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Determination of Phytosterols in Beebread from Different Botanical Origin

Farklı Botanik Orijinli Arı Ekmeğinde Fitosterollerin Belirlenmesi

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ABSTRACT Objective: The aim of this study is to determine sterol content of beebread, which is frequently used in Apitherapy due to its beneficial biological activities. **Material and Methods:** Palynological spectrum, lipid and sterol analyses of beebread samples collected by honeybees *(Apis mellifera L.)* from different geographical and botanical origins were analysed. Botanical origin of the samples was identified through pollen analysis and their sterol composition was determined by GC analysis. **Results:** The sterol contents of the beebread samples including citrus, clover, cotton, chestnut and sunflower changed between 4.85-1698.71 mg/kg. Citrus beebread contained higher amounts of campesterol, delta 5 avenosterol and 24-methylene cholesterol, while cotton beebread had the higher campesterol content. Δ5 avenasterol was the main constituent of clover beebread, whereas sitostanol was the major compound found in chestnut beebread and sunflower beebread contained higher amounts of β sitosterol compared to other sterols. Cholesterol level was the lowest in all the samples tested. **Conclusion:** The results revealed that beebread is a good source of sterols that is of paramount importance for both honeybee nutrition and human health.

Keywords: Sterol; stored pollen; beebread; cholesterol; honeybee

ÖZET Amaç: Bu çalışmanın amacı, faydalı biyolojik aktivitelerinden dolayı Apiterapide sıklıkla kullanılan arı ekmeğinin sterol içeriğini belirlemektir. **Gereç ve Yöntemler:** Farklı coğrafi ve bitkisel kökenli bal arıları *(Apis mellifera L.)* tarafından toplanan arı ekmeği örneklerinin palinolojik spektrum, lipid ve sterol analizleri yapıldı. Örneklerin botanik orijnleri polen analizleri ile sterol kompozisyonları GC-MS analizleri ile belirlenmiştir. **Bulgular:** Narenciye, yonca, pamuk, kestane ve ayçiçeği gibi arı ekmeği örneklerinin sterol içerikleri 4,85-1698,71 mg/kg arasında değişmiştir. Narenciye arı ekmeği yüksek miktarda kampesterol, delta 5 avenosterol ve 24-metilen kolesterol içerirken, pamuk arı ekmeğinde yüksek kampesterol içeriği tespit edildi. Δ5 avenasterol yonca arı ekmeğinin ana bileşeni iken sitostanol kestane arı ekmeğinde bulunan ana bileşikti ve ayçiçeği arı ekmeği diğer sterollere kıyasla daha yüksek miktarda β sitosterol içerdi. Kolesterol seviyesi, test edilen tüm örneklerde en düşük seviyedeydi. **Sonuç:** Elde edilen sonuçlar, arı ekmeğinin hem bal arısı beslenmesi hem de insan sağlığı için büyük önemi olan iyi bir sterol kaynağı olduğunu ortaya koydu.

Anahtar Kelimeler: Sterol; depolanmış polen; arı ekmeği; kolesterol; balarısı

Phytosterols which cannot be synthesized by the human body has been the focus of scientific research in recent years because of their positive health effects. They are a group of bioactive triterpene compounds that are synthesized by the plants. They are converted to the corresponding phytostanols (campesterol/campestanol, sitosterol/sitostanol) by chemical hydrogenation. More than 200 sterols have been identified in plants. Of these, sitosterol (24-α-etilkolesterol), kampesterol (24-α-etilkolesterol) and stigmasterol (Δ22, Δ24-α-etilkolesterol) constitute the majority of plant sterols.[1,2]

