ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

COMPARISON OF COMMERCIAL AND ANATOLIAN BEE VENOM IN TERMS OF CHEMICAL COMPOSITION

Anadolu Bal Arısı Arı Zehrinin ve Ticari Olarak Elde Edilen Arı Zehirlerinin Kimyasal İçerik Bakımından Karşılaştırılması

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ABSTRACT

We compared fresh bee venom samples produced by Anatolian beekeepers with commercial bee venom samples based on physicochemical analyses results. Sugar content analysis was conducted using HPLC-RID, moisture content analysis was performed using a moisture analyzer and melittin, apamin and phospholipase A_2 contents were analyzed via HPLC-UV. When we compared the commercial bee venom samples with the freshly collected Anatolian honey bee venom, it was determined that the apamin, melittin and phospholipase A_2 contents were generally lower in the commercial bee venom samples. Additionally, there was a statistically significant difference between the groups in terms of the moisture and phospholipase A_2 contents (p <0.5). When we evaluated the sugar profile analysis, other than in maltose and erlose no difference was found between the two groups. The results showed that the content quality of the fresh bee venom samples collected from Anatolian honey bees was higher than that of the commercially sold bee venom samples. This result clearly indicated that bee venom samples intended for use in apitherapy or for cosmetic purposes should be obtained fresh or kept under very good conditions.

Keywords: Bee venom, Apamin, Melittin, Phospholipase A_2, Anatolia

ÖZ

Bu çalışmada. Anadolu bal arısından taze olarak elde edilen ve ticari olarak satılan arı zehri örnekleri içerik analizleri bakımından karşılaştırıldı. Nem tayin cihazı kullanılarak nem içeriği, HPLC-UV kullanılarak Melittin, Apamin, Fosfolipaz A2 içeriği ve HPLC-RID kullanılarak şeker profil analizi gerçekleştirildi. Ticari arı zehri örnekleri Anadolu bal arısından taze olarak toplanan arı zehri örnekleri ile karşılaştırıldığında genel olarak apamin, melittin ve fosfolipaz A2 içeriğinin ticari olarak satılan arı zehri örneklerinde daha düşük olduğu; nem ve fosfolipaz A2 bakımından gruplar arasında istatistiki olarak önemli düzeyde farklılık olduğu belirlendi (P<.05). Şeker profil analizleri değerlendirildiğinde ise maltoz ve erloz dışında şeker içerikleri bakımından iki grup arasında bir farklılık olmadığı belirlendi. Sonuçlar Anadolu bal arısından elde edilen taze arı zehri örneklerinin ticari olarak satılan arı zehri örneklerinden içerik bakımından daha kaliteli olduğunu gösterdi. Bu sonuç özellikle apiterapi veya kozmetik amaçlı kullanılacak olan zehir örneklerinin taze olarak elde edilmesi veya çok iyi koşullarda muhafaza edilmesi gerektiğini açıkça ortaya koymuştur.

Anahtar Kelimeler: Arı zehri, Apamin, Melittin, Fosfolipaz A2, Anadolu

GENİŞLETİLMİŞ ÖZET

Amaç: Bal arısı zehri; bal arılarının zehir bezleri tarafından kolonilerini koruyabilmek için düşmanlarına karşı savunma amaçlı salgıladıkları ve iğneleri aracılığı ile zerk ettikleri protein. lipit ve düşük moleküllerden oluşan spesifik bir karışımdır. Bal arısı zehri ve içerdiği maddeler son zamanlarda geleneksel ve tamamlayıcı tıpta kozmetikte ve yeni ilaç geliştirme aşamalarında oldukça yaygın bir şekilde kullanılmaya başlanmıştır. Piyasadaki üretim talebinin artması sonrasında ticari amaçla ürünlere eklenen ilave maddelerin tespit edilmesi ve bal arısı zehrinin belirli bir standardının olmaması büyük bir problem haline gelmiştir. Birçok araştırmacı bal arısı zehrinin içerik analizini yapmış olmasına rağmen hala toplanması ve içeriği ile ilgili geçerli bir standart yöntem oluşturulmamıştır. Zehrin toplanması. depolanması ve içeriğinin. özellikle etken maddelerin stabilitesi ürünün kalitesi açısından oldukça önemlidir. Bu çalışmada Anadolu arısından taze olarak elde edilen arı zehri örnekleri ticari olarak satılan arı zehri örnekleri ile karşılaştırılmıştır.

