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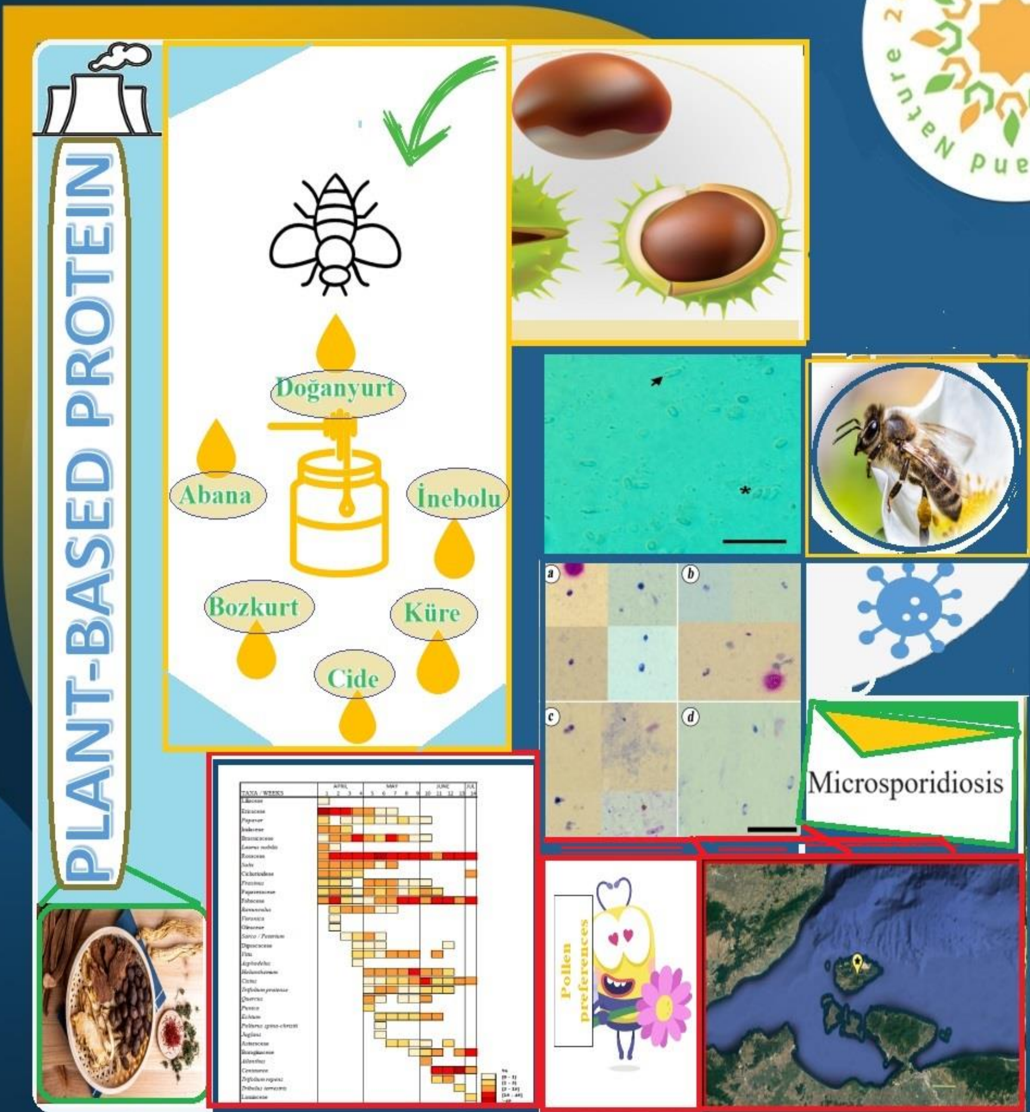

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The Journal of Apitheraphy and Nature accepts English-language original articles, reviews, and letters to the editor concerning various fields of research. Main topics include:

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A Record of Microsporidian Pathogen of the European wasp, Vespula vulgaris Linnaeus, 1758 (Hymenoptera: Vespidae) in Turkey

Avrupa Yaban Arısı Vespula vulgaris Linnaeus, 1758 (Hymenoptera: Vespidae)'in Türkiye'de Microsporidian Patojeni Kaydı

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Abstract

Microsporidia are common enigmatic pathogens of hymenopterans. Although these species are more concerned with Apidae (especially honeybees), they are also known to infect members of Vespidae. Apart from these species, many defined and undefined microsporidia infections were detected infecting Vespidae individuals in the literature. Especially *Vespula vulgaris* Linnaeus, 1758 (Hymenoptera: Vespidae) infected different microsporidian species like a *Nosema bombi*, *Vavraia culicis*, *Nosema vespula*, etc. Molecular identification-based microsporidian records in predator species such as *V. vulgaris* are highly suspicious. In such predator insects, microsporidian infections should be supported by characteristic visuals of the pathogen's life cycle. With this perspective this study is the first and only study that presents the life-cycle stages and spore morphometrics data of a microsporidium isolated from *V. vulgaris*. *V. vulgaris* samples were collected from July to September 2021 in Trabzon, Turkey. During the observations, 415 samples were examined, and five of them were infection positive (microsporidiosis prevalence 1.20%). Infection was found mostly in the midgut of the host, and infection was mostly chronic. Fresh mature spores were oval in shape and measured 4.57±0.54 (3.26–5.95; n=200) µm in length and 2.43±0.33 (1.43–3.35; n=200) µm in width. The current microsporidium has a *Nosema*-like disporoblastic merogony and sporogony.

Keywords: Hymenoptera, Microsporidium, *Nosema*, Pathogen, Wasp, *Vespula vulgaris*

Özet

Microsporidia, hymenopteranların yaygın gizemli patojenleridir. Bu türler daha çok Apidae (özellikle bal arıları) ile ilgili olsa da Vespidae üyelerini de enfekte ettikleri bilinmektedir. Bu türlerin dışında literatürde Vespidae bireylerini enfekte eden tanımlanmış ve tanımlanmamış birçok microsporidia enfeksiyonu tespit edilmiştir. Özellikle *Vespula vulgaris* Linnaeus, 1758 (Hymenoptera: Vespidae), *Nosema bombi, Vavraia culicis, Nosema vespula*, vb. gibi farklı mikrosporidian türleri ile enfekte olmuştur. *V. vulgaris* gibi yırtıcı türlerdeki moleküler tanımlamaya dayalı mikrosporidian kayıtları oldukça şüphelidir. Bu tür yırtıcı böceklerde mikrosporidia enfeksiyonları, patojenin yaşam döngüsünün karakteristik görselleriyle desteklenmesi gerekir. Bu bakış açısıyla bu çalışma, *V. vulgaris*'ten izole edilen bir microsporidium'un yaşam döngüsü aşamalarını ve spor morfometrik verilerini sunan ilk ve tek çalışmadır. *V. vulgaris* örnekleri Temmuz-Eylül 2021 tarihleri arasında Trabzon ilinde toplandı. Gözlemler sırasında 415 örnek incelendi ve bunlardan 5 örnekte enfeksiyon pozitif çıktı (mikrosporidiosis oranı %1.20). Enfeksiyon çoğunlukla konağın orta bağırsağında bulundu ve çoğunlukla kronikti. Taze olgun sporlar oval şekilliydi ve 4.57±0.54 (3.26–5.95; n=200) µm uzunluğunda ve 2.43±0.33 (1.43–3.35; n=200) µm genişliğinde ölçüldü. Mevcut microsporidium, Nosema benzeri bir dissporoblastik merogoni ve sporogoniye sahiptir.

Anahtar Kelimeler: Hymenoptera, Microsporidium, *Nosema*, Patojen, Yaban arısı, *Vespula vulgaris*

1. INTRODUCTION

Vespidae is one of the most significant insect families with more than 5000 species globally. This group, which has a cosmopolitan distribution on earth, spreads in Turkey with 298 records, 65 of which are endemic subspecies and species (Yıldırım & Gusenleitner, 2012; Yıldırım & Bekircan, 2020). These species consist of social and solitary forms. *Vespula vulgaris* Linnaeus, 1758 (Vespidae: Hymenoptera), a common wasp or European wasp, is a eusocial vespid that fed its larvae with masticated insect parts or glandular secretions by adult females (Goulet & Huber, 1993). Although this omnivorous wasp is native to the Euroasia, it is located in Argentina, Australia, and New Zealand and is listed as the world's worst invasive species (Gruber et al., 2019). This invasive species not only poses a significant danger to native species but also can sting humans to attack or in defense. With this behavior, exposure to stings is a real medical concern since some people can die from anaphylactic shock during their outdoor activities (Boeve et al., 2014). Due to these aforementioned, *V. vulgaris* is considered in the pest category.

Struggling with the *V. vulgaris* and other social wasps is generally done with chemical control methods like insecticides in bait formulation or direct insecticide application into nests (Rose et al., 1999). Although chemical control is an effective and fast-paced control method in the short term, it has disadvantages like insecticide use being expensive, labour-intensive, and potentially hazardous to non-target organisms. In addition, it should not be forgotten that this species, defined as a pest during its invasive periods, plays an important ecological role and acts as a regulator species on the other pest insect populations in nature, such as preying on flies and caterpillars (Boeve et al., 2014). For this reason, it is necessary to be more careful when chemical control of this species, and different control methods should be developed.

There is increasing awareness of the microbial control of pests worldwide, especially in the last decades. For this reason, the studies to determine the microbial communities and their effects on the fitness and performance of pests are increasing day by day. Similarly, this study tries to determine the natural pathogen and parasites of *V. vulgaris* and is the first attempt to assess the natural pathogens of Turkey's wild *V. vulgaris* populations.

2. MATERIALS and METHODS

Vespula vulgaris individuals were collected from July to September 2021 in Trabzon, Turkey. For the collection process, common plastic bottle yellowjacket traps were used (Erdoğan & Dodoloğlu, 2013). In these traps, fresh meat was used as bait not to catch the honey bees. Traps were placed in different locations far from each other to catch adult samples belonging to different nests. These traps were checked weekly, and the baits in the traps were replaced with new ones. The captured samples were labeled and brought to the laboratory as soon as possible after the necessary macroscopic examinations were made. In microscopic observations to determine the pathogens and parasites, wet smears were prepared using Ringer's solution dissected individuals and examined under the light microscope (Baki & Bekircan, 2018; Bekircan, 2020; Bekircan & Tosun, 2021). In order to determine the life cycles of possible pathogens, infection-positive smears were stained using the Giemsa stain protocol, which is frequently used in insect pathology (Yıldırım & Bekircan, 2020; Yıldırım et al., 2022). Zeiss AXIO microscope equipped with an Axicam ERc5s digital camera was used for photographing the infection-positive samples, and the ZEN 2.3 Blue Edition imaging software was used for measurement and analysis.

3. RESULTS and DISCUSSION

During the field study, 415 samples were collected from five different traps. In the light microscopic observations, microsporidiosis was determined in only five of the 415 samples, and with this result, infection prevalence was calculated as 1.20%. The infection was detected in a very limited region of the midgut of infected hosts. The number of mature spores, the first and most important finding of microsporidiosis, was very few (Figure 1). Fresh mature spores were oval in shape and measured 4.57±0.54 (3.26-5.95; n=200) µm in length and 2.43±0.33 (1.43-3.35; n=200) µm in width.

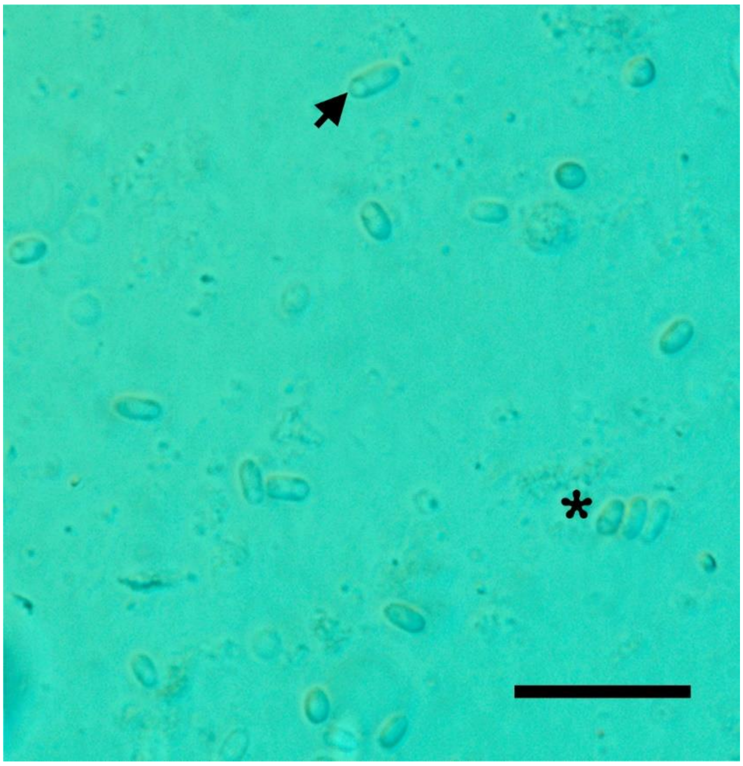

Figure 1. The light micrograph of the fresh oval spores. While the arrow indicates the mature spore's posterior vacuole, the asterisk shows chain formation with binary fission (Unite bar=20 µm).