Bee pollen is collected from the stamen of the flower by worker honeybees and mixed with vary gland and rolled it into small pellets. Bee pollen is the only natural source containing proteins, lipids, amino acids, starch, sterols, vitamins, and minerals and it is necessary for growth and development of brood and young bees.[3] The pollen collected by foraging workers is stored in the comb cells after mixing with a small amount of honey in order to prevent spoilage. The pollen undergoes some chemical changes to a product called "bee-bread".[4] Beebread is the most important nutrient for the growth and development of honeybee colonies. Also, honeybees need sterols for growth. Despite the biological importance of sterols, insects cannot synthesize sterols.[5] They are of particular importance to insects as they are components of cellular membranes, play essential role in insect development and are the starting material for the formation of skin replacing ecdysone.[6] Scientific studies showed that the pollen obtained from different plant sources were found to contain different amounts of sterol. In one of the first studies on it, sterol fractions in pollens of 15 plant species belonging to 11 families were analysed by Standifer et al. and 24-methylene-cholesterol was the main sterol detected in pollens of red clover, saguaro cactus, mustard, London-rocket, rye, timothy and sweet corn.[7] In addition, β-sitosterol was the highest sterol found in mule fat, juniper, heartsease, waterleaf, Scotch pine, European alder and Lombardy poplar. It was found that cholesterol is the main sterol of cottonwood plant. In another study, Xu et al. have found that lotus (*Nelumbo nucifera*) pollen contained campesterol, stigmatserol, b-sitosterol and b amyrin, Buckwheat (*Fagopyrum esculentum* Moench) pollen contained campesterol, stigmasterol and sitosterol.[8,9] In Citrus pollen cholesterol, 24-methylenecholesterol, campesterol, 24-methydesmosterol, 23-dehdyrositosterol, sitosterol and isofucosterol and in sunflower (*Helianthus annuus* L.) pollen 24-methylenecholesterol, 24-methylenecholestanol and isofucosterol has been identified as the most commonly found phytosterols.[9,10]

Phytosterols are reported to absorb from the intestinal tract esterification with fatty acids, to join to the lipoprotein structure in blood, and lower the low-density lipoprotein (LDL) and enhance high-density lipoprotein (HDL). In addition, reducing effect of blood vessel occlusion and the risk of coronary heart disease has been reported.[11,12] They have also shown to be effective in weight control because of the increasing effect of lipolysis, and have positive effect on the colon and prostate cancer by preventing the formation of malignant tumour development.[13] In recent years, due to its important role in the human diet, the sterol content of the beebread has gained importance. Therefore, the aim of this study is to determine sterol contents of beebread samp les from different geographical and botanical origin.

MATERIAL AND METHODS

BEEBREAD SAMPLES

A total of eleven beebread samples were collected from apiaries located in different monofloral honey production regions in Turkey between June and October of 2017. The type of flora and the sampling locations were as follows: cotton samples from Adana and Urfa, citrus samples from Adana and Mersin, chestnut sample from Zonguldak, sunflower sample from Edirne and clover samples from Urfa and Adıyaman (Figure 1). Beebread samples were hand collected from honeycombs and stored in a deep-freezer at -20°C before analyses. The pollen samples were collected by the same honeybee race (*Apis mellifera* L.).

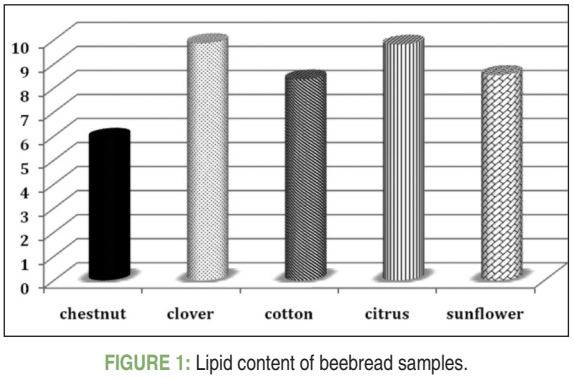

FIGURE 1: Lipid content of beebread samples.

REAGENTS AND CHEMICALS

All chemicals and reagents used in the study were purchased from Sigma-Aldrich-Fluka Co. Ltd. (Steinheim, Germany), unless otherwise stated. Trimethyl chlorosilane, potassium hydroxide and anhydrous sodium sulphate were purchased from Merck (Darmstadt, Germany). 5α-cholastene-3-β-ol was obtained from Supelco (Bellefonte, U.S.A).

