ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

MELISSOPALYNOLOGY ANALYSIS, PHYSICOCHEMICAL PROPERTIES, MULTI-ELEMENT CONTENT AND ANTIMICROBIAL ACTIVITY OF HONEY SAMPLES COLLECTED FROM BAYBURT, TURKEY

Türkiye'nin Bayburt İlinden Toplanan Bal Örneklerinin Melissopalinolojik Analizi, Fizikokimyasal Özellikleri, Multi-Element İçeriği ve Antimikrobiyal Aktivitesi

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ABSTRACT

The aim of this study was to determine the plant sources, physicochemical properties, element content and antimicrobial effects of honey samples collected from 10 different regions of the province of Bayburt, Turkey. The melissopalynological analysis showed that the pollen samples of 67 plant taxa belonged to 34 plant families in honey samples and were found with different rates and TPN-10 values was found between 16024 and 90126. In addition to these, the amount of ash was between 0.13% and 0.32%, the electrical conductivity value was between 0.36 and 0.69 mS/cm, the moisture content was between 16.1% and 18.9% and the fructose/glucose ratio was between 0.92 and 1.18. As a result of physicochemical analysis, it was determined that the data obtained were in compliance with the standard values defined in by the Turkish Food Codex Communiqué on Honey (No: 2012/58). Elemental analysis performed with ICP-MS showed that the K element was the highest (261.34-1863.05 mg/kg) in all honey samples in total among the 42 elements. In addition to these, the antimicrobial effects of honey samples and minimum inhibition concentration values (MIC) were determined by the agar well diffusion (AWD) method and and microbroth dilution method respectively.

Key words: Honey, Botanical origin, Element content, Melissopalynological analysis

ÖZ

Bu çalışmada, Türkiye'nin Bayburt ilinin 10 farklı bölgesinden toplanan bal örneklerinin bitki kaynakları, fizikokimyasal özellikleri, multi-element içeriği ve antimikrobiyal aktivitesinin belirlenmesi amaçlanmıştır. Melissopalinolojik analizler neticesinde bal örneklerinde 34 familyaya ait olan 67 bitki taksonun polen örneğine farklı oranlarda rastlanılmış ve TPS-10 değeri 16024-90126 arasında saptanmıştır. Fizikokimyasal analizler sonucunda ise kül miktarı %0,13-%0,32 arasında; elektriksel iletkenlik değeri 0,36-0,69 mS/cm arasında; nem miktarı %16,1- %18,9 arasında ve früktoz/glikoz oranı 0,92-1,18 arasında bulunmuştur. Fizikokimyasal analizler neticesinde çalışılan tüm numunelerin Türk Gıda Kodeksi Bal Tebliği (Tebliğ No: 2012/58)'nde verilen standart değerlerle uyumlu olduğu saptanmıştır. Multi-element analizleri neticesinde K elementi bütün bal örneklerinde en yüksek konstrasyonda (261,3496-18,630555 mg/kg) belirlenen ilk elementti. Bunlara ek olarak, bal örneklerinin antimikrobiyal etkileri ve minimum inhibisyon konsantrasyon değerleri (MİK) sırasıyla agar difüzyon metodu ve mikrobroth dilüsyon metodu ile belirlenmiştir.

Anahtar Kelimeler: Bal, Botanik orijin, Element içeriği, Melissopalinolojik analiz

GENİŞLETİLMİŞ ÖZET

Amaç: Bal, birçok faydalı özelliği nedeniyle yüzyıllardır insanlar tarafından kullanılan geleneksel bir gıda maddesidir. Bu çalışmada, Türkiye'nin Doğu Karadeniz Bölgesinde yer alan Bayburt ilinden toplanan farklı bal örneklerinin bitkisel kaynaklarının ve kalitesinin tespit edilmesi amaçlanmıştır. Melissopalinolojik analizlerde balların polen kaynağı ve 10 gr baldaki toplam polen sayısı (TPS-10) hesaplanmıştır. Fizikokimyasal analizlerde ise balların yüzde kül içeriği, elektriksel iletkenliği, nem oranları ve şeker oranları (früktoz, glikoz) incelenmiştir. Bununla birlikte örneklerin multi-element profillerine ek olarak beş adet Gr (+), beş adet Gr (-) bakteri ve bir adet mayaya karşı antimikrobiyal aktiviteleri araştırılmıştır.

Gereç ve Yönrem: Bal örneklerinin bitkisel kaynaklarının ve TPS-10 değerlerinin hesaplanmasında ışık mikroskobu kullanılmıştır. Yüksek performanslı sıvı kromotografi (HPLC) cihazı kullanılarak balların şeker profilleri incelenmiştir. ICP-MS cihazı kullanılarak Li, Be, B, Na, Mg, Al, Si, P, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Ru, Rh, Pd, Ag, Cd, In, Sn, Sb, Te, Cs, Ba, Hf, Ir, Pt, Au, Hg, Tl, Pb ve Bi elementlerinin konsantrasyonları hesaplanmıştır. Ayrıca bal örneklerinin agar difüzyon yöntemi ile antimikrobiyal aktivitesi ve mikrobroth dilüsyon yöntemi ile minimum inhibisyon konsantrasyonları tespit edilmiştir.

