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Fatty Acid and Proximate Composition of Bee Bread

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Summary

Palynological spectrum, proximate and fatty acid (FA) composition of eight bee bread samples of different botanical origins were examined and significant variations were observed. The samples were all identified as monofloral, namely *Castanea sativa* (94.4 %), *Trifolium* spp. (85.6 %), *Gossypium hirsutum* (66.2 %), *Citrus* spp. (61.4 %) and *Helianthus annuus* (45.4 %). Each had moisture content between 11.4 and 15.9 %, ash between 1.9 and 2.54 %, fat between 5.9 and 11.5 %, and protein between 14.8 and 24.3 %. A total of 37 FAs were determined with most abundant being (9Z,12Z,15Z)-octadeca-9,12,15-trienoic, (9Z,12Z)-octadeca-9,12-dienoic, hexadecanoic, (Z)-octadec-9-enoic, (Z)-icos-11-enoic and octadecanoic acids. Among all, cotton bee bread contained the highest level of ω-3 FAs, *i.e.* 41.3 %. Unsaturated to saturated FA ratio ranged between 1.38 and 2.39, indicating that the bee bread can be a good source of unsaturated FAs.

Key words: bee bread, fatty acid composition, proximate composition, monofloral pollen, pollen analysis

Introduction

Pollen and nectar are essential components of honeybee, *Apis mellifera* L., diet. Nectar provides carbohydrates, while pollen supplies protein, lipid and vitamins. Pollen collected by foraging worker bees is combined with honeybee secretions (*1*). Bee bread is processed pollen stored and packed in the honeycomb cells following the addition of various enzymes and nectar or honey as it undergoes lactic acid fermentation. Generally, the methods employed for quantification of nutritional dissimilarities amongst the levels of hive-stored and collected pollen have been proven difficult. There is a limited number of studies in the literature regarding the nutritional properties attributed to the stored pollen. The reported results are contradictory, indicating either no significant change or marginally increased nutrition (*2*). Bee pollen collection is a fairly new development. The pollen trap is used to scrape off the pollen from the legs of bees as they enter the hive. The scientific studies revealed various beneficial therapeutic and nutritional properties of the bee pollen and enabled the scientists to identify its antimicrobial, antioxidant, antiradical, anticancer, and anti-inflammatory activities (*3*). The main constituents of the bee pollen are carbohydrates (13–55 %), crude proteins (10–40 %), crude fibre (0.3–20 %) and lipids (1–10 %) (*4–6*).

As bee pollen contains all the essential amino acids required for the human organism, it is referred to as 'the only perfectly complete food' (*7*). Notwithstanding, there are only few papers published on the FA composition of the bee bread. Human and Nicolson (*8*) reported only 18 fatty acids in bee bread originating from an indigenous South African bee plant. Čeksterytė *et al.* (*9*) identified 22 fatty acids in the bee bread (containing >45 % rape or willow pollen) collected in the spring and summer seasons. (Z)-octadec-9-enoic and (5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoic acids were the most abundant unsaturated FAs, constituting around 15 % of total fatty acids. In another

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study, Čeksterytė and Jansen (*10*) reported the highest content (27–43.8 %) of (9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid (ω-3) among 22 FAs identified in spring rape and willow bee bread.

Fatty acids are of high importance in fertility and health of the honeybees. Unsaturated FAs have also many beneficial health effects such as reducing triglyceride (*11*) and cholesterol levels in blood and show anti-inflammatory and antithrombotic activities (*12*). Current literature suggests that pollen and bee bread are good sources of polyunsaturated FAs (PUFAs) that are crucial for human nutrition. PUFAs cannot be synthesized in human body endogenously and must be obtained from food. In this respect, bee bread can be considered as a potential source of PUFAs in human diet. However, in particular, scientific research exploring various properties of bee bread is scarce and additional research into this topic is highly required. Therefore, the aim of the current study is to obtain and compare data on the FA content, pollen and proximate composition of bee bread samples harvested in Turkey.

Materials and Methods

Bee bread samples

Eight bee bread samples were obtained from apiaries located in different monofloral honey production regions in Turkey between June and October of 2014. The pooled samples were collected from minimum three beehives in apiaries with 50–100 colonies. Bee bread samples were hand collected from honeycombs and kept at –20 °C before the analyses. The type of flora and sampling locations were as follows: cotton from Adana and Urfa, citrus from Adana and Mersin, chestnut from Zonguldak, sunflower from Edirne and clover from Urfa and Adıyaman.

Reagents and chemicals

The used reagents were purchased from Sigma-Aldrich-Fluka Co. Ltd. (Steinheim, Germany), unless otherwise stated. Anhydrous sodium sulphate and potassium hydroxide were obtained from Merck (Darmstadt, Germany). The standard reference mixture, Supelco-18919, of fatty acid methyl esters (FAMEs) was purchased from Supelco (Bellefonte, PA, USA). The standard mixture contained the following 37 FAMEs: butanoic, hexanoic, octanoic, decanoic, undecanoic, dodecanoic, tridecanoic, tetradecanoic, pentadecanoic, hexadecanoic, heptadecanoic, octadecanoic, icosanoic, heneicosanoic, docosanoic, tricosanoic, tetracosanoic, (Z)-tetradec-9-enoic, (Z)-pentadec-10--enoic, (9Z)-hexadec-9-enoic, *cis*-10-heptadecenoic, (E)-octadec-9-enoic, (Z)-octadec-9-enoic, (Z)-icos-11-enoic, (Z)-docos-13-enoic, (Z)-tetracos-15-enoic, octadeca-9,12-dienoic, (9Z,12Z)-octadeca-9,12-dienoic, octadeca-6,9,12-trienoic, icosa-11,14-dienoic, (11Z,14Z,17Z)-icosa-11,14,17-trienoic, (5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoic, docosa-13,16--dienoic, (9Z,12Z,15Z)-octadeca-9,12,15-trienoic, (11Z,14Z, 17Z)-icosa-11,14,17-trienoic, (5Z,8Z,11Z,14Z,17Z)-icosa-5, 8,11,14,17-pentaenoic and docosa-4,7,10,13,16,19-hexaenoic acids.

