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Bee Products and Antioxidant Activities: Bee Pollen and Bee Bread

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Abstract

Pollen is one of the food sources used by honey bees (*Apis mellifera* L.) for nutritional purposes. While honey bees use nectar as a carbohydrate, they use pollen as a protein source. Pollen grains collected from plants are mixed with bee secretion and turn into pellets. Honey bees coat the pollen inside the hive with honey and beeswax. The coated pollen is fermented with the effect of various enzymes and microorganisms to form as bee bread. With the biochemical change, the chemical composition of bee bread differs according to pollen. Bee pollen and bee bread have a broad biological spectrum. For this reason, it exhibits many biological activities, especially antioxidant activity. Studies have shown that these effects of bee pollen and bee bread are provided by the polyphenols they contain. In addition, when the literature is examined, it is stated that these beneficial effects vary depending on the botanical and geographical origin of bee pollen and bee bread.

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Arı Ürünleri ve Antioksidan Aktiviteler: Arı Polen ve Arı Ekmeği

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Özet

Polen, bal arılarının (*Apis mellifera* L.) beslenme amaçlı kullandıkları besin kaynaklarından biridir. Bal arıları karbonhidrat kaynağı olarak nektarı kullanırken, polenden protein kaynağı olarak yararlanmaktadırlar. Bitkilerden toplanan polenler arıların salgısıyla karıştırılarak pelet haline gelir. Bal arıları poleni kovan içinde bal ve bal mumu ile kaplar. Kaplanmış olan polenler çeşitli enzimler ve mikroorganizmaların etkisiyle fermente olarak arı ekmeğini oluşturur. Biyokimyasal değişikliklerle beraber arı ekmeğinin kimyasal bileşimi polene göre farklılaşır. Arı poleni ve ekmeği geniş biyolojik spektruma sahiptir. Bu nedenle antioksidan aktivite başta olmak üzere birçok biyolojik aktivite sergilemektedir. Arı poleni ve arı ekmeğine bu etkileri içerdiği polifenollerin sağladığı yapılan çalışmalarda belirtilmiştir. Bunun yanı sıra literatür incelendiğinde bu yararlı etkilerin arı polenin ve arı ekmeğinin elde edildiği bitkisel kaynak ve bulunduğu coğrafi orjine bağlı olarak değiştiği belirtilmektedir.

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Introduction

While the energy required by the body is released as a result of oxygenated breathing, molecules called free radicals are also formed. Free radicals are highly reactive and carry unpaired electrons in their outer orbits [1]. Radiation, pesticides, viruses, ultraviolet rays, industrial wastes, heavy metals, dirty food products, polluted air, chemicals, and residues are free radical sources. Reactive oxygen species constitute the important group of free radicals and are constantly produced as a result of metabolic activities. Superoxide radical, hydrogen peroxide radical, hydroxyl radical are the most common radical groups. There is an antioxidant system in the body that can neutralize reactive oxygen species. However, as these components are formed in much higher amounts than the antioxidant system of the body, "oxidative stress" occurs in the body [2]. In case of oxidative stress, many diseases such as cancer, aging, cardiovascular problems, diabetes, and gastrointestinal disorders emerge as a result of undesired reactions such as cell damage, DNA damage in the body [3-5]. Antioxidants are molecules with high health benefits that prevent the formation of chain reactions of many diseases, especially cancer, which can occur in the body by reacting with free radicals. Antioxidants are divided into two as endogenous and exogenous according to their sources. Endogenous antioxidants are synthesized within the body, while exogenes are taken from outside the body. Endogenous antioxidants are also divided into two as enzymatic and nonenzymatic [6].

Phenolic compounds are biologically active components in aromatic ring structure that carry at least one hydroxyl group. Polyphenols are divided into two as phenolic acid and flavanoids. Phenolic compounds with different structure have been defined and many biological activities have been determined. Studies have shown that phenolic compounds exhibit antioxidant, anti-inflammatory, antiviral, anticarcinogenic, antimutagenic activities. Phenolic acids and flavanoids are divided into main subgroups [7].

Natural biologically active substances have attracted a great deal of attention from the past to the present and have been used in traditional medicine. Bee products naturally contain bioactive components, especially phenolic components and flavanoids, in their structures. Chemical contents of bee products vary according to climate, geographic conditions and botanical origin Bioactive properties such as antioxidant, antimicrobial activity have increased the interest in bee products, especially honey, pollen, propolis, royal jelly, bee venom, and bee bread [8]. In this review, we aimed to evaluate the studies on the chemical and phenolic contents, antioxidant activities of bee pollen and bee bread.

