MELLIFERA RESEARCH ARTICLE

Chemical Content and Bioactive Properties of Drone Larvae (Apilarnil)

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A B S T R A C T

In this study, nutrients, amino acid content and bioactive properties of drone larvae (apilarnil) were determined. According to HPLC-UV analysis, 16 amino acids were determined in apilarnil. Only tryptophan was not detected from the essential amino acids. The highest amino acid amount was lysine amino acid (7198 mg /100g) and the lowest amino acid was methionine (500 mg/100g). According to the results of the research, moisture; ash; protein carbohydrate and lipid contents of apilarnil were found to be 4.43, 4.07, 48.75, 21.62 and 21.13 g/100 g apilarnil respectively. To determine the bioactive properties of apilarnil, the Folin-Ciocalteu method for phenolic content, phosphomolybdenum method for antioxidant activity and DPPH method for antiradical activity were used. The total phenolic content of apilarnil was 834.05 (mg GAE/100 g) and the antioxidant activity was 90.91 mg AAE/g. Antiradical activity inhibition level of drone larva (apilarnil) was found to be 81.61%. As a result, the beneficial biological activity of apilarnil may be due to its antioxidant activity.

Keywords: Apilarnil, drone larvae, phenolic, antioxidant, antiradical, amino acid

Introduction

Drone larvae (apilarnil) is one of the honey bee products that are not known much in our country. Apilarnil is a bee product obtained by lyophilizing after the collection of drone larvae at the age of 3-7 days from drone cels. It has a homogeneous, milky, yellowish gray color and a bitter taste. After the larval cells are closed, the nutrient composition of the larva changes during the pupal phase. Therefore, it is appropriate to collect apilarnil in the larval stage where the highest quality nutritional form is preserved.Since the larvae will die during harvesting, the larvae should be consumed or processed quickly because the protein in its structure may be disrupted [1].

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Studies on drone larvae mainly focused on the reproductive functions of farm animals such as broilers, pigs and rams [2-6]. However, there are also studies on the chemical content of drone larvae [7-11]. Apilarnil contains approximately 25-35% dry matter, 9-12% protein, 6-10% carbohydrate, 5-8% lipid, 2% ash and 3% unidentified substance [12,13]. The protein content of apilarnil is higher than other nutrients. The variety and richness of the pollen sources on which honey bees feeds affect the vitamin content of apilarnil. Vitamins (A, B1, B6, choline etc.) and minerals (Ca, P, Na, Zn, Mn, Fe, Cu and K) contained in larvae increase the quality of drone [14]. Apilarnil has a high level of antioxidant properties. This feature is due to the rich polyphenols in the structure [15]. In addition, apilarnil has been found to be rich in male sex hormones, especially testosterone, and has an androgenic effect that enhances male sex features [16,17]. Furthermore, apilarnil has been shown to be a natural anabolic agent in male individuals because it increases body muscle weight [2]. Apilarnil, a powerful source that stimulates oxidative processes that produce energy due to its strong catabolic effect in the body. It prevents glycogen loss in muscles In order to achieve the desired performance [1]. Determination of the chemical content of apillarnil, which has been found to have beneficial biological activities, is necessary to explain these activities. Therefore, in this study, the nutrients, amino acid content and bioactive properties determined of drone larvae (Apilarnil) produced in Turkey.

Materials and Methods

The drone larvae samples (n=6) used in the study were obtained from hives in Erciyes University Agricultural Application and Research Center in May 2018 and collected at the age of 7 days. Collected samples were frozen at -20 °C. It was then dried by lyophilization and pulverized.

Nutrient content

Determination of ash, crude fat and crude protein in apilarnil samples was carried out using standard analytical procedures, Association of Official Analytical Chemists [18], 920.153, 991.36, and 960.52 respectively. Moisture content was determined using a vacuum oven at 60 °C and weighing until a constant weight. The results were expressed in grams per 100 g

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of fresh weight. The ash content was determined gravimetrically following incineration in an oven at 550°C and weighing until constant weight. Nitrogen determination was performed using micro-Kjeldahl method. Then a conversion factor of 6.25 was used for converting percentage of nitrogen in the sample into percentages of protein. Energy content was determined by at-water method. All analyses were carried out in triplicate [18].

