder plants (180 species), melliferous plants (80) and medicinal plants (48 species) (Duisenov and Aitmatova, 2016).

Figure 1. A: Map of Kyrgyzstan and location of Sary-Chelek Biosphere Reserve total area: 238.33 km²; B: location of apiaries (UNEP-WCMC, 2024)

One of the main plants in the Sary-Chelek nature reserve is the walnut (*Juglans regia* L.) (1574 ha). Along with walnuts, apple trees (*Malus* Mill.), hawthorn (*Crataegus* L.), maple (*Acer*), barberry (*Berberis* L.)-honeysuckle (*Caprifoliaceae* Juss.) and other species also flourish in the forest. Along with walnut forests, local apple forests are concentrated in the southern part of the reserve and occupy about

400 hectares. Small massifs of pear forests (about 2 ha) are preserved in the tracts of Kelte-Sai. In the rich flora of Sary-Chelek, there are many endemic and rare species, with a narrow range of species listed in the Red Book. Above all, the Schrenk's spruce (*Picea schrenkiana* Fisch. & C.A. Mey.) is under special protection. *Eremurus* plants spread on stony-rubble soils of river terraces, and large eremurus *(Eremurus robustus* Regel*)* reaches a height of more than 1 m (Ionov and Lebedeva, 2002). A total of 13 species of plants and 15 species of animals are registered in the Red Book of the Kyrgyz Republic (Duisenov and Aitmatova, 2016).

Intensive use of the walnut-fruit forests has led to concerns that unsustainable patterns of land use have been a major cause of forest loss and degradation (Cantarello et al. 2014). Sustainable use and conservation management of protected areas is therefore very important. Biosphere Reserves are 'learning places for sustainable development' (UNESCO, 2022). Beekeeping has positive environmental impacts and is one of form of sustainable land use. Pollination is essential for the wild and cultivated flora and is performed by honeybees in the process of nectar collection. This process promotes biodiversity by facilitating the flourishing of diverse plant species and preserving ecological equilibrium.

Botanical authenticity is the main factor influencing honey prices, as it has a direct impact on the quality of honey. Moreover, honey's origin must be clearly stated on the label, and quality schemes are prescribed based on its geographical and botanical origin (Tsagkaris et al., 2021). The consistency of honey also depend on the botanical origin of honey (Smanalieva and Senge, 2009). In Europe, 117 species of monofloral honey have been described, of which 111 are floral and 6 are honeydew, including 15 species that have commercial value. Detailed characteristics are given for the following types of monofloral honey: *Thymus, Tilia* L., *Taraxacum* F.H. Wigg*., Rosmarinus* L*., Robinia pseudoacacia* L*., Rhododendron* L*., Lavandula* L*., Citrus* L. etc. (Oddo et al., 2004).

Producing monofloral honey in a natural landscape is challenging because, throughout the beekeeping season, multiple melliferous plants bloom at the same time. Despite this, recent studies of the botanical origin of honey samples collected in various regions of the Kyrgyz Republic in 2018 and 2019 have revealed the following monofloral honey varieties: sainfoin (*Onobrychis* Mill.), thyme (*Thymus*), eremurus (*Eremurus*), blueweed (*Echium* L.), forget-me-not (*Myosotis* L.), sage (*Salvia*), dandelion (*Taraxacum* F. H. Wigg.), cotton (*Gossypium* L.), toadflax (*Linaria* Mill.), and apple (*Malus*) (Ishenbaeva et al., 2021). Mountain honey from the Sary-Chelek Biosphere Reserve in Kyrgyzstan lacks scientific studies detailing its physicochemical characteristics and botanical origin. It should be noted that earlier research on the identification of melliferous plants from the Sary-Chelek Biosphere Reserve has been evaluated based on botanical expeditions but not through palynological analysis. Therefore, our research question was: Which melliferous plants (honey and pollen plants) in Sary-Chelek are the most important for beekeeping? Thus, the purpose of this research is to identify the botanical origins of melliferous plants and assess their potential for beekeeping, thereby effectively using the bee forage base and contributing to an increase in honey quality. The next objective was to determine the physicochemical parameters and describe the sensorial properties of honey collected from the Sary-Chelek Biosphere Reserve for detailed characteristics.

MATERIALS AND METHODS

Honey samples

About 17 honey samples were obtained directly from beekeepers of transition zone the Sary-Chelek Biosphere Reserve (Arkyt village) (latitude: 41.89 north longitude: 71.95 east) and buffer zones (latitude: 41.85 N, 72.00 E), collected in 2019, 2020 and 2021 (Table 1). Also, samples were collected from villages around the protected area. All investigated honey samples were stored in a refrigerator at a temperature of 4 °C until the analysis. Analysis of the samples was carried out in 2019–2021.