Gereç ve Yöntem: Bu çalışmada 2018 yılında toplanan; 2 adet ticari bal arısı zehri ve 3 adet özel olarak üretilmiş Anadolu bal arısı zehri kullanıldı. Çalışmada kullanılan tüm örnekler elektroşok yöntemi ile elde edildi. Nem tayin cihazı kullanılarak % nem içeriği belirlendi. HPLC-UV kullanılarak % Melittin, % Apamin, % Fosfolipaz A2 içeriği ve HPLC-RID kullanılarak şeker profil analizi gerçekleştirildi. Sonuçlar MINITAB programında Tukey testi ile karşılaştırıldı.

Bulgular: Ticari arı zehri örnekleri Anadolu bal arısından taze olarak toplanan arı zehri örnekleri ile karşılaştırıldığında genel olarak apamin. melittin ve fosfolipaz A2 içeriğinin ticari olarak satılan arı zehri örneklerinde daha düşük olduğu; nem ve fosfolipaz A2 bakımından gruplar arasında istatistiki olarak önemli düzeyde farklılık olduğu belirlendi ($P<.05$). Şeker profil analizleri değerlendirildiğinde ise maltoz ve erloz dışında şeker içerikleri bakımından ise iki grup arasında bir farklılık bulunamamıştır.

Sonuç: Sonuçlar taze olarak Anadolu bal arısından elde edilen arı zehri örneklerinin ticari olarak satılan arı zehri örneklerinden içerik bakımından daha kaliteli olduğunu gösterdi. Bu sonuç özellikle apiterapi veya kozmetik amaçlı kullanılacak olan zehir örneklerinin taze olarak elde edilmesi veya çok iyi koşullarda muhafaza edilmesi gerektiğini açıkça ortaya koymuştur. Ticari olarak üretimi yapılan ve kozmetik ya da apiterapi gibi sebeplerle kullanılacak olan arı zehri içeriğinin kalite standartlarının belirlenmesi ve uygulamaya konulması ürünlerin etkinliği ve güvenilirliği açısından oldukça önemlidir. Bu zamana kadar arı zehrinin gıda ya da ilaç kategorisinde değerlendirilmemesinden ve Türkiye'de yaygın üretiminin olmamasından dolayı ürünün toplanması ya da içerik analizi ile ilgili herhangi bir standardizasyon yapılmamıştır. Bal arısı zehrinin önemi ve piyasadaki geleceği göz önüne alındığında ileriki safhalarda yaşanabilecek problemlerin öngörü ile engellenebilmesi için bu konuda kapsamlı çalışmalar yapılması gerekmektedir.

Anahtar Kelimeler: Arı zehri, apamin, melittin, fosfolipaz A2, Anatolia

INTRODUCTION

Beekeeping is an important sector for the Turkish economy. According to FAOSTAT 2017 (Anonym, 2017) data, the total number of hives and total honey yield of Turkey are the second largest in the world. However, in terms of the honey yield per hive and the production and diversity of other bee products, it is far behind other countries. According to the statistics, total honey production in 2017 was 114,471 tons and the total number of hives was 7,991,072, with a yield of 14.32 kg / hive. Beeswax production was 4,488 tons and royal jelly production was 228 kg in 2016. According to official records, the production of bee venom has not yet begun. However, it is important to note that some beekeepers have recently started to produce bee venom.

In recent years, traditional and complementary medicine have been applied together with modern medicine. Many people tend to use herbal and other natural products for their beneficial effects and to avoid medication or surgical intervention due to side effects. Bee products have an important place among these natural products. Every product to be used for health purposes should be used in a controlled manner. Therefore, investigation into the use of natural products for health problems has begun and sound evidence of the mechanism of action of natural products is being found.