POLLEN ANALYSIS

A 10 g of stored pollen sample was weighed and dissolved in 20 ml of distilled water (20-40°C). This solution was centrifuged for 10 min at 1.000 g. The supernatant liquid was poured off. The sediment was re-dissolved in 20 ml of distilled water to completely dissolve the remaining sugar crystals then centrifuged for 5 min at 1.000 g. The sediment was taken up on absorbent paper to remove excess water. Then it was spread on a slide over an area of about 20 mm. The slide with the sediment of pollen was dried on a heating plate at 40°C. The glycerine jelly was liquefied by warming to 40°C. The cover slips (22x22 mm) were warmed on the heating plate. One drop of glycerine jelly was united onto the cover slip and placed on the slide. The pollen grain exine and shape were visualized under light Microscope Nicon Eclipse E 600 and photographed. Pollen grains were identified using reference collection and with the help of microphotographs from the literature. About 500 pollen grains were counted in each sample. The frequency of pollen grains of each melliferous taxon is expressed as percentage of the total pollen sum (Table 1).

LIPID AND STEROL ANALYSIS

The lipid content of the stored pollen samples was determined using ISO 659 standard method.[14] The samples were homogenized in a stainless steel warming blender. A 2 g of sample was weighed accurately into a glass beaker and 100 mL 4 N HCl were added. Then the content was heated at 100°C and stirred for 15 minutes. The sample solution was then cooled to room temperature and washed with 25 mL distilled water for three times. Sample was filtered through filter paper and the filter paper was dried at 105°C in an oven for 1 hour. The oil from the pollen samples was extracted using diethyl ether at 50°C for 3 hours by automated Soxhlet extractor (VELP Scientific, Italy). The oil extracts was kept in amber vials prior to fatty acid analysis.

Sterols were analysed as their trimetylsilyl esters (TMSE) according to the ISO 12228:1999 method.[15] Briefly, 0.5 g of oil sample was weight into test tube and 1 mL internal standard (5α-cholestan-3β-ol (1000 mg/L) was added and saponified with 10 mL saturated methanolic KOH at 80°C for 1 h. Then the solution was extracted with

TABLE 1: Pollen analysis of beebread samples.

Sample no	Geographical origin	Botanical origin	Polen (%)	Other pollens (3-15%)
1	Urfa	*Trifolium pratense, T. repens*	86.2	Fabacea
2	Adıyaman	*Trifolium pratense, T. repens*	85.6	Fabaceae
3	Adana	*Gossypium hirsutum*	65.6	Fabaceae, Lamiaceae
4	Urfa	*Gossypium hirsutum*	66.2	Fabaceae Asteraceae, Lamiaceae
5	Zonguldak	*Castanea sativa*	94.4	Fabacea
6	Adana	*Citrus spp.*	54.4	Fabaceae, Brassicaceae, Lamiaceae, Rhamnaceae, Rosacece
7	Adana	*Citrus spp.*	61.4	Fabaceae, Brassicaceae, Lamiaceae, Rhamnaceae, Myrtaceae
8	Mersin	*Citrus spp.*	51.3	Brassicaceae, Fabaceae
9	Mersin	*Citrus spp.*	48.6	Rosaceae, Myrtaceae, Rhamnaceae
10	Mersin	*Citrus spp.*	62.4	Fabaceae, Rosacaea, Boraginaceae
11	Edirne	*Helianthus annuus*	45.4	Fabaceae, Rosaceae, Apiaceae

