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α-Amylase Inhibition Properties of Bee Pollen and Bee Bread (Perga)

Arı Poleni ve Arı Ekmeği (Perga) nin α-Amilaz Enzimi Üzerine İnhibisyon Etkisi

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ABSTRACT

Bee pollen is a valuable bee product with its nutritional and bioactive values according to its protein, lipid, amino acid, vitamin and phenolic content. When bee pollen is converted into bee bread, it becomes more easily digestible in the human digestive system, because of the partial breakdown of the pollen wall, the exine layer, with the effect of fermentation. Therefore, bee bread has a richer nutrient content available than bee pollen. Diabetes mellitus is a metabolic and degenerative disease with long-term effects and inhibition of α-amylase is important for the treatment of this disease. In this study, methanol extract of pollen and perga samples was prepared. Total phenolic content, antioxidant capacity and α-amylase inhibition activity of the extract was determined. Total phenolic content and antioxidant capacity of pollen and perga extract was determined as 5.57-6.93 mg GAE/g and 74.56-97.66 µmol $FeSO_4.7H_2O$/g sample, respectively. IC_{50} value of bee pollen and perga for α-amylase inhibition was found to be 4.21 and 3.57 mg/mL, respectively. It could be concluded that perga may be more effective on Diabetes mellitus.

Key Words

Bee pollen, perga, phenolics, α-amylase.

ÖZ

Arı poleni ve perga önemli arı ürünleridir. Arı poleni içerdiği protein, yağ, amino asit, vitamin ve fenolik içeriğine göre sahip olduğu besin ve biyoaktif değerleriyle değerli bir arı ürünüdür. Arı poleni arı ekmeğine dönüştürüldüğünde insan sindirim sisteminde daha kolay sindirilebilir hale gelir bunun sebebi fermentasyonun etkisiyle polenin eksin tabakasında kısmen bir parçalanma olmasıdır. Bu nedenle arı ekmeği arı polenine göre daha zengin besin içeriğine sahiptir. Diyabet uzun süreli etkileri olan metabolik ve dejeneratif bir hastalıktır ve α-amilazın inhibisyonu bu hastalığın tedavisi için önem arz etmektedir. Bu çalışmada polen ve perga örneklerinin methanol ekstraktları hazırlandı. Ekstraktların toplam fenolik madde miktarı, antioksidan kapasitesi ve α-amilaz inhibisyon aktiviteleri belirlendi. Polen ve perga örneklerinin toplam fenolik madde miktarı ile antioksidan kapasite miktarları ise sırasıyla 5.57-6.93 mg GAE/g ve 74.56-97.66 µmol $FeSO_4.7H_2O$/g sample olarak belirlendi. Polen ve perga ekstraktlarının α-amilaz inhibisyonu için IC_{50} değerleri sırasıyla 4.21 ve 3.57 mg/mL olarak bulundu. Sonuç olarak Perganın Diabetes mellitus üzerinde daha etikili olabileceği ifade edilebilir.

Anahtar Kelimeler

Arı poleni, perga, fenolikler, α-amilaz.

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INTRODUCTION

Bee pollen is a bee product that is well-known with its nutritional and bioactive values according to its protein, lipid, vitamin and phenolic content. Due to its nutritional properties and health effects, it has been used in traditional medicine, alternative diets and supplementary foods for many years. Pollen is the main source of protein required for bees to grow and complete their development. Pollen also contains valuable nutrients for human metabolism. It is a rich source of protein and carbohydrates, but it is a rich store of vitamins and minerals. Pollen also contains all of the amino acids necessary for the human body [1].

The collected bee pollen is mixed with honey and bee saliva inside the honeycombs to produce bee bread [2]. Lactic acid fermentation is realized with the help of lactic acid bacteria, and bee pollen is transformed into bee bread. When bee pollen is converted into bee bread, it becomes more easily digestible in the human digestive system because of the partial breakdown of the pollen wall, the exine layer, with the effect of fermentation [3]. In addition, bee bread becomes richer with the addition of new nutrients to the bee bread when compared with bee pollen. High lactic acid content and other metabolites protect bee bread against degradation caused by molds and other microorganisms. It is known that bee bread is a very important food source for bees and humans with its protein, lipid, minerals, vitamins, flavonoids and essential amino acids. The main components of bee bread are approximately 20% protein, 3% lipids, 24-53% carbohydrates. In addition, bee bread contains over 25 different macro and micro elements such as iron, calcium, phosphorus, potassium, copper, zinc and magnesium [4]. Both bee pollen and bee bread has antioxidant activity. It is stated that bee bread is used to treat health problems such as anemia, hepatitis, diabetes and gastrointestinal problems [4].