Amino acid content

The analyzes were carried out using High Performance Liquid chromatograph (Perkin-Elmer Corp. Norwalk, USA), equipped with a UV detector. The amino acid content of the apilarnil was determined by the method of Jensen et al. [19]. Briefly, 0.2 g of sample was weighed into 15 mL of flask and 8 ml of 6.0 N HCl was added, sealed well and hydrolysed at 110 ^{o}C for 24 h. After this procedure, 1 mL with drawn and 45% vacuum dried. The sample was redissolved with stirring with 5 mL of 0.02 N HCl and centrifuged at 5,000 rpm. For derivatization, 20 µL of the aminoacid standard solution was placed in vial and dried in a vacuum oven at 65 °C for 2 hours. 30 µL of methanol-water-TEA (2:2:1) were then added and dried at 65 °C for 10 minutes. Then, 30 µL of derivatizing reagent methanol-water-TEA (7:1:1) (v/v) was added, vortexed for 30 h and allowed to stand at room temperature for 20 min. The resulting solution was vacuum dried at 65 °C for 15 min. Diluent containing 5% acetonitrile was added, vortexed for 15 h. The sample was then injected into HPLC.

Total phenolic content

The powdered samples were weighed to 0.5 g in the weighing vessel. It was dissolved with 5 mL of purified water. 0.1 mL of the prepared solution was taken and diluted with 5 mL of purified water and vortexed. 0.5 mL of Folin-ciocalteu reagent was added and mixed well for 3 minutes by vortexing. 1 mL of a solution of 35 g of Na_2CO_3 in 1 L of water prepared in a separate beaker was added to the mixture. Incubate for 1 hour at room temperature and in the dark. After incubation, spectrophotometer was measured at 725 nm.

Antioxidant activity

0.5 g of the powdered samples were weighed in the weighing vessel. It was dissolved with 5 mL of purified water. Sulfuric acid was added and the final volume was completed to 200 mL. 0.4 mL of samples were taken. 4 mL of prepared reagent solution was added. Mix well with vortex. Incubated in a hot water bath at 95oC for 1 hour 30 minutes. As a result of

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the incubation process, the tubes were taken into a container filled with tap water and kept waiting for 5 minutes and cooled. Spectrophotometer was measured at 695 nm wavelength.

Antiradical Activity

The powdered samples were weighed to 0.5 g in the weighing vessel. It was dissolved with 5 mL of purified water. It was vortexed and mixed well and 0.3 mL of DPPH (0.1 mM) was added. 2.4 mL of 99% ethanol was added and vortexed. The mixture was stirred for 30 min. It was kept at room temperature and in the dark. The spectrophotometer was measured at 517 nm.

Statistical analysis

All chemical assays were carried out in triplicate and the data were expressed as means ± standard deviations (SD).

Results and Discussion

The nutrient content of drone larvae is given in Table 1. As shown in Table 1, energy value was 472 kcal/100g; moisture; ash; protein carbohydrate and lipid contents were 21.13 g / 100g. 4.43 g/100g, 4.07 g/100g, 48.75 g/100g (NX6.25), 21.62 g/100g, respectively.