Melissopalynological analysis

Pollen analysis was conducted according to a method established by the International Commission of Bee Botany. The method consists of the preparation of microscopic preparations with a fixed amount of honey, followed by the identification and counting of real pollen grains (Louveaux et al. 1978, GOST 31766-2012, Von der Ohe et al. 2004). In particular, 10 g of honey was dissolved in 20 mL of water, and the resulting solution is centrifuged at 1000 rpm for 10 min. The supernatant was carefully

drained, and 20 mL of distilled water was added to the precipitate and stirred. The suspension was centrifuged for 5 min at 1000 rpm. The supernatant was decanted, and the centrifuge tube was placed on filter paper at an angle of 45° to remove residual liquid. The precipitate was thoroughly mixed with a microbiological loop, transferred to a slide, and covered with a coverslip. Pollen grains were identified using a microscope (XS-208A) at 100–400×magnification. For each pollen type, the fraction was calculated according to equation (1). The frequency of the occurrence of pollen grains in a particular plant species was calculated according to the following formula:

$$Xp = \frac{A}{n} \cdot 100 \quad (1)$$

where A is the number of pollen grains of a single species in all counting fields; n is the total number of pollen grains counted in all counting fields (about 500–800 pollen were counter in each sample); and 100 is the conversion coefficient of relative fractions into percent. Thus, the pollen taxa were determined according to Burmistrov et al. (1990), Kurmanov & Ishbirdin (2013) and Moore and Webb (1991).

Table 1. List of honey samples from Sary-Chelek Biosphere Reserve and their botanical origins according to melissopalynology

No	Botanical origin	Location	Location coordinates of apiaries	Collecting year
1	Sage honey	Avletim, Rajon Aksy, Kirgisistan	41°38′0″N 71°56′0″E	2019
2	Polyfloral honey	Arkyt, Rajon Aksy, Kirgisistan	41°48′20″N 71°57′30″E	2019
3	Polyfloral honey	Bioshere Reserve area Rajon Aksy, Kirgisistan	41°53′24″ N,71° 57′ 36″E	2019
4	Polyfloral honey	Bioshere Reserve area Rajon Aksy, Kirgisistan	41°53′24″ N, 71° 57′ 36″E	2019
5	Thyme honey	Bioshere Reserve area Rajon Aksy, Kirgisistan	41°53′24″ N, 71° 57′ 36″E	2019
6	Eremurus honey	Bioshere Reserve area Rajon Aksy, Kirgisistan	41°53′24″ N, 71° 57′ 36″E	2019
7	Polyfloral honey with forget-me-not	Arkyt, Rajon Aksy, Kirgisistan	41°48′20″N, 71°57′30″E	2020
8	Polyfloral honey with sage	Bioshere Reserve area Rajon Aksy, Kirgisistan	41°53′24″ N, 71° 57′ 36″E	2020
9	Thyme honey	Bioshere Reserve area Rajon Aksy, Kirgisistan	41°53′24″ N, 71° 57′ 36″E	2020
10	Sage honey	Avletim, Rajon Aksy, Kirgisistan	41°′24″ N, 71°85 57′ 36″E	2020
11	Sage honey	Bioshere Reserve area, Kyla-Köl, Rajon Aksy, Kirgisistan	41°85′23″ N, 71° 99′ 88″E	2020

12	Eremurus honey	Bioshere Reserve area Kyla-Köl, Rajon Aksy, Kirgisistan	41°85′23″ N, 71° 99′ 88″E	2021
13	Thyme honey	Bioshere Reserve area Kyla-Köl, Rajon Aksy, Kirgisistan	41°85′23″ N, 71° 99′ 88″E	2021
14	Thyme honey	Bioshere Reserve area Rajon Aksy, Kirgisistan	41°53′24″ N, 71°57′ 36″E	2021
15	Polyfloral honey	Arkyt, Rajon Aksy, Kirgisistan	41°48′20″N 71°57′30″E	2021
16	Polyfloral honey	Bioshere Reserve area, Iiri Köl, Rajon Aksy, Kirgisistan	41°85′23″ N, 72° 00′ 63″E	2021
17	Polyfloral honey	Bioshere Reserve area, Iiri Köl, Rajon Aksy, Kirgisistan	41°85′23″ N, 72° 00′ 63″E	2021

According to the international melissopalynological nomenclature, dominant pollen in honey is defined as pollen occurring more than 45% of the total count; accompanying or secondary pollen are pollen occurring in the range 16–45%; important minor pollen are pollen occurring in the range 3–15%; and minor pollen are <3%. It should be noted that many pollen types, such as *Robinia pseudoacacia* L., *Citrus* L., *Tilia* L., *Lavandula* L., *Rosmarinus* L. can be underrepresented in honey. For declaration as monofloral honey collected from this plant flowers, the minimum percentage of the taxon that gives the honey the name should be 15% for acacia and 10% for citrus. Also, pollen from plants such as *Brassica napus* L., *Castanea sativa* Mill., and *Eucalyptus* L'Her. can be overrepresented in honey. For classify honey as monofloral chestnut and eucalyptus honey is required over 70–90% pollen from *Castanea sativa* and *Eucalyptus* and 60-80% rape (Brassica napus L.) pollen grains (Thrasyvoulou et al. 2018). If the ratio "Honeydew Elements/Pollen Grains (HDE/PG)" exceeds 3, the sample is considered honeydew (Louveaux 1978).

