

## An Evaluation on Bee Bread: Chemical and Palynological Analysis

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### A B S T R A C T

Beebread is important protein source for honeybees in early spring. Chemical composition of beebread may vary according to geographical origin. In this study, proximate and fatty acids composition of five Citrus beebread samples were determined obtained from different geographical origins. Moisture content of citrus beebread samples varied between 11.0-16.4 %, ash 1.86-2.4 %, fat 7.0-13.4 %, and protein between 18.6-21.6 %. A total of thirty-seven fatty acids (FAs) were identified and of these palmitic, stearic, arachidic, oleic, eicosenoic, erucic, and linoleic acids were the most abundant in all of the samples. Beebread sample ratios of unsaturated/saturated FAs were ranged between 1.28 and 2.23 indicating that citrus beebread is a good source of unsaturated FAs. The pollen, proximate and fatty acid composition of beebread may vary significantly according to its geographical origin and citrus beebread is good source of unsaturated fatty acids.

Keywords: Citrus, beebread, pollen, fatty acid, pollen analysis, chemical composition

### Introduction

Pollen, which is one of the bee products, is the reproduction units that occur in male organs of flowering plants [1]. When pollens are collected from plants by bees, they are usually adhered to with some saliva to form pellets. The pollen is carried in the corbicula and brought to the hive. This new product differentiated from flower pollen is called "bee pollen". Bee pollen is the most important nutrient that provides the protein, lipid, sterol, vitamins and minerals necessary for honeybees to grow sufficiently after larvae and for the adequate development of their tissues, muscles, glands and other organs [2]. When honeybees carry the pollen they collect into the beehive, they store it in the form of a bee bread (perga) in the honeycomb cells. While the bee bread is produced in the hive, it is mixed with pollen, honey and other bee

secretions and subjected to lactic acid fermentation. The mixture turns into bee bread in about two weeks, so that the bee bread, which is a fermented product, can be kept in the hive for a long time [3,4]. Bee bread is the source of protein, fat and vitamins for the feeding of bees. Although the contents of bee pollen and bee bread are similar, there are some differences. Bee bread contains less protein than bee pollen, but bee bread proteins are easier to digest [4,5].

Lipids are important to honeybees primarily as a source of energy with some components of lipids involved in the synthesis of reserve fat and glycogen and the membrane structure of cells [6]. Pollen is virtually the only source of lipid in the honeybee diet. Lipid components such as fatty acids are important in honeybee

development, nutrition and reproduction. The bee pollen and bee bread have been studied for fatty acid composition. Soldberg & Remedios [7] reported that ether extracts of pollen consisted mainly of linoleic, linoleic and arachidonic acids. Human & Nicolson [8] investigated the amino acid and fatty acid composition of fresh, bee collected and stored pollen. In another study, Ceksteryte et al. [9] identified twenty-four fatty acids in the bee bread collected in spring and summer, and oleic and arachidonic acids were found to be the most abundant unsaturated fatty acids. Moreover, Ceksteryte, & Jansen [10] studied fatty acid composition in bee bread collected from various floral origins and linolenic acid was reported to be the highest within fatty acids identified in spring bee bread.

The Citrus genus is the most important of all Turkish plants to the beekeeping industry. At the beginning of spring, beekeepers migrate to the Mediterranean

region to prepare their bees for the season. Also, in early spring, Citrus nectar and pollen have great importance for the development of bees in the region [11]. Bees collect plenty of citrus pollen and use them to produce bee bread.

Bee bread is an important nutrient for bees and recently gained importance in human nutrition due to its rich nutrient content and useful biological properties such as hypolipidemic, antimicrobial, and antioxidant [12-14]. People nowadays takes into account the nutritional activities as versatile, to be protected from certain diseases and natural nutrition for treatment has come to the fore. Studies in the literature about the bee bread are quite a few. It is the first study on fatty acid composition of citrus bee bread from different geographical origin. Therefore, the aim of this study is to investigate the pollen content, fatty acid and proximate composition of Citrus bee bread samples collected from various geographic regions.