Pollen analysis

A mass of 10 g of bee bread sample was weighed into a centrifuge tube and mixed thoroughly with 20 mL of distilled water. The solution mixture was centrifuged at 1000×*g* for 10 min and the liquid part was discarded. The sediment was redissolved in 20 mL of distilled water and centrifuged. Then the sediment was placed on an absorbent paper to remove excess water, spread on a slide covering an area of about 20 mm and dried on a heating plate at 40 °C. The cover slips (22 mm×22 mm) containing a drop of glycerine jelly that liquefied by warming to 40 °C were warmed by a heating plate and then placed on the slide. The light microscope Eclipse E600 (Nikon, Tokyo, Japan) was used to visualize the pollen grain exine and shapes. Pollen grains were identified using reference collection and the microphotographs from the literature.

Chemical analysis

Chemical analysis of bee bread (ash, crude fat and crude protein) was carried out using standard AOAC methods 920.153, 991.36 and 960.52, respectively (*13–15*). Moisture content was measured using a vacuum oven model VO200 (Memmert GmbH+Co. KG, Schwabach, Germany) at 60 °C and weighing until a constant mass. The ash content was measured gravimetrically after incineration at 550 °C and weighing. The total protein content was calculated by multiplying the nitrogen content by nitrogen to protein conversion factor of 6.25. All analyses were made in triplicate and the results were expressed in g per 100 g of fresh bee bread.

Determination of oil content was carried out using the ISO 659:2009 standard method (*16*). The bee bread samples were homogenized using a stainless steel blender (Waring, Atlanta, GA, USA). A mass of 2 g of sample was weighed accurately into a glass beaker and mixed with 100 mL of 4 M HCl. Then the content was heated at 100 °C and stirred for 15 min. After cooling to room temperature the solution was washed three times with 25 mL of distilled water. The sample was filtered through a filter paper, which was dried at 105 °C in an oven for 1 h. The extraction of oil from bee bread samples was carried out with diethyl ether at 50 °C for 3 h by automated Soxhlet extractor (VELP Scientifica, Usmate (MB), Italy). The oil extracts were stored in amber vials prior to analysis of fatty acids.

Fatty acid analysis

Determination of FAMEs was performed using the ISO 12966-2:2011 standard method (*17*). Briefly, 0.1 g of bee bread oil was weighed into a test tube. After the addition of 5 mL of heptane and 0.5 mL of methanolic 2 M KOH, the tube content was mixed by vortexing for 1 min at room temperature. Then, the upper layer was dried with anhydrous sodium sulphate for gas chromatography analysis.

Chromatographic analysis was carried out by a gas chromatography (GC) system Clarus 500 (PerkinElmer, Shelton, CT, USA) equipped with an autosampler, split--splitless injector and a flame ionization detector. A 100-metre Supelco 2380 capillary column (Sigma-Aldrich, Belle-

fonte, PA, USA) with an internal diameter of 0.25 mm and 0.2 μm film thickness was used for chromatographic separation. Helium carrier gas flow rate was set at 1.2 mL/min. The injector and detector temperatures were set at 250 and 260 °C, respectively. The initial GC oven temperature was 165 °C, held for 5 min, increased to 240 °C at 5 °C/min and held at 240 °C for 10 min. A volume of 1.0 μL of sample was injected using the split injection mode (1:50). The peaks were identified by comparison of their relative retention times with a standard FAME mixture. The results were expressed as percentage of total FAMEs.

The resulting FAMEs were also confirmed by GC-MS through comparison of retention time and mass spectrometry data using the authentic reference standards. Confirmation analyses of individual FAMEs were performed under identical conditions. Chromatographic separation of compounds was carried out using an Agilent GC-MS system (Agilent Technologies, Palo Alto, CA, USA) equipped with Agilent 6890 gas chromatograph and Agilent 5973 mass spectrometer.

Chromatographic separation of fatty acids was achieved on a 30-metre DB-WAX capillary column (0.25 mm i.d., film thickness 0.25 μm; Agilent Technologies, Folsom, CA, USA). The carrier gas (helium) flow rate was 1.5 mL/min. The injection port temperature was set at 250 °C. The volume of the injected sample was 1 μL (split ratio 1:10). Initially, the GC oven temperature was maintained at 180 °C for 3 min. Then it was increased to 210 °C at a rate of 2 °C/min and after 20-minute isothermal run at 210 °C, finally increased to 240 °C at 10 °C/min and held for 5 min. The mass spectra were acquired in an electron-impact (EI) ionization mode at 70 eV in the mass scan range of m/z=35–550. The temperatures of electron ionization source and mass quadrupole analyser were 150 and 280 °C, respectively.