Honey bee venom (BV) is a bitter. colorless liquid produced by the venom glands in worker bees and stored in the venom sacs. Newborn bees have very little ability to produce venom. The venom glands begin to function soon after the transformation period from juvenile to adult and peak production level is reached within two or three weeks. The composition of the worker bee venom changes over time. This is probably related to the transition from 'house bee activity' to 'field bee activity'. A worker bee is capable of producing about 0.1 µg of dry venom during its life. Ten thousand bee stings (the yield of a small colony) are required to produce1 g of dried venom (Hider 1988).

Bee venom contains pharmacologically important active substances. The components of bee venom have been characterized as a mixture of proteins, peptides and low-molecular components. Although the composition of fresh and dried bee venom differs substantially from the volatile components, the overall biological activities are similar (Pak 2017).

Bee venom from *Apis mellifera* L. is a mixture of at least 18 complex active components. These components have a wide variety of properties. Bee venom has been used in traditional medicine to treat chronic inflammatory diseases such as rheumatoid arthritis and to relieve pain (Kang et al. 2002; Kwon et al. 2002; Son et al. 2007). A number of studies have been published recently indicating its antimutagenic, radioprotective, antinociceptive, anti-inflammatory and anticancer effects (Varanda and Tavares 1998; Kim et al. 2003; Lee et al. 2004; Son et al. 2007; Gajski and Garaj-Vrhovac 2009).

Bee venom includes melittin, apamin, secapin, procapine, histamine, adolapin, catecholamines and mast cell degranulating peptide components. The dominant enzymes are phospholipase A_2 followed by hyaluronidase, acid phosphomonoesterase, lysophospholypase and glucosidase. Bee venom also contains several physiologically active amines, fructose, glucose and phospholipids, all having effects on many cellular systems (Neuman and Habermann 1954; Habermann 1972; Gauldie et al. 1976; Stuhlmeier 2007).

Enzymes are proteins that catalyze specific reactions. For example, phospholipase A_2 is an enzyme that deacetylates to produce disophospholipids and long-chain fatty acids which catalyze the hydrolysis of natural lipids. Hyaluronidase catalyzes the hydrolysis of hyaluronic acid in the viscous mucopolysaccharide structure in the interstitial substrate of connective tissue (Banks and Shipolini 1986).

Apamine is a peptide component of bee venom and has anti-inflammatory properties (Son et al. 2007). The most important component of the chemical composition in bee venom is the melittin in the polypeptide structure. Melittin (MEL) is the main active ingredient of 40–50% of the total dry weight of bee venom. It is water-soluble, linear, cationic, hemolytic and amphipathic. It is a peptide with a weight of 2840 Da and consists of 26 amino acids. Melittin binds to the negatively-charged cell membrane and disrupts the integrity of the phospholipid double layers with increased penetration of atomic ions and molecules, ultimately leading to cell destruction. Due to this feature, it is an important component for use in cancer treatment (Sobral 2016; Rady et al. 2017).

A search of the literature revealed that bee venom can be used for many diseases. Moreover, it has been shown that bee venom not only has a protective effect in the treatment of disease but is also capable of producing biological and chemical effects against radiation energy (Varanda and Tavares 1998).

Apitherapy is an old medical treatment that includes the use of bee products such as honey, pollen, propolis, royal jelly and bee venom for medicinal purposes. In the United States, apitherapy has a 100-year history. Bee venom can also be used by injection in apitherapy acupuncture. The immune system is a complex mechanism responsible for recognizing and combating foreign invaders such as bacteria and viruses and also for eliminating cells undergoing malignant transformation. Thus, bee venom treatment is also a kind of immunotherapy.

Bee venom, when properly produced and stored, provides many extremely important benefits to human health. The active ingredients of bee venom which are widely used in apitherapy and cosmetics must be preserved and it should not contain any chemical additives. The aim of this study was to draw attention to the need for establishing standard criteria for handling bee venom.