Bulgular: Bu çalışmada, Bayburt ilinin farklı bölgelerinden toplanan 10 bal örneğinin melissopalinolojik analizi sonucu *Acanthaceae*, *Acereaceae*, *Apiaceae*, *Asteraceae*, *Asparagaceae*, *Berberidaceae*, *Brassicaceae*, *Boraginaceae*, *Caryophyllaceae*, *Campanulaceae*, *Caprifoliaceae*, *Chenopodiacea*, *Cupressaceae*, *Cucurbitaceae*, *Cyperaceae*, *Elaeagnaceae*, *Fabaceae*, *Fagacea*, *Geraniaceae*, *Hypericaceae*, *Liliaceae*, *Malvaceae*, *Lamiaceae*, *Onagraceae*, *Plantaginaceae*, *Papaveraceae*, *Poaceae*, *Polygonaceae*, *Rhamnaceae*, *Ranunculaceae*, *Rosaceae*, *Rubiaceae*, *Salicaceae* ve *Scrophulariacea* olmak üzere, toplam 34 familyaya ait bitki taksonlarının polenlerine farklı oranlarda rastlanılmıştır. Bal örneklerinin früktoz oranı %33,35 ile %43,36; glikoz oranı %33,50 ile %41,77; F/G oranı 0,92 ile 1,18 arasında olduğu belirlenmiştir. Bal örneklerinin nem içeriği %16,1-%18,9, kül miktarı %0,13-%0,32 arasında, elektriksel iletkenlik değeri 0,36-0,69 mS/cm arasında değişkenlik göstermiştir. Ek olarak, test edilen bal örneklerinin tümünün glikoz ve früktoz içeriğinin Türk Gıda Kodeksi Bal Tebliği (No: 2012/58)'nde verilen değerlerle uyumlu olduğu görülmüştür. Tüm bal örneklerinde farklı konsantrasyonlarda Al, B, Ba, Ca, Na, Cu, Fe, K, Si, Mg, Mn, Ni, P, Rb Sr ve Zn elementlerini farklı konsantrasyonlarda içerdiği tespit edilmekle birlikte K, Na, P ve Mg elementlerinin tüm numunelerde en yüksek konsantrasyonda belirlenen ilk dört element olduğu belirlenmiştir. Ayrıca Bayburt'tan toplanan bal örneklerinin minimum inhibitör konsantrasyonunun Gram pozitif bakterilere karşı %6,25-%25 (w/v) arasında değiştiği görülmüştür.

Tartışma ve Sonuç: Elde edilen sonuçlar değerlendirildiğinde oldukça farklı bitkilerin Bayburt ballarına kaynaklık ettiği ve bölge florasının ballı bitkiler açısından zengin olduğu söylenebilir. Fakat Bayburt bölgesi ballarına ağırlıklı olarak kaynak oluşturan bitkilerin net olarak belirlenebilmesi için daha ayrıntılı ve daha fazla sayıda örnekle çalışmaya gerek duyulmaktadır. Ayrıca incelenen tüm örneklerin fizikokimyasal kriterler açısından (şeker profili, elektriksel iletkenlik, nem oranı) Türk Gıda Kodeksi Bal Tebliği (No: 2012/58)'nde verilen değerler ile uyumlu olması balların temin edildiği arıcıların dikkatli bir üretim yaptığını göstermekle birlikte, balların kalitesi hakkında daha kesin yorum yapabilmek için balın kalitesi ve orijini hakkında fikir veren daha farklı parametrelerin (C4 şekerleri oranı; antibiyotik kalıntısı, prolin, diastaz, HMF, fenolik madde vb.) çalışılmasına gereksinim vardır.

INTRODUCTION

The activities related to beekeeping in Turkey are carried out quite intensively. In Turkey, especially honey, propolis, bee pollen and bee products such as bee milk (royal jelly) are produced and studied in relation to their chemical properties (Bayram et al. 2017, Ecem Bayram and Gerçek 2019, Can et al. 2015) as well as plant sources (Sorkun et al. 2018, Gok et al. 2015) are conducted. Honey, which is produced the most, is defined by the Turkish Food Codex Communique on Honey (No: 2012/58), as a natural product, which can be modified by combiningit with different nectar sources, the water content can be decreased and the storing methods in honeycombs to ripen after nectar collection, can all affect the properties of honey. Honey can be characterized as blossom honey or a honeydew honey according to the plant source that the honey bees collect from (Abu-Jdayil et al. 2002). Honey is

a highly variable and complex mixture that contains sugar and other components (Anklam 1998) and has a chemical composition of about 200 different substances (da Silva et al. 2016, Escuredo et al. 2013, Ferreira et al. 2009). Honey, which is mostly composed of sugar, is a food containing proteins (enzymes), organic acids, especially vitamins B6, thiamine, niacin, riboflavin and pantothenic acid, minerals (magnesium, manganese, phosphorus, potassium, sodium, zinc, pigments, and solid particles that are mixed in during harvest (Alqarni et al. 2014, Ciulu et al. 2011, Pontes et al. 2007). The sugar content of honey isdetermined by the region and climatc conditions, along with the flowers visited by foraging bees (Tornuk et al. 2013). Honey contains 25 different oligosaccharides together with the monosaccharides: fructose and glucose (Karadal and Yıldırım 2012). There is generally a positive correlation between the mineral content, color and electrical conductivity of honey (Karabagias et al. 2014). The electrical conductivity of honey is used to differentiate between blossom honey and honeydew honey (Sanz et al. 2005, Nombré et al. 2010). The electrical conductivity of honey may vary depending on floral origin, mineral content, proteins, organic acids and complex sugars (D'Arcy 2007).

The water content of honey is one of the most important factors affecting the storage and quality of honey (Gomez-Diaz et al. 2012). The chemical, physical and microbiological stability of honey depends primarily on the moisture ratio. The water content of honey is mostly in the range of 16.0-18.5% (Çınar and Ekşi 2012). The moisture of honey may differ depending on environmental conditions, the processes carried out by the beekeepers during the harvest period and also the year. Due to its hygroscopic property, the moisture of honey may be increased during different processing and under inadequate storage conditions (Karabagias et al. 2014). A high moisture ratio can accelerate crystallization in some honeys and increase the probablility of various yeasts to grow (Yanniotis et al. 2006).

The most important factor affecting the content of honey is the floral sources where nectars are collected because the changes in the source of honey affect its smell, taste and color (Kaya et al. 2005, Şahinler and Kaya 2001). For this reason, melissopalinological analysis which have recently been carried out to determine the floral source of honey all over the world have become increasingly important (Erdoğan et al. 2006, Azim and Sajid 2009, Gençay Çelemli et al. 2018, Sorkun et al. 2014, Ecem Bayram and Demir 2018). As a result of the melissopalinological analyses made on different types of honey, it is possible to determine the plant, from which the most sophisticated honey is produced along with the plants that provide the properties of odor flavor, light or-dark color and quick crystallization properties (Pınar et al. 2003). Each type of honey has a unique combination in terms of its components and properties due to the diversity of the vegetation and climatic conditions of the geographical region in which the honey is produced as well as different storage and processing methods (Gomez-Diaz et al. 2012). In addition to factors such as early harvest, which may be caused by the beekeeper, bees being fed with excess sugar syrup and not following the hygiene rules during honey production, plant diversity, and climate conditions of the environment, also affect the quality of honey in a positive or negative way. Among these factors, plant variety is the most important (Öner 1967, Accorti et al. 1987). The viscosity of honey varies depending on the chemical composition, sugars, moisture, enzymes, acids, vitamins and plant diversity of the region (Accorti et al. 1987).