Table 1. Nutrient content of drone laerva (Apilarnil) (n=6)

Nurition element	Mean±SD
Energy	472± 2.3kcal/100g
Moisture	4.43±0.5 g/100g
Ash	4.07±0.8 g/100 g
Protein	48.75±4.2 g/100 g (NX6.25)
Carbohydrate	21.62±1.2 g/100g
Lipit (acid hyrdolysis)	21.13±1.3 g/100g

The amino acid profile of the drone larva is given in Table 2. The amino acids alanine, aspartic acid, methionine, glutamic acid, phenylalanine, lysine, histidine, tyrosine, glycine, valine, leucine, isoleucine, threonine, serine, proline and arginine were determined in apilarnil as shown in the table. Leucine, isoleucine, valine, lysine,

methionine, phenylalanine and threonine amino acids were identified as essential amino acids and only tryptophan could not be detected from essential amino acids. The highest amount of amino acid lysine was 7198 mg/100g, while the lowest amount of amino acid methionine was 500 mg/100g. The amount of other amino acids of alanine, aspatic acid, glutamic acid, phenylalanine histidine, tyrosine, glycine, valine, leucine, isoleucine, threonine, serine, proline and arginine were found 1826 mg/100g, 3571 mg/100g, 5625 mg/100g, 1844 mg/100g, 990 mg/100g, 2021 mg/100g, 1663 mg/100g, 2269 mg/100g, 3258 mg/100g, 2016 mg/100g, 1303 mg/100g, 1610 mg/100g, 3918 mg/100g, and 3005 mg/100g, respectively.

Table 2. Amino acid content of apilarnil (UFLC-UV)

Amino acid	mg/100g	Amino acid	mg/100g
L-Alanine (Ala)	1826±2.8	Glycine (Gly)	1663±2.3
L-Aspartic acid (Asp)	3571±4.3	L-Valine (Val)	2269±2.4
L-Methionine (Met)	500±4.5	L-Leucine (Leu)	3258±1.6
L-Glutamic acid (Glu)	5625±2.3	L-Isoleucine (Ileu)	2016±2.4
L-Phenylalanine (Phe)	1844±3.4	L-Threonine (Thr)	1303±2.8
L-Lysine (Lys)	7198±2.8	L-Serine (Ser)	1610±1.4
L-Histidine (His)	990±1.5	L-Proline (Pro)	3918±2.6
L-Tyrosine (Tyr)	2021±1.6	L-Arginine (Arg)	3005±4.7

Total phenolic content and antioxidant and antiradical activity of apilarnil were 834 mg GAE/100 g, 90.91 mg AAE/g and 81.61 %, respectively.

Table 3. The bioactivity of apilarnil

Bioactivity parameters	Mean ±SD
Total phenolic content (mg GAE/100 g)	834.05± 4,07
Antioxidant activity (mg AAE/g)	90.91± 8,16
Antiradical activity(% İnhibition)	81.61± 0,14

Honey is the most well-known honey bee products. However, besides honey, there are other honey bee products that are nutritious and supportive to treatment. Pollen, royal jelly and propolis have become one of the most researched bee products in recent years. However, products such as bee bread (perga) and drone larvae (apilarnil) are unknown products with little study. Apitherapy; is a form of use of bees and bee products as a protective and complementary application method in the treatment of some diseases. However, chemical and biological properties must be known for successful application of these products.

Hu and Li have determined the weight, moisture, protein, fat, ash and amino acid content of worker bee larvae and pupae at the age of 7-20 days [20]. In their research, they analyzed the vitamin and mineral contents of larva and pupa. They found that the moisture content of pupae and larvae was 81.69-73.48%, protein content of them was 42.36-48.47%, fat level was 15.75-20.63% and ash content was 9.99-18.47%. They were detected 17 amino acids in dry matter, especially glutamate, aspartate, lysine and leucine content was found to be higher and ranged between 26.35-44.72%. In addition, vitamin C and vitamin D, such as high content of vitamins have been identified [20]. Compared to this study, the fat and protein content of apilarnil was higher and the ash content was lower in our study. In another study, Barnutiu et al. studied fresh apilarnil obtained from Transylvanian.The water content of apilarnil changed between 69.70-76.44% and an average of 72.06%. They reported that the ash content was less than 1%, and only 3 samples were above 1%. They found the total lipid content of the samples to be between 1.29-4.51% and 3.8% on average. They reported that the total protein content varied between 4.55-9.95% [8]. Since we used lyophilized drone larvae in our study, nutrient contents were found to be higher than this study results.