As of 2018 there had been 700 scientific studies on bee venom. However, there have not been many studies on bee venom content and standardization (Bogdanov 2015; Banks and Shipolini 1986; Moreno and Giralt 2015). Our aim with this study was to determine the changes in chemical content of bee

venom depending on the holding period. Hence, the goal was to establish standard criteria by determining the changes occurring in the quality of bee venom to be used for treatment purposes according to the storage conditions. In this study, apamin, melittin, phospholipase A_2 and sugar profile analyses of bee venom were conducted using HPLC, and the moisture content of bee venom was measured using a moisture analyzer.

The findings of this study will be useful in determining quality criteria for the bee venom intended for use in traditional and complementary medicine.

MATERIALS AND METHODS

Chemicals

Products purchased from Sigma–Aldrich and TCI used in the study included melittin from honey bee venom (Sigma-Aldrich, CAS = 20449-79-0), apamin (Sigma-Aldrich, CAS = 24345-16-2), phospholipase A_2 from honey bee venom (Sigma-Aldrich, CAS = 9001-84-7), D-(+) sucrose (TCI, CAS = 57-50-1), D-(+) glucose (TCI, CAS = 50-99-7), D-(-) fructose (TCI, CAS = 57-48-7), melezitose monohydrate (Sigma-Aldrich, CAS = 10030-67-8), D-(+) turanose (TCI, CAS = 547-25-1), D-(+) maltose monohydrate (TCI, CAS = 6363-53-7), maltotriose (Sigma-Aldrich, CAS = 1109-28-0), D-(+) trihalose dihydrate (Sigma-Aldrich, CAS = 6138-23-4), D- turanose (Sigma-Aldrich, CAS = 547-25-1) and erlose (Sigma-Aldrich, CAS = 13101-54-7). Those provided by Carlo Erba and Merck included triflor acetic acid (Carlo Erba, CAS = 76-05-1) and acetonitrile (Merck, CAS = 75-05-8). The water used throughout the study was purified using a Water Pro BT Purification System device from LABCONCO (Kansas City, MO, USA).

Sample collection and preparation

The bee venom used in this study (Fig. 1) included two commercial bee venoms and three bee venoms specially produced by Anatolian beekeepers, collected by electroshock in 2018 and kept at -18 °C in the dark.