In this study, it was aimed to determine the floral diversity, physicochemical characteristics, multi-elemental content and antimicrobial activity of honeys collected from Bayburt, Turkey.

MATERIAL AND METHODS

Honey samples

The 10 honey samples were obtained from apiaries in 10 different locations from Bayburt, Turkey in 2017. All samples were stored at room temperature prior to the analyses.

Microscopic analysis of honey samples

Pollen diagnosis

The pollen spectra of the honey samples were determined according to the methodology described by Louveaux et al. (1978). Accordingly, 10 g of honey samples were thoroughly mixed with a sterile glass rod were taken and transferred to a test tube to which 20 mL of distilled water was added. For the dissolving of the honey sample in water, the tubes were placed in a water bath at about 45°C for 10-15 minutes and then each tube was shaken by a stirrer. This dissolved honey water mixture was then

centrifuged at 3500 rpm for 45 minutes and the supernatant fraction was poured off. The precipitate remaining in the bottom of the tube was infused with a quantity of basic-fucose with added glycerin-gelatin taken from the needle tip with and this material was transferred onto the slide. The slide was heated to 30-40°C to allow the dissolving of the basic fuchsin added with the glycerin gelatin and then an 18x18 cover slip was placed on top of it. The preparation was kept this way for nearly 12 hours after which it became suitable for examination. The pollen preparations that were prepared to determine the botanical origin of the honey samples were examined with a Leica DM500 light microscope. To diagnose of the pollen grains, the microphotographs of the pollens in the literature and the reference preparations were utilized (Sorkun 2008). Then, the observed pollen types were classified into four categories: dominant pollen (≥ 45%, D), secondary pollen (16-44%, S), important minor pollen (3-15%, I) and minor pollen (<3%, M) (Louveaux et al.,1978).

***Total pollen numbe**r*

The total pollen number (TPN) of the honey samples was calculated according to the Moar (1985) by using tablets of *Lycopodium* spores (Stockmarr 1971). The honey samples were classified according to the number of pollen grains present in 10 grams of honey, into: group I (<20000), group II (20000-100000), group III (100.000–500.000), group IV (500.000–1.000.000) and group V (>1.000.000) (Louveaux et al. 1978).

Physicochemical analysis of honey samples

Ash content

For the ash content of the honey samples the method developed by Accorti et al. (1987), Sancho et al. (1991) and Piazza et al. (1991) was used. 1 g of each honey sample was weighed and placed in a porcelain bowl and this was placed in an oven set at 600 °C. The honey sample placed in the ash oven was burned for about 6 hr and then re-weighed after cooling. The amount of ash was expressed as a weight percent (g / 100 g).

Electrical conductivity

Electrical conductivity was determined by the relationship between percent ash content and electrical conductivity as following: EC (mS/cm) = 0.14 + 1.74 · A (in which A represents the ash content (g/100 g honey) (Sancho et al. 1991, Piazza et al. 1991).

Moisture content

The moisture ratio of the honeys was detected by using a portable refractometer (ATC 0-32) according to the method of Devillers et al. (2004) and Bogdanov (1997).

Sugar analysis by high performance liquid chromatography (HPLC)

The analyses of honey sugars were performed according to the harmonized methods of the International Honey Commission (2009). 5 g of each honey sample was dissolved in 40 mL of distilled water and then 25 mL of methanol was added to the solution. Afterwards, distilled water was used to bring the the final volume to 100 mL. The prepared solution was then analyzed by high performance liquid chromatography (HPLC) (Agilent Technologies 1200 Series, Germany) with a refractive index detector (HPLC-RID) using a carbohydrate column (Agilent Technologies Carbohydrate 5 µm, 4.6 x 250 mm, USA).

Determination of mineral profiles of honey samples by inductively coupled plasma mass spectrometry (ICP-MS)

ICP-MS analysis was performed at the Central Research Laboratory of Bayburt University. In this study all reagents used for the elemental analysis of samples were of analytical grade. The element standard solutions were prepared by diluting a stock solution of 1000 mg/L of Lithium (Li), Boron (B), Beryllium (Be), Magnesium (Mg), Sodium (Na), Silicon (Si), Aluminum (Al), Phosphorus (P), Copper (Cu), Calcium (Ca), Potassium (K), Vanadium (V), Chromium (Cr), Manganese (Mn), Cobalt (Co), Iron (Fe), Nickel (Ni), Zinc (Zn), Ga (Gallium), Selenium (Se), Arsenic (As), Cesium (Cs), Strontium (Sr), Indium (In), Rubidium (Rb), Rhodium (Rh), Ruthenium (Ru), Silver (Ag), Palladium (Pd), Cadmium (Cd), Platinum (Pt), Tin (Sn), Tellurium (Te), Lead (Pb) Antimony (Sb), Barium (Ba), Hafnium (Hf), Iridium (Ir), Gold (Au), Mercury (Hg), Thallium (Tl) and Bismuth (Bi). 0.5 g of each honey sample was weighed and 9 mL of suprapur nitric acid (Sigma Aldrich, Germany) (%65) and 1 mL of hydrogen peroxide (Sigma Aldrich, Germany) (30%) were added. After that, the digestion procedures were carried out in a microwave digestion system (Milestone, Ethos Easy, Italy) according to instrumental parameters. The final volume of the samples removed from the microwave was completed to 50 mL with ultra-pure water. Blank

solutions were prepared in the same way. The 42 elements in the honeys were determined using inductively coupled plasma mass spectrometry ICP-MS (7800 Series from Aigelent) (Oroian et al. 2015).