Sensorial analysis

The sensory analysis was applied according to ISO standards (ISO 5492, 2008; ISO 6658, 2005). This method takes into consideration all the sensory descriptors, such as appearance, consistency, colour, odor, taste: aroma and flavor. The samples were evaluated by 7 trained panellists. For the sensory analysis, a honey sample of 30–50 g in glass jars was used. First, honey samples were analysed visually. We assessed the appearance, physical condition/consistency, and color range of honey samples. Colour intensity was described using a honey color chart and descriptors of normal honey color: water white, extra white, white, extra light amber, light amber, amber, dark amber. During the visual analysis, attention was paid to the purity and transparency of honey, homogeneity, and the presence or absence of any inclusions. The odour has to be evaluated both immediately after swirling the honey and after 10 or 20 s. For the description of odour, the following descriptors were used: woody, chemical, fresh, floral, fresh fruit, warm, spoiled, and vegetable. Then, to assess the mouth sensations, 1 or 2 g of honey is sampled with a stainless steel spoon. The honey is allowed to dissolve in the mouth before being slowly swallowed, so that the taste (sweet, salty, acidic, or bitter), the aroma (intensity and quality), the persistence, any aftertaste, and other mouth sensations can be perceived. The descriptors of the sensorial properties of honey were adopted from (Piana et al. 2004).

Physicochemical measurement

Analysis of quality parameters such as moisture, free acidity and diastase activity was conducted according to GOST 19792-2017 (Honey Natural. Technical Conditions), which corresponds to the Harmonised Methods of the European Honey Commission (Bogdanov et al. 1997). The refractive index of honey samples was measured using a refractometer (Abbe 2WAJ, Wincom Company Ltd., China) at 20°C. The corresponding moisture content

(%) was calculated using the relationship between refractive index and water content. The free acid content was measured in a 10% (w/w) honey solution by acid-base titration with 0.1 M NaOH up to pH 8.1 using a pH meter (AD8000, ADWA Instruments, Hungary), and the results were expressed as the milliequivalents per kilogram of honey (meq/kg) (Bogdanov et al. 1997).

The determination of diastase (α-amylase) activity was performed using a spectrophotometer (Spectrophotometer UV-1800, China) and expressed as diastase number in Gothe units (GOST 34232-2017). Diastase number is defined as the amount of enzyme that will convert 0.01 g of starch to the prescribed endpoint in 1 h at 40 °C under the conditions of the test (Bogdanov et al, 1997).

Indicators such as reducing sugars and sucrose, qualitative reactions to HMF, and electrical conductivity were carried out according to GOST 19792-2017, as well as some quality indicators such as tetracycline, chloramphenicol and pesticides, which were investigated according to the Technical Regulations of the Customs Union "On the Safety of Food Products" (TR CU 021, 2011).

Statistical analysis

Statistical analyses were performed using SPSS software version 16.0 (SPSS Inc., Chicago, IL). Comparative analysis of physicochemical parameters between samples was carried out using analysis of variance (ANOVA). Duncan's multiple test (DMRT) is a post hoc test that was used to measure specific differences between pairs of means at a significance level of $p \leq 0.05$. Pearson correlation coefficient analysis was performed to determine the relationship between the physicochemical characteristics of honey at a significance level of $p \leq 0.05$ and $p \leq 0.01$.

RESULTS

Melissopalynological analysis

Pollen grains from 60 taxa of nectar sources and about 10 taxa of pollen sources were identified. The main pollen grains belong to 10 families: *Apiaceae, Asteraceae, Campanulaceae, Hypericaceae, Rosaceae, Lamiaceae, Liliaceae, Fabaceae Boraginaceae, and Plantaginaceae*. Most of the identified species are confined to thermophilic slopes, streamside communities, agricultural lands and deposits.