The mass spectra of compounds were identified by comparing the mass spectra obtained from their related chromatographic peaks with the Wiley and NIST mass spectral libraries (*18,19*).

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 least significant difference (LSD) test. Differences between the mean values at the 95 % confidence interval ($p<0.05$) were considered statistically significant.

Results and Discussion

Pollen content of the samples

Botanical origin of the bee bread samples was identified by pollen analysis. The results are presented in Table 1. All of the eight samples studied were unifloral: cotton (two), clover (two), citrus (two), chestnut (one) and sunflower (one). Chestnut bee bread contained 94.4 % *Castanea sativa* pollen, while the clover bee bread samples contained *Trifolium* spp. (*T. repens* and *T. pratense*) pollen >85 %. Cotton bee bread samples contained *Gossypium hirsutum* L. pollen at 65.6 and 66.2 %, citrus samples comprised *Citrus* spp. at 61.4 and 54.4 %, and sunflower sample contained *Helianthus annuus* L. at 45.4 %. Other pollen taxa found in the samples were Fabaceae, Lamiaceae, Brassicaceae, Rhamnaceae, Apiaceae, Myrtaceae and Rosaceae.

Characterization of honeybee products such as honey, bee pollen and bee bread is important for consumers. Honey with a pollen frequency >45 % is considered to be monofloral or unifloral. In monofloral honey, underrepresented pollen (*e.g.* citrus) frequency is minimum 10–20 % or 20–30 %, and overrepresented pollen (such as chestnut, eucalyptus) frequency is minimum 70–90 % (*20*). Similarly, the results of pollen analysis revealed that more than 45 % of total pollen detected in the bee bread samples was from monofloral source. In particular, chestnut and clover samples had the monofloral pollen contents of over 85 %. *Castanea sativa* is an important nectar and pollen source for a pollen forager bee (*21*) as it is abundantly available and easy to manage. The anemophilous plants like chestnut produce large quantities of small pollen grains and nectar foraging honeybees actively collect them mainly earlier in the day during the flowering season to strengthen and improve the colony life (*22,23*). All the above information explains the preference of chestnut pollen by honeybees and high rate of *Castanea sativa* representation in bee bread.

Frequency of *Trifolium* spp. in clover bee bread samples from Adıyaman and Urfa was 85.6 and 86.2 % respectively. *Trifolium* species classified under the Fabaceae family is among the most important pollen sources for honey-

Table 1. Results of palynological analysis of bee bread samples from different botanical origins (N=8)

	Sample	Geographical origin	Botanical origin	$\frac{w(\text{pollen})}{\%}$	w(other important pollen)=3–15 %
1	Clover	Urfa	*Trifolium pratense, T. repens*	86.2	Fabacea
2	Clover	Adıyaman	*Trifolium pratense, T. repens*	85.6	Fabaceae
3	Cotton	Adana	*Gossypium hirsutum*	65.6	Fabaceae, Lamiaceae
4	Cotton	Urfa	*Gossypium hirsutum*	66.2	Fabaceae, Asteraceae, Lamiaceae
5	Chestnut	Zonguldak	*Castanea sativa*	94.4	Fabacea
6	Citrus	Adana	*Citrus* spp.	54.4	Fabaceae, Brassicaceae, Lamiaceae, Rhamnaceae, Rosacece
7	Citrus	Mersin	*Citrus* spp.	61.4	Fabaceae, Brassicaceae, Lamiaceae, Rhamnaceae, Myrtaceae
8	Sunflower	Edirne	*Helianthus annuus*	45.4	Fabaceae, Rosaceae, Apiaceae

bees as they are available all year around with long flowering periods. Besides, they have dense populations and produce many flowers per inflorescence (*24*). This could be a reasonable explanation for the higher *Trifolium* spp. pollen content in the tested clover bee bread samples.

Citrus, cotton and sunflower pollen is not represented as much as chestnut and clover. Cotton (*Gossypium hirsutum* L., Malvaceae) is a valuable plant from which floral and extrafloral nectaries are collected by honeybees for honey production. However, honeybees seldom visit and obtain pollen from cotton plants (*25,26*). Cotton pollen is covered with sticky material (*27*) which makes its grooming from the body of bees painstakingly hard and it explains the avoidance of cotton pollen by honeybees (*28*). In another study, the repellency of cotton is attributed to the gossypol, which is a dimeric sesquiterpenoid (*25*).

Sunflower bee bread contained the lowest pollen content from *Helianthus annuus* (45.4 %). This could be explained by the fact that although honeybees are the most frequent visitors of the sunflowers, they rather collect nectar from sunflowers and are less attracted to their pollen compared to other pollen types (*29*). Furthermore, it is reported that the protein content of sunflower pollen is low in both quality and quantity, so it is considered to be poor pollen source for honeybees (*30*).

Although citrus trees are considered as the most significant floral source for the production of honey, they are rarely a good pollen source due to their low protein level. This could be one of the reasons for low representation of C*itrus* spp. pollen in the citrus bee bread samples (*31*).

Moreover, it is known that the nectar and pollen collecting behaviours of honeybees are different. There are several factors which have to be taken into consideration regarding pollen collection by honeybees; the need of the colonies for pollen, brood production, the rhythm of the colony life throughout the season, biological value of bee pollen for honeybees, age of the foragers, handling time and factors related to pollen (size, colour, floral shape and symmetry, pigmentation patterns, attractiveness, *etc.*) (*32*).