Antimicrobial activity and minimum inhibition concentration

Microorganisms and growth conditions

Five gram positive bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* BC 7231, *Staphylococcus aureus* NCTC 10788, *Enterococcus faecalis* NCTC 12697, *Bacillus cereus* BC 6830) five gram negative bacteria (*Escherichia coli* NCTC 9001, *Escherichia coli* BC 1402, *Pseudomonas aeruginosa* NCTC 12924, *Salmonella Typhimurium* RSSK 95091, *Yersinia enterocolitica* ATCC 27729) and 1 yeast-like fungus *Candida albicans* ATCC 10231 were used to determine *in vitro* antimicrobial activities of the honeys. The microorganisms used in the study were obtained from the Bayburt University, Vocational School of Health Services, Department of Medical Services and Techniques.

The bacterial strains and the yeast-like fungus were cultured for 24 hours at 37°C using Mueller Hinton Broth (MHB, Oxoid) and Sabouraud liquid medium (SDB, Oxoid), respectively. The suspensions were adjusted to a standard turbidity of 0.5 McFarland (10^6 CFU/ml) and used as inoculum (Sherlock et al. 2010).

Preparation of honey samples for antimicrobial activity assays

2 gr of each honey samples were transferred to 15 mL sterile falcon tubes. The prepared honey samples (50% w / v) were used for the determination of antimicrobial activity and MIC values (Lee et al. 2008).

Determination of antimicrobial activity

The agar-well diffusion (AWD) method was used to determine the in-vitro antimicrobial activity of the honeys. Mueller Hinton Agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for *Candida albicans* ATCC 10231 were sterilized at 121°C in the autoclave and cooled to 50°C at room temperature. Mediums were transferred to sterile petri dishes by taking 25 ml volumes and 500 µl inoculum were added to every petri dish. After the addition of the inoculum, the medium was cooled at 4°C for 1 hour (Oses et al. 2016). 8 mm diameter wells were cut into the mediums using a sterile cork borer drilled into the solidified petri dishes (Sherlock et al. 2010). After these processes 100 µl 50% (w/v) of the honey samples that were previously prepared with sterilized distilled water were transferred to wells and incubated at 37°C for 24 h for bacteria and 48 h for *Candida albicans* ATCC 10231 (Silici et al. 2010). The inhibition zones observed around the wells following the incubation period were measured with a vernier caliper and recorded. Each assay was carried out in duplicate (Oses et al. 2016).

In order to have a negative control in determining the *in vitro* inhibitory effect, an artificial honey formulation was prepared which reflects the approximate sugar composition of many honey samples. For this purpose, 2 g sucrose, 8 g maltose, 30 g glucose and 40 g fructose were dissolved in 100 mL distilled water and sterilized in autoclave for 15 min at 121°C (Taormina et al. 2001). The inhibition zone diameters of vancomycin and gentamicin antibiotics a positive control against the target pathogenic microorganisms were also determined by the disc diffusion method.

Determination of minimum inhibitory concentration (MIC)

The microbroth dilution method was used to determine MIC values (Huttunen et al. 2013). In this process, 96-well microtiter plates were used. Initially, 95 µl of sterile MHB medium for the bacterial strains and 95 µL sterile SDB medium for *Candida albicans* ATCC 10231 was added to all wells and then 5 µL of inoculum was added to all wells. Thus, 100 µL of medium plus an inoculum mixture was added to each well. Following these processes, 100 µL of diluted honey samples 50% (w/v) were added to the first wells and mixed thoroughly by pipetting. Then a sample of 100 µL was taken from the first well and transferred to the second well by micropipette. These processes were repeated for all eight wells and the concentrations of the honey samples were serially diluted in each well by serial dilution. The lowest concentration of the honey samples that inhibited pathogenic microorganisms was determined as the MIC value (Sarker et al. 2007).

RESULTS

The taxa and their pollen spectra detected as a result of the pollen analysis of 10 honey samples from Bayburt, Turkey are given in Table 1. As a result of the melissopalynological analysis, the pollen of plant taxa belonging to *Acanthaceae*, *Apiaceae*, *Acereaceae*, *Asteraceae*, *Asparagaceae*,

Boraginaceae, *Brassicaceae*, *Berberidaceae*, *Campanulaceae*, *Caryophyllaceae*, *Caprifoliaceae*, Chenopodiacea, Cucurbitaceae, *Cyperaceae*, *Cupressaceae*, *Fabaceae*, *Elaeagnaceae*, *Geraniaceae*, *Fagacea*, *Lamiaceae*, *Polygonaceae*, *Hypericaceae*, *Liliaceae*, *Malvaceae*, *Onagraceae*, *Plantaginaceae*, *Poaceae*, Papaveraceae, *Ranunculaceae*, *Rhamnaceae*, *Rosaceae*, *Rubiaceae*, *Salicaceae* and *Scrophulariacea* were found in different proportions from the honey samples. Pollen belonging to the taxa *Achillea* spp., *Aster* spp., *Campanula* spp., *Juniperus* spp., *Elaeagnus angustifolia*, *Astragalus* spp., *Coronilla* spp., *Medicago* spp., *Onobrychis* spp., *Trifolium* spp., *Lamium* spp., *Rumex* spp., *Ranunculus grandiflorus* and *Salix* spp. were observed at minor levels.

Table 1. Pollen spectrum of plant taxa of honey samples (S: secondary pollen, M: Minor pollen, I: important minor pollen)