## Material and Methods

### *Bee bread samples*

A total of five bee bread samples were collected from apiaries located in two major citrus growing provinces, Adana and Mersin (Mediterranean Region of Turkey) during 2014. Two of the samples were from Adana (Seyhan and Kozan) and three samples from Mersin (Erdemli, Silifke and Tarsus). Bee bread samples were collected manually from honeycombs and stored in a deep-freezer at -20 °C before analyses.

### *Chemicals*

All chemical reagents were purchased from Sigma-Aldrich-Fluka Co. Ltd. (Steinheim, Germany), unless otherwise stated. N,O-Bis (trimethylsilyl) trifluoroacetamide,

trimethyl chlorosilane, 5 $\alpha$ -cholastene-3- $\beta$ -ol and fatty acid methyl esters (FAME) mixture was obtained from ABCR (Karlsruhe, Germany), Merck (Darmstadt, Germany), Alfa Aesar (Karlsruhe, Germany) and Supelco (Bellefonte, U.S.A), respectively. Potassium hydroxide and anhydrous sodium sulphate were purchased from Merck (Darmstadt, Germany).

### *Pollen analysis*

A 10 g of bee bread 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 discarded. The sediment was redissolved in 20 ml of distilled water to

completely dissolve the remaining sugar crystals then centrifuged for 5 min at 1.000 g. The excess water in the sediment was removed by placing it on an absorbent paper. 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 at 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 Nikon 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 taxon is expressed as percentage of the total pollen sum [15].

#### *Chemical analysis*

Determination of ash, crude fat and crude protein in bee bread samples was carried out using standard analytical procedures, Association of Official Analytical Chemists [16], 920.153, 991.36, and 960.52 respectively. Moisture content was determined using a vacuum oven at 60 °C and weighing until a constant weight. The results were expressed in grams per 100 g of fresh weight. The ash content was determined gravimetrically following incineration in an oven at 550°C and weighing until constant weight. Nitrogen determination was performed using micro-Kjeldahl method. Then a conversion factor of 6.25 was used for converting percentage of nitrogen in the sample into percentages of protein. All analyses were carried out in triplicate.

Determination of oil content in bee bread samples was performed using the standard method of ISO 659 [17]. The bee bread samples were homogenized in a stainless steel Waring blender. A of 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. Diethyl ether was used for the extraction of oil from bee breads at 50 °C for 3 h using automated Soxhlet extractor (VELP Scientifica Ser 148, Italy). Oil extracted from bee bread samples was kept in amber vials prior to fatty acid analysis.

#### *Determination of Fatty Acid Composition*

The ISO 12966-2 [18] standard method was used for the determination of fatty acid methyl esters (FAME) in oils of bee bread samples. In brief, 0.1 g bee bread oil was weighed into test tube with screw cap and 0.5 mL of methanolic 2 M KOH and 5 mL heptane were added and vortexed. Then, the upper layer was dried with anhydrous sodium sulphate for gas chromatography analysis. Chromatographic analysis was carried out by a Perkin Elmer Clarus 500 (Perkin-Elmer, Shelton, CT, USA) gas chromatograph equipped with an auto sampler, split-splitless injector and a flame ionization detector (FID). Chromatographic separation was achieved on a Supelco 2380 capillary column (100 m x 0.25 mm i.d., 0.2 µm film thickness). Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The injector and detector temperatures were set at 250 °C and 260 °C respectively.

The temperature programme was started and held at 165 °C for 5 min then increased to 240 °C at a rate of 5°C/min and finally held at 240 °C for 10 min. The injection volume was 1.0 µL and the split ratio was set at 1:50. Identification of the peaks was performed by comparing their relative retention times with those of the standard FAME mixture. The results were expressed as percent of total FAMES.