hexane and dried with anhydrous sodium sulphate. A 0.5 mL of dried hexane extract was silylated with bis (trimethylsilyl) trifluoroacetamide/ trimethyl chlorosilane (4:1) and 250 µL of dry pure pyridine at 60°C for 15 min. The sterol composition was determined using Gas Chromatography (Perkin Elmer, Autosystem GLX, Shelton, U.S.A.) equipped with a flame ionization detector (FID). Chromatographic separation of sterols was achieved on a SE-54 capillary column (30 m x 0.32 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas at a flow rate of 0.8 mL/min. The injector and detector temperatures were set at 280°C and 300°C, respectively. The oven temperature program was started and held at 60°C for 2 min then increased up to 220°C at a rate of 40°C/min and held at 220°C for 1 min and finally increased to 310°C at a rate of 5°C/min and held there for 10 min. Individual sterols were identified on the basis of retention times and by comparison with mixture of sterol TMSE analysed under the same conditions.

STATISTICAL ANALYSIS

All chemical assays were performed in triplicate. The obtained data were expressed as mean value±standard deviation. The data were compared using one-way analysis of variance (ANOVA) followed by Duncan test. Differences between the mean values at the 95% confidence interval ($p<0.05$) were considered statistically significant.

RESULT

The lipid content of the beebread samples studied varied 5.93 and 11.55% (Figure 1). The cholesterol content in stored citrus pollen samples was found between 8.73 and 22.47 mg/kg. The results showed statistically significant differences between the cholesterol content of citrus samples from different geographic origins ($p<0.01$) (Table 2). Cholesterol is the sterol that was determined at the lowest level (between 8.3 and 39.2 mg/kg) in the stored citrus pollen samples studied. The samples collected from Adana contained a higher amount of cholesterol (between 13.6 and 22.5 mg/kg) than the samples obtained from Mersin (8.7 mg/kg). Similarly, the stored citrus pollen samples from Adana contained more 24-methylene cholesterol (199.2-244.2 mg/kg) than the Mersin samples (640.9-575.5 mg/kg). However campesterol content of these samples was similar to one another. On the other hand, the samples from Mersin contained no campestanol whereas content of campestenol in Adana samples ranged between 91.8 and 467.9 mg/kg. In the citrus samples stigmasterol content was between 10.4 and 25.1 mg/kg. D5-avenosterol amount ranging between 154.3 and 1199.4 mg/kg was the most inconstant in the citrus samples studied. There were no significant difference between b-sitosterol content of the Mersin citrus samples whereas b-sitosterol in Adana samples varied between 369.4 and 1169.0 mg/kg. Sitostanol content of all the citrus samples changed between 18.6 and 126.5 mg/kg. The amount of unknown compound determined in Adana citrus samples (566-591 mg/kg) was more than two-fold higher than in Mersin samples (243-287 mg/kg). Briefly, cholesterol, campestanol, stigmasterol, b-sitosterol and sitostanol contents of citrus samples from Mersin were not statistically different ($p> 0.05$), but there were statistically significant differences between the sterol contents of all Adana samples ($p <0.05$).

The stored clover pollen samples collected from Adana and Urfa region did not show significant differences in terms of cholesterol and sitostanol content ($p> 0.05$) whereas there were statistically significant differences in the contents of the other sterols tested ($p <0.05$). The biggest difference in individual sterol contents of Adana and Urfa samples were in campestanol, D5-avenosterol and b-sitosterol content. The difference in sterol content of the clover samples collected from the two different geographic regions was statistically significant ($p <0.05$). Sitostanol was determined in Adıyaman sample and campestanole was present in Urfa sample only. The biggest difference between the stored clover pollen samples was observed in the content of campesterol and D5-avenosterol. Cholesterol (10.13 mg/kg) was the lowest and D5-avenosterol (1851.60 mg/kg) was the highest amount of sterol determined in both of the samples.

TABLE 2: Sterol content of beebread samples from different botanical and geographical origin (mg/kg).