Family	Taxon	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
Acanthaceae	*Acanthus* spp.	M						M			
Apiaceae	*Bupleurum falcatum*	M		M		M			M		
	Chaerophyllum spp.			M			M				
	Eryngium billardierei	M			M			M		M	
	Pipinella spp.	M							M		M
Asteraceae	*Achillea* spp.	I	I	I	I	I	I	S	S	I	I
	Anthemis spp.	M	M	M	M	M	M	M		I	
	Aster spp.	I	I	M	I	I	I	I	I	I	I
	Centaurea spp.	M	M	M	M	I	M	M	M	M	M
	Cirsium spp.	M			M					M	
	Crepis spp.						M				M
	Helichrysum spp.										M
	Scorzonera spp.					M					
	Tanacetum spp.	M		M	M		M				M
	Taraxacum spp.	M	M	M	M	M	M	M	M	M	M
	Xeranthemum spp.	M	M	M		M				M	M
Asparagaceae	*Ornithogalum sphaerocarpum*	M									
Berberidaceae								M			
Boraginaceae	*Alkanna* spp.			M	M		M			M	M
	Anchusa spp.							M			
	Cerinthe minör		M								
	Onosma spp.	M		M		I		M	M	M	M
	Symphtum spp		M		M		M				
Brassicaceae	*Alyssum* spp.	M	M			M		M	M		
	Aethionema spp.		M	M		M					M
	Lepidium spp.	M		M			M		I	M	
	Isatis spp.	M									
Campanulaceae	*Campanula* spp.	M	M	I	I	M	M	M	M	M	M
Caryophyllaceae	*Dianthus* spp.		M				M				
	Minuartia spp.									M	
Caprifoliaceae	*Silene caryophylloides*			M					M		
Chenopodiacea										M	
Cucurbitaceae		M									
Cupressaceae	*Juniperus* spp.	I	I	M		I	I	I	I	I	I
Cyperaceae	*Carex* spp.				M						
Elaeagnaceae	*Elaeagnus angustifolia*	M	I				I	I		I	
Fabaceae	*Astragalus* spp.	I	I	I	I	I	I	M	I	I	I
	Coronilla spp.	I	I	I	M			I		M	M
	Medicago spp.	I	I	I	I	M	I	I	I	I	I
	Melilotus spp.	M	M	M	M	M	M	M	M	M	M
	Onobrychis spp.	I	I	I	S	I	S	I	I	S	S
	Trifolium spp.			M		I	I		I	I	M
	Vicia spp.		M								M
Fagaceae	*Quercus* spp.					I			I		
Geraniaceae	*Geranium* spp.			I			M				
Hypericaceae	*Hypericum* spp.				I						
Lamiaceae	*Lamium* spp.	I	I	I	I	I	I	I	I	I	I
	Mentha spp.	M	M	M	M	M	M	M	M	M	M
	Nepeta spp.									M	
	Salvia spp.			I			I				

	Stachys spp	M						M			M
	Teucrium spp.	M	M	M	M	M	M	M	M	M	M
	Thymus spp.	M	M	M		M	M	M	M		M
Liliaceae				M							
Malvaceae							M				
Onagraceae	*Epilobium angustifolium*	M	M	M	M	M	M	M	M	M	M
Papaveraceae	*Papaver* spp.					I					
Plantaginaceae	*Plantago* spp.						M	M			
Polygonaceae	*Rumex* spp.	M	I	M	I	M	I	I	I	I	I
Ranunculaceae	*Ranunculus grandiflorus*	I		I	I	M	I	M	M	M	I
Rhamnaceae		M									
Rosaceae	*Potentilla* spp.	M		M		I	M			I	
	Pyrus spp	M	M		M			M			
	Rosa spp.	M			I	M			M		M
	Rosa canina			M		M	I		M		M
	Cerasus angustifolia	M		M	M			M		M	I
	Rubus spp.			M		M					M
Rubiaceae	*Asperula* spp.				M				M		M
	Galium spp.		M				M				M
Salicaceae	*Populus* spp.				M				I		
	Salix spp	I	I	I		I		I			I
Scrophulariacea	*Verbascum* spp.		I		I					I	
	Scrophularia spp.										M
Solanaceae		M						M			

TPN-10 values of the samples were also determined by light microscope. TPN values of 10 grams of honey were determined between 16024 and 90126 (Table 2). According to the results of TPN, the B6, B9 and B10 samples had low pollen counts and the other seven honey samples had average pollen counts.

Table 2. TPS-10 values of honey samples

Sample Code	TPN	Grup	Pollen Content
B1	65630	Grup II	Normal
B2	41608	Grup II	Normal
B3	56789	Grup II	Normal
B4	78965	Grup II	Normal
B5	34572	Grup II	Normal
B6	19904	Grup I	Low
B7	90126	Grup II	Normal
B8	21225	Grup II	Normal
B9	18906	Grup I	Low
B10	16024	Grup I	Low

The percent ash content and electrical conductivity of the 10 honey samples were detected and the results are presented separately in Table 3. It was determined that the amount of ash was between 0.13% and 0.32% and the conductivity value varied between 0.36 mS / cm and 0.69 mS / cm in honey samples examined in our study. In addition to this, it was determined that the moisture content of the honey samples varied between 18.9% - 6.1-% (Figure 1-Table 3).

In the present study, the fructose content of the honey samples was 33.35% to 43.36%; the glucose ratio was 33.50% to 41.77% and the fructose/glucose ratio was between 0.92 and 1.18. The results of the sugar analysis of the honey samples are given in Table 3.

Table 3. Physicochemical properties (ash content, electrical conductivity, sugar content) of honey samples

Sample Code	Ash Content (%)	Electrical Conductivity (mS/cm)	Moisture content (%)	Sugar Content			
				Fructose (%)	Glucose (%)	F/G	F+G (%)
B1	0.17	0.43	18.8	39.45	36.35	1.09	75.80
B2	0.27	0.60	18.6	40.04	36.90	1.09	76.93
B3	0.19	0.47	18.3	41.20	38.24	1.08	79.44
B4	0.26	0.50	16.1	41.03	41.17	1.00	82.20
B5	0.13	0.36	16.6	33.35	36.10	0.92	69.45
B6	0.21	0.50	17.3	41.42	35.50	1.17	76.92
B7	0.12	0.37	18.9	40.89	36.04	1.13	76.94
B8	0.16	0.41	17.6	43.36	38.70	1.12	82.06
B9	0.32	0.69	18.3	42.62	36.24	1.18	78.86
B10	0.27	0.60	16.6	44.41	39.14	1.13	83.54