Protein, fat, moisture and ash contents

The proximate compositions of the studied samples are given in Table 2. The moisture fractions of the samples were between 11.4 and 15.9 %. The mass fractions of ash were 1.9 to 2.5 %, the fat from 5.9 to 11.5 % and protein from 14.8 to 24.3 %.

The bee bread samples studied were obtained from regions with different climatic conditions. For example, while the Mersin and Adana regions are under the influence of Mediterranean climate, continental climate is found in the other regions. Moreover, the sample collection points were at different altitudes from the sea level. Therefore, changes determined in the moisture levels of the samples could be ascribed to the altitude and different climatic conditions. Clover bee bread samples had the highest protein content (22.6 and 24.2 g/100 g) and cotton appeared to have the lowest content of protein (14.8 and 15 %). Clover bee bread also had the highest fat content along with citrus. Our results showed that the protein and lipid content varies according to the botanical origin of the bee bread. Herbert Jr and Shimanuki (*33*) reported similar findings for the seven bee bread samples they studied but their data spread out over a wider range than ours. They found moisture content ranging between 18.8 and 28.0 %, protein content between 19.3 and 26.5 %, ash content between 2.1 and 3.2 %, and lipid content between 3.9 and 6.7 %.

Table 2. Proximate composition of the bee bread samples (N=8)

Sample*	w(moisture) / %	w(ash) / g/100 g	w(protein) / g/100 g	w(fat) / g/100 g
1	$(12.60\pm0.30)^{b}$	$(2.03\pm0.01)^{c}$	$(15.01\pm0.01)^{b}$	$(9.23\pm0.02)^{f}$
2	$(13.16\pm0.01)^{c}$	$(1.97\pm0.01)^{b}$	$(14.82\pm0.01)^{a}$	$(7.49\pm0.02)^{b}$
3	$(15.82\pm0.01)^{f}$	$(2.04\pm0.01)^{c}$	$(19.71\pm0.01)^{e}$	$(10.47\pm0.04)^{g}$
4	$(12.33\pm0.02)^{b}$	$(2.02\pm0.01)^{c}$	$(18.59\pm0.02)^{c}$	$(9.15\pm0.03)^{e}$
5	$(15.32\pm0.01)^{e}$	$(2.62\pm0.01)^{e}$	$(20.53\pm0.02)^{f}$	$(5.93\pm0.02)^{a}$
6	$(14.05\pm0.03)^{d}$	$(1.93\pm0.02)^{a}$	$(19.41\pm0.02)^{d}$	$(8.54\pm0.03)^{d}$
7	$(15.89\pm0.01)^{f}$	$(2.52\pm0.01)^{d}$	$(22.66\pm0.02)^{g}$	$(8.19\pm0.02)^{c}$
8	$(11.41\pm0.01)^{a}$	$(2.54\pm0.01)^{d}$	$(24.26\pm0.01)^{h}$	$(11.55\pm0.05)^{h}$

The groups in the same column with different letters in superscript are statistically different ($p<0.05$)
*geographical and botanical origin of the samples are given in Table 1

Fatty acid composition

A total of 37 FAs including 20 saturated and 17 unsaturated were identified in the bee bread samples obtained from different botanical origins (Table 3). The results of fatty acid determination included both free acids and products of glyceride hydrolysis. Thirty-one of the total identified fatty acids were common to all eight samples. Only six of them were detected in one or more of the samples. There were statistically significant differences in the amounts of 34 fatty acids determined in the samples ($p<0.05$), while the amount of the remaining three fatty acids, (Z)-tetradec-9-enoic, icosa-11,14-dienoic and docosa-4,7,10,13,16,19-hexaenoic acids did not vary significantly ($p>0.05$). The bee bread samples contained quite high levels of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFAs). MUFAs belonging to ω-9 fatty acid family were (Z)-icos-11-enoic, (Z)-docos-13-enoic, (E)-octadec-9-enoic, (Z)-tetracos-15-enoic and (Z)-octadec-9-enoic acids. Eleven PUFAs, four from ω-3 and seven from ω-6 family, were identified in all the samples. (9Z,12Z,15Z)-octadeca-9,12,15-trienoic is the most abundant PUFA from ω-3 family, while (9Z,12Z)-octadeca-9,-12-dienoic acid is the most abundant ω-6 fatty acid in the samples. On average, the major saturated FAs in decreasing order of abundance in the samples were hexadecanoic, octadecanoic and icosanoic acids. The most abundant unsaturated FAs found were (9Z,12Z)-octadeca-9,12-dienoic, (Z)-octadec-9-enoic and (9Z,12Z,15Z)-octadeca-9,-12,15-trienoic acids.