Margaoan et al. compared queen and drone larvae in terms of quality parameters.. They identified carbohydrates by HPLC-IR and stated that seven carbohydrate compounds were identified n queen and drone larvae, predominantly glucose, fructose and sucrose. They determined lipid profile by Soxhlet method, total protein content by Kjeldahl method and free amino acids by LC-MS analysis. They identified a total of 31 amino acids in apilarnil and reported that nine of them were essential to humans. They found the moisture content of the bee larva to be 73.25 ± 0.02 and the protein content to 9.47 ± 0.13%. In addition, the total amount of essential amino acid in apilarnil 655.86 reported that the total amount of amino acids is 1830.07 [21]. In another study, the amino acid content of larvae of worker, queen and drone collected from different regions were determined [22]. Samples were obtained from four different regions of Russia and analyzed in five independent studies. As a result

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of this study, amino acid contents of bee larvae were found as 37.57 - 40.57% in drone larvae; 35.06 - 38.42% in queen bee larva and 35.61 - 35.71% in worker bee larva. Essential amino acid contents in drone larvae were 15.45 - 16.28%, 18.92 - 19.01% for queen larvae, 15.95-16.96% for worker larvae, respectively, and the total amino acid contents were between 39.73-42.77%, 51.25-54.79% and 44.74-47.70%, respectively [22].

In a comparative study on bioactivity of bee products; the antioxidant activities of multifloral honey, bee pollen, royal jelly and propolis were 58.89, 42.37, 59.02 and 267.37 mgAAE/g, respectively, while their antiradical activities (% inhibition) were reported as 28.44, 89.66.5.72 and 96.14, respectively [23]. When we compare the results of this research with our research, antioxidant and antiradical activities of apilarnil were found to be higher than other bee products lower than propolis.

Conclusion

In this study, it was determined that apilarnil produced in our country is an important food source.In addition, it was found that apilarnil shows antioxidant and antiradical activity. The antioxidant activity detected in apilarnil can be held responsible for the biological properties determined in scientific studies. However, the determination of the chemical composition that is the source of these effects is important to determine the possible beneficial biological effects. Therefore, there is a need for further research on the determination of the detailed chemical composition and biological activity of drone larvae.

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Erkek Arı Larvalarının (Apilarnil) Kimyasal İçeriği ve Biyoaktif özellikleri

Öz: Bu çalışmada, erkek arı larvasının (apilarnil) besin elementleri, aminoasit içeriği ve biyoaktif özellikleri belirlenmiştir. HPLC-UV ile yapılan analiz sonuçlarına göre apilarnil'de 16 amino asit belirlenmiştir. Esansiyel amino asitlerden yalnızca triptofan tespit edilememiştir. En yüksek amino asit miktarı 7198 mg/100g olan lizin amino asidi iken en düşük metionin amino asidi olup 500 mg/100g olarak belirlenmiştir. Elde edilen sonuçlara göre apilarnilin nem, kül, protein, karbonhidrat ve lipit içeriği sırasıyla 4.43, 4.07, 48.75, 21.62 ve 21.13 g/100 g dır. Apilarnilin biyoaktif özelliklerini belirlemede fenolik madde içeriği için Folin-Ciocalteu metodu, antioksidan

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aktivite için fosfomolibden metodu ve antiradikal aktivite için DPPH metodu kullanılmıştır. Apilarnilin toplam fenolik madde içeriği 834,05 (mg GAE/100 g) iken antioksidan aktivitesi 90,91 mg AAE/g bulunmuştur. Erkek arı larvasının (apilarnil) antiradikal aktivite inhibisyon düzeyi % 81.61 olarak tespit edilmiştir. Sonuç olarak, apilarnilin faydalı biyolojik aktivitesi antioksidan aktivitesinden kaynaklanıyor olabilir.

Anahtar Kelimeler: Apilarnil, arı larvası, fenolik, antioksidan, antiradikal, aminoasit

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