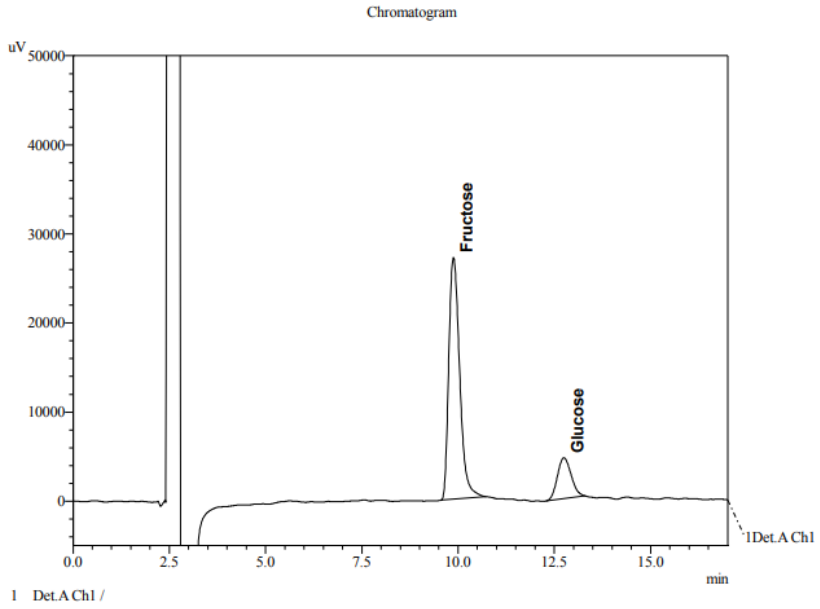

Figure 1. A representative chromatogram of carbohydrates contents determined in Bayburt honey by using HPLC-RI

In the present study, elements such as Mg, B, Al, Si, Na, P, Ca, Mn, K, Ni, Fe, Zn, Cu, Sr, Rb, Ba were found in different concentrations in all of the honey samples. K, Na, P and Mg were the first four elements determined at the highest concentration in all of the samples (Table 4). In addition, elements such as V, Ga, As, Pd, Ag, Se, Rh, In, Sb, Cs, Te, Hf, Ir Pt, Au, Hg Tl and Bi were not found in honey from the Bayburt region.

Table 4. Multi-element contents of honey samples (mg/kg)

Elements	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
7 Li	nd	0.07	nd	nd	nd	nd	nd	0.01	nd	0.02
9 Be	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.63
11 B	1.51	3.12	3.67	2.19	0.60	0.44	2.8	4.46	4.27	5.17
23 Na	98.61	38.82	24.72	366.38	42.27	147.34	53.49	27.73	33.13	26.03
24 Mg	21.59	15.50	12.81	30.64	35.43	15.02	22.99	10.41	16.21	18.13
27 Al	0.42	0.55	0.83	0.71	6.476	1.53	0.58	1.25	1.07	1.35
28 Si	7.24	4.46	5.70	8.73	55.53	7.26	6.82	5.63	8.78	9.38
31 P	55.88	90.28	78.26	68.81	123.55	69.97	90.56	87.92	116.25	105.38
39 K	261.34	532.66	625.00	521.35	1863.05	349.94	500.10	646.47	859.23	783.67
44 Ca	13.29	13.59	14.04	14.20	17.95	12.66	21.40	10.66	16.04	14.72
51 V	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
52 Cr	nd	nd	nd	nd	nd	0.02	nd	nd	nd	nd
55 Mn	0.24	0.19	0.30	0.28	0.45	0.94	0.70	0.25	0.33	0.39
56 Fe	29.49	1.02	2.28	3.92	3.09	0.74	1.48	1.44	1.77	14.60
59 Co	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.01
60 Ni	0.09	0.01	0.01	0.02	0.08	0.08	0.01	0.01	0.02	0.01
63 Cu	0.14	0.14	8.61	0.10	0.29	0.13	0.12	0.10	0.15	0.13
66 Zn	5.73	0.80	6.22	1.43	0.87	0.57	0.51	0.61	0.60	57.84
71 Ga	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
75 As	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
78 Se	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
85 Rb	0.10	0.23	0.21	0.30	1.22	0.21	0.36	0.28	0.38	0.33
88 Sr	0.36	0.24	0.13	0.30	0.27	0.19	0.32	0.14	0.27	0.24
101 Ru	nd	nd	nd	0.01	0.01	0.01	nd	0.01	0.01	nd
103 Rh	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
105 Pd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
107 Ag	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
111 Cd	nd	0.04	nd	nd	0.01	0.03	nd	nd	0.01	0.05
115 In	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
118 Sn	0.13	0.03	nd	0.05	nd	nd	nd	nd	nd	nd
121 Sb	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
125 Te	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
133 Cs	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
137 Ba	0.08	0.07	0.12	0.06	0.11	0.05	0.05	0.08	0.12	0.12
178 Hf	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
193 Ir	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
195 Pt	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
197 Au	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
201 Hg	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
205 Tl	nd	nd	nd	nd	nd	nd	nd	nd	nd	6.10
208 Pb	nd	0.07	0.06	0.01	0.05	0.04	nd	0.02	0.01	0.14
209 Bi	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

*ND: not detected

In this study, the AWD method was used to determine the antimicrobial properties of the honey samples collected from the province of Bayburt in 2017. The obtained results showed that *Bacillus cereus* BC 6830, *Staphylococcus aureus* NCTC 10788, *Staphylococcus aureus* BC 7231 and *Staphylococcus aureus* ATCC 25923 strains were susceptible among the Gram (+) bacteria (Table 5). But it has been observed that *Enterococcus faecalis* NCTC 12697 strain was much less sensitive compared to other Gram (+) bacteria. However, among the selected microorganisms, Gram (-)

bacteria were highly resistant to honey samples and in most wells, indicated by not observing an inhibition zone. In addition to this, it was observed that only three honey samples had a weak antifungal effect against the *Candida albicans* ATCC 10231 strain.

In order to determine MIC values, the microbroth dilution method was used and it was observed that the minimum inhibitor concentration of honey samples collected from Bayburt which ranged from 6.25% to 25% (w/v) against Gram positive bacteria. However, there was no observed inhibitor effect against Gram negative bacteria and *Candida albicans* ATCC 10231. Based on these results, it can be concluded that honey samples collected from the Bayburt province have antibacterial effects, especially against Gram positive bacteria. In addition to this, the antibacterial effect of these honey samples against Gram negative bacteria and fungi like the *Candida albicans* ATCC 10231 strain is neglible.