Various life cycle stages were observed in Giemsa stained microscopic smears. Meronts were spherical or ovoid in shape. While the spherical meronts were measured 2.46-2.72 × 2.72-2.90 (n=8) µm, ovoid meronts were measured as a 3.08-3.34×2.20-2.55 (n=6) µm (Figure 2a). Similarly, sporonts were spherical and elongated oblong and measured 3.34-4.22 × 2.64-3.60 (n=6) µm and 3.52-5.45 × 1.84-2.64 (n=5) µm, respectively (Figure 2b). Sporoblasts, which mature into a spore and originate from the divided diplokaryotic sporonts, were elongated in shape. And sporoblasts were measured 5.01-7.56 × 2.99-3.43 (n=4) µm (Figure 2c). Finally, mature Giemsa stained spores were measured again (3.90±0.55 (2.99–5.10; n=30) µm in length and 2.14±0.40 (1.49–2.90; n=30) µm in width) (Figure 2d).

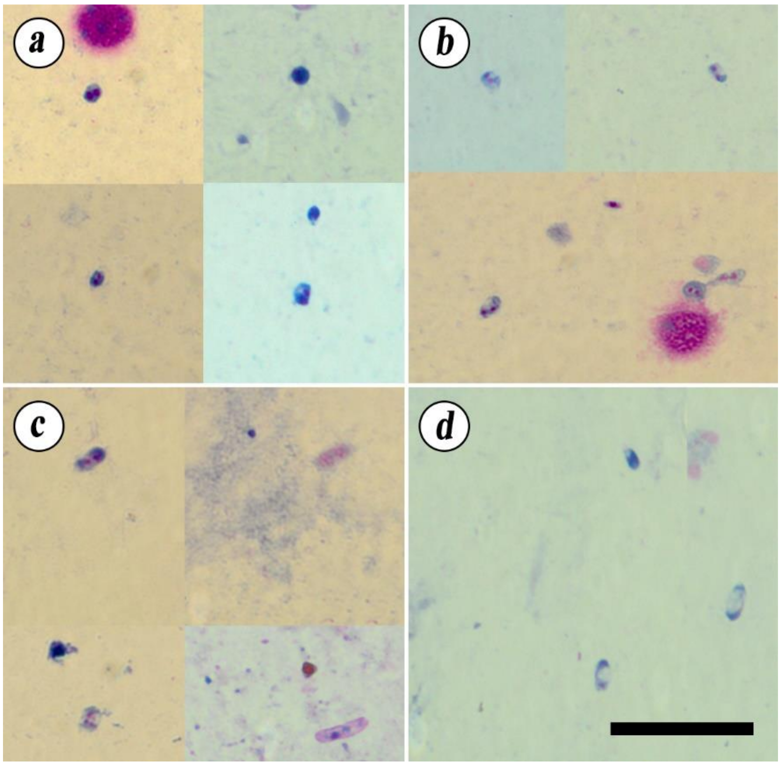

Figure 2. Light micrographs of the Giemsa stained life-cycle stages. **a**: Spherical and oval binucleate meront (midgut); **b**: Binucleate oval sporont (midgut); **c**: Sporoblast (midgut); **d**: Mature oval spores (midgut) (Unite bar=20 µm).

The approach to using pathogens and parasites is widely agreeable worldwide for pest control, especially in the last quarter. Therefore, the number and scope of studies on detecting and identifying pathogens and parasites are increasing day by day (Felden et al., 2020; Lester et al., 2015; Rose et al., 1999). Similarly, this research is the first and only study conducted to determine natural pathogens and parasites of European wasps, *Vespula vulgaris* Linnaeus, 1758 (Hymenoptera: Vespidae) in Turkey. The genus *Vespula* is exposed to various diseases and pathogens like other hymenopterans. To date, nearly 50 fungi, 12 bacteria, seven nematodes, four protozoans, and two virus species have been detected from different individuals of this genus (Felden et al., 2020).

Microsporidia are common enigmatic pathogens of hymenopterans. The most reputed microsporidia species infecting hymenopterans are *Vairimorpha* (*Nosema*) *ceranae* and *Vairimorpha* (*Nosema*) *apis* (Graystock et al., 2013; Plischuk et al., 2009; Tosun & Bekircan, 2021). Although these species are more concerned with Apidae (especially honeybees), they are also known to infect members of Vespidae (Gabín-García et al., 2021; Lester et al., 2014; Rose et al., 1999). Apart from these species, many defined and undefined microsporidia infections were detected infecting Vespidae individuals in the literature. Especially *V. vulgaris* infected different microsporidian species like a *Nosema bombi*, *Vavraia culicis*, *Nosema vespula*, etc. (Felden et al., 2020; Lester et al., 2014; Lester et al., 2015; Quinn et al., 2018).

Modern microsporidian taxonomy is built on classical morphological approaches (spore shape, structure, size, host type, etc.) and molecular-based methods (Vega & Kaya, 2012; Weiss & Becnel, 2014). However, in microsporidian pathogens isolated from *V. vulgaris*, there is almost no description based on this basis. For instance, a base sequence uploaded to NCBI GenBank by Da Silva et al. in 1994 entered the literature as *Nosema vespula* (Accession no: U11047) and is widely used in molecular comparisons for species identification (Bekircan et al., 2016; Biganski et al., 2020; Vossbrinck & Debrunner-Vossbrinck, 2005). However, there is no study prepared with taxonomic characters (like spore structure or life-cycle) proving that this mysterious record is indeed a microsporidian infection. The records of microsporidiosis detected in these studies only on a molecular basis are suspicious because microsporidium spores and DNAs that settle in the predator's digestive tract after consuming an insect with microsporidiosis by predator insects such as *V. vulgaris* may cause false infection positivity in these molecular analyses. The study of Quinn et al. in 2018 with metatranscriptomic analysis on *V. vulgaris* revealed results confirming this claim. *Vavraia culicis* (Weiser, 1947), a microsporidium infecting mosquitoes, was detected in the *V. vulgaris* samples analyzed in this study and in the same publication, the authors stated that this would not be possible (Quinn et al., 2018). Therefore, microscopic findings such as mature spore structure and life-cycle stages should be detected before molecular analysis, especially in studies to identify microsporidian pathogens of predatory insects.

The current study is the first and only study that presents the life-cycle stages and spore morphometrics data of microsporidium isolated from *V. vulgaris*. Therefore, it was impossible to compare the current microsporidium with the other isolates previously isolated from *V. vulgaris* in the literature in terms of the classical morphological approach (based on nuclear arrangement, spore shape, structure, and size, etc.). It would not be unreasonable to compare the current microsporidium with *V. ceranae, V. apis*, and *Nosema bombi*, which were detected by molecular techniques in *V. vulgaris* and caused infection in other Hymenoptera individuals according to these taxonomical characters. The spore shape and dimensions traditionally have been significant taxonomic characteristics used in Microsporidia taxonomy (Canning & Vávra, 2000; Sprague et al., 1992). The spore dimension of the current microsporidium (4.57±0.54 × 2.43±0.33 µm) clearly differentiates from the *V. apis*. The spore dimensions of *V. apis*, *V. ceranae*, and *Nosema bombi* are 5-7 × 3-4 µm, 4.7 × 2.7 µm, and 4.20-5.39 × 2.13-3.50 µm, respectively (Fries, 1993; Fries et al., 1996; McIvor & Malone, 1995; Zander, 1909). In addition, the current microsporidium (in midgut) differs from *Nosema bombi* (in Malpighian tubules) with the infection site, which is another important taxonomic characteristic (Sprague et al., 1992). The current microsporidium is similar to *V. ceranae* in terms of spore morphology and infection site. However, the type of infection they develop in their hosts is quite different from each other. While *V. ceranae* creates systemic infection in its hosts, the current microsporidium creates a chronic infection in *V. vulgaris* (Chen et al., 2009). Unfortunately, this feature of the current microsporidium has prevented adequate sample availability for us to carry out the detailed molecular and electron microscopical studies necessary for species identification.

The current microsporidium's life-cycle type, detected in the examinations of Giemsa stained preparations, is disporoblastic development (diplokaryotic merogony and sporogony) as in the Nosema genus (Nägeli, 1857, Weiss & Becnel, 2014). Although the life cycle stages and types are similar to that of the genus *Nosema*, this claim is contradictory since we could not support it from a molecular point of view. However, there are records of Nosema-like spores detected in the genus *Vespula* in the literature, which could not be amplified the SSU-rRNA locus by the primers (Gabín-García et al., 2021). Because of all these shortcomings, it would be more appropriate to express the microsporidium detected in this study as *Microsporidium sp*. in order not to cause systematic confusion.

4. CONCLUSION

This manuscript is the first and only research to reveal a microsporidium species' life cycle and spore morphology that causes infection in *Vespula vulgaris* Linnaeus, 1758 (Hymenoptera: Vespidae).

DECLARATIONS

The authors declare that they have no conflicts of interest.

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Pollen Preferences of Honey Bees (Apis mellifera L.) on Marmara Island

Marmara Adasında Bulunan Bal Arılarının (Apis mellifera L.) Polen Tercihleri

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Abstract

Islands have a special importance in terms of biodiversity. At the same time, it is important to follow the feeding behavior of pollinators that ensure the continuation of biodiversity throughout the island. In this study, it was aimed to follow the pollen collection activities of honey bees (*Apis mellifera* L.) on Marmara Island and to determine the pollen sources. Pollen sampling was carried out weekly during the 14 weeks from the first week of April to the first week of July when honey bees intensively carried out pollen collection activities. The sampled pollen grains were classified according to their color and examined by light microscopy. A total of 34 taxa were identified from the pollen grains. The taxa represented in significant proportions were; Rosaceae, Fabaceae, and *Centaurea*, Ericaceae, Brassicaceae, *Cistus*, *Helianthemum*, *Vitis* and *Salix*. They constitute a total of 79.37%. Rosaceae family pollen has been found as the most preferred taxa by honey bees for 8 weeks. The first two weeks of May was the most productive period in terms of pollen species in Marmara Island and the amount of pollen brought to the hives decreased in early July. In this study, it was tried to shed light on the pollen collection preferences of honey bees and pollination periods of honey bee used plants under conditions of Marmara Island.

Keywords: *Apis mellifera*, Honey bees, Pollen preferences, Marmara Island

Özet

Adalar biyoçeşitlilik açısından özel bir öneme sahiptir. Aynı zamanda ada genelinde biyoçeşitliliğin devamını sağlayan polinatörlerin beslenme davranışlarının takip edilmesi önemlidir. Bu çalışmada Marmara Adası'nda bal arılarının (*Apis mellifera* L.) polen toplama faaliyetlerinin takip edilmesi ve polen kaynaklarının belirlenmesi amaçlanmıştır. Bal arılarının yoğun olarak polen toplama faaliyeti gerçekleştirdiği Nisan ayının ilk haftasından Temmuz ayının ilk haftasına kadar 14 hafta boyunca haftalık olarak polen örneklemesi yapılmıştır. Örneklenen polen taneleri renklerine göre sınıflandırılmış ve ışık mikroskobu ile incelenmiştir. Polen tanelerinden toplam 34 takson tespit edilmiştir. Önemli oranlarda temsil edilen taksonlar; Rosaceae, Fabaceae ve *Centaurea*, Ericaceae, Brassicaceae, *Cistus*, *Helianthemum*, *Vitis* ve *Salix*'tir. Bunlar toplamda %79.37'lik bir oranı oluşturmaktadır. Rosaceae familyası polenleri, 8 hafta boyunca bal arıları tarafından en çok tercih edilen takson olarak bulunmuştur. Marmara Adası'nda Mayıs ayının ilk iki haftası polen türleri açısından en verimli dönem olmuş ve Temmuz ayı başında kovanlara getirilen polen miktarı azalmıştır. Bu çalışmada Marmara Adası koşullarında bal arılarının polen toplama tercihlerine ve bal arılarının kullandıkları bitkilerin tozlaşma periyodlarına ışık tutulmaya çalışılmıştır.

Anahtar Kelimeler: *Apis mellifera*, Bal arıları, Polen tercihleri, Marmara Adası

1. INTRODUCTION

Honey bees live by forming colonies. Worker bees dominate within the hive. These worker bees, which have taken on different roles, collect pollen and nectar intensively during the spring and summer seasons to raise their offspring and survive the winter. Pollen is collected directly from plants and stored in specialized baskets called corbicula and brought into the hive. The collected pollen is stored as bee bread. Pollen pellets collected from different plant sources at different times are used in brood rearing (Goodman, 2003). Pollen is an important source of protein, especially for larval bees. The decisions of honey bees regarding pollen collection activities are influenced by various factors such as the number of eggs or larvae in the hive, weather conditions, the genetic structure of the bees, available resources in the environment, and the amount of stored pollen (Dreller et al., 1999; Latshaw & Smith, 2005).

The pollen production of colonies can be monitored through pollen traps at the hive entrance. This allows for gathering information about the pollen preferences of honey bees, as well as the timing and weather conditions under which pollen collection activities occur. Under favorable conditions, honey bees initiate their pollen collection activities early in the morning and reach their maximum levels around 10 a.m. The majority of the daily collected pollen is usually gathered in the morning hours before noon. During the afternoon, honey bees focus more on collecting water and nectar. On rainy days, pollen collection activities almost cease, but they quickly resume and reach maximum levels shortly after the rain (Ngo et al., 2021).

Studies have shown that temperature is the most significant factor in pollen collection activities. Pollen collection increases during spring and especially summer when the weather is warmer. Cloudy, windy, and humid conditions have a negative impact on flight and, consequently, on pollen collection activities (De Mattos et al., 2018).

Flora is shaped by climate, topography, and biogeographic location and varies from region to region. Since floral elements are the natural resources of honey bees, their diet is also directly affected by the structure of the flora. For this reason, the palynological content of any product (e.g., honey, pollen, propolis) taken from one colony is not always compatible with the same product taken from a different region, and it is necessary to determine the resources honey bees use in different areas. However, the islands are very important regions in terms of biodiversity, and it is known that different bee varieties can be found on the islands.