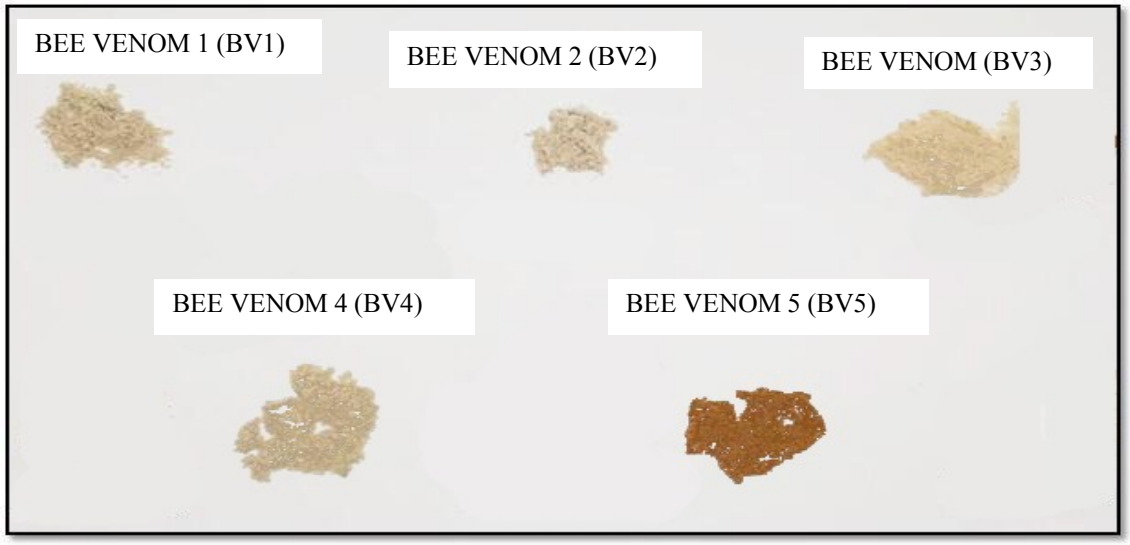

Figure 1. Bee venom samples
Şekil 1. Arı zehiri örnekleri

For the analyses, samples were prepared by diluting 5 mg of each of the bee venoms with 10 mL of ultrapure water and then filtering them for HPLC UV analysis. Three replicates of each sample were studied.

Moisture content analysis

The moisture content of all samples was determined by an infrared heated moisture analyzer using a modified AOAC 934.01 method.

HPLC-UV analysis: Melittin, apamin, phospholipase A_2 content

The HPLC technique used in this study has been fully described previously (Zenon and Kokot, 2009). The Supelcosil lc-318 5 µm. 4.6 × 250 mm column (Supelcosil HPLC Products) was used. The bee venom was separated by linear gradient 5% B – 80% B at 30 min (eluent A – 0.1% TFA in water; eluent B – 0.1 % TFA in acetonitrile: water (80:20)). The flow rate of the mobile phase was maintained at 1 mL/min with an injection volume of 40 µL at a separation temperature of 25 °C. The analysis was monitored at 220 nm. The HITACHI HPLC system consisted of a quaternery 5160 pump, a 5260 auto sampler, a 5450 RI dedector, a 5410 UV dedector and a 5310 column oven. Control of the instrumentation, data acquisition and data reporting was performed by using Chromaster computer software. The concentrations of the analyzed honeybee venom constituents were calculated from the standard calibration curve equations.

HPLC-RID analysis: Sugar profile analysis

The sugars in the 0.5-g bee venom samples were extracted using an acetonitrile-water solution and Carrez I-II and then analyzed by centrifugation and filtration for HPLC-RID analysis. Three replicates of each sample were studied.

The DIN 10758 modified method was applied. The isocratic analysis used the acetonitrile: ultra-pure water mobile phase. The HITACHI HPLC system consisted of a quaternery 5160 pump, a 5260 auto sampler, a 5450 RI dedector, a 5410 UV dedector and a 5310 column oven. The Thermo Scientific Hypersil C18 column (250 × 4.0 mm id, 5-µm particle size) was used. Control of the instrumentation, data acquisition and data reporting was performed using Chromaster computer software. The concentrations of the analyzed honeybee venom constituents were calculated from the standard calibration curve equations.

RESULTS

The HPLC-UV studies demonstrated that a very good separation of apamine, phospholipase A_2 and melittin had been achieved by using the Supelcosil LC -318 (Supelcosil HPLC Products -250*4.6, 5-µm particle size) column and gradient elution (Fig. 2).