As mentioned above honey can be declared a monofloral honey if the amount of pollen from one plant is more than 45% of the total amount (Louveaux et al. 1978). The exception is plants that intensively release pollen. Examples are chestnuts, rapeseed and forget-me-not flowers. By contrast, some plants produce less pollen; their nectarines and pollen petals are arranged in such a way that pollen cannot get into the nectar. Therefore, for honey from such plants, the number of “main” pollen grains is set significantly lower; for example, honey from false acacia should contain from 16% to 40% “main” pollen grains (Louveaux et al. 1978). According to the melissopalynological nomenclature, the minimum percentage of pollen required for the characterization of monofloral sage (*Salvia officinalis L.*) and thyme (*Thymus vulgaris* L.) honey must contain at least 15% pollen from these flowers (Thrasyvoulou et al. 2018). As for monofloral eremurus honey, the proper amount of pollen is unknown but probably must be at least 45%. Based on melissopalynological analysis, in investigated honey samples were identified blueweed (*Echium vulgare* L.), bellflowers (C*ampanula* L.), sainfoin (*Onobrychis*) and fruit trees (*Rosaceae)*—apple, plum, honeysuckle and others—as accompanying pollen and sources of nectar. Further, the investigated honey samples contained pollen from *carboniferous* plants that do not secrete nectar but give pollen: *Hypericum L., Sanguisorba officinalis* L*., Artemisia* L*., Plantago L., Poaceae* Barnhart*, Cannabis sativa L., Humulus* L*. and Galium verum* L. Pollen of the *Rosaceae, Amaryllidaceae (Allium* L.) and *Apiaceae* families was found in all honey collected from different locations in the Sary-Chelek reserve. Only nine honey samples were identified as monofloral, and eight samples were polyfloral honey. The results of pollen analysis for three monofloral honey and two polyfloral honey samples are presented in Table 2.

Organoleptic properties

Table 3 lists the sensorial parameters of the investigated samples. Visual analysis consists of three parameters: appearance, colour and consistency. The components of gustatory and olfactory analysis are odour, aroma, and flavour.

Table 2. The quantity and taxonomy of pollen in samples of honey collected in Sary-Chelek biosphere reserve

Honey samples	S	PH	PH	PH	TM	ERE	PH+ FMH	PH+S	TM	S	S	ERE	TM	TM	PH	PH	PH
Pollen types	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Allium sp.	0.7	4.5	4.5	3.2	1.9	2.5	0.3	4.8	1.2	4.2	4.2	1.4		4.2	4.8	1.8	3.1
Amoria hybrida	0.7													0.9	0.4		
Amoria repens	0.4						1.5	0.6	2.5		0.4	0.7		0.9			
Apiaceae	1.8	13.5	13.5	17.0	13.0	7.0		19.9	21.3	0.7	1.2	1.4	4.4	2.9	19.9	15.2	36.0
Apiaceae: Angelica sp.	2.9							8.4	4.2	12.9			22.0		8.4		0.9
Arctium sp.	0.4							0.6							0.4		1.5
Archangelica																	15.0
Artemisia sp.	0.4			1.0	12.0			0.6				0.7	1.9	2.9	0.4		0.6
Asteraceae	0.4				2.4						12.0		2.5				2.4
Berberis sp.													0.6				
Bistorta major	0.4															0.3	
Boraginácеae					5.3		4.0										
Brassicaceae	0.4				0.9		0.7		12.9								0.9
Galium sp.																1.8	
Campanula sp.	1.1	17.3	17.3					12.7							12.7	26.0	0.6
Cannabis sp.						1.0	1.5										
Caryophyllaceae	0.4	3.0								0.7							
Centaurea sp.	1.4			1.0			0.3	0.6			2.1	0.7	3.7		0.4	0.3	
Cirsium sp.				2.5	0.4			0.6			2.4				0.4		
Chaerophyllum sp.	1.4		3.0					3.0	1.2						3.0		
Chenopodiaceae				2.5			0.3										0.6
Cynoglossum sp.	1.4				2.4											1.8	
Conium maculatum								0.6				0.7		0.9	0.4		
Coriandrum sativum																	0.9
Convolvulus arvensis											1.2						
Echium vulgare	17.5	25.7	25.7	21.0	2.6			0.6	4.2	27.6		0.7	13.7	0.9	0.4	39.4	4.8
Eremurus fuscus				3.2	7.3	67.0	18.0	1.2	0.5		2.1	50.0	1.9	16.3	3.6	0.5	5.8
Euphorbia sp.					0.4		0.3										
Fabaceae					0.9	1.0	0.7			0.7			0.6				0.6
Fumaria sp.							0.7						0.6				
Galium sp.	1.1				0.4						0.8						1.5
Geranium sp.	0.4												0.6				
Heracleum sp.		1.5	1.5		2.9	3.9		1.2	0.5			0.7		4.2	1.3		0.9
Hedysarum sp.				1.0	0.4			0.6	1.2		2.0	1.4		0.9	0.4		
Hypericum sp.	49.4	7.4	7.4		1.4	8.5		7.8						5.3	7.8	1.8	0.6
Humulus sp.				3.2	2.9												
Lamiaceae				3.2	6.3		0.7				21.0	13.8	2.4	14.7			2.4
Lamium sp.					0.9												1.5
Lappula sp.					0.4		2.8										
Leonurus sp.	0.4	1.5	1.5					1.2		0.7				0.9	1.3	0.6	
Liliaceae					0.9		0.3				0.8		0.6			0.6	
Linaria vulgaris					0.9			0.6	0.5			0.7		0.9	0.4		
Lotus sp.	0.7									0.7	0.4		0.6				
Malus sp.				3.2	0.4		1.5	0.6					1.9	0.9	0.4		
Malvaceae	0.4																
Medicago sp.										0.7			0.6				
Mentha sp.	0.4										0.8		0.6				
Melilotus sp.		1.5	1.5				1.5	1.2	2.5	7.2		0.7	0.6	0.9	1.3	0.6	0.6
Myosotis sp.							47.0					0.7			1.3		
Nepeta sp.					2.4												
Onobrychis sp.	2.5	7.4	7.4	18.0	0.4	1.0		8.4	0.5	8.8	0.4	1.4		8.9	8.4	1.8	3.1
Oryganum vulgare		3.0	3.0		1.9			1.2	0.5		4.2		2.4	8.9	3.0	1.8	
Pedicularis sp.										0.7							
Pimpinella sp.				1.0				0.6	0.5			0.7			0.4		
Polygonum sp.	0.4										1.2						0.9
Poaceae							0.3	1.2	0.5								
Phacelia sp.					1.9												