Table 5. Inhibition zone diameters obtained by agar well diffusion (AWD) assay (mm)

Microorganisms	Diameter of Inhibition Zones (mm)												
	B1	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**	**B10**	**AH**	**V**	**G**
Bacillus cereus BC 6830	16±1	15±1	12±1	17±1	16±1	12±1	19±1	18±1	15±1	11±1	-	18±1	18±1
Enterococcus faecalis NCTC 12697	-	10±1	-	-	11±1	-	-	11±1	-	-	-	20±1	17±1
Staphylococcus aureus NCTC 10788	15±1	13±1	18±1	19±1	14±1	18±1	12±1	15±1	14±1	13±1	-	13±1	21±1
Staphylococcus aureus BC 7231	21±1	18±1	16±1	15±1	20±1	13±1	17±1	17±1	18±1	16±1	-	14±1	27±1
Staphylococus aureus ATCC 25923	13±2	14±1	16±1	13±2	18±1	15±1	12±1	16±1	15±1	12±1	-	-	19±1
Escherichia coli NCTC 9001	-	-	-	-	-	-	-	-	-	10±1	-	-	15±1
Escherichia coli BC 1402	-	-	-	-	-	-	-	11±1	-	-	-	-	22±1
Pseudomonas aeruginosa NCTC 12924	-	-	-	11±1	-	-	-	-	-	-	-	-	18±1
Salmonella typhimurium RSSK 95091	-	-	-	-	12±1	-	-	-	-	-	-	12±1	15±1
Yersinia enterocolitica ATCC 27729	-	10±1	-	-	-	-	-	11±1	-	-	-	-	16±1
Candida albicans ATCC 10231	-	-	11±1	-	13±1	-	-	-	12±1	-	-	-	-

* AH: Artificial Honey; V: Vancomycin; G: Gentamicin

Table 6. Minimum inhibition concentration (MIC) values obtained by microbroth dilution method (% w/v)

Microorganisms	Minimum inhibition concentration (MIC) (% w/v)										
	B1	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**	**B10**	**AH**
Bacillus cereus BC 6830	6.25	12.5	12.5	12.5	6.25	12.5	6.25	6.25	12.5	25	-
Enterococcus faecalis NCTC 12697	-	-	-	-	25	-	-	-	-	-	-
Staphylococcus aureus NCTC 10788	6.25	25	6.25	6.25	12.5	6.25	12.5	6.25	12.5	12.5	-
Staphylococcus aureus BC 7231	6.25	6.25	6.25	12.5	12.5	12.5	6.25	6.25	6.25	12.5	-
Staphylococus aureus ATCC 25923	12.5	12.5	6.25	12.5	6.25	6.25	12.5	6.25	12.5	25	-
Escherichia coli NCTC 9001	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli BC 1402	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa NCTC 12924	-	-	-	-	-	-	-	-	-	-	-
Salmonella typhimurium RSSK 95091	-	-	-	-	-	-	-	-	-	-	-
Yersinia enterocolitica ATCC 27729	-	-	-	-	-	-	-	-	-	-	-
Candida albicans ATCC 10231	-	-	-	-	-	-	-	-	-	-	-

*AH: Artificial Honey

DISCUSSION

The melissopalynological analysis results obtained indicate that all examined honey samples show multifloral honey characteristics. The source of honey is always plants and the raw material is called nectar (Zander and Koch 1994). Thanks to pollen analysis, the plant resources of honey and the plants providing nectar and pollen for bees at different times can be determined. On the other hand, this analysis allows plants that cause as well as bad smells, bitterness and rapid crystallization to be identified as well as plants that provide pleasant odor, aroma, taste and late crystallization (Andrada et al. 1998). The content of honey varies depending on the plant source of the collected nectar, the geographical characteristics of the area it is collected in and climatic factors (Anklam 1998). The melissopalynologic analysis of the honey samples collected by Sorkun et al. (2014) and Kaya et al. (2005) from different regions of Turkey were made in a manner similar to the present study. In a study on honey samples produced in the Ardahan region of Turkey, the pollen types of *Asteraceae*, *Apiaceae*, *Boraginaceae*, *Betulaceae*, *Brassicaeae*, Caryophyllaceae, Campanulaceae, *Cistaceae*, *Cyperaceae*, *Chenopodiaceae*, *Dipsacaceae*, *Euphorbiaceae*, *Ericaceae*, *Fagaceae*, *Fabaceae*, *Geraniaceae*, *Liliaceae*, *Lamiaceae*, *Poaceae*, *Onagraceae*, *Polygonaceae*, *Rhamnaceae*, *Pinaceae*, *Rutaceae*, *Rosaceae*, *Solanaceae* and *Salicaceae* were determined (Sorkun 2014). The pollen belonging to the taxa of *Apiaceae*, *Anthriscus*, *Pimpinella anisum*, *Cardamine, Centaurea*, *Compositae*, *Ericaceae* and *Dianthus* spp. were dominant in the honey samples collected from the Burdur region in a research carried out by different researchers (Taşkın and İnce 2009). Unlike the present study, Çenet et al. (2015) reported that they observed dominant pollen from *Zea mays*, *Styrax officinalis* and *Trifolium* spp. plants in the honey samples from Muğla, Turkey. These differences put forward by the researchers suggest the richness in diversity of plants and the content of honey produced in Turkey.

It can be said that *Achillea* spp. (B7, B8) and *Onobrychis* spp. (B4,B6,B9,B10) taxa, which were detected secondarily in some honey samples; and *Aster* spp. (in 9 sample) , *Juniperus* spp. (in 9 sample), *Astragalus* spp. (in 9 sample), *Medicago* spp. (in 9 sample), *Lamium* spp. (in all sample), *Rumex* spp. (in 7 sample), *Ranunculus grandiflorus* (in 6 sample) and *Salix* spp. (in 6 sample) taxa, identified as the minority in at least five honey samples, are important plant species originating from the honey samples produced in the Bayburt province. However, it is thought that other plant taxa (Table 1) detected at minor proportions besides these taxa are also important contributors to the formation of the characteristic of the honey samplesof the characteristic of the honey samples.