## Results

The results of pollen analyses showed that bee bread samples contained *Citrus* spp. pollen at a range of 62.87-98.44 %. Other pollens belonging to Asteraceae, Fabaceae and Brassicaceae families were observed in the samples at minor and rare levels (Table 1)

Table 1. Results of palynological analysis of citrus beebread samples (n=5)

Sample	Geographical origin	% pollen
SeC	Seyhan (Adana)	78.02
KC	Kozan (Adana)	85.43
TC	Tarsus (Mersin)	62.87
EC	Erdemli (Mersin)	98.44
SiC	Silifke (Mersin)	89.39

The moisture content of bee bread samples collected from Adana region were between 15.82-16.43 % and the samples from Mersin region contained lower moisture ranging between 11.01 and 12.34 %. Similarly, Adana bee bread samples contained higher fat content ranged between 10.47 and 13.46 % than Mersin samples ranged between 7.06 and 9.22 %. The ash and protein content of Adana samples were between 1.86-2.03 and 19.71-19.83 respectively whereas Mersin samples had slightly higher content of ash and

## Statistical analysis

All chemical assays were carried out in triplicate and the data were expressed as means  $\pm$  standard deviations (SD). One-way analysis of variance (ANOVA) followed by least significant difference (LSD) was used to comparison of the data. Differences between means at the 95 % (P < 0.05) confidence level were considered statistically significant.

protein at a range of 2.03 to 2.42 and 18.60 to 21.60 respectively (Table 2). Compared to the bee bread samples from different geographical origin, statistically significant differences were determined in moisture, protein and fat content (p> 0.05).

A total of 37 fatty acids identified and quantified in *Citrus* bee bread samples (Table 3). The quantities of these twelve fatty acids determined were similar and there was no statistically significant difference between the samples (p>0.05). The saturated fatty acids including palmitic, stearic and arachidic determined in high amounts were common to all samples. The common unsaturated ones were oleic, cis eicosenoic, erucic and linoleic acids. Palmitic acid to saturated fatty acids was found in the range of 17.83-29.59 % in the bee bread samples studied. Of these unsaturated fatty acids, the amount of oleic acid ranged from 11.75 to 18.86%, cis eicosenoic acid from 2.24 to 8.51 %, erucic from 0.39 to 5.80 % and linoleic acid ranged from 14.19 to 32.41% in all of the citrus bee bread samples. There were no differences in terms of palmitic, stearic, erucic and oleic acids in the samples from different region. However, butyric, arachidic and oleic acids were found in

greater amounts in Adana samples. Unsaturated/saturated ratio of fatty acids in

all samples was determined in the range of 1.28 and 2.23.

Table 2. Chemical composition of citrus bee bread samples (n=5)

Bee bread samples	Moisture	Ash	Protein	Fat
SeC	15.82±0.01 <sup>d</sup>	2.03±0.01 <sup>b</sup>	19.71±0.01 <sup>b</sup>	10.47±0.04 <sup>c</sup>
KC	16.43±0.02 <sup>e</sup>	1.86±0.01 <sup>a</sup>	19.83±0.02 <sup>c</sup>	13.46±0.03 <sup>d</sup>
EC	11.01±0.04 <sup>a</sup>	2.42±0.01 <sup>c</sup>	21.60±0.02 <sup>e</sup>	7.06±0.34 <sup>a</sup>
MC	12.34±0.02 <sup>c</sup>	2.42±0.01 <sup>c</sup>	20.09±0.02 <sup>d</sup>	9.15±0.03 <sup>b</sup>
SiC	11.86±0.01 <sup>b</sup>	2.03±0.01 <sup>b</sup>	18.60±0.02 <sup>a</sup>	9.22±0.07 <sup>b</sup>

Data: Arithmetical mean ±SD. <sup>a-e</sup> The groups in the same row with different letters are statistically significant (p < 0.05).

## Discussion

Citrus honey is regarded as one of the best unifloral honey due to its unique taste and floral aroma [19]. It is also economically important for migratory beekeeping in Anatolia as well as local beekeeping in the Mediterranean region. In a several studies, citrus pollens found as "under represented" in citrus honeys from different countries [11,20]. It is attributed to anther maturity does not always coincide with the maximum secretion of nectar [21]. However, Citrus pollen was represented predominantly in bee bread samples analyzed in this study. The honeybees need to increase their population, in early spring in order to have a productive season. In this season, the flora is not diverse and rich yet and therefore, the vast majority of the beekeepers move their hives to the Mediterranean region where plenty of citrus pollen and nectar available for the development of bees.