Plantago sp.		1.5	1.5						0.5			1.4	0.6	0.9	1.3	0.6	0.6
Prunus sp.													2.4				
Ribes sp.														0.9			
Rosaceae		5.7	5.7	7.7	6.3	7.0	5.3	0.6	0.5		12.0	20.0	7.0	0.9	0.4	1.8	0.6
Rubus idaeus		4.5	4.5	2.5		1.0		3.6									
Salvia officinalis	9.4			3.2	5.3			9.0		19.9	21.0	1.4		0.9	9.0	0.6	0.6
Sanguisorba officinalis	1.8							6.0	0.5	5.9				5.1	7.8		3.1
Salix sp.										0.7							
Salidago sp.	1.4			1.0				0.6						0.9	0.4	0.3	
Sedum sp.							0.3										
Serratula sp.											0.4						
Sinapis sp.							1.5										0.9
Spiraea sp.													1.9				
Stachys sp.				2.5													
Taraxacum sp.													0.6				
Thymus vulgaris	0.4	0.7	0.7	1.0	8.7			0.6	37.0	0.7	6.3	0.7	18.3	18.9	0.4	1.8	3.1
Trifolium mediums					1.9		2.8			4.2			0.6			0.6	
Veronica officinalis L.					3.9		2.8						1.9				3.1
Vicia sp.								0.6		0.7					0.4		
Undetermined species	0.0	1.3	1.3	1.1	0.0	0.1	2.1	0.1	1.2	1.9	2.1	0.0	3.7				
Broken pollen grains	0.0	0.0	0.0		0.0	0.0	2.8		4.4	0.0	1.0	0.1	0.8				
	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

*PH-polyfloral honey

Physicochemical parameters and safety indicators

The physicochemical parameters and safety indicators of honey are shown in Table 4. The moisture content, diastase number, free acidity, reducing sugars, sucrose, qualitative reaction for HMF, and electrical conductivity of the studied honey samples meet the requirements of the International Standards and Technical Regulations of the Customs Union (Codex Alimentarius, 2001; GOST 1979–2017, "Natural Honey. Technical Conditions). Safety indicators in terms of antibiotics and organic pesticides also meet all norms and requirements of the Technical Regulations of the Customs Union and international standards.

Table 3. Results of organoleptic analysis for honey types from Sary-Chelek

Name of honey	Visual analysis			Olfactory and gustatory analysis			Mechanical impurities and signs of fermentation
	appearance	consistency	colour	odour	aroma	flavour	
Sage honey *(Salvia officinalis L) (n =3)*	homogeneous	liquid	Dark amber	floral	fragrant flowers	sweet, tart	absent
Polyfloral honey with sage *(Salvia officinalis L) (n=3)*	homogeneous	liquid	amber	floral	pronounced, strong	sweet	absent
Eremurus honey *(Eremurus fuscus O.Fedtsch) (n=2)*	turbid	beginning of crystallisation	Dark amber	vegetal	weak	sweet	absent
Polyfloral honey with Forget-me-not honey (*Myosotis sylvatica) (n=3)*	homogeneous	crystallized	Dark amber	vegetal	weak	sweet	absent
Thyme honey **(***Thymus vulgaris L*) (n=4)	homogeneous	creamy	amber	fruity	fragrant, strong	sweet	absent
Polyfloral honey with eremurus (*Eremurus fuscus O.Fedtsch)(n=2)*	homogeneous	beginningofcrystallisation	light yellow	fruity	pronounced	sweet	absent

Table 4. Physicochemical and food safety parameters of honey samples from the Sary-Chelek biosphere reserve of Kyrgyzstan