	Cholesterol	24-metilen cholesterol	Campesterol	Campestanol	Stigmasterol	Δ5-avenasterol	β-sitosterol	Sitostanol	Unknown
Citrus-M*	8.73±0.16^{ab*}	199.21±0.46^{b}	457.30±0.30^{e}	0.00±0.00^{a}	287.03±0.82^{g}	355.75±1.24^{f}	65.04±0.08^{b}	18.65±0.03^{b}	25.11±0.41^{a}
Citrus -Er	8.76±0.13^{ab}	244.23±2.34^{c}	559.29±10.23^{g}	0.00±0.00^{a}	23.90±2.43^{d}	154.31±1.28^{c}	65.80±0.88^{b}	22.37±0.58^{b}	242.90±19.03^{c}
Citrus-A1	20.29±0.75^{e}	575.55±0.71^{i}	561.16±7.07^{g}	291.79±2.90^{e}	10.44±0.81^{b}	1199.43±2.30^{j}	1169.01±4.07^{i}	126.51±0.50^{d}	565.73±0.86^{f}
Citrus-A2	22.47±0.15^{f}	623.89±4.41^{j}	638.27±1.01^{h}	467.96±2.27^{f}	22.91±0.20^{d}	919.27±17.93^{h}	727.35±4.64^{g}	84.15±5.70^{c}	580.76±14.21^{g}
Citrus-A3	13.63±0.88^{c}	640.94±5.43^{k}	489.72±1.25^{f}	91.83±0.77^{b}	15.10±0.49^{c}	994.33±2.38^{i}	369.43±5.23^{c}	87.01±3.11^{c}	591.17±1.66^{g}
	14.78	456.74	541.15	283.86	71.88	724.59	479.33	67.74	761.13
Cotton A	8.60±0.16^{ab}	268.52±3.44^{e}	118.90±3.72^{c}	1698.71±11.73^{g}	29.33±0.84^{e}	60.49±1.24^{a}	35.03±0.20^{a}	16.40±0.03^{b}	265.81±2.77^{c}
Cotton-U	9.67±0.65^{ab}	257.18±1.48^{d}	103.70±0.20^{b}	148.60±0.26^{c}	4.85±0.12^{a}	478.94±2.22^{g}	825.91±3.53^{h}	18.32±0.67^{b}	315.16±0.03^{d}
	9.13	262.85	111.3	923.66	17.09	269.72	430,47	17.36	290.49
Trifolium-U	10.13±1.14^{b}	320.21±0.20^{f}	94.12±0.42^{b}	146.67±0.45^{c}	20.36±0.07^{d}	188.25±2.71^{d}	442.78±2.53^{e}	0.00±0.00^{a}	441.04±2.35^{e}
Trifolium-A	39.17±0.18^{g}	485.39±4.96^{h}	451.53±4.60^{e}	0.00±0.00^{a}	137.52±3.27^{f}	1851.60±3.68^{k}	404.52±1.37^{d}	355.78±0.91^{e}	740.26±1.61^{h}
	24.65	402.8	272.83	17.09	78.94	1019.93	423.65	177.89	590.65
Chestnut-E	8.31±0.02^{a}	167.24±2.57^{a}	288.45±1.08^{d}	229.82±2.65^{d}	30.86±0.48^{e}	216.25±1.70^{e}	619.19±1.46^{f}	914.48±0.12^{i}	213.01±0.14^{b}
	8.31	167.24	272.83	17.09	78.94	1019.93	423.65	177.89	590.65
Sunflower-E	15.25±0.30^{d}	474.58±2.90^{g}	59.28±0.05^{a}	223.47±0.99^{d}	9.38±0.46^{b}	112.08±1.02^{b}	1024.23±1.47^{j}	87.62±0.39^{c}	334.75±0.89^{d}
	15.25	474.58	59.28	223.47	9.38	112.08	1024.23	87.62	334.75

*: Different letters (a-i) in the same column represent different statistical groups (P <0.05). Values are mean ± SD. is given.