Many researchers indicate that foraging patterns and abundance of native pollinators are altered in the presence of honey bees on the islands (Gross, 2001; Hansen et al., 2002; Roubik, 1978; Schaffer et al., 1983; Vaughton, 1996; Wenner & Thorp, 1994). For this reason, it is important to know the pollination ability or pollination preferences of the honey bees in a region, especially on an island. Also, honey bees (*Apis mellifera* L.) forage in 2·3 km meanly and the circle enclosing 95% of the colony's foraging activity had a radius of 6 km (Visscher & Seeley, 1982), but later studies reported the long-range foraging for the efficient source e.g., 9.5 km (Beekman & Ratnieks, 2000). The distances become even more important when the place is an island as the bee cannot rest on the sea surface.

This study aims to determine the timing of the main pollen flow period for honey bees in a special island ecosystem, to reveal the important pollen sources used by honey bees, to determine the pollination periods of pollen sources and to prepare a beekeeping pollen calendar for the blooming period for Marmara Island.

2. MATERIAL and METHODS

2.1. Study Area

The Marmara Islands are located in the southwest of the Marmara Sea. The largest of these islands is Marmara Island, which has an area of 117 km^2 with an altitude of 0-800 m. The coast of Marmara Island is approximately 5.5 km from the coast of the nearest Avşa Island, and the mainland is approximately 20 km to the north and 9.5 km to the south (Figure 1).

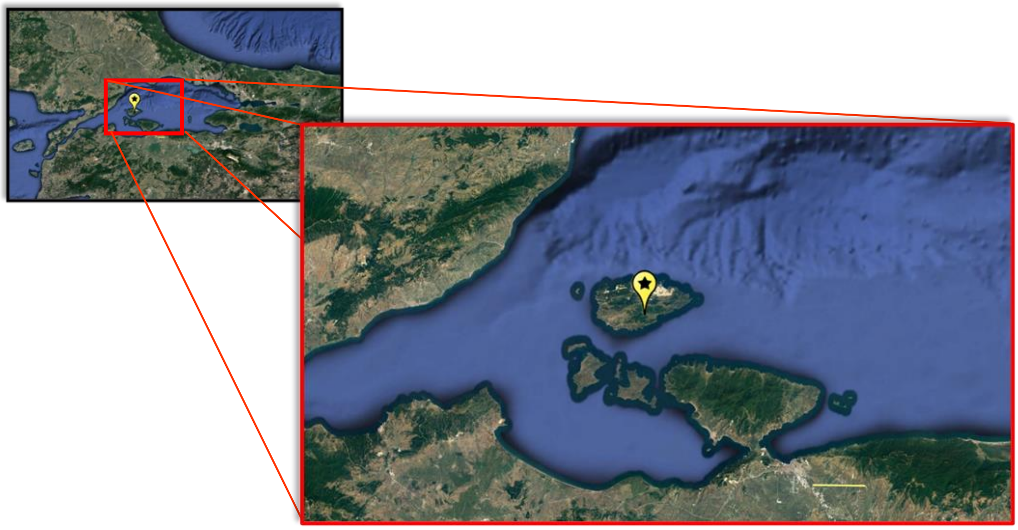

Figure 1. Location map of Marmara Island (Anonymous, 2023).

Among the Islands, Marmara Island receives the highest amount of rainfall. The coldest month on the island is January, and the hottest month is July with an average temperature of 24.6 °C. The island experiences a Mediterranean climate, but the northern parts are influenced by moist air masses coming from the Black Sea. As a result, the northern region is more humid compared to the southern region. The southern part mainly consists of maquis formations (Tunçdilek et al., 1987). The characteristic species found on the southern slopes include; *Ficus carica* L., *Pistacia terebinthus* L., *Cercis siliquastrum* L., *Olea europaea* L., *Quercus coccifera* L., *Rhus coriaria* L., *Juniperus oxycedrus* L., along with *Sarcopoterium spinosum* L., *Cistus* sp., *Rubus fruticosus* L., *Spartium junceum* L., *Rosa canina* L., *Jasminum fruticans* L., and *Thymus vulgaris* L. among these formations. An increase in *Matricaria*, *Papaver rhoeas* L., and *Eryngium cucrulea* is observed in the region with the spring rains (Tunçdilek et al., 1987). In the northern region, the characteristic plants include; *Pinus brutia* Ten., *Arbutus andrachne* L., *Arbutus unedo* L., *Olea europaea* L., *Erica arborea* L., *Myrtus communis* L., *Pistacia terebinthus* L., *Strax officinalis* L., *Phillyrea latifolia* L., *Quercus coccifera* L., *Rhus coriaria* L., *Laurus nobilis* L., *Buxus* sp., and *Cercis siliquastrum* L. (Tunçdilek et al., 1987). Additionally, *Castanea sativa* Mill. can be observed at elevations of 300-350 meters on the northern slopes (Coşkun, 1999).

2.2. Sampling and Pollen Analyses

Sampling was carried out in selected 7 Langstroth-type hives of honey bees (*Apis mellifera* L.) with brood and pollen drawers located in the Topağaç region of the Marmara Island (40°36’14.90” N 27°38'12.03" E). From April 4th, sampling was continued weekly until the amount of pollen in the drawers dropped below the sampling level. Sampling was terminated after July 10th as no hives were brought 500 pollen pellets weekly. For this reason, sampling was carried out for a 14-week period when honey bees were intensively collecting pollen on Marmara Island.

Color classification and calculation method was followed for pollen preparations and calculations of percentage values (Almeida-Muradian et al., 2005; Bilisik et al., 2008; Mărgăoan et al., 2014). The collected samples were stored in a refrigerator at +4 °C for diagnosis, and 83 samples were collected within the specified dates. After the sampling process was completed, 500 randomly selected pollen grains were separated from each pollen sample and classified based on their colors (Kirk, 1994). A piece of each pollen load from each color was mixed with glycerin-jelly, and stained using basic fuchsine, and the unacetolysed pollen grains were examined (Wodehouse, 1935). Pollen identifications were made using light microscopy and compared with the reference slide collection of the Bursa Uludag University Palynology Laboratory. From this data the percentages of the each taxon of pollen grains were calculated (Almeida-Muradian et al., 2005; Bilisik et al., 2008; Mărgăoan et al., 2014).

For the preparation of pollen calendar; taxonomic groups detected in over 45%, 16-45%, 3-16%, 3-1%, less than 1% respectively, of the 500 pollen grains analyzed per sample, which modified from Louveaux et al. (1978) and Lau et al. (2019) relative abundance values.

3. RESULTS

As a result of the study, 34 different taxa were identified from pollen pellets. Among these, 13 plant families, 15 genera, and 6 species were determined. Parallel with the floristic opulence, May had the highest pollen diversity, with 22 taxa foraged by honey bees. On a weekly basis, 19 different taxa were used as pollen sources in the 6th week (2nd week of May) of the sampling period. The 5th week was the second highest, with 17 different taxa (Figure 2).

Nine taxa, exceeding 3% of the overall total, were evaluated as pollen species highly preferred by honey bees for the region as follows: Rosaceae (29.6%), Fabaceae (11.69%), *Centaurea* (8.33%), Ericaceae (6.88%), Brassicaceae (6.76%), *Cistus* (4.81%), *Helianthemum* (4.67%), *Vitis* (3.58%), and *Salix* (3.05%) and these pollen types comprised 79.37% of the overall (Table 1). From these, Rosaceae, *Centaurea*, and Ericaceae pollen were found in more than 45% and reported as weekly dominated pollen types (Figure 2).

During the sampling period, Rosaceae was the most preferred honeybee pollen source on Marmara Island. From the first week of sampling until the last week, honey bees have consistently preferred and utilized Rosaceae members as an efficient pollen source. Rosaceae members account for the highest total percentage with 29.6%, and honey bee foragers mostly prefer Rosaceae pollen 2-3, 5-9, and 14th weeks as a primary source with high constancy (26.73-52.23%). The second week of May, which corresponds to the 6th week of the sampling period, is observed to have the highest weekly percentage of Rosaceae pollen (>45%) as the preferred pollen source, with a weekly rate of 52.23% (Figure 2, Table 2).

Table 1. Total percentages of pollen pellets collected from honey bee hives on Marmara Island

TAXA	TOTAL %
Rosaceae	**29.60**
Fabaceae	**11.69**
Centaurea	**8.33**
Ericaceae	**6.88**
Brassicaceae	**6.76**
Cistus	**4.81**
Helianthemum	**4.67**
Vitis	**3.58**
Salix	**3.05**
Boraginaceae	2.72
Ranunculus	2.55
Fraxinus	2.04
Cichorioideae	2.03
Papaveraceae	1.95
Echium	1.72
Trifolium pratense	1.42
Iridaceae	1.24
Trifolium repens	0.83
Quercus	0.79
Papaver	0.68
Laurus nobilis	0.63
Asteraceae	0.47
Sarco / Poterium	0.45
Ailanthus	0.32
Tribulus terrestris	0.18
Punica	0.15
Lamiaceae	0.12
Dipsacaceae	0.11
Paliurus spina-christii	0.07
Oleaceae	0.06
Veronica	0.03
Juglans	0.03
Liliaceae	0.03
Asphodelus	0.01

The second most preferred pollen type, with a total percentage of 11.69%, was represented by the Fabaceae family (Table 1). Fabaceae was preferred throughout the entire sampling period as Rosaceae (Figure 2). The highest weekly percentages were observed in the 11-12 and 14th weeks (20.55-28.50%) of the sampling (2-3rd weeks of June and the first week of July) (Table 2). According to the generated pollen calendar, Rosaceae and Fabaceae were consistently used as pollen sources by honey bees for 14 weeks (Figure 2).

Centaurea was the third most preferred pollen species, utilized as a pollen source during the last four weeks of the sampling period, represented 8.33% of the total pollen (Table 1). Honey bees collected *Centaurea* pollen from the 11th week until the end of the sampling period, the 14th week (Figure 2). The highest preference for *Centaurea* pollen was observed in the 13th week as a week's dominated pollen type (47.52%) on the last week of June (Table 2).

Pollen loads of the Ericaceae family constitute 6.88% of the total (Table 1). Pollen collection from Ericaceae by honey bees was intense in the early weeks and ceased in the 7th week of the sampling period (Figure 1). The percentage, initially recorded as 47.64% (week's dominated pollen type with >45%) in the first week (early April), gradually decreased as the weeks progressed, reaching a weekly value of 0.62% in the 7th week (Table 2). Ericaceae pollen was found in over 45% of the first week of April as a week's dominated pollen (Figure 2).

Brassicaceae was preferred by honey bees from the first week to the 10th week of the sampling period (Figure 1). The highest intensity was observed in the 4th week, with a weekly value of 31.61%. The percentage increased until the 4th week, reaching the highest value, then decreased in the 5th and 6th weeks. It experienced a second peak in the 7th week, with 18.22%. In the 10th week, the end of the sampling period, and the first week of June, it concluded with 0.44% (Table 2). It constitutes 6.76% of the total pollen load (Table 1).

Cistus pollen loads were observed starting from the 5th week of the sampling period, the first week of May (Figure 1). Honey bees visited *Cistus* and collected its pollen from the 5th week until the 14th week. The highest value, with a percentage of 37.65%, was reached in the first week of June (the 10th week of the sampling period). It constitutes 4.81% of the total pollen load (Table 1).

Helianthemum pollen pellets constitute 4.67% of the total pollen load (Table 1). Honey bees started visiting *Helianthemum* in the 5th week of the sampling period and continued until the 12th week (from the first week of May to the third week of June) (Figure 1). The highest weekly percentage, with a value of 20.03%, was observed in the 9th week of the sampling period (the last week of May) (Table 2).

Vitis, which refers to grapevine, has been used as a pollen source by honey bees starting from the 4th week of the sampling period (last week of April) (Figure 1). The highest weekly percentages were observed in the 8th week (12.15%) and 9th week (11.62%). In the 10th week, *Vitis* was not preferred, and the use of *Vitis* as a pollen source ended in the 11th week with a percentage of 9.79% (Table 2). *Vitis* accounts for 3.35% of the total pollen load (Table 1).

Salix pollen pellets were present from the first week of the sampling period until the 7th week. They were used as a pollen source by honey bees from the first week of April until the 3rd week of May (Figure 1). The most preferred week for *Salix* was the 3rd week of the sampling period, with a percentage of 10.79% (Table 2). *Salix* accounts for 3.05% of the total pollen load (Table 1).

The number of pollen species that are not among the most preferred taxa but are significantly preferred by honey bees and exceed 1% of the total is 8. These are Boraginaceae (%2.72), *Ranunculus* (%2.55), *Fraxinus* (%2.04), Cichorioideae (%2.03), Papaveraceae (%1.95), *Echium* (%1.72), *Trifolium pratense* L. (%1.42), and Iridaceae (%1.24) (Table 1). Among the taxa that constitute less than 1% of the total, the following taxa are included: *Trifolium repens* L. (0.83%), *Quercus* (0.79%), *Papaver* (0.68%), *Laurus nobilis* L. (0.63%), Asteraceae (0.47%), *Sarcopoterium/Poterium* type (0.45%), *Ailanthus* (0.32%), *Tribulus terrestris* L. (0.18%), *Punica* (0.15%), Lamiaceae (0.12%), Dipsacaceae (0.11%), *Paliurus spina-christii* L. (0.07%), Oleaceae (0.06%), *Veronica* (0.03%), *Juglans* (0.03%), Liliaceae (0.03%), and *Asphodelus* (0.01%) (Table 1).