Physicochemical parameters	Sage honey (*Salvia officinalis*)	Polyfloral honey with sage (*Salvia officinalis. L*)	Eremurus honey (*Eremurus fuscus* O.Fedtsch)	Polyfloral honey with Forget-me-not honey (*Myosotis sylvatica*)	Thyme honey (*Thymus vulgaris L*)	Polyfloral honey with eremurus (*Eremurus fuscus O.Fedtsch*)	Norm
	(n = 3)	(n = 3)	(n=2)	(n = 3)	(n=4)	(n = 2)	
Moisture content, %	18.2±0.65[b]	16.6±0.5[a]	17.8±0.2[b]	18.5±0.7 [b]	18.6 ± 0.5[b]	17.5 ± 0.4 [a]	< 20
Diastase number, Gothe unit	31.3±1.8[d]	23.9±1.4[c]	14.3±0.3[b]	9.8 ±1.3[a]	41.1 ± 0.9[e]	15.3 ± 0.4[b]	> 8
Free acidity, meq/kg	22.4±1.3 [b]	26.2 ± 1.5 [c]	26.4 ±1.8 [c]	20.4 ±1.4 [a]	22.4 ± 0.8 [b]	24.0 ±1.0 [b]	< 40
Reducing sugars, (dw), %	84.3±0.7 [c]	81.8±0.3 [b]	80.6±1.5[a]	88.6±0.7[d]	80.9±1.2[a]	83.2±0.6 [c]	>60
Sucrose (dw), %	3.0±0.7	3.1±0.6	1.9±0.2	3.23±1.2	3.7±0.7	2.9±0.1	<5
Qualitative reaction for HMF	negative	negative	negative	negative	negative	negative	<40 meq/kg
Electrical conductivity, mS/cm	0.9±0.1[b]	0.5±0.1 [b]	0.2±0.0[a]	0.2±0.0[a]	0.8±0.1 [b]	0.2±0.0[a]	***
Antibiotics: Tetracycline, mg/kg	<0.0025	<0.0025	<0.0025	<0.0025	<0.0025	<0.0025	< 0.01
Chloramphenicol, mg/kg	*	*	*	*	*	*	not allowed
Organochlorine pesticides: sum of isomers of hexachlorocyclohexane, α, β, γ isomers, mg/kg	**	**	**	**	**	**	< 0.005
DDT (dichlorodiphenyltrichloroethane) and its metabolites 4,4-DDT; 4,4-DDE, mg/kg	**	**	**	**	**	**	< 0.005

Means ± standard deviations within a row with small superscripts differ significantly ($p < 0.05$);* below of detection limits <0.000025, ** Below detection limits 0.005; Norm -TR TS 021/2011 - Technical Regulations of the Customs Union. ***EC > 0.8 for honeydew honey and honeydew nectar mixtures, EC < 0.8 for nectar honey

Table 4 Pearson correlation coefficients of physicochemical parameters of honey samples

	Moisture content, %	Diastase number, Gothe unit	Free acidity, meq/kg	Reducing sugars (dw), %	Sucrose (dw), %	Electrical conductivity, mS/cm
Moisture content, %	1					
Diastase number, Gothe unit	.241	1				
Free acidity, meq/kg	-.422	-.012	1			
Reducing sugars, (dw), %	.353	**-.523***	**-.523***	1		
Sucrose (dw), %	.460	.394	-.047	.268	1	
Electrical conductivity, mS/cm	.144	**.657****	.214	-.289	**.688****	1

**. Correlation is significant at the 0.01 level (2-tailed), *. Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

Botanical origin and sensorial properties

In this section, the pollen content of five representative honey samples and their organoleptic characteristics are discussed. Nine of the samples were monofloral honey, three of sage, four of thyme, and two of eremurus, and eight samples were polyfloral honey. This information about the predominant nectar sources in the region will help beekeepers plan harvests to keep particularly good sources separate (Solak et al. 2023).

Pollen grains from 29 different taxa are identified in sample №1. The pollen grains of *Hypericum* were dominant. *Hypericum*, *Sanguisorba officinalis*, *Galium* and *Artemisia* are plants that do not produce nectar, and therefore, their amounts were excluded from the total amount of pollen. After recalculation, the part of *Echium vulgare* increased to 37% and *Salvia officinalis* to 15.4%. According to the definition of monofloral honey, the pollen of common *Echium vulgare* must represent not less than 45%, and in monofloral sage honey, *Salvia officinalis* pollen must consist of a minimum of 15%. Therefore, sample N1 can be attributed to monofloral sage honey.

The important sensory factors in assessing the quality of honey are colour and flavour, which also contribute to the definition of the honey's botanical origin. The colour of the sage honey was dark amber and honey has a fragrant odour, fragrant flowery aroma and sweet-tart flavour. The flavour is defined as “the complex combination of gustatory, olfactory and trigeminal sensations perceived during tasting”. Flavour could be affected by kinaesthetic, tactile, thermal and/or painful effects (ISO 5492, 2008). The physical characteristics, chemical composition, production technology, storage time, and temperature all affect the consistency of honey. Sage honey (sample N1) and polyfloral honey with sage (sample N8), were in a liquid state. That is in line with the literature, which states that sage honey rarely crystallises since it has a high content of fructose (Kenjerić et al. 2006).