**: A: Adana; M: Mersin; Er: Erdemli; E: Edirne; U: Urfa.

Chestnut samples contained the highest level of sitosterol and b-sitosterol while cholesterol was present at the lowest level. The amount of the sterols tested in chestnut samples in descending order was as follows; sitostanol, b-sitosterol, campesterol, campestanol, D5-avenosterol, unknown compound, 24-methylene-cholesterol, stigmasterol and cholesterol. The content of the sterols determined in sunflower stored pollens were between 9.4 and 1024.2 mg/kg. Stigmasterol and cholesterol ratio was the lowest while the sample contained the highest amount of b-sitosterol.

There was a statistically significant difference in cholesterol contents (between 8.3 and 39.2 mg/kg) of all the samples tested ($p < 0.01$). However, unlike the others the clover samples contained higher levels of cholesterol. The level of 24-methylene cholesterol was the highest in stored sunflower pollen and followed by citrus, clover and cotton, respectively. However, chestnut sample contained the lowest amount of 24-methylene cholesterol. All of the citrus samples contained higher amounts of campesterol than the other samples studied and it was followed by chestnut, clover and cotton samples.

The lowest campesterol content was determined in stored sunflower pollen samples.

Mersin citrus and clover samples did not contain any campestanol whereas Adiyaman cotton samples contained the highest amount of campestanol. Stigmasterol content was the lowest in both sunflower and the Urfa cotton samples. Clover (Adıyaman) sample contained the highest proportion of D5-avenosterol among the all beebread samples studied. Sun-

flower beebreads and one of the citrus samples had the largest amount of b-sitosterol content. The lowest amount of sitostanol was determined in the cotton samples whereas the chestnut sample contained the highest amount. An unknown compound was also detected in large amounts (between 213 and 740 mg/kg) in all the samples studied.

DISCUSSION

The lipid extracted from the pollen is reported to come mainly from pollen coat or pollenkit. However, the lipid contents of flower pollens' are highly variable.[16] The lipid contents of beebread samples studied in this study varied between 5.93 and 11.55%. Similarly, in a review published by Roulston and Cane, lipid contents of dry pollens obtained from 62 plant species were reported to vary significantly (i.e. eucalyptus contained 0.8% lipid and dandelion pollen 18.9%).[17] In addition, it was reported that the lipid concentration of the species from Myrtaceae family was 1.43%, the mean lipid concentrations for Fabaceae species such as clover, faba beans and robinia as 6.7%, and species of the Brassicaceae family, such as mustards and canola contain 10.7% lipids. The lipid content of the same genus of plant pollens may also vary within their own, for example the species of genus Eucalyptus (*E. camaldulensis, E. bridgesiana*) is reported to vary between 0.43-4.6%.[18] Other than that, it is emphasized that the lipid contents of the pollens from different countries are diverse; pollen lipid concentration of 16 plant species collected from four different regions of the USA was 9.2% whereas it was 3.2% for 15 different species grown in Scandinavia.[7,18]

Sterols are the compounds found in all vegetable and animal tissues. 24-methylene cholesterol and isofucosterol were reported to be the major sterol compounds in flower pollens.[19] On the other hand, cholesterol, stigmasterol, and sitosterol have been reported as pollen sterols by Faraq et al. In this study, citrus stored pollen samples were found rich in campesterol, b sitosterol, 24 methylene cholesterol and delta 5 avenasterol content compared with other sterols determined.[20] Takatsuto et al. reported the high abundance of cholesterol, 24-methylenecholesterol, campesterol, 24 methyl desmosterol, 23 dehydrositosterol, sitosterol and isofucosterol in both *Citrus unshiu* Marcov and *Citrus sinensis* Osbeck pollens.[9] In the present study, b sitosterol was determined as the main sterol in sunflower stored pollen. In another study, 24-methylene cholesterol, 24- methylenecholestanol and isofucosterol determined as the main sterols of sunflower pollen. The same researcher group identified brassin steroids (brassinolide, castaterone and narcastasterone) in sunflower pollen.[9] Clover beebread contained the highest percentage of delta 5 avenasterol. However, in another study sterol contents of pollens obtained from 15 plant species belonging to 11 family were determined and 24 methylene cholesterol was found as the main sterol in red cover (*Trifolium pratense* L.), mustard, sweet corn, timothy and London rocket.[7]