Chemical Content

Bee pollen

Pollen grains are plant powders that contain male gametes in their structure. When honey bees (*Apis mellifera*) visit flowers to collect nectar, plant pollen grains stick to their body covers. They pelletize plant pollen that clings to their bodies by processing it with oral secretions [9]. Pollen is an important protein source for honey bee larvae and young worker bees. In addition to its protein content, it contains carbohydrates, mineral substances, various vitamins (such as B2, B3, B5, C, E), fatty acids, flavonoids, carotenoids and some enzymes [10,11]. The chemical content of pollen generally varies depending on its botanical origin, geographical origin, climatic conditions, soil type and beekeeping activities [12]. Storage conditions also affect the content of pollen [13]. Bee pollen contains 40-85% carbohydrates in dry weight. Fructose is the predominant sugar in pollen, followed by glucose and sucrose. Oligosaccharides and polysaccharides are important ingredients of pollen. Carbohydrates are most abundant groups in pollen, followed by proteins. It is on the average 14-30% in dry weight [14]. Pollen contains almost all amino acids. Aspartic acid and glutamic acid are the main amino acids found in pollen. The presence of proline and glutamic acid is associated with the quality of bee pollen and the amount of glutamic acid higher than 20 mg / g is accepted as an indicator of freshness [15,16]. As a result of the studies, approximately 20 fatty acids have been identified in the pollen and it has been stated that linolenic acid and palmitic acid are the major fatty acids [16]. It has been determined by studies that the ratio of total unsaturation of fatty acids to saturation (TUFA / SFA) is between 1.57-1.92 [17]. The lipid content has been reported to be in the range of about 1-10% by dry weight. Bee pollen is a rich source of vitamins. It contains B group vitamins, nicotinamide and niacin are the most common B group vitamins. Vitamins A, C and E were also detected. Bee pollen contains important minerals such as K, Na, Ca, Mg, P, Zn, Cu, Mn [16,18]. Pollen can be considered as a potential food supplement because it contains various nutrients.

Bee bread

It is formed by the accumulation of pollen pellets in the hive [19]. Pollen pellets brought to the hive are protected with honey and beeswax and become bee bread after about 2 weeks with the effect of enzyme, microorganism, humidity and temperature [20-22]. Bee bread contains vitamins, carbohydrates, protein, lipids and microelements. The nutritional composition of bee bread varies depending on the honey plants of the region, climate conditions and seasonal changes [22]. The chemical content of bee bread is different from the pollen. The fact that bee bread contains high amounts of lactic acid distinguishes it from pollen [20,23]. The protein content of bee bread is similar to that of pollen and ranges from about 14.1 to 37.3 g/100 g. Bee bread contains amylase, phosphatase and glucose oxidase enzymes. Bee bread is rich in aspartic acid, glutamic acid and proline [15]. Since bee bread contains more free amino acids in its composition, it is a better digestible nutrient than pollen and has a wide biological spectrum [22,24]. Studies have shown that bee bread contains Ca, Mg, P, K, Fe, Zn and Mn macro and micro elements in varying rates depending on the floral pollen source [25]. Bee bread contains about 24-34% carbohydrates [15]. It has been reported to contain 57.51% fructose, 42.59% glucose and 3.37% maltose as carbohydrates [26]. The sugar content of bee bread is higher than the pollen and the starch content is low [24]. In a study on the chemical content of bee bread belonging to Colombia, total lipid amounts were found to be varying between 1.65-5.50%. This variability has been linked to the amount of fatty acid, carotene and vitamin of the pollen which is the source of bee bread [27]. Bee bread is a rich source of vitamin K. It also contains more than 3% lactic acid. Bee bread obtained from birch pollen has been reported to contain 6 times more lactic acid than pollen [24].

Phenolic Content

Bee pollen

Among other ingredients, pollen contains phenolic compounds and flavonoids. It is stated that these components show significant protecting activity against pathogen development and environmental factors that cause the formation of reactive oxygen species such as biological and UV radiation, high temperature. Therefore, they are very important antioxidants [28]. Campos et al. (2003) [29] examined some pollen samples and detected quercetin, myricetin and luteolin flavonoid aglycons in the structure. In a study on bee pollen of South Brazil, it was found that rutin and myricetin phenolics are found in high amounts [30]. 22 pollen samples from Northeastern Brazil were examined and as a result isorhamnetin 3-O- β neohesperidoside was detected in all samples. In addition, isoquercetin, myricetin, tricetin, quercetin, luteolin, selagin, kaempferol and isorhamnetin are other phenolic compounds detected [31]. In the study on the phenolic content of bee pollen collected from Serbia, a total of 37 different polyphenols were found, 19 flavonol glycosides and 18 polyphenols. Quercetin-3-O galactoside has been identified as the predominant compound. Quercetin-3-O ramoside, isorhamnetin-3-O-rutinoside, rutin-3-O-rutinoside were other specified compounds [13]. Fanali et al. (2013) [32] identified 9 different polyphenols in the bee pollen they examined. These polyphenols are p-coumaric acid, myricetin, ferulic acid, cinnamic acid, quercetin, hesperidin, naringenin and kaempferol. Bee pollen extracts from Egypt have been found to be rich in 3,4-dimethoxycinnamic acid [33]. In a study examining the phenolic content of Anzer pollen, it was determined that all pollen samples had vanillic acid, caffeic acid, syringic acid, p-OH benzoic acid, p-coumaric acid, rutin, ferulic acid. In addition, it was stated that they contain high amounts of quercetin except one of the pollen samples [34]. Li et al. (2015) [35] identified that bee pollen contains 4 flavonoid glycosides. Quercetin-3-O- β -D-glucosyl- (2 \rightarrow 1)- β -glucoside, kaempferol-3,4-di-O- β -D-glucoside, 5,7,4-trihydroxy-3-methoxyflavone-3-O- β -D-sorofocyt and kaempferol-3-O- β -D-glucosyl- (2 \rightarrow 1)- β -D-glucoside were compounds identified.