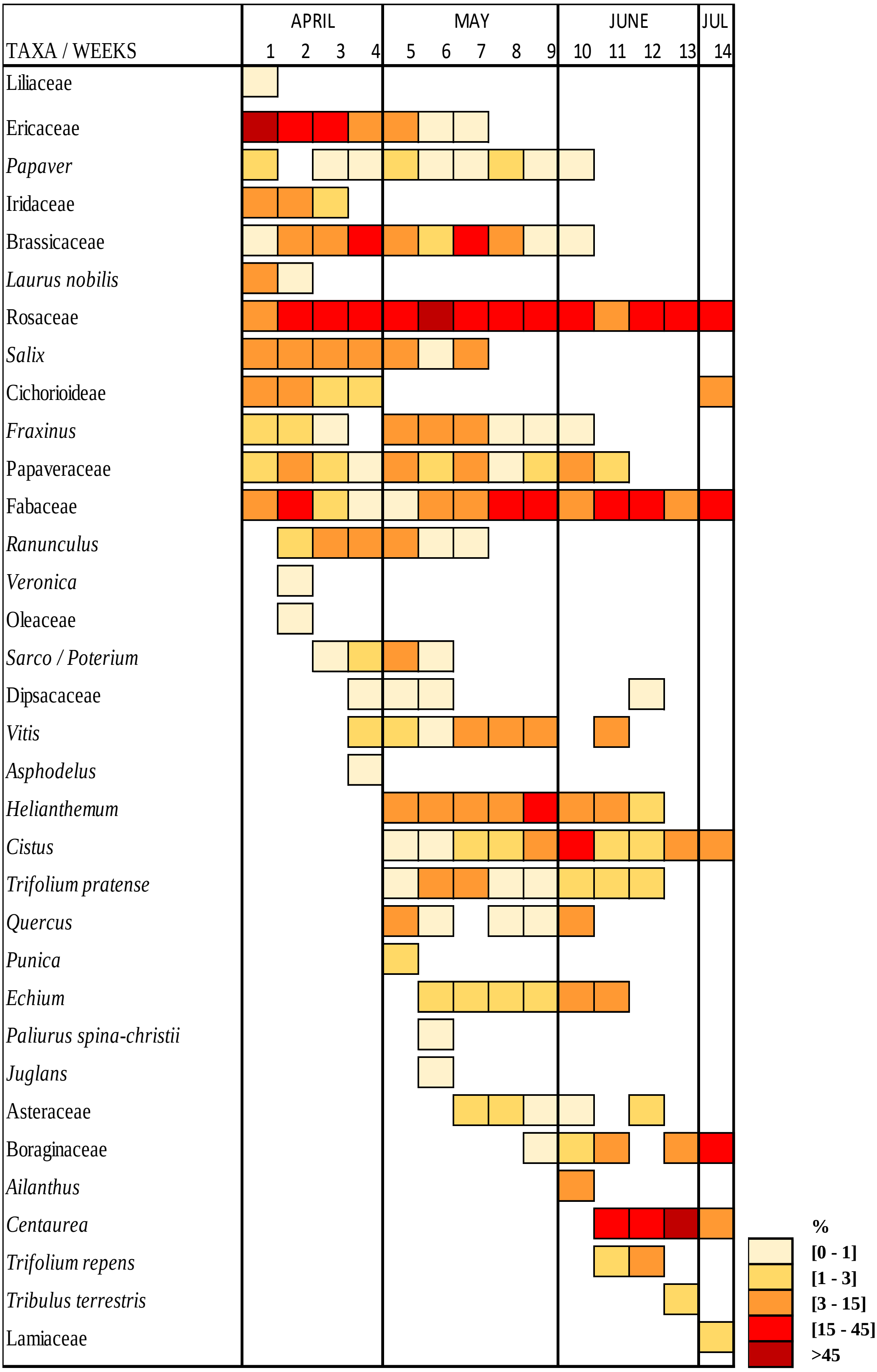

Figure 2. Pollen calendar, weekly and monthly variation of pollen types preferred by honey bees on Marmara Island

Table 2. Weekly mean pollen frequency of beehives in Marmara Island

TAXA / WEEKS	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Liliaceae	0.43													
Ericaceae	47.64	18.23	18.11	6.98	4.49	0.30	0.62							
Papaver	2.42		0.74	0.27	2.10	0.36	0.32	2.59	0.06	0.66				
Iridaceae	9.61	5.17	2.61											
Brassicaceae	0.09	7.07	10.79	31.61	14.52	2.40	18.22	9.09	0.36	0.44				
Laurus nobilis	8.31	0.48												
Rosaceae	7.04	26.73	35.49	31.01	37.30	52.23	30.41	38.39	39.90	17.58	12.76	21.49	28.67	35.40
Salix	3.53	6.87	10.79	9.13	8.04	0.85	3.52							
Cichorioideae	9.33	11.63	1.87	1.48										4.06
Fraxinus	2.36	1.63	0.17		6.96	6.38	9.26	0.83	0.65	0.33				
Sarco / Poterium			0.40	1.21	4.38	0.27								
Dipsacaceae				0.47	0.15	0.03						0.84		
Papaveraceae	1.21	4.15	2.50	1.07	4.26	2.31	3.90	0.61	1.35	4.70	1.22			
Helianthemum					3.14	5.95	6.27	11.95	20.03	10.17	5.24	2.62		
Vitis				2.75	1.35	0.76	11.72	12.15	11.62		9.79			
Ranunculus		1.50	14.20	13.42	5.84	0.39	0.38							
Veronica		0.48												
Oleaceae		0.82												
Cistus					0.60	0.15	1.20	2.34	5.67	37.65	2.62	2.52	8.19	6.38
Asphodelus				0.10										
Trifolium pratense					0.19	9.11	3.81	0.11	0.76	1.22	2.27	2.41		
Quercus					3.93	0.79		0.36	0.70	5.25				
Punica					2.10									
Echium						2.16	2.93	1.65	2.95	8.13	6.29			
Paliurus spina-christii						0.97								
Juglans						0.46								
Asteraceae							2.40	2.07	0.14	0.33		1.68		
Boraginaceae									0.73	2.38	4.72		5.05	25.15
Ailanthus										4.53				
Fabaceae	8.03	15.24	2.33	0.50	0.67	14.12	5.04	17.87	15.10	6.63	28.50	20.55	8.00	21.08
Centaurea											25.00	37.84	47.52	6.19
Trifolium repens											1.57	10.06		
Tribulus terrestris													2.57	
Lamiaceae														1.74
WEEKLY TOTAL	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

4. DISCUSSION

In early spring, honey bees prefer early-blooming plants such as Liliaceae, Ericaceae, Iridaceae, *Laurus nobilis*, *Fraxinus*, *Salix*, and Cichorioideae. Among the preferred taxa in April are Liliaceae, Ericaceae, *Papaver*, Iridaceae, Brassicaceae, *Laurus nobilis*, Rosaceae, *Salix*, Cichorioideae, *Fraxinus*, Papaveraceae, Fabaceae, *Ranunculus*, *Veronica*, Oleaceae, *Sarco*/*Poterium*, Dipsacaceae, *Vitis*, and *Asphodelus* (Figure 2). The highest pollen diversity is observed in May during the study period, with a total of 22 taxa being used as pollen sources. In May, honey bees prefer Ericaceae, *Papaver*, Brassicaceae, Rosaceae, *Salix*, *Fraxinus*, Papaveraceae, Fabaceae, *Ranunculus*, *Sarco*/*Poterium*, Dipsacaceae, *Vitis*, *Helianthemum*, *Cistus*, *Trifolium pratense*, *Quercus*, *Punica*, *Echium*, *Paliurus spina-christii*, *Juglans*, Asteraceae, and Boraginaceae as pollen sources. In June, the preferred taxa include *Papaver*, Brassicaceae, Rosaceae, *Fraxinus*, Papaveraceae, Fabaceae, Dipsacaceae, *Vitis*, *Helianthemum*, *Cistus*, *Trifolium pratense*, *Quercus*, *Echium*, Asteraceae, Boraginaceae, *Ailanthus*, *Centaurea*, *Trifolium repens*, and *Tribulus terrestris.* As the number of eggs and larvae decreases within the hive, there is also a decrease in the pollen requirement and the amount of pollen entering the hive. In July, the preferred taxa are mainly Rosaceae, Cichorioideae, Fabaceae, *Cistus*, Boraginaceae, *Centaurea*, and Lamiaceae (Figure 2).

Rosaceae and Fabaceae family pollen grains are mostly preferred by honey bees than other taxa (Reis et al., 2023; Toopchi-Khosroshahi & Lotfalizadeh, 2011; Topal et al., 2023). However, when we consider identified members of the Asteraceae family within the family, we see that Asteraceae is also represented with a value of 10.83%. Honey bees show a strong preference for the Asteraceae. Similarly, when *Cistus* and *Helianthemum* are considered as the Cistaceae family, they represent 9.48% of the total. The Cistaceae is particularly preferred by honey bees as a pollen source, especially during the summer months, and an attractive pollen source for honey bees (Dimou et al., 2014). If *Echium* is classified under Boraginaceae, it can be seen that Boraginaceae, with a value of 4.44%, was also able to have an important position among the taxa exceeding 3%. This may be a result of the different amino acid compositions of pollen or the need-oriented pollen content of some species compared to others (e.g. Cook et al., 2003; Kim & Smith, 2000; Pernal & Currie, 2001).

During the spring, woody plants, including trees and shrubs, are heavily used as pollen sources by honey bees. If we exclude Rosaceae and Ericaceae, the proportion of trees and shrubs used as pollen sources is 7.14% of the total, and most likely, many members of the Rosaceae and Ericaceae, which are used as pollen sources in the early period, are also woody plants.

Specifically, *Salix*, and *Fraxinus* are also important early spring blooming woody perennials honey bees prefer (Forcone et al., 2011) as in our study.

As predicted, Vitis cultivated in the region was heavily favored by honeybees. However, *Olea*, which is also cultivated on the island, was not represented in our study and was not preferred by honey bees. It is known that *Olea* is anemophilous, meaning its pollination occurs through wind dispersal. However, it is also noted that sometimes it can undergo entomophilous pollination (Marcucci et al., 2008). Nevertheless, the honey bees in the region do not use *Olea* members as a pollen source. This situation may be parallel to the fact that the region where olive trees are dense is far from the colonies. Also, according to our observations, *Melia azaderach*, *Salvia*, and *Arbutus* are used as the main nectar sources by honey bees. However, this was not observed in the pollen pellets.

Although various types of pollen were collected by honey bees, the amount of pollen and brood decreased in the colonies due to the heat in the region in July, and sampling was terminated when there was not enough pollen in the drawers. Honey bees may not work below 13 °C and rarely above 38 °C (Abou-Shaara, 2014). Honey bee foragers forage less pollen in the hottest period, and a decrease in pollen collection activity is observed at high ambient temperatures (Cooper et al., 1985). In our study, the decrease in pollen in the drawers in July was attributed to this.

On the other hand, an interesting deficiency was noted in the study results. It is known that there are small chestnut tree communities in the northern part of Marmara Island, where Euro-Siberian phytogeography dominates. However, according to the results of our study, honey bees did not engage in foraging activities in June from the hives located in the south of the island to the north for the resource (*Castanea sativa*) to which they are addicted. The hives are located in the southern part of Marmara Island, and in the case of the Marmara Sea, the north-facing slopes (far from honey bee colonies used for this study outside of the range) are included in the Euxine belt of the European-Siberian phytogeographic region, and the south-facing slopes are included in the Mediterranean phytogeographic region. Therefore, although Marmara Island is a small island, it is understood that honey bees do not travel the distance from the south to the north of the island. This can also be evaluated from another perspective as follows: Most likely, the honey bees in the location where we worked did not engage in foraging activities by flying from the island to another island or land.

5. CONCLUSION

In conclusion, a pollen calendar has been suggested to be used for the beekeepers and researchers based on the pollen types preferred by the honey bees in Marmara Island. In this calendar, Rosaceae, Fabaceae, and *Centaurea* pollen loads characterize the southern part of the region, particularly Marmara Island, and are frequently recorded. Knowing the species that bees utilize in different regions is important for regional or migratory beekeepers. However, since the study area is an island and these types of pollination studies are important for explaining the biogeography and biodiversity of the islands, much more detailed studies are needed. In addition, according to other studies conducted in nearby regions (Bilisik et al., 2008), it has been noted that the pollen flow in the region ends slightly earlier and there was a period in which there was a negligible amount of pollen in the hives. This situation in our study can be explained by the fact that the southern slope of the island is very dry after June under the influence of the hot Mediterranean climate. Further research is needed to understand the potential impact of annual weather conditions or the conditions within the hive on this phenomenon.

DECLARATIONS

The authors declare that they have no conflicts of interest.

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Plant-Based Foods and Latest Developments

Bitki Bazlı Gıdalar ve Güncel Gelişmeler

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Abstract

In recent years, plant-based foods have been widely preferred due to their benefits to human health and economy, as well as their reduction of greenhouse gas emissions and their benefits to the environment. In order to meet the increasing need for plant protein, research has increased and different plant protein sources such as seaweed, spirulina, sugar beet leaves, alfalfa and hemp seeds have begun to be used in developed products. There are many studies on the benefits of an herbal diet, and many herbal products have been developed in recent years. However, most of the developed products have deficiencies in terms of appearance, texture, taste and nutritional value, and new methods need to be investigated to improve this situation. In addition, it would be wrong to say that all of the developed vegetable protein products are superior to animal sources in terms of nutritional value. In this review, plant protein sources were examined and studies on their health effects and bioavailability and the latest technological developments were evaluated.