Diabetes is a metabolic and degenerative disease with long-term effects. Diabetes is the main cause of a number of complications, such as blindness, heart attack, lower limb amputation, and kidney failure [5]. Type-2 diabetes is the dominant form of diabetes. Inhibition of pancreatic amylase can be effective in controlling postprandial sugar levels in patients with diabetes [5]. Therefore, in this study, the antioxidant properties of bee pollen and bee bread harvested from the same hive was determined. The inhibition of α-amylase with methanol extract of bee pollen and bee bread was also compared.

MATERIALS and METHODS

Materials

Bee pollen and perga samples were supplied from local beekeepers in Bilecik city, Turkey in 2020. Methanol, gallic acid, α-amylase and ethanol were purchased from sigma Aldrich, USA. All other reagents were analytical grade.

Methods

Preparation of Bee Pollen and Perga Extracts

Extraction of samples with methanol (absolute) was carried out by simple maceration technique separately. 1:10 (g/v) ratio was used for the extraction. Frozen bee pollen and perga samples was powdered by grinding and 2 g of this fine powder was mixed with 10 mL of methanol. Extraction was carried out for 48 h on a magnetic stirrer under constant stirring at 150 rpm. Finally, mixtures were separately filtered and filtrates were stored at +4^{O}C.

Determination of Botanical Origin

Samples are prepared for analysis by making minor changes in Barth et al.[6]. Prepared preparations were examined with Nikon Eclipse E400 microscope. 500 pollen was counted in each preparation, counts were made in 3 replications. Reference preparations and related sources were used for the diagnosis of pollen grains [7-10]. According to this; (> 45%) dominant (D), (16-45%) secondary (S), (3-15%) minor (M) and (1-3%) artifacts (E).

Determination of Total Phenolic Content and Flavonoid Content

Total phenolic content of bee pollen (BPE) and perga (PE) extracts was determined by using Folin-Ciocalteu method [11, 12]. Gallic acid was used as standard. Results were expressed as mg GAE/mL. Total flavonoid content of the samples was determined by using aluminum chloride method [13] quercetin as standard. Results were expressed as mg QE/mL.

Determination of Antioxidant Activity

Antioxidant activity of bee pollen and perga extract was determined by using ferric reducing antioxidant power (FRAP) [14]. Briefly, 3 mL freshly prepared FRAP reagent and 100 µL of methanol extract was mixed and incubated for 4 min at 37 °C and the absorbance was read at 595 nm. FRAP values were expressed as µmol $FeSO_4.7H_2O$ equivalent of g sample.

Determination of α-Amylase Inhibition

α-Amylase activity was assayed in the presence of soluble starch as substrate. Reducing ends were determined according to DNS method described [15]. Reaction mixture containing 300 µL of 1% soluble starch and 300 µL of enzyme solution was incubated for 30 min at 35ºC. Equal volume of DNS reagent was added into tubes and kept in a boiling water bath. Absorbance of the tubes was recorded at 550 nm against a blank sample. All characterization assays were performed triplicate. IC_{50} value of the extracts was determined at five different extract concentrations at standard assay condition and dose response curve was generated. Acarbose was used as reference inhibitor [16].

RESULTS and DISCUSSION

In this study, bee pollen and perga samples were collected from the same hive and biochemical properties were determined. Microscopic pollen analysis were performed. The predominant type of pollen was found *Trifolium pretense* both pollen and perga. It is clear that perga and pollen samples were the same as with each other. The results were shown in Table 1.

The total phenolic, flavonoid and antioxidant capacity of pollen and perga samples and their inhibition properties of α-amylase enzyme were compared. As a result, the total phenolic, flavonoid, antioxidant capacity and inhibition properties of α-amylase enzyme was found as 5.57 mg GAE/g, 2.11 mg QE/g, 64.56 µmol $FeSO_4.7H_2O$/ g and 4.21 mg/mL, respectively for pollen extract. Total phenolic, flavonoid, antioxidant capacity and inhibition properties of α-amylase enzyme was found as 6.93 mg GAE/g, 2.27 mg QE/ g, 83.62 µmol $FeSO_4.7H_2O$/ g and 3.57 mg/ mL, respectively for perga extract (Table 2).