The sample №8 contained pollen from 31 different taxa. Pollen grains of *Apiaceae (19.8%)*, *Campanula* sp. (12.7 %), and *Salvia officinalis (*9%) were dominant. Thus, this sample was attributed to polyfloral honey with sage. Polyfloral honey with sage has a pleasant taste and amber colour, while pure sage honey has a sweet-tart flavour.

Sample №6 was harvested in May, and in this sample was identified pollen from 10 taxa. *Eremurus* pollen was dominant, with an amount of 67%. The high percentage of *Eremurus* pollen allows us to judge that the honey is monofloral Eremurus honey. Pollen of *Hypericum*, *Apiaceae*, and *Rosaceae* were accompanying pollen. The distinctive aroma and deep/dark amber colour of this honey are its defining features (see Table 2). It should be noted that the leaves and roots of *Eremurus* are used in traditional medicine to treat disorders of the liver, stomach, constipation, and diabetes (Beiranvand & Beiranvand, 2022). One of the most important sensory factors in assessing the quality of honey is colour, which also contributes to the definition of the honey's botanical origin. The colour of the investigated samples ranged from light yellow to dark amber. Eremurus honey has a sweet, pleasant flavour and a pronounced, strong aroma with a floral smell. Eremurus honey and forget-me-not honey were beginning to crystallise.

Pollen from 32 taxa was identified in the sample №5. Pollen grains of *Thyme vulgaris*, *Apiaceae*, *Eremurus and Lamiaceae* prevailed. After the exclusion of pollen of pollen-bearing species *such as Artemisia*, *Hypericum*, *Galium*) from the calculations, the share of thyme increases up to 20 %. In sample № 9, 13, and 14 were found 37 %, 18 %, and 14 % *Thyme vulgaris* pollen. In European monofloral thyme honey, thyme pollen is less represented, e.g., at least 15%. Therefore, the studied sample can be attributed to monofloral thyme honey. In contrast to Greek samples, which display a highly variable pollen content of 40.2% ± 16.4, Italian honey samples' melissopalynological pattern was more typical for under-represented thymus pollen (26.6% ± 10.0) (Persano Oddo and Piro 2002). Recent studies show, that thyme honey in a concentration of 12.5% (v/v) in twenty hours eliminates bacterial strains resistant to antibiotics, including vancomycin-resistant *E. faecalis*, ESBL *K. pneumoniae*, and *P. Aeruginosa* (Özkök et al, 2016). According to Ishenbaeva et al. (2021), sage and thyme honey collected from Kyrgyzstan in a concentration of 50% (v/v) have exhibited the strongest antibacterial effect relative to *Escherichia coli*. Against *Staphylococcus aureus*, thyme honey had the strongest effect.

Polyfloral honey with forget-me-not. 23 taxa were identified in sample №4. The pollen of *Myosotis sylvatica* was dominant. After the exclusion of nectarless plants from the calculations, the proportion of forget-me-nots reached 47%.

However, for forget-me-not honey, the pollen content of *Myosotis sylvatica* should be over 90%. Therefore, this sample can be attributed to polyfloral honey with forget-me-not (*Myosotis sylvatica*).

Polyfloral honey. Pollen from 29 taxa was identified in sample №6 and the sample can be attributed to polyfloral honey. Pollen grains of the *Eremurus fuscus* and *Rosaceae* families (fruit trees) were dominant.

Physicochemical parameters and safety indicators

Moisture content is the most important quality criterion for honey and should not exceed 20% (Codex Alimentarius, 2001). The moisture content of the studied honey samples varied in the range of 16.6%–18.6% (w/w).

Diastase (amylase) is a glycoprotein with a molecular weight of 24,000 to 25,000 Da and α-amylolytic properties. It is less sensitive than other enzymes to high temperatures. Many honeys have diastase numbers ranging from 8 to 24 on the Schade or Gothe scale (Serrano et al. 2004, Huidobro et al. 1995). The diastase numbers varied between the studied honey samples. There was a statistically significant difference ($p < 0.05$) in the diastase activity means. It should be noted that diastase activity depends on the botanical composition of honey, i.e., on the leading, main honey-bearing plant. High diastase activity was found in samples of thyme honey *(Thymus vulgaris L)* (41.1 ± 2.9 Gothe) and in samples of sage honey (*Salvia officinalis L*) (31.3 ± 2.2 Gothe). The other species of honey samples tested had relatively low diastase activity (9.8–23.9 Gothe). It has been proven that the families of *Lamiáceae* (thyme, sage), in contrast to other families and melliferous species, impart high diastase activity in honey. The difference between the means of diastase activity was statistically significant ($p < 0.05$). The diastase number of the honey samples from the Sary-Chelek reserve collected in 2015 ranged from 17.0±0.1 to 37.9±5.1 in Schade units (Kadyrova and Smanalieva 2017, Mazhitova and Smanalieva 2022). For comparison, the diastase number of European thyme honey was in the range of 15.0 ÷ 44.4 Gothe units (Persano Odo and Piro 2002). Diastase activity of honey from Argentina was found between 11.2 and 25.8 Schade units (Tosi et al. 2008), acacia honey from China has a 18.30 and longan honey 22.63 DN units. Pasias et al. (2017) reported for Greece honey collected from local experienced beekeepers in Lamia the diastase activity from 7.0–22.0 DN units. Honey in Kyrgyzstan is harvested and marketed by the beekeepers themselves. This has two advantages: unprocessed honey is fresher and not heat-treated for subsequent liquefaction and filling purposes. Therefore, honey collected from Kyrgyzstan has high diastase activity.