A total of 35 and 32 fatty acids were identified in cotton (*Gossypium hirsutum* L.) bee bread samples from Adana and Urfa, respectively. Undecanoic and tridecanoic acids were not detected in the cotton bee bread, while hexanoic, octanoic and pentadecanoic acids were only present in the cotton sample from Adana. The ratios of major FAs found in the cotton bee bread samples were

Table 3. Fatty acid composition of bee bread samples

Saturated fatty acid		Sample 1	2	3	4	5	6	7	8
		w(fatty acid)/%							
C4:0	Butanoic	(0.75±0.01)^{c}	(0.65±0.01)^{b}	(1.29±0.46)^{e}	(0.37±0.01)^{a}	(1.16±0.01)^{e}	(1.03±0.04)^{d}	(1.06±0.02)^{d}	(1.30±0.41)^{e}
C6:0	Hexanoic	(0.08±0.00)^{a}	–	(0.35±0.01)^{b}	–	–	(0.23±0.07)^{b}	–	–
C8:0	Octanoic	(0.10±0.01)^{d}	–	(0.12±0.01)^{d}	(0.02±0.00)^{a}	(0.04±0.00)^{b}	(0.34±0.03)^{e}	–	(0.08±0.01)^{c}
C10:0	Decanoic	(0.07±0.01)^{c}	(0.02±0.00)^{a}	(0.05±0.01)^{b}	(0.16±0.00)^{d}	(0.04±0.00)^{b}	(0.04±0.00)^{b}	(0.04±0.00)^{b}	(0.04±0.00)^{b}
C11:0	Undecanoic	–	–	–	–	(0.07±0.00)	–	(0.07±0.00)	–
C12:0	Dodecanoic	(0.14±0.00)^{a}	(0.05±0.01)^{a}	(0.29±0.01)^{b}	(0.11±0.00)^{a}	(0.07±0.04)^{a}	(6.15±0.10)^{c}	(0.06±0.00)^{a}	(0.14±0.02)^{a}
C13:0	Tridecanoic	–	–	–	–	–	(0.07±0.01)	–	–
C14:0	Tetradecanoic	(1.29±0.04)^{e}	(0.36±0.01)^{bc}	(0.51±0.01)^{d}	(0.21±0.01)^{a}	(0.41±0.01)^{c}	(0.38±0.01)^{c}	(0.30±0.00)^{b}	(1.26±0.04)^{e}
C15:0	Pentadecanoic	(0.14±0.00)^{b}	–	(0.21±0.02)^{c}	(0.14±0.01)^{b}	(0.53±0.01)^{d}	(0.15±0.00)^{b}	(0.13±0.01)^{b}	(0.21±0.02)^{c}
C16:0	Hexadecanoic	(29.63±0.42)^{d}	(26.34±0.51)^{c}	(38.69±0.31)^{e}	(22.32±0.33)^{a}	(24.58±0.29)^{b}	(27.18±0.14)^{c}	(24.71±0.25)^{b}	(28.87±0.38)^{d}
C17:0	Heptadecanoic	(0.23±0.02)^{a}	(0.20±0.01)^{a}	(0.46±0.03)^{c}	(0.32±0.02)^{b}	(0.91±0.01)^{d}	(0.35±0.04)^{b}	(0.35±0.03)^{b}	(0.51±0.03)^{c}
C18:0	Octadecanoic	(3.21±0.05)^{e}	(1.31±0.02)^{a}	(6.27±0.12)^{f}	(2.33±0.03)^{d}	(2.37±0.10)^{d}	(1.59±0.09)^{b}	(1.91±0.05)^{c}	(3.39±0.11)^{e}
C20:0	Icosanoic	(0.73±0.03)^{b}	(0.80±0.04)^{b}	(3.23±0.07)^{f}	(1.04±0.03)^{c}	(1.41±0.06)^{d}	(1.12±0.02)^{c}	(0.61±0.02)^{a}	(1.64±0.03)^{e}
C21:0	Heneicosanoic	(0.04±0.00)^{a}	(1.17±0.04)^{d}	(0.05±0.00)^{a}	(1.70±0.04)^{e}	(0.37±0.00)^{c}	(0.08±0.01)^{b}	–	–
C22:0	Docosanoic	(0.55±0.02)^{b}	(0.44±0.01)^{b}	(0.08±0.01)^{a}	(0.89±0.05)^{c}	(1.20±0.04)^{d}	(2.60±0.18)^{e}	(0.13±0.01)^{a}	(0.60±0.03)^{b}
C23:0	Tricosanoic	(0.28±0.01)^{a}	(1.07±0.21)^{c}	–	(5.61±0.07)^{d}	(1.02±0.03)^{c}	(0.58±0.04)^{b}	–	(0.62±0.06)^{b}
C24:0	Tetracosanoic	(0.03±0.00)^{a}	(0.05±0.00)^{cd}	–	(0.04±0.00)^{ab}	(0.33±0.01)^{e}	(0.06±0.01)^{d}	–	–
w(total)/%		37.28	32.48	51.59	35.25	34.51	41.97	29.37	38.67
Unsaturated fatty acid		1	2	3	4	5	6	7	8
C14:1n5	(Z)-tetradec-9-enoic	(0.41±0.01)^{b}	(0.56±0.02)^{c}	(1.06±0.07)^{f}	(0.76±0.01)^{e}	(0.02±0.00)^{a}	(1.35±0.02)^{g}	(0.65±0.01)^{d}	(1.05±0.01)^{f}
C15:1	(Z)-pentadec-10-enoic	(0.03±0.01)^{a}	(0.05±0.01)^{b}	(0.14±0.01)^{e}	(0.09±0.00)^{c}	(0.26±0.01)^{f}	(0.12±0.00)^{d}	(0.05±0.00)^{b}	(0.07±0.01)^{c}
C16:1n7	(9Z)-hexadec-9-enoic	(0.19±0.01)^{e}	(0.11±0.01)^{c}	(0.11±0.01)^{c}	(0.14±0.00)^{d}	(0.12±0.00)^{cd}	(0.05±0.00)^{a}	(0.08±0.00)^{b}	(0.13±0.01)^{d}
C17:1	*cis*-10-heptadecenoic	(0.11±0.01)^{a}	(0.18±0.00)^{c}	(0.21±0.02)^{d}	(0.26±0.01)^{e}	(0.49±0.01)^{f}	(0.11±0.01)^{a}	(0.15±0.01)^{b}	–
C18:1n9t	(E)-octadec-9-enoic	(0.03±0.00)^{a}	(0.05±0.00)^{a}	(0.01±0.00)^{a}	(0.04±0.00)^{a}	(0.57±0.05)^{c}	(0.05±0.00)^{a}	(0.02±0.00)^{a}	(0.35±0.01)^{b}
C18:1n9c	(Z)-octadec-9-enoic	(17.25±0.41)^{f}	(10.41±0.67)^{b}	(16.32±0.20)^{e}	(11.70±0.08)^{c}	(21.25±0.13)^{g}	(3.90±0.04)^{a}	(12.60±0.16)^{cd}	(13.43±0.23)^{d}
C20:1n9c	(Z)-icos-11-enoic	(2.16±0.05)^{a}	(2.58±0.14)^{b}	(6.28±0.44)^{g}	(3.39±0.07)^{d}	(4.17±0.03)^{f}	(3.02±0.04)^{c}	(3.55±0.03)^{de}	(3.71±0.03)^{e}
C22:1n9	(Z)-docos-13-enoic	(1.90±0.02)^{c}	(2.57±0.04)^{de}	(3.66±0.35)^{f}	(5.43±0.05)^{g}	(2.69±0.11)^{e}	(0.11±0.01)^{a}	(1.37±0.01)^{b}	(2.44±0.05)^{d}
C24:1n9	(Z)-tetracos-15-enoic	(0.24±0.01)^{a}	(0.46±0.01)^{c}	(0.33±0.02)^{b}	(0.22±0.01)^{a}	(0.56±0.03)^{d}	(0.87±0.03)^{e}	(0.85±0.03)^{e}	(0.35±0.03)^{b}
C18:2n6t	Octadeca-9,12-dienoic	(0.03±0.00)^{a}	(0.04±0.00)^{ab}	(0.22±0.01)^{f}	(0.15±0.01)^{e}	(0.03±0.00)^{a}	(0.05±0.01)^{bc}	(0.06±0.01)^{c}	(0.11±0.01)^{d}
C18:2n6c	(9Z,12Z)-octadeca-9,12-dienoic	(36.96±0.23)^{g}	(8.05±0.48)^{b}	(14.95±0.43)^{d}	(23.79±0.25)^{e}	(31.26±0.07)^{f}	(14.84±0.14)^{d}	(6.26±0.07)^{a}	(10.35±0.44)^{c}
C18:3n6	Octadeca-6,9,12-trienoic	(0.04±0.01)^{a}	(0.28±0.01)^{b}	(2.76±0.07)^{c}	(0.05±0.00)^{a}	(0.06±0.00)^{a}	(0.12±0.01)^{a}	(0.23±0.02)^{b}	(0.06±0.01)^{a}