Keywords: Plant-based foods, Plant-based proteins, Animal foods, Greenhouse gas, Sustainability

Özet

Bitki bazlı gıdalar son yıllarda insan sağlığına faydaları ve ekonomik olmalarının yanında sera gazı emisyonlarını azaltması, çevreye sağladığı yararlar sebebiyle de çok tercih edilmektedir. Bitkisel proteine artan ihtiyacı karşılamak için araştırmalar artmış ve deniz yosunu, spirulina, şeker pancarı yaprağı, yonca, kenevir tohumu gibi farklı bitkisel protein kaynakları da geliştirilen ürünlerde kullanılmaya başlanmıştır. Bitkisel beslenmeni yararlarına dair pek çok çalışma mevcuttur ve son yıllarda bitkisel kaynaklı pek çok ürün geliştirilmiştir. Fakat geliştirilen ürünlerin çoğunda görünüm, doku, lezzet ve besin değeri açısından eksikler vardır ve bu durumun iyileştirilmesi için yeni yöntemlerin araştırılması gerekmektedir. Ayrıca geliştirilen bitkisel protein ürünlerinin hepsinin besin değeri açısından hayvansal kaynaklardan üstün olduğunu söylemek yanlış olacaktır. Bu derlemede bitkisel protein kaynakları irdelenmiş ve sağlık etkileri ve biyoyararlanımlarına dair çalışmalar ile son teknolojik gelişmeler değerlendirilmiştir.

Anahtar Kelimeler: Bitki bazlı gıdalar, Bitkisel proteinler, Hayvansal gıdalar, Sera gazı, Sürdürülebilirlik

1. INTRODUCTION

The world population is expected to reach 10 billion by 2050. Accordingly, the United Nations states that protein demand will double and if no precautions are taken, there will be a protein shortage (Aimutis, 2022). In terms of quantity, quality and sustainable supply, traditional protein production methods cannot meet the future needs of human life (Liu et al., 2023).

The human body consists of macronutrients (carbohydrates, protein, fat) and micronutrients (vitamins, minerals, water). Protein, a macronutrient, has functions in the body such as muscle building, cell repair and immune response control (Kumar et al., 2022; Nasrabadi et al., 2021).

In addition, the omnivorous diet that most people follow is harmful to the planet in terms of climate crisis, decrease in biodiversity and pollution.

However, a diet consuming vegetable proteins will reduce these negative effects and greenhouse gas emissions by up to 80% (Pye et al., 2022). At the same time, less agricultural land, water and energy are needed for the production of these proteins, and less greenhouse gas emissions occur compared to animal husbandry (Milião et al., 2022; Nasrabadi et al., 2021).

While the low-cost and sustainable production of plant-based protein attracts attention, many studies are trying to produce plant-based substitute meat. However, studies in this field need innovation and development. Products developed with synthetic biology technology create conditions for large-scale production of essential components in plant-based foods. The safety of technology and products must be ensured during the production process. Potential biosecurity risks need to be fully estimated in order to establish independent, objective technical systems for production safety or nutritional assessment. Taken together, combining existing technology with synthetic biology technology needs scientific support for the production of high-quality plant-based meat (Liu et al.,2023).

In this review, plant protein sources were examined and studies on their health effects and bioavailability and the latest technological developments were evaluated.

2. PLANT-BASED PROTEINS

Recently, plant proteins have been found attractive by consumers due to their more economical, sustainable and positive health effects compared to animal protein sources (Nasrabadi et al., 2021). However, despite this interest and the increased tendency to try new products compared to the past, individuals who have completely stopped consuming animal protein constitute 1% of society (Maciel et al., 2022).

Studies examining consumers' attitudes towards plant proteins have explained the reasons why individuals do not consume them as not having sufficient knowledge about these proteins and the products produced (Drolet-Labelle et al., 2023).

Some studies have revealed that individuals do not find products produced using plant protein sources healthy and do not want to consume them because they are artificial and overly processed.

In addition, there are some who find it unhealthy due to the high salt content used to enrich its taste. Most consumers think that vitamins and minerals such as iron and B12 found in animal meat are not found in any plant food, and therefore none of them can replace meat (Drolet-Labelle et al., 2023; Rizzo et al., 2023). In addition to those who defend the disadvantages of plant protein sources, there are those who state that they are more environmentally friendly and easily digestible compared to animal proteins, and that they feel lighter after consuming them (Drolet-Labelle et al., 2023).

When the distribution of consumers according to sociodemographic characteristics is examined; It has been revealed that women (Drolet-Labelle et al., 2023), individuals with higher education levels, and vegans or vegetarians are more willing to try alternative protein sources. Although there are studies arguing that age is not a factor, there are studies revealing that young people are more open to new tastes (Rizzo et al., 2023).

Cereals and pseudocereals such as wheat, corn, rice, sorghum, quinoa, buckwheat, amaranth; Legumes such as beans, peas, soybeans and oilseeds such as sesame, flaxseed, canola, pumpkin seeds, rapeseed and sunflower seeds can be given as examples of vegetable protein sources (Kumar et al., 2022). Apart from these, there are also different plant protein sources such as seaweed, spirulina, sugar beet leaves, alfalfa and hemp seeds.

2.1. Legumes and Oilseeds

When looking at the protein content of legumes, it is seen that broad beans are 30%, mung beans are 28%, lentils are 20-30%, and chickpeas are 18-25% (Webb et al., 2023). Among the oilseeds, sunflower seeds, flaxseeds, pumpkin seeds, sesame, almonds, cashews and canola contain 16-36% protein (Hoehnel et al., 2022).

2.2. Cereals and Pseudocereals

In a study examining the protein content of grains, it was revealed that oats contain 64% protein, brown rice 65%, wheat 81%, and potatoes and peas 80% (Kumar et al., 2022). Grains such as quinoa, buckwheat, chia and amaranth are called pseudocereals and contain 12-19% more protein than other grains. Its amino acid balance is better and it is rich in lysine (Hoehnel et al., 2022).

Chia seeds are very rich in dietary fiber. However, it contains less lysine compared to other pseudocereals (Hoehnel et al., 2022). Researchers state that chia seeds can be a good protein alternative in meat derivatives because their protein content is close to lentils, peas and chickpeas.

Buckwheat is poor in leucine and rich in lysine. Apart from this, it is gluten-free and is frequently used in the development of gluten-free products since it contains low amounts of prolamin. It is also less allergenic compared to other grains (Jin et al., 2022). Although the non-nutritive elements it contains, such as tannin, phytic acid and saponin, negatively affect the utilization of proteins, its bioavailability is higher than most grains and is 80%. Nowadays, methods such as fermentation, sprouting, and enzymatic hydrolysis are used to increase this rate (Jin et al., 2022).

2.3. Macro and Micro Algae

Macroalgae such as spirulina and seaweed; Microalgae such as Rhodomonas salina, Tetraselmis chui and Phaeodactylum tricornutum and duckweed are examined in this group (Coleman et al., 2022). A study has shown that microalgae Rhodomonas salina has a crab aroma, Phaeodactylum tricornutum has a mussel aroma, and Tetraselmis chui microalgae has crab and fish aromas, and these can be used as seafood alternatives (Coleman et al., 2022).

Seaweeds are known to have high calcium, magnesium, phosphorus, potassium, sodium and iron minerals. They have an umami taste, called the 5th taste, due to the aspartic and glutamic acids they contain. Recently, the consumption of seaweed-based food products has increased significantly in the world due to this umami taste. It is also a prebiotic and has properties that regulate the intestinal microbiome (Raja et al., 2022). Seaweed is a food frequently consumed in Asian countries. It is even stated that mothers who have just given birth consume seaweed soup to provide protein, vitamin A and mineral support for both themselves and their babies (Torun & Konuklugil, 2020).

Seaweeds, called edible macroalgae, are divided into three groups: red (Rhodophyta), green (Chlorophyta) and brown (Ochrophyta, Phaeophyceae). Although the protein content of seaweeds varies depending on the type, it is around 30-50% and their bioavailability is 75%. When looking at the amino acid balance, it is seen that it is rich in phenylalanine, tyrosine and theronin amino acids, which are limited in plant proteins. But it is poor in methionine and lysine (Raja et al., 2022). The addition of seaweed to cheese improves its nutritional value and sensory properties by increasing the concentration of calcium it contains. There are studies showing that adding seaweed to probiotic yoghurt enriches the yoghurt in terms of calcium, potassium, sodium, magnesium and iron (Kandil, 2019).

When seaweed powder is added to ice cream, there is no change in the amount of fat but a significant increase in the amount of protein. This addition also makes the ice cream more creamy and delicious (Raja et al., 2022). The possibility of heavy metal deposits such as cadmium, arsenic, lead and copper in seaweed is a disadvantage of seaweed. Another disadvantage is that since they are rich in iodine, excessive consumption may cause disease effects (Raja et al., 2022).

2.4. Clover

Although alfalfa is an important protein source for cattle due to its nutrition, the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) reported that the soluble and insoluble protein found in its leaves has a balanced amino acid composition and is an important source that can be used in human nutrition.

It is suggested by some researchers that clover can limit some diseases such as high cholesterol, diabetes, and atherosclerosis due to the antioxidants it contains (Hadidi et al., 2023).

2.5. Other Plant-based Protein Sources

The cactus called *Pereskia aculeata*, also known as the "flesh of the poor", found in Brazil and Florida state of the North American continent, has a very high protein content. Its protein content is 25%, higher than legumes. *Jatropha curcas* L. is a small tree species found in almost all countries with tropical climates. Jatropha seed has high protein quality as it contains all essential amino acids except lysine.

Glutelin and globulin are the main components that make up the proteins of this plant. 60% of its protein consists of essential amino acids. In addition, lysine content constitutes 5-6% of the total protein content and can be used to enrich lysine-poor grain foods (Milião et al., 2022). The leaves of sugar beet, another source of vegetable protein, are rich in protein and contain 19.4-22.8% protein in their dry weight. It has a balanced amino acid composition consisting of leucine, valine, phenylalanine, lysine, theronin, isoleucine and methionine.

Although bamboo shoots vary depending on species and maturity, they have low fat, high protein, dietary fiber, vitamin and mineral content.

The variety with the highest protein amount is Yushania alpina, which contains 33.4% protein. It contains 17 types of amino acids, 8 of which are essential amino acids. Although it is poor in lysine, the most abundant amino acid is tyrosine (Milião et al., 2022).

Hemp seeds contain 25-30% protein and have high digestibility. Since it has a good balance of essential amino acids, it is a suitable ingredient for meat derivatives. It also contains arginine, which ensures normal blood pressure by ensuring vasodilation of the vessels (Milião et al., 2022).

3. HEALTH EFFECTS and BIOAVAILABILITY

Red meat is rich in nutrients such as fat, high-quality protein, minerals and multivitamins that the body needs to support growth and development. It especially contains 8 types of essential amino acids that the human body cannot synthesize. Therefore, compared to plant protein, animal protein has a higher biological potential. Although meat is rich in nutrients, attention should be paid to its health risks. Additionally, excessive consumption of processed red meat can lead to inflammatory bowel disease and functional gastrointestinal disorders. The World Cancer Research Fund/American Institute for Cancer Research reported that consumption of red meat and processed meat has a possible association with the risk of colon cancer (Zhao et al., 2018).

Cholesterol intake increases with animal-based nutrition, posing a problem for individuals with lactose intolerance or animal protein allergy (Aimutis, 2022). Diets rich in animal protein also have negative effects on the intestinal microbiota and fecal content (Gratz et al., 2020).

In plant-based nutrition, there are studies showing that high-quality plant protein consumption is associated with low mortality and reduces the risk of diverticulitis, cataract, heart failure, hypertension and cancer development (Brown, 2023; Herpich et al., 2022; Keaver et al., 2021; Key et al., 2022; Kumar et al., 2022; Neary et al., 2022; Shaghaghian et al., 2022). Again, since herbal nutrition improves glycemic control and reduces the risk of complications in individuals with Type II DM, the American Association of Clinical Endocrinologists recommends plant-based nutrition for individuals with Type II DM (Pye et al., 2022). In addition, since it has hypoallergenic properties, it is seen as a protein supplement for children and adults who are allergic to cow's milk (Kumar et al., 2022).

There are also studies showing the positive health effects of vegetable protein intake in individuals with chronic kidney failure (CKD) (Brown, 2023; Stanford et al., 2023). In a study including 5316 adults, it was observed that every 20 g increase of vegetable protein reduced the incidence of CKD by 16% (Herpich et al., 2022). In addition, since plant sources have high phytic acid, phosphorus bioavailability is low, which positively affects chronic renal failure (Burstad et al., 2023).

In a study investigating the effect of herbal nutrition on neurological and psychiatric disorders, an improvement in pain, anxiety, stress and depressive symptoms was observed (Herpich et al., 2022; Pye et al., 2022). Supporting this, in a study including 333 healthy participants between the ages of 8-79, it was found that positive mood was directly proportional to the herbal diet index score in children (Ma et al., 2023). However, studies on this subject are contradictory and more research is needed. Vegetable proteins also have positive effects on the feeling of fullness and muscle synthesis rate.

In a study involving 24 adult men to measure the satiety effect of vegetable minced meat, participants were called to two clinic appointments at least a week apart and were offered bolognese pasta containing vegetable minced meat or ground beef and asked to eat until they were full. As a result of the study, it was observed that participants reached saturation by consuming less plant-based bolognese pasta compared to ground beef, supporting the hypothesis (Muhlhausler et al., 2023).

In the study investigating the effects of vegetable proteins on the muscle formation mechanism, the participants, all of whom were 24 men, were divided into two groups and one group was given 230 g of vegetable meat containing 40 g of wheat and chickpea proteins, while the other group was given 174 g of chicken breast to consume. At the end of the study, a similar muscle protein synthesis rate was observed in individuals consuming plant protein products as those consuming chicken (Kouw et al., 2022).