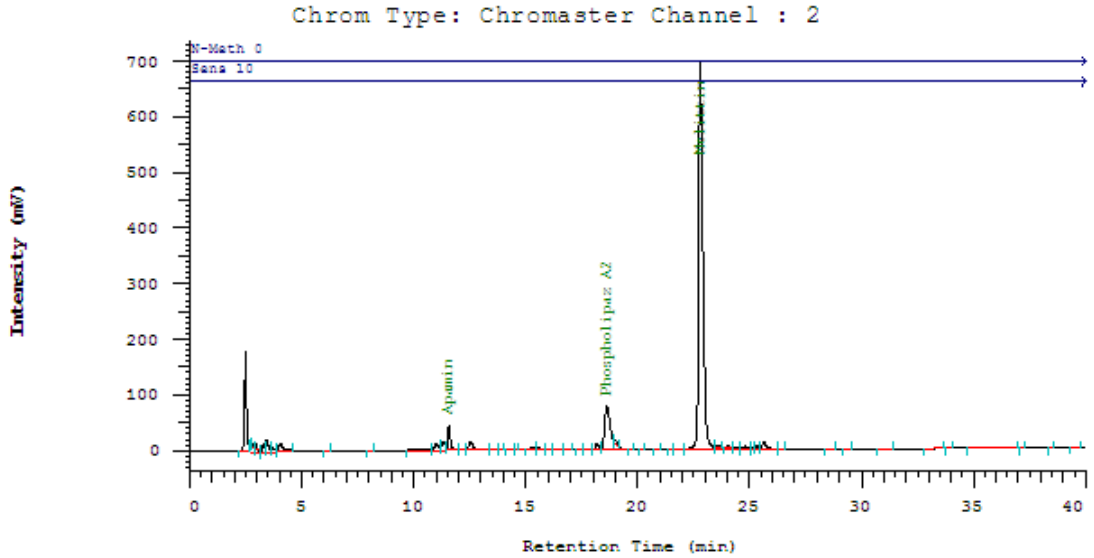

Figure 2. Chromatogram of main bee venom components.

Şekil 2. Arı zehri ana bileşen kromatogramı.

The HPLC-RID studies demonstrated that there was a good separation of fructose, glucose, saccarose, turanose, maltose, isomaltose, erlose and melezitose using the APS-2-HYPERSIL (thermo scientific -250*4, 5-µm particle size) column and gradient elution.

The results of the analysis of the bee venom are given in Tables 1 and 2. These results showed that the apamine, melittin and phospholipase A_2 contents of the commercial bee venoms were lower than those of the Anatolian bee venom. Analysis of variance (ANOVA) was applied using the statistical MINITAB software package. The differences in means (averages) between groups was confirmed by the Tukey test.

Table 1. Content analysis of bee venoms: Moisture, apamine, phospholipase A_2 and melittin

Tablo 1. Arı zehri içeriği nem. apamin. fosfolipaz A2. melittin analizleri

Sample Type	Sample Code	Number of Repetitions	Moisture Content %	Apamine (%)	Phospholipase A_2 (%)	Melittin (%)
Fresh Anatolian Bee Venom	BV1	3	10.47±1.54^{b}	2.61±0.07^{a}	10.83±0.21^{ab}	46.85±0.82^{a}
	BV2	3	9.53±1.67b	2.09±0.11^{b}	10.52±0.15^{b}	36.95±0.36^{b}
	BV3	3	8.91±0.28b	2.63±0.24^{a}	11.00±0.18^{a}	38.92±0.09^{ab}
Commercial Bee Venom	**BV4**	3	13.93±0.63^{a}	0.91±0.02^{d}	9.08±0.11^{c}	18.76±0.13^{d}
	BV5	3	9.68±0.52^{b}	1.60±0.01^{c}	6.90±0.06^{d}	25.3±0.44^{c}

The same letters are not significantly different (P <0.05)

When we examined the sugar profile, no difference was found between the two groups (Table 2). However, the glucose content of the BV5 sample was higher than the other samples, so it was believed that the product might have been adulterated.

Table 2. Sugar profile analysis of bee venoms
Tablo 2. Arı zehri şeker profil analizleri

	Sample code	Number of Replicates	Fructose (%)	Glucose (%)	Sucrose (%)	Turanose (%)	Maltose (%)	isomaltose (%)	Erlose (%)	Melezitose (%)
Fresh Anatolian Bee Venom	BV 1	3	2.97±0.12^{c}	0.07±0.06^{d}	1.37±0.40^{a}	0.67±0.35^{a}	0.03±0.06^{b}	0.30±0.30b	0.17±0.21^{a}	0.10±0.10^{b}
	BV 2	3	3.77±0.06^{b}	2.93±0.06^{c}	1.90±0.52^{a}	0.03±0.06^{b}	0.27±0.46^{ab}	0.33±0.21ab	0.60±0.10^{a}	0.03±0.06^{b}
	BV 3	3	7.93±0.15^{a}	5.67±0.06^{b}	2.03±0.06^{a}	0.07±0.06^{b}	0.03±0.06^{b}	0.23±0.06b	0.37±0.12^{a}	1.30±0.10^{a}
Commercial Bee Venom	**BV 4**	3	1.07±0.06^{d}	0.03±0.06^{d}	0.10±0.10^{b}	0.03±0.06^{b}	0.63±0.06^{a}	0.77±0.06a	0.23±0.12^{a}	0.03±0.058^{b}
	BV 5	3	0.37±0.06^{e}	36.53±0.12^{a}	0.13±0.06^{b}	0.03±0.06^{b}	0.03±0.06^{b}	0.03±0.06b	0.23±0.40^{a}	0.17±0.15^{b}