Table 3. – continued

Unsaturated fatty acid		Sample							
		1	2	3	4	5	6	7	8
		w(fatty acid)/%							
C20:2n6	Icosa-11,14-dienoic	(0.08±0.00)^{a}	(0.28±0.01)^{b}	(0.09±0.01)^{a}	(0.94±0.06)^{e}	(0.76±0.01)^{d}	(0.04±0.00)^{a}	(0.55±0.03)^{c}	(1.73±0.10)^{f}
C20:3n6	(11Z,14Z,17Z)-icosa-11,14,17-trienoic	(2.09±0.03)^{c}	(0.03±0.00)^{a}	(0.06±0.00)^{a}	(0.02±0.00)^{a}	–	(2.10±0.05)^{c}	(2.46±0.05)^{d}	(0.40±0.05)^{b}
C20:4n6	(5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoic	(0.02±0.00)^{a}	(0.18±0.02)^{b}	(0.07±0.01)^{a}	(0.05±0.00)^{a}	(0.26±0.01)^{c}	(0.17±0.01)^{b}	(0.31±0.03)^{c}	(0.19±0.03)^{b}
C22:2n6	Docosa-13,16-dienoic	(0.31±0.01)^{a}	(0.42±0.05)^{b}	(1.16±0.35)^{e}	(0.44±0.03)^{b}	(0.62±0.03)^{c}	(0.41±0.02)^{b}	(0.77±0.04)^{d}	(0.58±0.02)^{c}
C18:3n3	(9Z,12Z,15Z)-octadeca-9,12,15-trienoic	(0.17±0.01)^{a}	(40.70±0.32)^{f}	(0.15±0.01)^{a}	(16.85±0.16)^{b}	(0.29±0.01)^{a}	(29.81±0.39)^{d}	(39.18±0.22)^{e}	(25.38±0.83)^{c}
C20:3n3	(11Z,14Z,17Z)-icosa-11,14,17-trienoic	(0.32±0.01)^{a}	(0.41±0.02)^{b}	(0.44±0.01)^{b}	(0.21±0.00)^{a}	(1.83±0.06)^{e}	(0.43±0.06)^{b}	(0.94±0.09)^{d}	(0.67±0.01)^{c}
C20:5n3	(5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenoic	(0.10±0.01)^{b}	(0.05±0.01)^{a}	(0.13±0.01)^{b}	(0.12±0.01)^{b}	(0.05±0.00)^{a}	(0.05±0.01)^{a}	(0.10±0.00)^{b}	(0.23±0.01)^{c}
C22:6n3	Docosa-4,7,10,13,16,19-hexaenoic	(0.29±0.03)^{e}	(0.12±0.01)^{cd}	(0.10±0.01)^{bc}	(0.04±0.00)^{a}	(0.06±0.00)^{b}	(0.16±0.03)^{d}	(0.07±0.01)^{ab}	(0.10±0.00)^{bc}
w(total)/%		62.74	67.54	48.26	64.68	65.35	57.76	70.29	61.34
w(unsaturated FAs)/*w*(saturated FAs)		1.68	2.08	0.94	1.83	1.89	1.38	2.39	1.59