One indicator of honey quality is the content of free acids. This characteristic is related to the organic acids naturally present in honey that are in equilibrium with lactones, esters and certain inorganic ions such as phosphates, sulphates and chlorides. As per the EU Directive (Council EU 2001) and Codex Alimentarius (2001), honey may contain up to 50 meq/kg of free acidity. The free acidity of the investigated samples ranged from 20.4 to 26.4 meq/kg; the studied types of honey by this indicator also meet the requirements of the standard. In our previous research, the free acids of honey from Kyrgyzstan were in the range of 13.6–32.1 meq/kg (Mazhitova and Smanalieva, 2022). Regarding the reducing sugars and sucrose contents of the samples, the studied honey did not deviate noticeably from interstate and international standards. In all samples, the mass fraction of sucrose did not exceed 5.0%.

The electrical conductivity is dependent on the honey's botanical origin (Pita-Calvo and Vazquez 2017), which is closely related to the amounts of organic acids and mineral salts in the honey (Sanz et al. 2005). Therefore, many researchers have stated a positive correlation ($r = 0.934$) between the ash content and the electrical conductivity of the honey samples (Smanalieva, 2008; Majewska et al. 2019). Honeydew honey exhibits higher electrical conductivity (0.735–1.295 mS/cm) compared to flower honey (0.196–0.798 mS/cm) (Krauze and Zalewski 1991). The sage honey exhibited the highest electrical conductivity (0.9 mS/cm), while the Eremurus honey (*Eremurus fuscus* O. Fedtsch) showed the lowest electrical conductivity (0.2 mS/cm). The differences between the means of electrical conductivity of the samples were statistically significant ($p < 0.05$). The sage honey's electrical conductivity is higher than the EU Directive's minimal requirement of 0.8 mS/cm for honeydew and chestnut honey. Exceptions are given for honey from strawberry trees (*Arbutus unedo* L.), bell heather (*Erica* L.), eucalyptus, lime (*Tilia*), ling heather (*Calluna vulgaris*), manuka or jelly bush (Leptospermum), and tea trees (*Melaleuca*) (Council EU 2001). For blossom honey,

samples from Kosovo have electrical conductivity ranging from 0.04 to 0.71 mS/cm (Durmishi 2023). According to Gurbüz et al. (2020), the electrical conductivity of Turkish honey varieties is measured between 0.15 and 0.31 mS/cm.

The correlation analysis between moisture content, diastase number, free acid content, and electrical conductivity was carried out to determine the association between the physicochemical characteristics of honey (Table 4). A statistically significant negative correlation was observed between the reducing sugars and the diastase activity ($r = -0.523$, $p < 0.05$) and between the reducing sugars and the free acidity ($r = -0.523$, $p < 0.05$). However, a statistically significant positive correlation was observed between the diastase number and the electrical conductivity ($r = 0.657$, $p < 0.01$), as well as between the electrical conductivity and sucrose ($r=0.688$, $p<0.01$). This finding is in line with the findings of other authors. Adgaba et al. (2017) found significant differences in eight parameters (total dissolved sugar, electrical conductivity, acidity, total ash, colour, and microelement content) between different honey origins. The linear relationship between electrical conductivity and diastase number could be explained by the fact that some microelements like K^+, Na^+, Mg^{2+}, and Ca^{2+} can interact with α-amylase (diastase) and increase their activity (Farooq et al. 2021).

Conclusion

Pollen analysis of 17 honey samples collected from different locations on the Sary-Chelek Biosphere Reserve allowed us to determine the characteristic species of melliferous plants for this region. The abundance of flowering plants in the vicinity of the apiary during the entire honey harvest period allows for the collection of polyfloral honey, but it is also possible to collect some valuable and rare monofloral honey such as sage, thyme, and eremurus. The organoleptic and physiochemical parameters of eremurus honey were described for the first time. On the other hand, research shows the benefits of beekeeping in protected areas. The obtained results make a significant contribution to the determination of the botanical and geographical origin of honey produced in Kyrgyzstan and can be used for establishing standards for monofloral honey such as sage, thyme, and eremurus honey.

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