The biological activity of bee bread is higher than the obtained pollen due to fermentation of the outer membrane [17]. The outer wall of the pollen is called exine. This layer consists of a structure called sporopollen, which is very strict and very durable. The inner layer is made of cellulose and is in the structure of the typical plant cell wall. Therefore, when the pollen enters the digestive system, the digestion is inadequate due to the exine layer. When the pollen is fermented this outer membrane is opened. Therefore nutrients in obtained product, bee bread or perga, has higher bioavailability. Studies show that perga has higher antioxidant capacity than pollen as well [17, 18].

It is stated that the total amount of phenolic content of pollen samples varied between 9.27 and 18.37 mg GAE/g, total flavonoid content ranged from 3.16 to 6.42 mg QE/ g [1]. It is stated that the total phenolic content of pollen samples extracted using different solvents ranged from 4.35 to 74.86 mg/g [19]. In a study, the total phenolic content and antioxidant activity of pollen and perga samples were compared. Accordingly, ıt was stated that total flavonoid content of perga and pollen samples varied between 1.81 and 3.74 mg QE/g and 2.62 and 4.44 mg QE/g respectively [18]. In a study total phenolic and flavonoid content and antioxidant capa-

Table 1. Microscopic pollen analysis of bee pollen and bee bread (perga).

	Predominant type of pollen (>45%)	Secondary pollen (15-45%)	Important Minor Pollen (3-15%)	Minor Pollen (<3%)
Bee Pollen	Trifolium pretense (53.50%)	Cistaceae (20.66%)	Echium spp. (10.70%) Rosaceae (10.33%)	Poaceae (2.58%) Apiaceae (0.73%) Papaveraceae (0.73%) Brassicaceae (0.36%) Asteraceae (0.36%)
Bee Bread (Perga)	Trifolium pretense (70.39%)	-	Cistaceae (10.10%) Asteraceae (9.38%) Rosaceae (6.13%)	Scabiosa spp. (1.08%) Papaveraceae (0.72%) Poaceae (0.72%) Anchusa spp. (0.36%) Oleaceae (0.36%) Chenopodiaceae (0.36%) Sanguisorba spp. (0.36%)

Table 2. Biochemical properties of bee pollen and bee bread (perga)

	Total phenolics mg GAE/g	Total flavonoids mg QE/g	Antioxidant capacity µmol $FeSO_4.7H_2O$/g sample	α-amylase Inhibition IC_{50} mg/mL
Bee Pollen	5.57±0.12	2.11±0.04	64.56±0.41	4.21±0.01
Perga	6.93±0.09	2.27±0.05	83.62±0.33	3.57±0.01
Acarbose	-	-	-	5.93±0.01

city of bee pollen samples was determined and results were reported as 6.33 mg GAE/g, 1.88 mg QE/g and 72.38 µmol $FeSO_4.7H_2O$/g respectively [20]. Total phenolic and flavonoid content and antioxidant capacity of bee bread collected from Malaysia were reported to be 14.19 mg GAE/g, 3.92 mg QE/g and 0.94 mmol Fe^{2+}/L, respectively [21]. It is reported that total phenolic and flavonoid content was found as 8.9±3.1 mg GAE/g and 3.2±1.0 mg QE/g beebread, respectively [22]. Antioxidant capacity was reported as 46.1±13.0 µmol Trolox E/g bee bread [22]. It is stated that total phenolic content of pollen samples varies between 0.41-7.38 mg GAE/g and antioxidant capacity varies between 8.69-75.65 µmol $FeSO_4.7H_2O$/g [23].

In this study, the in vitro inhibition effect of α-amylase enzyme associated with Diabetes mellitus was investigated. In literature the effects of bee pollen and perga on Diabetes mellitus are expressed in *in vivo* studies [2, 24]. It is reported that the effect of pollen on the inhibition of two enzymes important for Diabetes mellitus [25]. According to this study, the total phenolic content of the pollen extract prepared using aqueous ethanol solution was determined as 1.79 mg GAE/g and the IC_{50} value of α-amylase enzyme was found to be 4.51 mg/mL [25]. All of the findings were found to be compatible with the literature.

Conclusion

Pollen and bee bread are two important bee products. Pollen is converted into bee bread by the lactic acid fermentation in honey combs. It is stated that nutrients in bee bread are more available than counter pollen samples. This is due to the breakdown of exine layer of pollen wall. In this study, total phenolic and flavonoid content and antioxidant activity of methanol extract of pollen and bee bread was compared. α-Amylase inhibition property of both extracts was also determined. It was determined that methanol extract of bee bread contains more phenolic and flavonoid content than counter pollen extract. Due to its richer phenolic content, bee bread methanol extract showed better amylase inhibition as well. It can be concluded that bee bread is better than bee pollen for the patient suffering from Diabetes mellitus.

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