Values in the same row with different letters in superscript are statistically significant ($p<0.05$)
*geographical and botanical origin of the samples are given in Table 1; FA=fatty acid

significantly different from each other. (9Z,12Z)-octadeca--9,12-dienoic, hexadecanoic and (Z)-octadec-9-enoic acids were detected in the cotton sample from Adana at a higher level than in that from Urfa. On the contrary, (9Z,12Z,15Z)--octadeca-9,12,15-trienoic acid content of the cotton sample from Urfa was extremely high (40.7 %) and in that from Adana it was found only in traces (0.17 %). The dominant fatty acid in the cotton sample from Adana was (9Z,12Z)-octadeca-9,12-dienoic acid (36.9 %), while (9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid was the most abundant in the cotton sample from Urfa (40.7 %), which also had the second highest unsaturated fatty acid content (67.5 %), after the clover sample from Urfa (70.3 %). Total number of FAs identified in the citrus bee bread samples from Adana and Mersin was 33 and 34, respectively. Citrus bee bread from Adana was the only sample containing higher mass fraction of saturated FAs (51.6 %) than unsaturated FAs (48.3 %). Hexadecanoic acid fraction was the highest (38.7 %) in these samples, while tricosanoic acid was present only in the citrus bee bread sample from Mersin at 5.6 %.

Each of the clover bee bread samples obtained from the Urfa and Adana provinces contained 31 FAs that are mostly unsaturated. Fatty acid profiles of the samples were slightly different from one another. Undecanoic and heptadecanoic acids were barely detected in the clover bee bread sample from Urfa, while two of the saturated fatty acids, octanoic and tricosanoic acids, were found only in the clover bee bread sample from Adana.

Thirty-four fatty acids were identified in the chestnut bee bread sample. (11Z,14Z,17Z)-icosa-11,14,17-trienoic acid (ω-6), detected in all other samples, was not present in chestnut bee bread. However, the chestnut sample contained the highest mass fraction (1.8 %) of (11Z,14Z,17Z)--icosa-11,14,17-trienoic acid (ω-3) among the samples.

The most abundant fatty acid in sunflower bee bread was (9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid (29.8 %), followed by hexadecanoic acid (27.2 %). Sunflower bee bread contained the largest number of fatty acids. Only undecanoic acid was missing from this sample. It was the only sample with the highest dodecanoic acid content (6.15 %); other samples contained this acid at very low levels (between 0.05 and 0.29 %). Additionally, tridecanoic acid was detected solely in the sunflower bee bread.

The major FAs found in all bee bread samples were hexadecanoic (22.3–38.7 %), (9Z,12Z)-octadeca-9,12-dienoic (6.3–37 %), (Z)-octadec-9-enoic (3.9–21.2 %), octadecanoic (1.3 to 6.3 %) and (9Z,12Z,15Z)-octadeca-9,12,15-trienoic (0.2–40.7 %) acids. However, there were statistically significant ($p<0.01$) differences in the types and mass fractions of FAs detected in the bee bread samples obtained from the different botanic origins.

A total of four ω-3 fatty acids, including (9Z,12Z,15Z)--octadeca-9,12,15-trienoic, (11Z,14Z, 17Z)-icosa-11,14,17--trienoic, (5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenoic and docosa-13,16-dienoic acids, were detected in bee bread samples and their mass fractions ranged from 0.04 to 40.70 %. The total ω-3 fatty acid content was the highest (41.3 %) in the cotton bee bread sample from Urfa and the lowest (0.8 %) in the citrus bee bread sample from Adana. Seven ω-6 fatty acids, including octadeca-9,12-dienoic, (9Z,-12Z)-octadeca-9,12-dienoic, octadeca-6,9,12-trienoic, icosa--11,14-dienoic, (11Z,14Z,17Z)-icosa-11,14,17-trienoic, (5Z,-

8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoic and docosa-13,16--dienoic acids, were detected in the samples investigated in this study. The content of total ω-6 fatty acids varied between 9.3 and 39.5 %. The highest mass fraction of total ω-6 fatty acids (39.5 %) was determined in the cotton bee bread sample from Adana, but the lowest in the cotton from Urfa (9.3 %). Five ω-9 FAs found in the samples were: (Z)-octadec-9-enoic, (E)-octadec-9-enoic, (Z)-docos--13-enoic, (Z)-icos-11-enoic and (Z)-tetracos-15-enoic acids. The total ω-9 content of the bee bread samples was between 9.6 (sunflower from Edirne) and 28.1 % (citrus from Adana). The unsaturated to saturated FA ratio ranged from 1.38 to 2.39. Total unsaturated FA content was higher than that of saturated FAs in all of the bee bread samples, except in the citrus sample from Adana.