Bee Bread

Phenolic contents of bee bread samples from Poland, Russia and Latvia regions were determined by GC MS. Amounts of p-coumaric acid, kaempferol and isorhamnetin were found to be significant [36]. Phenolic content of bee bread obtained from Georgia was determined by HPLC method and flavonoid compounds were determined as rutin, quercetin and naringenin. In addition, a decrease in total flavonoid content was observed during storage [37]. A study was conducted on flavonoid content and antitumor activity of bee bread samples taken from the hives of the northeast region of Portugal. As a result of the study, thirty-two compounds were identified, as the main phenolic compounds; flavonol derivatives which are quercetin, kaempferol, myricetin, isorhamnetin and herbacetin have been identified. Isorhamnetin-O-hexosyl-O-rutinoside, isorhamnetin-O-pentosyl-hexoside and quercetin-3-O-ramnoside were the most common flavonol species [38]. Bakour et al. (2019) [22] found

isorhamnetin-O-hexoxyl-O-rutinoside as the main component by identifying flavonoid glycoside derivatives of bee bread in the Moroccan region. When the studies conducted within the scope of bee pollen and bee bread phenolic content are evaluated, it is seen that although similar compounds are found, the types and amounts of these components vary from sample to sample. It is thought that this difference may arise from the geographical area where pollen and bee bread is taken, floral diversity, and bee breeds.

Antioxidant Activity

Bee pollen

The antioxidant activity of pollen has been associated with the polyphenol content and many studies have been conducted to determine antioxidant activity. The antioxidant activity of Anzer pollen was investigated in a study. As a result, the antioxidant activity of the pollen was correlated with the phenolic content of the pollen. [39]. Antioxidant activities of bee pollen samples collected from different regions of Turkey were investigated within scope of a study. As a result, it was determined that the total phenolic concentration was 21.23-27.66 mg GAE/ g and DPPH radical scavenging activity was between 69.49-77.93% [40]. It is known that bee pollen is used in apitherapy applications due to its many biological activities. Therefore, DPPH free radical removal activity, superoxide radical scavenging analysis of linden plant origin bee pollen was tested *in vitro* and *in vivo*. Analysis of superoxide dismutase activity, total anti-oxidant capacity was performed and as a result, an increase in superoxide dismutase enzyme activity and total antioxidant activity was observed depending on the dose. Significant reduction in MDA content was also determined. It has been reported that bee pollen may be a potential natural source for drug development [41]. Some studies on the antioxidant activity of bee pollen are summarized in Table 1.

Bee bread

Due to the wide nutritional properties of bee bread, studies have been carried out to determine its biological activity. DPPH, total phenolic content and total flavonoid content of bee bread purchased from different regions of Ukraine were analyzed in a study. Bee breads have been determined to have high amounts of total phenolic and total flavonoid content. These values were calculated as 12.36-18.24 mg GAE/g dry weight and 13.56-18.24 µg/g dry weight, respectively [23]. -In a study some functional properties of Lithuanian bee breads were tested using different solvents. It is stated that 100% normal aqueous extract shows the best antioxidative properties. DPPH activity of 10% ethyl alcohol extract was highest. It has been reported that bee bread can be used in the food and pharmaceutical industries due to its inhibition activity against reactive oxygen species [20]. Antioxidant properties of bee bread are given in Table 2.