It has been pointed out in different studies that the TPN-10 value can be used as a criterion in determining the authenticity of honey (Başoğlu et al. 1996). In this study, the seven honey samples were found in Group II and the three honey samples in Group I. Gencay Celemli et al. (2018) reported that the value of the TPS-10 number for 100 honey samples from the Kars region of Turkey were found to be a minimum of 226 and a maximum of 481157 with an average of 31678.

Electrical conductivity is a good criterion for determining the botanical source of the honey and for separating different honey varieties. (Grujić and Komićla 2012, Bogdanov and Martin 2002, Nombré et al. 2010). This value depends on the amount of organic acid, protein, sugar and mineral matter according to some references (Çınar and Ekşi 2012). It is generally reported that the electrical conductivity of honeydew honey is greater than that of flower honey (Çınar and Ekşi 2012). It has been reported in the Turkish Food Codex Communiqué on Honey (No: 2012/58), that the value of electrical conductivity should be lower than 0.8 mS/cm in blossom honey and higher than 0.8 mS/cm in honeydew honey. Results indicate that the honeys examined within the scope of our study are flower honey and support the results of the palynological analysis of the study. In the same way, Güzel (2014) reported that the electrical conductivity values of 76 honey samples collected from the Ardahan region of Turkey were found to be a minimum of 0.16 mS/cm, a maximum of 0.26 mS/cm, with a mean of 0.02 mS/cm. Guler et al. (2007) concluded that the electrical conductivity of pure blossom honey is higher than that of honey produced by sucrose syrup.

Moisture is one of the most important parameters affecting the physical characteristics of honey such as viscosity and crystallization (Escuredo et al. 2013). According to the Turkish Food Codex Communiqué on Honey (No: 2012/58), the water content of honey should be less than 20%. Our results indicate that the moisture content of the honeys obtained in the present study is among the normal criteria.

The basic composition of honey is carbohydrates. Approximately 70-80% of carbohydrates are composed of glucose and fructose monosaccharides (Ecem Bayram and Demir 2018). The ratio of glucose to fructose in honey depends on the source of nectar. The mean fructose/glucose ratio is 1.2: 1 (White 1980). The fructose/glucose ratio in the honey sample gives information on the rate of the crystallization of the honey. Crystallization is fast when the fructose/glucose ratio is between 1.0 and 1.2, and crystallization takes longer when this ratio is 1.3 or more (Ruoff et al. 2006).

In this study, all of the honey samples tested were found to have a glucose and fructose content consistent with the values given in the Turkish Food Codex Honey Communiqué (No: 2012/58). Furthermore, when the crystallization ratings of the honey samples were evaluated, it can be said that all the honey samples have quick crystallization properties. Similarly, studies on sugar profiles have been made on honey samples from different origins. Can et al. (2015), reported that the fructose/glucose ratio of honeys from different regions in Turkey was between 1.16 and 2.44. In a different study, the amount of glucose in Spanish honeys was reported as 19.3-31.2%; the fructose ratio was 23.2%-39.9%; the fructose plus glucose value was 42.5-71.1%, and the fructose / glucose value was between 1.13 and 1.36 (Soria et al. 2004).

The multi-element content of honey is quite low and this ratio varies depending on the botanical source of the honey, the climatic conditions of the area it is obtained from and the extraction effect. Any significant deficiency in the elemental content of soil, rock and water affects the elemental content of the plant growing in this region, which directly affects the nectar and pollen, hence the mineral content of the honey. For this reason, the content of metal ions in honey can contribute to the determination of the geographic origin of honey since it is in harmony with the environmental conditions (Hernández et al. 2005). The increase in the mineral content of honey results in a darker color and strong aroma (Escuredo et al. 2013, Karabagias et al. 2014), which makes it more attractive for the consumers as the honeys rich in minerals are considered to have health benefits. K was the first element determined at the highest concentration in all of the honey samples (261.34-1863.05 mg/kg) and these results were consistent with previous studies (Terrab et al. 2003, Chua et al. 2012, Oroian et al. 2015). Concentrations of Na, K, Ca, Mg, Cu, Fe, Mn, Zn and Co were 118, 296, 51, 33, 1.8, 6.6, 1.0, 2.7, respectively and 1.0 mg/kg in honey samples from South-Eastern Anatolia (Turkey). In contrast, it was found that the amount of other elements (Ca, Na, Cu, Mg, Mn, Fe, and Co) excluding K and Zn (694.28, 7.52 mg / kg, respectively) were lower in Bayburt honeys on average. Oroian et al. (2015) reported that K has the highest concentration in all honey types, regardless of botanical origin. These results may indicate that the amounts of other elements apart from potassium differ according to the botanical or geographic origin of the honey.

Depending on its plant source, honey can have important effects on human health. The antimicrobial property of honey was first identified by Van Ketel in 1892 (Dustmann 1979). The antimicrobial effects of

honey are caused by more than one factor. Hydrogen peroxide (H_2O_2), a component produced in honey due to glucose oxidase enzyme activity secreted by bees, can be considered as the first among these factors. In addition to this, defensin-1 produced by honey bees, various phenolic compounds, pH parameters and high osmolality also contribute to the antimicrobial effect of honey (Cooper et al. 2002, Kwakman et al. 2010; Szweda, 2017.)

CONCLUSION

By defining the characteristics of honey, types of honey can be classified according to their floral and geographic origins and can be presented to the consumer as special products with unique characteristics (Sancho et al. 1991). When the results of this study are evaluated, it can be said that a variety of different plant species are the source of Bayburt honey and the flora of the region is rich in honey plants. However, it is necessary to work out in detail and with more examples, in order to clearly determine the plants that are the complete source of the honey produced in Bayburt. In addition, the fact that all of the honey samples examined conform to the Turkish Food Codex Communique on Honey (No: 2012/58) in terms of physicochemical criteria (sugar profile, electrical conductivity, moisture) indicates that the beekeepers who produce honey in this region carry out a quality and hygienic production. In addition, when looking at the element profile of the honey samples, it can be said that the element diversity and content are rich. These results indicate that honey from Bayburt has the quality that may be preferred by the consumer and can be used as a good nutritional supplement.

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