According to our results, cotton bee bread samples from Adana contained hexadecanoic, tetradecanoic, octadecanoic (saturated acids) and (Z)-octadec-9-enoic, (9Z,-12Z)-octadeca-9,12-dienoic, (Z)-icos-11-enoic, (11Z,14Z,17Z)--icosa-11,14,17-trienoic and (Z)-docos-13-enoic (unsaturated acids), while samples from Urfa had hexadecanoic, octadecanoic, heneicosanoic, tricosanoic (saturated acids), and (Z)-octadec-9-enoic, (9Z,12Z)-octadeca-9,12-dienoic, (9Z,12Z,-15Z)-octadeca-9,12,15-trienoic, (Z)-icos-11-enoic and (Z)--docos-13-enoic (unsaturated acids) in the higher mass fractions than other fatty acids. Although having the same botanical origin, the geographical origin affected the quantity of fatty acids to a large extent. For example, cotton samples from Adana contained 36.96 % (9Z,12Z)-octadeca-9,12-dienoic acid and cotton samples from Urfa 8.05 %.

Similarly, the citrus samples from both Mersin and Adana contained all the following: butanoic, hexadecanoic, octadecanoic, icosanoic (saturated) acids, and (Z)-octadec-9-enoic, (9Z,12Z)-octadeca-9,12-dienoic, octadeca-6,9,-12-trienoic, (Z)-icos-11-enoic, (Z)-docos-13-enoic, (Z)-tetradec-9-enoic, heneicosanoic and docosa-13,16-dienoic (unsaturated) acids at significantly different percentages. Besides, the Mersin sample, but not the Adana sample, contained tricosanoic and tetracosanoic acids. On the other hand, hexanoic acid was only detected in the Adana sample. It should be highlighted that cotton populations in Adana and Urfa where the beehives are located are very alike. Adana and Mersin are the commercial cotton and citrus production regions having similar citrus populations. Therefore, the differences in the compositions of the samples from these locations are not only related to the intensity of plant populations, but also to the preferences of honeybees. Szczęsna (*34*) published similar findings to ours, namely, Australian eucalyptus pollen contained (9Z,12Z)-octadeca-9,12-dienoic and (9Z,12Z,15Z)--octadeca-9,12,15-trienoic acids, whereas Italian eucalyptus pollen contained a higher mass fraction of (9Z,12Z)-octadeca-9,12-dienoic acid. (9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid was dominant in Brassicaceae pollen in the sample from Poland (*34*). In Korean and Chinese pollen samples (9Z,12Z)-octadeca-9,12-dienoic, hexadecanoic and octadeca-6,9,12-trienoic acids were detected at the highest mass fractions (*34*). In another study, Isidorov *et al.* (*35*) compared the chemical composition of bee bread samples from different countries and they contained large amounts of unsaturated fatty acids (linoleic and α-linolenic).

Omega-3 and ω-6 polyunsaturated fatty acids are both required for the body to function. Humans cannot synthesize them and therefore they must be obtained from the diet (*36*). Omega-3 fatty acids provide many beneficial effects such as anti-inflammatory function and prevention of cardiovascular diseases. Omega-6 fatty acids are also beneficial to human health. However, they have opposite effects on inflammatory response and cardiovascular health. Because they compete for the same enzymes to produce signalling molecules, they have opposing physiological functions. For example, while ω-6-derived molecules are proinflammatory, ω-3-derived signalling molecules are anti-inflammatory. Furthermore, they compete to incorporate into cell membranes. Therefore, the balance of ω-6/ω-3 fatty acids is important for human health. Modern Western diets have ω-6/ω-3 ratio of 15:1 or 20:1. It was concluded that while very high ω-6/ω-3 ratio promotes the pathogenesis of many diseases, a reduced ω-6/ω-3 ratio can prevent these diseases. In addition to the ratio 2:1, the ratio 3:1 suppressed inflammation in patients with rheumatoid arthritis, and the ratio 5:1 had a beneficial effect on asthma (*37*). Therefore, the optimal ratio may vary because chronic diseases are multigenic and multifunctional. Simopoulos (*38*) concluded in his review that a lower ratio of ω-6/ω-3 fatty acids is more desirable for reducing the risk of many diseases.

Conclusion

The pollen content, fatty acid composition, and chemical composition of bee bread samples from different botanical origins vary. Preferred or readily available plants for the bees as pollen source are also present in the bee bread samples, whereas others can be found in smaller amounts as a result of selective low preference.

The total amount of unsaturated fatty acids (FAs) is higher than the sum of saturated FAs found in all the samples except citrus sample from the Adana region. The results obtained in the current study confirmed that the bee bread can be considered as a good source of unsaturated FAs. The fatty acid content of bee bread is very important for the honeybees and PUFAs are essential for a healthy body development and productivity. However, unsaturated FAs are not essential just for the bees but also for the human nutrition. The unique results of this study can thus be used as a reference for research into the bee and also human health. The findings can also provide a scientific basis for the nutritional value assessment of the bee bread, thereby making contribution to the food composition database.

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