Bee bread samples showed differences in the chemical analysis. Despite being both Mersin and Adana provinces located in the Mediterranean region with high-density citrus orchards, moisture content of the bee bread samples collected from Mersin was lower than the samples collected from Adana. This can be explained as the

influence of environmental factors on the chemical composition of bee bread. Similarly, fat content of bee bread samples from Adana was found lower than Mersin samples. Nevertheless, the protein content of citrus bee bread samples collected from Mersin was higher than the beebread samples of Adana. In a study, the moisture contents ranged between 18.8 and 28.0%, protein content between 19.3 and 26.5%, ash content ranged from 2.1 to 3.2 % and lipid content changed between 3.9 and 6.7% of bee bread samples [22]. In another study, HTF (hot-water fraction), WSF (water soluble fraction) and ESF (ethanol soluble fraction) of bee bread from Lithuania were extracted and the protein contents of these fractions were 2.29 mg/ml, 9.59 mg/ml and 1.94 mg/ml, respectively [11]. A review by Roulston and Cane [23] of ether extractable material from dry pollen of 62 plant species showed the percent lipid in pollen to vary from 0.8 % (*Eucalyptus marginate*), to 18.9 % for dandelion (*Taraxacum officinale*). According to the obtained data, differences in the chemical composition of bee bread samples may vary depending on botanical origin, environmental and geographical factors and extraction methods.

Table 3. Fatty acid composition of citrus beebread samples from different regions.