4. OVERVIEW of TECHNOLOGICAL DEVELOPMENTS and VEGETABLE PROTEIN SOURCES

Although plant proteins have positive health effects and are environmentally friendly, they also have some disadvantages such as having an unusual taste and difficult digestibility (Shaghaghian et al., 2022; Drolet-Labelle et al., 2023). However, today the food industry is trying to prevent these disadvantages by using various technological methods; herbal cheeses, meat, milk, etc. It produces alternatives to animal sources with similar taste and nutritional value (Maciel et al., 2022).

Various meat substitutes have been developed by the food industry from past to the present. These are divided into 1st and 2nd generation meat substitutes. 1st generation meat substitutes include products produced by cooking defatted soybean meal, soy protein concentrate or gluten at low moisture, and these products have a fibrous and spongy texture. Second generation meat substitutes are products obtained as a result of high moisture cooking and aim to imitate meat in terms of appearance, nutritional values, aroma and taste. New generation meat analogues can be cited as examples of 2nd generation meat substitutes (Penna Franca, 2022).

As a high-value food ingredient, fat contributes to many sensory qualities of meat products, such as physical appearance, taste, juiciness and chewiness. The use of oil imparts sensory as well as certain technological properties to the masses of food products. Therefore, the structure of fat in meat is important to improve the overall performance of plant-based meat (Liu et al., 2023).

Enzymes are known to improve the functional properties of proteins. However, high temperature and pressure; It is the most common processing technology to create fibrous meat structure.

However, high temperatures cause enzymes to denature and stop working. Therefore, it is necessary to improve the thermal stability of vegetable protein-based meat-related enzymes (such as glutamine transaminase and laccase) and obtain engineering enzymes that are heat resistant and have high enzyme activity. These modifications are grouped into 4 groups: physical, chemical, biological and other (amyloid fibrillization and complexity) (Liu et al., 2023; Nasrabadi et al., 2021). Unlike animal products, plant-based meats are deficient in vitamins B2, B12, iodine, zinc, calcium, potassium, selenium and other nutrients (McClements & Grossmann, 2021). These deficiencies need to be eliminated in plant-based meats.

Proteins from plants such as soybeans, wheat, and peas are the main raw materials used in plant-based meat production. The amino acid composition of these plant protein sources does not meet nutritional requirements. In order for a plant-based meat product to be considered a well-balanced source of amino acids for human nutrition, plant protein and other proteins must be combined. Proteins such as algae protein and edible mushroom protein can be used for this.

The digestibility and bioavailability of plant protein is limited due to the presence of anti-nutritional factors. Phytic acid is commonly found in legumes, but the presence of phytic acid affects the absorption of minerals as well as proteins, so efforts should be made to reduce or eliminate phytic acid in plant-based meats (Şahin, 2023).

In a study conducted to reduce phytic acid content, Aspergillus sojae, Aspergillus ficuum were used and the effects of fermentation on antinutritional factors in lupine flour were examined. It was determined that the phytic acid content of lupine flour fermented with Aspergillus sojae and Aspergillus ficuum was significantly reduced. The use of Aspergillus ficuum showed the best effect and the phytic acid content decreased by 73% (Olukomaiya et al, 2020).

When looking at products developed other than meat substitutes; At the top is tempeh, which is made with soybeans and uses legumes and coconut waste in different parts of Asia. This product is considered a good source of positive effects on human health due to the high protein and isoflavonoids in soy. 100 g of fresh soybean tempeh contains 20.8 g protein, 13.5 g carbohydrates, 8.8 g fat and 234 mg potassium (Romulo & Surya, 2021).

In a study measuring the liking of cashew fiber on individuals, volunteers developed coxinha, mini burgers, kibbeh and sausages using cashew fiber and presented them to the participants. Participants also stated after the tasting that the use of cashew fiber in products developed as an alternative to minced meat, chicken, fish and crab would attract a lot of attention (Maciel et al., 2022).

In another study, hamburger patty alternatives were created using mycoprotein, soy, pea and insect protein and were examined in terms of their environmental impact, nutritional value and texture.

As a result of the study, meatballs made from peas and soy are more environmentally friendly; pea and insect protein patties are sensory better; It has been determined that soy and mycoprotein have higher nutritional value (Smetana et al., 2021). In another similar study, vegetable protein hamburger patties were developed using soy and pumpkin seed flour and revealed that these hamburger patties had 10 times less impact on the environment than meat-based patties (Baune et al., 2022). Again, herbal mayonnaises are produced using various products such as chia seed oil, peanut, sesame oil, chickpea aquafaba, pea and soybean extract (de Menezes et al., 2022).

Apart from these, soy protein is added to products such as bread and pasta to increase the water retention capacity and extend the shelf life; rice protein concentrate to improve nutritional value and cooking quality (Kumar et al., 2022), legumes and pseudocereals are added to prolong the feeling of fullness (Hoehnel et al., 2022). In plant-based meat production, wheat gluten and legume proteins are used due to their viscoelastic structure; Quinoa is also used as a binder, oil alternative and gelling agent (Shaghaghian et al., 2022). Plant-based meat production uses umami compounds such as glutamate, inosinate and guanylate to create meat taste. Legumes are one of the main sources of vegetable protein due to their high protein content and nutritional value.

The presence of undesirable tastes such as bean-like taste and grassy taste in legumes prevents the application and consumption of legume protein and products. There are some methods to eliminate or reduce the undesirable aroma. One method is to use a modification of the protein by controlled biological treatment. For example, fermentation is widely used to improve the palatability and nutritional value of plant proteins. The metabolic effects of strains in the fermentation process can contribute to the production of new aromatic compounds, some of the undesirable aroma compounds can be eliminated or their content can be reduced (Sajib et al., 2023).

One study found that fermentation of pea protein isolates using Lactobacillus casei reduced bitterness intensity after 48 h (Arteaga et al., 2021). However, there is a small amount of literature showing that fermentation leads to a significant reduction in bean aroma, which is the main odor of plant proteins. Therefore, a more in-depth investigation of the relevant ingredient is required to prevent the occurrence of off-flavors or to significantly reduce its content in order to increase consumers' acceptability of plant proteins. Adding spices for odor masking is used to mask off-flavors and make plant-based meats resemble the flavor of the original meat (Mittermeier-Kleßinger et al., 2021).

Natural spices like basil and fennel can flavor plant-based meats and mask other flavors. In addition, monosaccharides, amino acids, and aroma nucleotides, which are the precursors of the Maillard reaction, are added to plant-based meat products to ensure that the Maillard reaction occurs during the preparation and cooking process. Again, yeast extract, in addition to being resistant to high temperatures, can cause Maillard reactions with the amino compounds in the ingredients with reducing sugars in the ingredients under high-temperature cooking conditions, and the taste of the product can be closer to meat (Liu et al., 2023). It would be wrong to say that all of the developed plant protein sources are superior to animal sources in terms of nutritional value. In support of this, in a study in which vegetable cheeses were developed using products such as coconut oil, modified starch, modified potato starch, coconut cream and tapioca starch, it was found that the protein values of these cheeses were lower than traditional cheeses (Grasso et al., 2021).

Another study compared the nutritional value of plant-based meat alternatives in supermarkets in Ireland and the United Kingdom with traditional meat products and found that these products had a better nutritional profile than hamburger meat, sausages, and pork, but less so than ground beef, beef, and chicken breast. It has been found that it has a worse nutritional profile than processed meat products (Lindberg et al., 2022).

The development of the field of synthetic biology has allowed them to use cell engineering, which is important for plant-based meat. These engineering processes can transform important components of cells such as color, taste and flavor. It can help plant-based meats resemble real meat in terms of appearance characteristics (Shleikin & Medvedev, 2014).

Hemoglobin or myoglobin, the molecule that carries iron in animals, is responsible for the color and flavor of meat. Therefore, hemoglobin can be used as a functional food ingredient in meat analogues. Thanks to synthetic biology, cell factories that can synthesize hemoglobin have been designed. Thus, an alternative production method was created other than extraction from plant tissue or animal blood (Zhao et al., 2018). It is thought that in future studies, synthetic biology may help develop specific functional proteins that have water retention and fiber formation functions in meat (Liu et al., 2023).

5. CONCLUSION

Although plant-based foods are popular due to their benefits to human health and their economic and sustainability, they also have some disadvantages such as having an unusual taste and difficult digestibility. With the increase in people's health needs in recent years, plant-based meat products have gradually come into the public interest. However, many plant-based meats available in limited quantities on the market today contain too many additives. These foods often contain high levels of sugar, salt or additives such as spices, coloring agents, emulsifiers and preservatives. Over-processing can turn original high-quality and healthy plant-based meat into ultra-processed foods. Therefore, more experimental research is still needed on how to control the degree of processing of plant-based meat products.

The sustainability of food systems is a global challenge due to the detrimental effects of food production on nitrogen and phosphorus cycling, biodiversity loss and climate change. This need has led to a shift in the food industry towards plant-based foods. There is a concern that plant-based foods may be nutritionally deficient, but they also have health-promoting benefits such as dietary fibers and nutraceuticals that are not found in most animal products.

The effects of long-term consumption of plant-derived meats on human health are not known today. Although recent studies have compared the digestibility and gastrointestinal digestion process of animal meats with plant-based meats in an in vitro digestion model, these results still have some limitations as the human body is a more complex environment.

Plant-based meat is a promising solution to partially alleviate meat supply problems. Improvements are needed in the texture, taste and nutrition of plant-based meats. However, there are deficiencies between plant-based meat and real meat in various aspects such as appearance, texture, taste, nutrition, and new methods need to be investigated to improve this situation.

In addition, countries may need to halt the expansion of agricultural land, reduce the number of livestock, introduce a meat tax and develop incentive schemes that support sustainable consumer products to mitigate climate change. A shift to predominantly plant-based diets and diversification of plant-based protein intake may be needed to protect human and environmental health. Much work, including sensory evaluation, will be needed in the future to develop targeted plant-based products that are tasty, affordable and healthy.

DECLARATIONS

The authors declare that they have no conflicts of interest.

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Mineral Composition and Heavy Metal Contents of Chestnut Honey Collected from Kastamonu Region

Kastamonu Bölgesinden Toplanan Kestane Balının Mineral Madde ve Ağır Metal İçerikleri

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Abstract

Chestnut honey, which is known for its apitherapic properties besides its nutritional attributes, is one of the main groups of monofloral honey types. Kastamonu is the most important province because where constitutes two-thirds of the chestnut forest flora of the Black Sea region. Kastamonu Chestnut Honey, produced in apiaries within the chestnut forests, has been registered as a geographical indication and designation of origin. The nutritional value of honey is related to its nutritional and chemical content. Kastamonu chestnut forests have a different distribution with their scattered structure from the coastline to 1000-1200 meters altitude. Therefore, this study was planned with the prediction that chestnut honey produced in different districts has a different composition. The aim of the study is to contribute to official regulations regarding the product, product standardization, and national branding studies by determining mineral and heavy metal contents based on the region where the chestnut honey is produced. As a result of the study, the main concentrations of the elements in the chestnut honey samples were affected by the geographical location, because the apiaries where honey samples were collected in chestnut forests in Kastamonu districts had different altitudes, climates and soil properties, and different secondary flora. The most abundant elements in the samples were potassium (1410.0-6581.5 ppm) sodium (0-204.4 ppm), calcium (125.3-287.5 ppm), and magnesium (24.73-61.88 ppm). Iron (1.32-9.54 ppm), zinc (1.34-20.84 ppm), and manganese (1.39-18.69 ppm) were also found in moderate and others in trace levels. Because manganese is found at moderate levels in all chestnut honey samples and it is found at higher levels than other honey types, it can be recommended that Mn, as fingerprints to detect imitation and adulteration of chestnut honey. While Cd, a heavy metal, was found in only one sample, Ni was not detected in any sample. Whereas Pb concentration varied between 1.84 ppm and 3.38 ppm for the samples, the contents of Al concentrations were quite high (2.94-13.94 ppm). Because there wasn't a maximum limit for heavy metal contents of honey types in the Turkish Food Codex Contaminants Regulation, no evaluation could have been made regarding the suitability of these heavy metal contents. It is thought that heavy metal contamination limits in honey types should be included in the regulations and especially secondary sources such as metallic containers used for storage during handling processes and harvesting of honey for heavy metal contamination should be reviewed.