Table 1. Antioxidant activities of bee pollen

Geografial origin/ Floral source	Experimental model	Results	Reference
Portugal, Spain/-	TPC	18.55-32.15 mgGAEs /g pollen	Pascoal et al., 2014 [42]
	TFC	3.71-9.25 mg CAEs/g pollen	
	DPPH	2.98-6.69 mg/g extract	
	TBARS	0.35-3.7 mg/g extract	
Brazil/-	DPPH (EC50 µg/mL)	104.5 (Ethanol extract) 212.0 (Methanol extract) 41.9 (Ethyl acetate extract)	Silva et al., 2006 [43]
-/B. napus subsp. napus L.	DPPH TPC	86.25% inhibition 1383.67 mg/kg	Fatrcová-Šramková et al., 2013 [44]
-/Helianthus annuus L.	DPPH TPC	47.97% inhibition 691.67 mg/kg	Fatrcová-Šramková et al., 2013 [44]
-/P. somniferum L.	DPPH TPC	75.93% inhibition 817.33 mg/kg	Fatrcová-Šramková et al., 2013 [44]
Serbia/Helianthus annuus L.	TPC	244.44 mg/kg dry weight (methanol extract pollen) 200.58 mg/kg dry weight (ethanol extract of pollen)	Kostić et al., 2019 [13]
Portugal	β- carotene bleaching (EC50)	4.6 ±0.9 mg/mL	Feás et al., 2012 [45]
Alagoas state (Brazil)/-	DPPH (EC50)	3.0 ±0.7 mg/mL	
Parana state (Brazil)/-	β- carotene bleaching method	83.3% antioxidant activity (70% ethanol extract)	Carpes et al., 2007 [46]
Spain/Cistus ladanifer, Cistus albidus	DPPH (IC50)	81% antioxidant activity (60%ethanol extract)	
Sonoran desert/Mesquite	FRAP ([Fe ²⁺ +mM])	196.7 µg/mL (Ethanol extract)	Izuta et al., 2009 [47]
Sonoran desert/ Mimosa	FRAP ([Fe ²⁺ +mM])	2.89 (Methanol extract) 1.98 (Ethanol extract)	LeBlanc et al., 2009 [48]
		3.96 (Methanol extract) 3.22 (Ethanol extract)	LeBlanc et al., 2009[48]

TPC-Total phenolic content

TFC-Total flavonoid content

DPPH-2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

TBARS-thiobarbituric acid reactive substances

FRAP-ferric ion reducing antioxidant parameter

Table 2. Antioxidant activities of beebread

Geografial origin/ Floral source	Experimental model	Results	Reference
Northwest Lithuania	TPC DPPH	394 mgGAE /100 g 85-93% antioxidant activity	Bartkiene et al., 2019 [49]
Lithuania-Kėdainiai district	TPC DPPH ABTS ORAC	21.2 mgGAE/g 1.14 mg TE/g 4.86 mg TE/g 626.30 mg TE/g	Čeksterytė et al., 2016 [50]
Poland	TPC TAS (antioxidant activity)	35.18 mg GAE/g 1.11 mmol/L	Markiewicz-Żukowska et al., 2013 [19]
Ukraine-Kyiv	TFC TPC DPPH FRAP (µmol trolox/g)	13.56-18.24 µg QE/g 12.36-25.44 mg GAE/g 15.78 mg TEAC/g 11.075 (water extract) 46.473 (ethanol extract)	Ivanišová et al., 2015 [23] Borykca et al., 2016 [51]
Commercial		39.875 (methanol extract)	
	ABTS(µmol trolox/g)	40.339 (water extract) 137.934 (ethanol extract) 86.948 (methanol extract)	
Colombia	FRAP TEAC TPC	46.1 µmol trolox/g 61.5 µmol trolox/g 8.9 mg GAE/g	Zuluaga et al., 2015 [27]
Northeast Portugal	DPPH (EC50)	0.02-13.0 mg/mL	Tomás et al., 2017 [52]
Morocco	ABTS (EC50) DPPH (EC50)	0.98 mg/mL 0.50 mg/mL	Bakour et al., 2019 [22]
Ukraine	Total antioxidant activity	33.61-82.39 %	Hudz et al., 2017 [53]
Transylvania-Romania	FRAP TEAC	0.521 mmol Fe ²⁺ /L 0.21 mmol Trolox/L	Stanciu et al., 2008 [26]

TPC-Total phenolic content
TFC-Total flavonoid content
DPPH-2,2-diphenyl-1-picrylhydrazyl radical scavenging activity
TBARS-thiobarbituric acid reactive substances
FRAP-ferric ion reducing antioxidant parameter
mg GAE-mg of gallic acid equivalents
mg TE-mg Trolox equivalents
ORAC-oxygen radical absorbance capacity
ABTS-ABTS⁺ scavenging capacity
QE-Quercetin equivalents
TEAC-Trolox equivalent antioxidant capacity

Conclusion

Bee pollen and bee bread are very important bee products with their wide chemical content and biological activity properties. These features vary depending on their botanical and geographical source. Although there are similarities in the studies examined, some products were found to be more valuable according to region or floral origin. Considering the floral and regional diversity in the world, it is anticipated that more studies can be done in the coming years by seeing that studies on determining the phenolic compounds and antioxidant activities of bee pollen and bee bread are not sufficient.

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