The same letters are not significantly different (P <0.05).

DISCUSSION

We analyzed and compared three dried fresh Anatolian BV and two dried commercial BV samples. Chemical analyses were generally carried out via HPLC and focused on phospholipase A_2, melittin and apamine. Since the stated molecules are the major biologically active components of BV, we conducted our study through the analysis of those proteins and peptides. Additionally, we analyzed the sugar profile of the samples using HPLC.

According to the Russian BV standard presented by Bogdanov (2015), the humidity content of dried bee venom should be less than 12%. Although the moisture value in the commercial sample BV4 was above this limit, suitable values were observed in the other samples.

Melittin is one of the most important indicator components in BV and it is important to determine the standard values as found in the literature if it is to be used for apitherapeutic or cosmetic purposes (Banks and Shipolini 1986). In previous studies, the value of the melittin component was reported as 40–50% (Ali 2012; Banks and Shipolini 1986; Bogdanov 2015; Kye-Sung and Ki-Rok 2009; Moreno and

Giralt 2015; Zhou et al. 2010;). In other previous studies, the highest melittin reported was determined in Polish BV samples (70.15%) (Rybak-Chmielewska and Szczêsna 2004), while the lowest melittin content was seen in Romanian BV samples (27.66%) (Ionete et al. 2013). According to our results, the melittin value of the Anatolian BV samples was found to be 36.95%–46.85%, whereas in the commercial BV samples it was determined as 18.76%–25.3%. In the current study, the highest melittin content was found in fresh Anatolian BV1 (46.85%), while the lowest was found in the commercial BV4 sample (18.76%).

In the previous studies, the apamine content of BV has been reported as 2–3% and the phospholipase A_2 component in the range of 10–12% (Banks and Shipolini 1986; Bogdanov 2015; Moreno and Giralt 2015). When our data were compared with the literature, the amounts of apamine and phospholipase A_2 in commercial honey bee venom were found to be lower than the literature average.

The sugar profile analyses in recent studies have generally focused on fructose and glucose content analyses, with the average range reported as 2–4% The highest glucose content in the current BV samples was determined as 36.53% in the commercial BV5 sample, which is quite a bit higher than found in the literature (Ali 2012; Bogdanov 2015). When we compared the sugar profile analyses of the commercial and Anatolian BV groups, no differences were observed except for maltose and erlose.

The results of the physicochemical analyses showed that Anatolian BV samples contained higher amounts of apamin, phospholipase A_2 and melittin than the commercial samples.

As a result of our findings, it was recommended that BV should be used fresh and that the collection and storage conditions during the BV production process must be upgraded and standardized to improve the quality of the product.

CONCLUSION

In this study the main components of bee venom, the sugar profile and the moisture content were analyzed. According to the results, a difference in chemical content was observed between the commercial and the Anatolian bee venom. Moreover, the color of the BV5 sample was darker than the other bee venom samples. Therefore, bee venom samples should be analyzed before use. Because of its great financial value, bee venom is frequently adulterated with additives in the commercial sector. In addition, the bee venom intended for use in apitherapy and for cosmetics should be stored in the dark at -18 °C. The aim of this study was to draw attention to the necessity of establishing standard criteria for bee venom production. We believe that this study will light the way for further research seeking to standardize bee venom and to determine its storage conditions and shelf life.

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