Keywords: Chestnut honey, Mineral content, Heavy metal, Food security

Özet

Besleyici özelliklerinin yanı sıra apiterapik özellikleriyle de bilinen kestane balı, monofloral bal türlerinin ana gruplarından biridir. Kastamonu, Karadeniz bölgesinin kestane ormanı florasının üçte ikisini oluşturması nedeniyle en önemli kestane balı üretimi açısından önemli bir lokasyondur. Kestane ormanları içerisindeki arılıklarda üretilen Kastamonu Kestane Balı, coğrafi menşe işareti ile tescillenmiştir. Balın besin değeri, besinsel ve kimyasal içeriğiyle ilgilidir. Kastamonu kestane ormanları kıyı şeridinden 1000-1200 metre yüksekliğe kadar dağınık yapısıyla farklı bir dağılıma sahiptir. Bu nedenle farklı ilçelerde üretilen kestane balının farklı bileşime sahip olduğu öngörüsüyle bu çalışma planlanmıştır. Çalışmanın amacı kestane balının üretildiği bölgeye göre mineral ve ağır metal içeriklerini belirleyerek ürüne ilişkin resmi düzenlemelere, ürün standardizasyonuna ve ulusal markalaşma çalışmalarına katkı sağlamaktır. Çalışma sonucunda Kastamonu ilçelerindeki kestane ormanlarından bal örneklerinin toplandığı arılıkların farklı rakım, iklim ve toprak özelliklerine sahip olması nedeniyle kestane balı numunelerindeki elementlerin ana konsantrasyonlarının coğrafi konumdan etkilendiği ortaya çıkmıştır. Numunelerde en yüksek bulunan elementler potasyum (1410.0-6581.5 ppm), sodyum (0-204.4 ppm), kalsiyum (125.3-287.5 ppm) ve magnezyum (24.73-61.88 ppm) olarak belirlendi. Demir(1.32-9.54 ppm), çinko(1.34-20.84 ppm) ve manganez (1.39-18.69 ppm) orta düzeyde, diğerleri ise eser düzeylerde bulundu. Manganezin tüm kestane balı örneklerinde orta düzeyde ve diğer bal türlerine göre de daha yüksek düzeyde bulunması nedeniyle kestane balında taklit ve tağşişin tespit edilmesi amacıyla Mn'nin parmak izi olarak kullanılması önerilebilir. Ağır metallerden Cd yalnızca bir örnekte bulunurken, Ni hiç bir örnekte tespit edilmemiştir. Örneklerde Pb konsantrasyonu 1.84 ppm ile 3.38 ppm arasında değişirken Al konsantrasyonu oldukça yüksekti (2.94-13.94 ppm). Türk Gıda Kodeksi Bulaşanlar Yönetmeliği'nde bal türlerinin ağır metal içerikleri için üst sınır bulunmadığından bu ağır metal içeriklerinin uygunluğu konusunda bir değerlendirme yapılamamıştır. Bal türlerinde ağır metal kontaminasyon limitlerinin mevzuata dahil edilmesi, özellikle ağır metal kontaminasyonunun bal hasadı ve depolama gibi proseslerde kullanılan metalik kaplar gibi ikincil kaynakların gözden geçirilmesi gerektiği düşünülmektedir.

Anahtar Kelimeler: Kestane balı, Mineral madde içeriği, Ağır metal, Gıda güvenliği

Abbreviations: TURKSTAT,Turkish Statistical Institute

1. INTRODUCTION

Turkey has a very rich genetic diversity in terms of secretion source trees such as pine and fir, which are considered important nectar sources by bees, and forest trees such as acacia, linden, maple and chestnut. This diversity has not been found even in countries with high honey yield (Kumova & Korkmaz, 2005). In addition, the climatic conditions and plant diversity of the seven regions of our country are different. Therefore, Turkey is very rich in bee genetic resources due to its diverse climate pattern. Scientific studies indicate that there are five different bee races (*Apis mellifera anatoliaca, Apis mellifera meda, Apis mellifera caucasica, Apis mellifera syriaca, Apis mellifera carnica*) in Turkey (Kandemir et al., 2006; Kukrer & Bilgin, 2020; Yıldız & Karabağ, 2022). Moreover, the local ecotypes registered by the Ministry of Agriculture and Forestry are the Caucasian and Anatolian Bee races, as well as the Efe, Gökçeada, Trakya, Hatay and Yığılca honey bee ecotypes (Anonymous, 2020). This situation increases the diversity of monofloral secretion honey produced in our country as well as polyfloral flower honey.

While the nutritional content, flavor and aroma of honey are generally determined by pollen and nectar sources in the environment, processing conditions, the equipment used from the hive to the consumer, and environmental factors are also effective in its physicochemical qualities. The nutritional, physicochemical, bioactive and apitherapeutic qualities of mono and polyfloral honey with various pollen contents collected from different locations also vary, and there are many studies in the literature on these issues (Cunningham et al., 2022; Habryka et al., 2021; Moldakhmetova et al., 2023). However, the number of studies on the mineral compositions and especially heavy metal contamination of honey is quite low (Bilandžić et al., 2019; Frazzoli et al., 2007; Güneş, 2021; Kanbur et al., 2021; Pavlin et al., 2023).

The mineral content of honey varies depending on the nectar yield of the plants and pollen composition. The main concentrations of elements in honey are also affected by the soil, air, water, etc. so the composition and content of elements in honey are affected by the geographical origin of the honey. The most abundant mineral in honey is potassium (45-85% of the total mineral content). Other major elements are sodium, calcium, and magnesium. Copper, iron, zinc, and manganese are found in moderate levels. Honey also contains lower levels (<1 µg/g) of trace elements (Kılıç Altun et al., 2017; Sager, 2017; Solayman et al., 2016). Since chestnut honey is rich in minerals, the composition of the honey varies according to its monofloral character, and the higher the chestnut nectar level of the honey, the higher its biological value (Rodriguez-Flores et al., Taş-Küçükaydın et al., 2023). The proximity of industrial zones, urbanization status, exhaust gases, asphalt resources in the environment (such as main roads close to the apiary), and the equipment used in the process of delivering honey to the consumer may also affect the variety and level of heavy metals that may contaminate honey (Bosancic et al., 2020; Manouchehri et al., 2021).

Chestnut honey, which has distinctive features in a certain geography, is one of the main groups of monofloral honey types. It can be distinguished more easily from other kinds of honey, especially due to its taste, color and aroma (Hendek Ertop & Atasoy, 2018). It is known for apitherapy properties besides the nutritional attributes such as high antioxidant activity, B and C vitamin contents. In the Black Sea region, Kastamonu is the most important province for chestnut honey production because of that Kastamonu province constitutes two-thirds of the chestnut forest flora of the Black Sea region.

Chestnut forests in Kastamonu, where apiaries where chestnut honey are produced, have a different distribution with their scattered structure from the coastline to 1000-1200 meters altitude. As well as the climatic characteristics of these locations, their secondary flora, which is a source of pollen and nectar except chestnuts, also differs. Therefore, this study was planned with the prediction that chestnut honey produced in different districts has a different composition. Some mineral and heavy metal contents of chestnut honey obtained from 15 different locations in Kastamonu province were examined. It aims to contribute to official regulations regarding the product, product standardization, and national branding studies by determining mineral and heavy metal contents based on the region where the honey is produced.

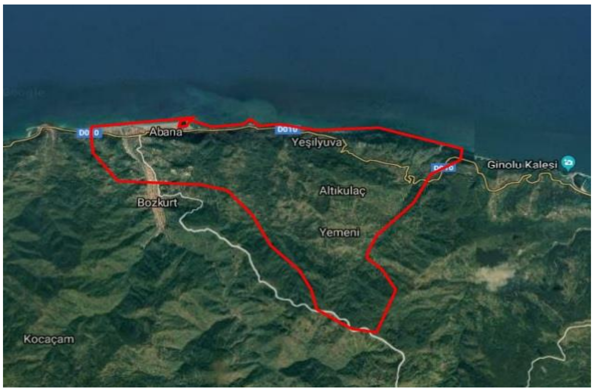
2. MATERIALS and METHODS

2.1. Material

The chestnut honey samples used in the study were collected in 15 production locations in Abana, Bozkurt, Doğanyurt, Cide, Küre, and İnebolu Districts, which are stated in Table 1. These Districts are located in the area of İnebolu and Küre Forest Enterprise Directorate. 50% of Turkey's chestnut production is provided by the chestnut forests located in the İnebolu Forest Enterprise area (Anonymous, 2018). The research area is based on the Greenwich prime meridian; It is located between 32°04'55"-34°13'02" eastern longitudes and 41°04'07"-41°57'08" northern latitudes. In this area, chestnut forests have a scattered distribution, starting from the coastline and reaching an altitude of 1600 m. As the material supply area, 15 different chestnut honey production locations in Abana, Bozkurt, Doğanyurt, Cide, Küre and İnebolu Districts, which are located in the İnebolu and Küre Forest Enterprise Directorate area and have dense chestnut forests, were determined. The districts where the samples were obtained were identified as CBC (Geographical Information). Map images taken from the database are shown in Figure 1.

Table 1. Code and locations collected of the honey samples

Code	Location	Village
A	Abana	Akçam
B1	Bozkurt1	Darsu
B2	Bozkurt2	Işığan
B3	Bozkurt3	Günvakti
C1	Cide1	Kovanören
C2	Cide2	Başköy
C3	Cide3	Ağaçbükü
C4	Cide4	Kayaardı
D1	Doğanyurt1	Yukarı Mescit
D2	Doğanyurt2	Gözalan
İ1	İnebolu1	Yuvacık
İ2	İnebolu2	Gökbel
K1	Küre1	İkizciler
K2	Küre2	Uzunöz
K3	Küre3	Başören



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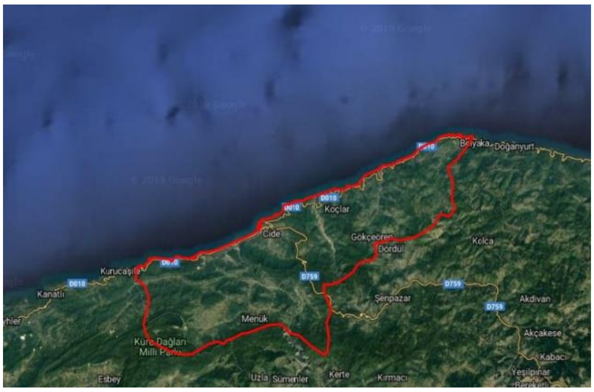

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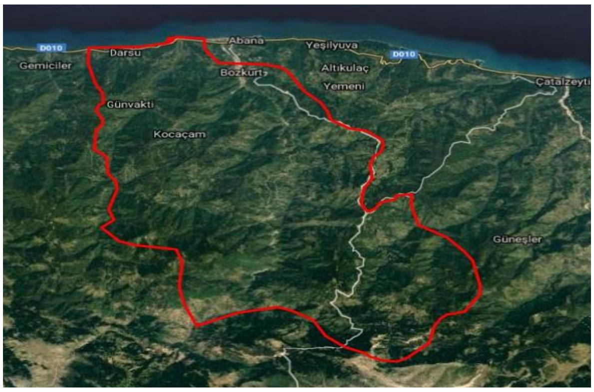

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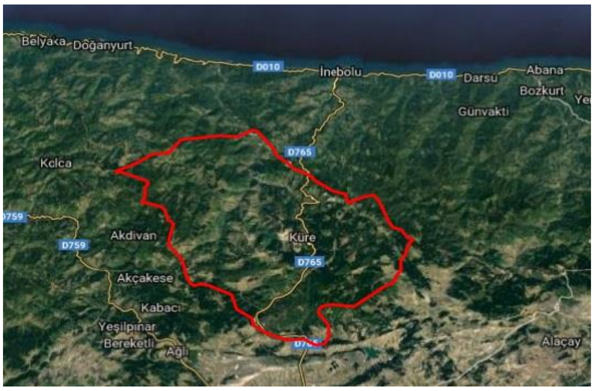

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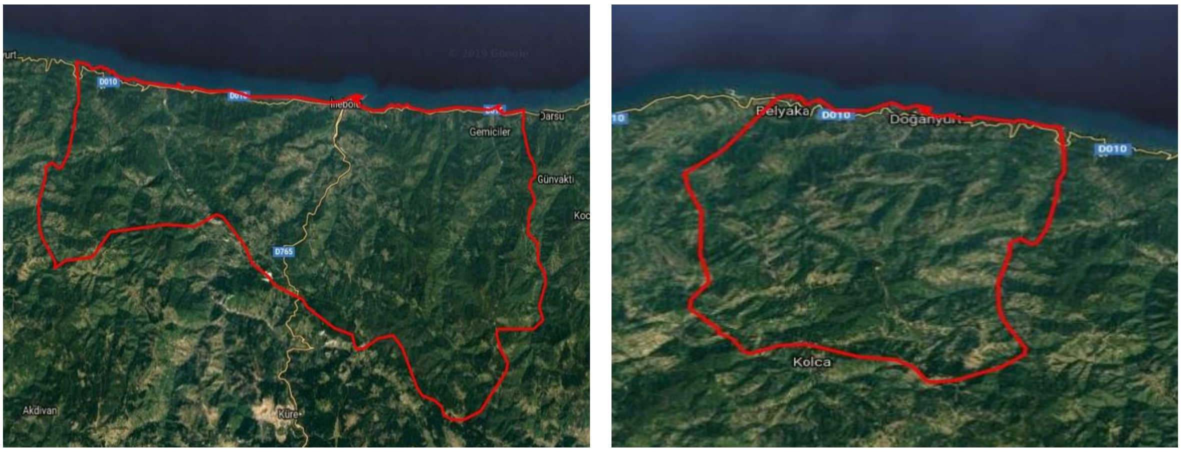

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Figure 1. Geographical regions where chestnut honey samples were obtained (a: Abana; b: Bozkurt, c: Cide, d: Küre, e: İnebolu, f: Doğanyurt)

2.2. Mineral Content

The mineral content was measured by using the microwave (CEM MARS6, USA) nitric acid digestion procedure followed by the inductively coupled plasma-optical emission spectrometry (ICP-OES) (SpectroBlue, Germany) method.

The sample (approximately 1 mL) was transferred directly to PTFE flasks after adding 10 mL of HNO_3 (67% v/v) and 1 mL H_2O_2 (30% v/v) and then subjected to the following digestion program: the temperature was raised to 200 °C (15 min) and kept at 200 °C for 15 min. After cooling at room temperature, sample solutions were transferred into 50 mL polyethylene flasks and the volume was completed with ultrapure water. The digested samples were filtered through microfilters and analyzed with ICP-OES (Al Khalifa & Ahmad, 2010). Multi-element standard stock solution (Merck, Germany) provided for ICP-OES was used in the preparation of calibration standards. The measurements were carried out in triplicate, and the dilution factor was taken into consideration in the calculations.

2.3. Statistical Analysis

Statistical evaluation of the analysis results was made using the SPSS 17.0.1 software (SPSS Inc., Chicago, Illinois, US).