In our study, chestnut beebread has been found to contain higher percentage of sitostanol as compared with other sterols. On the other hand, Guo et al. identified other sterols in chestnut flower pollen including cholesterol, (3b, 24R)-ergost-5-en-3-yl, sitgmatserol, b sitosterol, (3 b)-stigmasta-5, 24 (28) dien-3yl and estradiol.[21] In floral bud and anthers of cotton (*G. hirsutum* cv. Stoneville213) plant sitosterol, stigmatserol, 24 methyl-cholest-5-en-3-betaol were determined as the major sterols.[22]Also, sitosterol, isofucosterol and 24 methylene-cholesterol were determined in hand collected and corbicular almond pollens by Loper et al.[23]

Honey bee (*Apis mellifera* L.) benefit from pollen as dietary sources of protein, fatty acids, sterols, vitamins and some carbohydrates. The quality and quantity of stored pollen affect brood rearing and longevity, and thus the productivity of honeybees.[24,25] Svoboda et al. compared the sterol contents of the pollens collected from 7 different areas and prepupal bees gathered from the colonies in each area.[26] In prepupal bees, 24 methylene cholesterol was identified as the major sterol, sitosterol and isofucosterol were present to a lesser extent

and the cholesterol was in trace amounts. Indeed, 24 methylene cholesterol was isolated from body of queen bees earlier by Barbier and Schindler.[27] At a later study, Herbert et al. reported that 24 methylene cholesterol affected the survival of honey bees at the highest degree.[28] However, it should be noted that the stored pollens used in nutrition of bees do not belong to a single family, as revealed by the pollen analysis. Even though a certain type is dominant, pollen supplement from different plant species at different rates, provides variations and substitutes missing sterols. In other words, the sterols required for feeding honeybees can be obtained from pollen mixtures.

Sterols are beneficial both for honeybee and human health. They are the membrane components regulating permeability and fluidity of phospholipid bilayers.[29] In western countries, daily intake of sitostanol from an average diet is 20-50 mg and both sitosterol and kampasterol are 300 mg, in order to benefit from cholesterol lowering effect, daily intake of about 1 g sterol is recommended.[30,31] The importance of phytosterols in human health was investigated on laboratory animals in many research studies. For example b-sitosterol has been shown to inhibit the development of colon cancer cells and altered lipid membrane.[32] Besides, it showed anti-inflammatory and antipyretic effects.[33,34] Cholesterol lowering effect of campesterol was reported by Thuluva et al. In addition, stigma sterol showed anti-osteoarthritic activity.[35,36]

In conclusion, flower pollens collected from plants by honeybees are stored in the hive to feed the young bees. Sterol content of these stored pollen significantly differed from each other in terms of variety and quantity. However, sterols needed by honeybees can be met as monofloral pollen is not the only source for their diet. The present study also showed that sterol and lipid content of the stored pollens of the same species collected from different geographical areas were significantly different from each other. The reason for this is, because various types of pollens from different plants contribute in different proportions to the pollen stored in the honeycombs. Also, it is important to note that the pollen stored in the honeycombs is rather rich in sterol content and valuable for human nutrition.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Sibel Silici, Muammer Kaplan, İlknur Demirtaş;* ***Design:*** *Muammer Kaplan, İlknur Demirtaş;* ***Control/Supervision:*** *Muammer Kaplan, Sibel Silici;* ***Analysis and/or Interpretation:*** *Muammer Kaplan, İlknur Demirtaş, Sibel Silici;* ***Literature Review:*** *Muammer Kaplan, Sibel Silici;* ***Writing the Article:*** *Muammer Kaplan, Sibel Silici;* ***Critical Review:*** *Muammer Kaplan, Sibel Silici;* ***References and Fundings:*** *Muammer Kaplan, Sibel Silici;* ***Materials:*** *Sibel Silici.*

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