The difference between the data averages subjected to analysis of variance (ANOVA) was determined by performing the Tukey multiple comparison test at the $p<0.05$ significance level.

3. RESULTS and DISCUSSION

Within the scope of the study, Sodium (Na), calcium (Ca), potassium (K) and magnesium (Mg) concentrations as the major elements of honey samples obtained from 15 different locations were determined and the average values on a location basis (Table 2).

Table 2. Content of major elements of the samples

Sample code	Na (ppm)	Ca (ppm)	K (ppm)	Mg (ppm)
A	56.03^{f}	173.7^{f}	**1410.0^{l}**	41.75^{d}
B1	nd	141.5^{h}	3041.4^{k}	**24.73^{j}**
B2	84.30^{d}	225.7^{c}	4172.5^{g}	46.31^{b}
B3	115.00^{b}	180.7^{f}	3405.7^{j}	29.12^{i}
C1	32.33^{h}	198.2^{e}	4000.9^{h}	32.32^{h}
C2	13.13^{i}	236.0^{b}	4778.4^{e}	35.14^{g}
C3	84.50^{d}	225.0^{c}	4846.2^{de}	37.23^{ef}
C4	58.73^{ef}	213.8^{d}	5821.6^{b}	43.74^{c}
D1	**204.4^{a}**	**125.3^{i}**	3368.5^{j}	28.89^{i}
D2	98.00^{c}	197.2^{e}	4726.0^{ef}	36.68^{f}
İ1	61.50^{e}	**287.5^{a}**	4935.3^{d}	**61.88^{a}**
İ2	4.70^{j}	142.7^{h}	4607.9^{f}	34.82^{g}
K1	**3.90^{j}**	145.6^{h}	5664.8^{c}	38.52^{e}
K2	39.83^{g}	182.3^{f}	**6581.5^{a}**	42.44^{cd}
K3	61.03^{ef}	155.7^{g}	3568.6^{i}	31.53^{h}

*nd: Non determined

** a–l: Means with different superscripts in the same column are significantly different ($p < 0.05$).

The difference between the major element contents of chestnut honey samples between the locations was found to be statistically significant ($p<0.05$). It is clear that K is the most abundant mineral in all honey samples, followed by Ca, Na, and Mg as the second, third and fourth major minerals, respectively. In line with most of the previous studies (Chua et al., 2012; Pisani et al., 2008, Vanhanen et al., 2011), the mineral of potassium covered about 70% of the total elements of the honey samples, ranging from 81.93% to 96.08% in this study. According to the results, the main concentrations of the elements in the chestnut honey samples were affected by the geographical location. The reason for this was that although the apiaries where honey samples were collected were in chestnut forests, Kastamonu districts had different altitudes, climates and soil properties, and different secondary flora.

Besides the major minerals, some elements such as Mn, Zn and Fe were also detected in honey samples. These elements were found in moderate levels (Table 3).

Table 3. Content of other elements found in moderate levels.in the samples

Sample code	Mn (ppm)	Zn (ppm)	Fe (ppm)
A	**1.39^{m}**	3.63^{k}	**9.54^{a}**
B1	10.13^{g}	**1.34^{l}**	**1.32^{j}**
B2	17.12^{b}	5.10^{j}	1.72^{i}
B3	11.25^{f}	7.45^{gh}	2.98^{gh}
C1	5.52^{l}	8.69^{f}	3.33^{f}
C2	6.89^{j}	14.70^{d}	4.10^{e}
C3	7.08^{ij}	5.98^{i}	4.71^{d}
C4	13.47^{d}	7.06^{h}	4.02^{e}
D1	6.37^{k}	16.35^{c}	8.39^{b}
D2	9.84^{gh}	6.72^{hi}	2.77^{h}
İ1	9.54^{h}	12.82^{e}	4.27^{e}
İ2	12.62^{e}	7.36^{h}	3.00^{gh}
K1	14.72^{c}	8.21^{fg}	4.76^{d}
K2	**18.69^{a}**	**20.84^{a}**	3.23^{fg}
K3	7.39^{i}	18.49^{b}	6.89^{c}

*nd: Non determined

** a–m: Means with different superscripts in the same column are significantly different ($p < 0.05$).

The difference between the other major element contents of chestnut honey samples according to locations was found to be statistically significant ($p<0.05$). In a conducted study by Küçük et al. (2007), when the comparison of mineral contents of chestnut, rhododendron, and multi-floral honey samples was carried out, the Mn concentration of the chestnut honey (9.69 ppm) was found quite high than the others (2.14 and 0.59 ppm, respectively). In another study (Kolaylı et al., 2008), similarly the Mn concentration of the chestnut honey (17.20 ppm) was found quite high than the Anzer honey (2.30 ppm) and Bayburt honey (1.20 ppm). This situation can be interpreted as Mn content being a good fingerprint in detecting imitation and adulteration of chestnut honey. Fe concentration varied between 1,32 ppm- 9,54 ppm, and Zn concentration varied between 1,34 ppm and 20,84 ppm in this study. This level is high in the findings of several researchers (Grembecka & Szefer, 2013; Kolaylı et al., 2008), who reported the amount of Zn and Fe content in chestnut honey.

Minor elements Co, Cu, Ga, Cr and Ag concentrations of the honey samples obtained from different locations were examined. Cu, Ga and Cr were found in trace levels. Co and Ag were not determined in any location (Table 4).

Table 4. Content of minor elements of the samples

Sample code	**Co (ppb)**	**Cu (ppb)**	**Ga (ppb)**	**Cr (ppb)**	**Ag (ppb)**
A	nd*	**11832.30**^{a}	4820.50^{fg}	33.50^{cd}	nd
B1	nd	1360.20^{d}	**1696.70**^{i}	nd	nd
B2	nd	**508.20**^{f}	3186.80^{hi}	30.60^{d}	nd
B3	nd	612.90^{f}	**4179.60**^{gh}	33.53^{cd}	nd
C1	nd	7874.70^{b}	6505.10^{de}	nd	nd
C2	nd	1317.00^{d}	6028.10^{ef}	30.80^{d}	nd
C3	nd	4194.00^{c}	6546.10^{cde}	44.20^{ab}	nd
C4	nd	3819.70^{c}	6750.80^{bcde}	41.10^{abc}	nd
D1	nd	nd	8832.70^{a}	33.70^{cd}	nd
D2	nd	nd	8183.7^{ab}	nd	nd
İ1	nd	nd	**9185.00**^{a}	nd	nd
İ2	nd	nd	8060.9^{abc}	nd	nd
K1	nd	nd	8337.30^{a}	33.90^{cd}	nd
K2	nd	nd	8980.60^{a}	35.30^{bcd}	nd
K3	nd	nd	7657.70^{abcd}	50.80^{a}	nd

*nd: Non determined

** a–h: Means with different superscripts in the same column are significantly different ($p < 0.05$).

Copper in the chestnut honey was detected in the A, B and C coded samples. It means Cu was determined in the close locations of the same districts. This can be probably due to the use of pesticides in the farmlands, which are located outside the forest area. Although a trace amount of copper is essential for the production of melanin which is responsible for pigmentation in the skin, and for the formation of hemoglobin, the tolerable daily intake of copper is limited to 3 mg/60 kg adult body weight (Joint FAO/WHO, 1999).

The heavy metals (Cd, Pb, Ni and Al) concentrations of the honey samples obtained from different locations were determined (Table 5).

Table 5. Content of heavy metals of the samples

Sample code	Cd (ppb)	Pb (ppb)	Ni (ppb)	Al (ppb)
A	nd*	nd	nd	8230.20[e]
B1	nd	nd	nd	**2938.90[l]**
B2	nd	nd	nd	5451.60[ij]
B3	nd	940.30[e]	nd	4731.90[k]
C1	**11.10**	1007.40[e]	nd	8178.50[e]
C2	nd	1365.10[cde]	nd	7148.70[fg]
C3	nd	1298.60[de]	nd	7585.70[f]
C4	nd	nd	nd	6476.40[h]
D1	nd	1714.70[cd]	nd	8949.60[d]
D2	nd	1203.30[e]	nd	6987.30[gh]
İ1	nd	1829.10[c]	nd	**13944.30[a]**
İ2	nd	**835.90[f]**	nd	4913.80[jk]
K1	nd	1355.60[cde]	nd	5623.40[i]
K2	nd	2396.80[b]	nd	9901.30[c]
K3	nd	**3378.40[a]**	nd	13021.90[b]

*nd: Non determined

** a–l: Means with different superscripts in the same column are significantly different ($p < 0.05$).

In this study, while the Cd element was detected in only one sample (C1), the Ni element was not detected in any chestnut honey sample. Because there wasn't a maximum limit for both of them in the Turkish Food Codex Contaminants Regulation (2011), no evaluation could have been made regarding the suitability of these heavy metal contents. Indeed there is no limit regarding the heavy metal contents value determined for honey types in the current Turkish Food Codex Contaminants Regulation (2011). The Contaminants Regulation was revised and published as a draft text in 2022. According to the Draft Regulation, the maximum Pb limits were indicated as 0.15 ppm for floral honey and 0.1 ppm for secretion honey starting from the date of 31.12.2023. Pb concentration varied between 1.84 ppm and 3.38 ppm for the samples. According to the Pb content results obtained in this study, while all honey samples are in compliance with the current regulation, only 4 chestnut honey samples will be in compliance with the Contaminants Regulation after December 31, 2023.

The change of Al concentration in chestnut honey samples, due to location was evaluated. As a result of the study, it was determined that the contents of Al concentrations were quite high, and there are great differences in terms of Al concentration between locations (between 1.32 ppm-13.94 ppm). Some portion of Al content might be attributed to secondary sources such as metallic containers used for storage during handling processes and harvesting of honey (Pisani et al., 2008). This metal contamination is usually accompanied by Cr contamination from the same sources (Chua et al, 2012).

The total multi-elemental content of the chestnut honey samples was presented using a column chart as in Figure 2. The total mineral contents of all samples varied widely between 1720.9 ppm and 6910.1 ppm. The locations with the lowest total mineral content were districts A (Abana) and B (Bozkurt). Locations in K (Küre) and C (Cide) districts generally presented high mineral substance content.

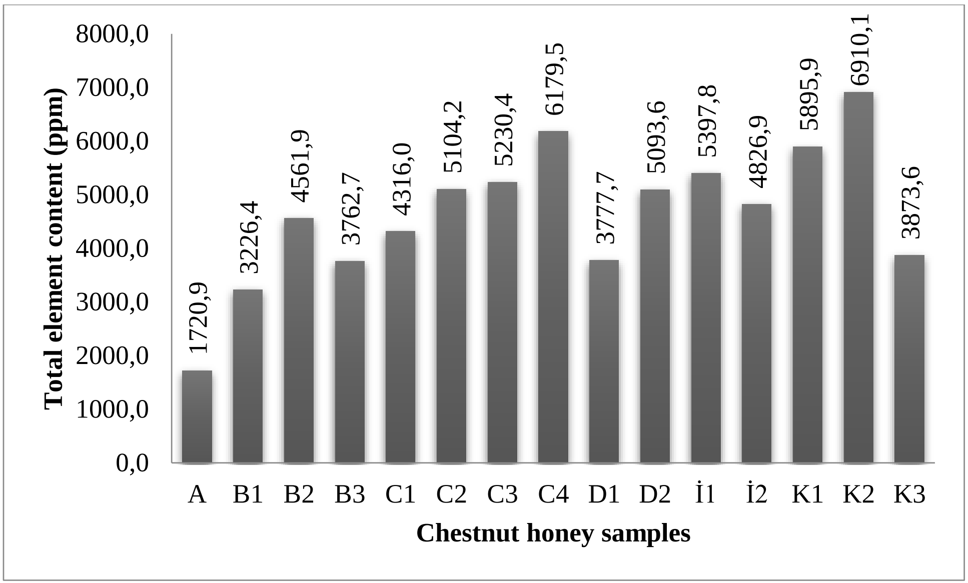

Figure 2. Total element contents of the chestnut honey samples (ppm)

4. CONCLUSION

In this study, 15 chestnut honey, which is widely produced in Kastamonu, samples were collected from Abana, Bozkurt, Doğanyurt, Cide, Küre, and İnebolu districts, and their mineral composition and heavy metal contents were examined. The mineral composition of honey varied widely depending on district and location. It was determined that especially Mg and K concentrations of chestnut honey were significantly high. Mn, Fe and Zn concentrations of chestnut honey examined in the study were found to be higher than other honey types. For this reason, it can be recommended to use some minor elements, especially Mn, as fingerprints to detect imitation and adulteration of chestnut honey. As a result of the study, it was determined that the location-based changes in heavy metals were statistically significant as a result of variance analysis. However, heavy metal findings could not be compared since there are no limit values for heavy metal residues in honey in the Contaminants Regulation in force based on the Turkish Food Codex. More studies are needed on heavy metal contaminants in other bee products, especially honey. Nowadays, as clean agricultural areas decrease and urbanization increases, the possibility of contaminating heavy metals such as arsenic and lead into the hive is increasing in the location where the bees are located. Furthermore, in this study, the strained honey samples collected from different locations were used that were prepared from natural comb honey by the producers. It is thought that the reason for the high lead and aluminum levels in honey samples is due to the equipment used in the strained honey production process. In addition, aluminum content may be contaminated from different environmental sources. According to the studies, heavy metal contents of some species in the flora affect the honey contents (for example, the density of *Mentha* species is increased in the amount of Cr). And this effect varies according to honey types (secretion, monofloral, multifloral, etc.). These interactions should be re-evaluated with more comprehensive studies. Additionally, the limit values for other heavy metal contaminants in the Turkish Food Codex, Draft Contaminants Regulation should be re-evaluated.

DECLARATIONS

The authors have no conflicts of interest to declare.

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