Saturated Fatty Acids		Fatty acid contents (%)				
		Adana Province		Mersin Province		
		SeC	KC	TC	EC	SiC
C4:0 Butyric acid		1.34±0.01 <sup>c</sup>	1.87±0.03 <sup>d</sup>	0.37±0.01 <sup>a</sup>	0.83±0.01 <sup>b</sup>	1.30±0.07 <sup>c</sup>
C6:0 Caproic acid		0.36±0.00	0.09±0.01	-	0.13±0.01	-
C8:0 Caprylic acid		0.09±0.02 <sup>b</sup>	0.09±0.01 <sup>b</sup>	0.02±0.00 <sup>a</sup>	0.29±0.01 <sup>c</sup>	0.06±0.01 <sup>b</sup>
C10:0 Capric acid		0.05±0.01	0.67±0.06	0.16±0.01	-	0.11±0.01
C11:0 Undecanoic acid		-	0.09±0.00	-	-	0.08±0.00
C12:0 Lauric acid		0.28±0.00 <sup>abc</sup>	0.60±0.02 <sup>d</sup>	0.11±0.01 <sup>ab</sup>	0.08±0.00 <sup>a</sup>	0.47±0.02 <sup>bc</sup>
C13:0 Tridecanoic acid		-	0.07±0.00	-	-	-
C14:0 Myristic acid		0.52±0.01 <sup>ab</sup>	0.65±0.02 <sup>ab</sup>	0.22±0.01 <sup>a</sup>	0.56±0.01 <sup>ab</sup>	0.85±0.04 <sup>b</sup>
C15:0 Pentadecanoic		0.20±0.01 <sup>bc</sup>	0.09±0.06 <sup>a</sup>	0.14±0.01 <sup>ab</sup>	0.28±0.01 <sup>c</sup>	0.13±0.01 <sup>ab</sup>
C16:0 Palmitic acid		29.59±0.18 <sup>b</sup>	17.83±8.39 <sup>a</sup>	22.49±0.29 <sup>a</sup>	23.20±0.06 <sup>a</sup>	27.18±1.34 <sup>a</sup>
C17:0 Heptadecanoic acid		0.49±0.01	0.49±0.00	0.33±0.01	0.24±0.00	0.32±0.00
C18:0 Stearic acid		6.56±0.05 <sup>b</sup>	3.27±0.06 <sup>a</sup>	2.35±0.03 <sup>a</sup>	2.57±0.01 <sup>a</sup>	3.34±0.00 <sup>a</sup>
C20:0 Arachidic acid		3.35±0.01	6.08±0.04	1.07±0.01	0.91±0.00	1.40±0.06
C21:0 Heneicosanoic		0.45±0.02 <sup>b</sup>	0.08±0.00 <sup>a</sup>	1.80±0.01 <sup>c</sup>	0.08±0.01 <sup>a</sup>	0.24±0.01 <sup>ab</sup>
C22:0 Behenic acid		0.09±0.00 <sup>a</sup>	0.60±0.05 <sup>b</sup>	0.41±0.02 <sup>ab</sup>	1.25±0.02 <sup>c</sup>	0.36±0.07 <sup>ab</sup>
C23:0 Tricosanoic ac		0.43±0.01 <sup>a</sup>	2.16±0.08 <sup>a</sup>	5.79±0.12 <sup>b</sup>	0.51±0.02 <sup>a</sup>	0.55±0.02 <sup>a</sup>
C24:0 Lignoceric acid		0.13±0.01	1.29±0.08	0.04±0.00	-	0.28±0.01
<b>Total</b>		<b>43.93</b>	<b>36.02</b>	<b>35.3</b>	<b>30.93</b>	<b>36.67</b>
Unsaturated Fatty Acids	$\omega$	AC	KC	MC	EC	SC
C14:1 Myristoleic acid	$\omega$ -5	1.18±0.01 <sup>b</sup>	1.17±0.06 <sup>b</sup>	0.76±0.01 <sup>ab</sup>	1.58±0.01 <sup>b</sup>	0.67±0.01 <sup>a</sup>
C15:1 cis-pentadecanoic acid		0.16±0.01	0.40±0.02	0.09±0.01	0.11±0.01	0.48±0.02
C16:1 Palmitoleic acid	$\omega$ -7	0.12±0.01 <sup>a</sup>	0.12±0.01 <sup>a</sup>	0.15±0.00 <sup>b</sup>	0.22±0.00 <sup>c</sup>	0.13±0.00 <sup>a</sup>
C17:1 cis-Heptadecanoic acid		4.21±0.01	8.77±0.08	0.27±0.01	0.15±0.02	0.26±0.02
C18:1n9t Elaidic acid	$\omega$ -9	0.07±0.03	0.27±0.02	0.03±0.01	0.06±0.01	0.17±0.04
C18:1n9c Oleic acid	$\omega$ -9	16.29±0.19 <sup>ab</sup>	18.86±0.05 <sup>a</sup>	11.75±0.06 <sup>a</sup>	21.53±0.03 <sup>b</sup>	13.06±0.63 <sup>a</sup>
C20:1 cis-Eicosenoic	$\omega$ -9	8.51±0.12	8.58±0.06	2.24±0.59	2.57±0.01	2.32±0.09
C22:1n9 Erucic acid	$\omega$ -9	3.69±0.02 <sup>b</sup>	4.22±1.66 <sup>b</sup>	5.54±0.05 <sup>b</sup>	0.39±0.02 <sup>a</sup>	5.80±0.31 <sup>b</sup>
C24:1 Nervonic acid	$\omega$ -9	0.34±0.01 <sup>ab</sup>	0.34±0.01 <sup>ab</sup>	0.23±0.01 <sup>a</sup>	0.84±0.03 <sup>c</sup>	0.40±0.01 <sup>b</sup>
C18:2n6t Linolelaidic acid	$\omega$ -6	0.18±0.02	1.67±0.02	0.16±0.01	0.18±0.01	0.05±0.01
C18:2n6c Linoleic acid	$\omega$ -6	14.90±0.42 <sup>a</sup>	14.19±0.07 <sup>a</sup>	24.45±0.28 <sup>ab</sup>	32.41±0.09 <sup>b</sup>	28.53±1.38 <sup>b</sup>
C18:3n6 g-Linolenic acid	$\omega$ -6	2.80±0.05 <sup>b</sup>	0.08±0.01 <sup>a</sup>	0.06±0.01 <sup>a</sup>	0.07±0.01 <sup>a</sup>	0.10±0.00 <sup>a</sup>
C20: 2 cis-11,14-Eicosadienoic	$\omega$ -6	0.09±0.01 <sup>a</sup>	1.40±0.69 <sup>bc</sup>	0.96±0.06 <sup>ab</sup>	0.81±0.03 <sup>ab</sup>	2.14±0.09 <sup>c</sup>
C20: 3n6 cis-8,11,14-Eicosatrienoic acid	$\omega$ -6	0.07±0.01 <sup>a</sup>	0.51±0.03 <sup>a</sup>	0.03±0.01 <sup>a</sup>	1.85±0.20 <sup>b</sup>	0.02±0.01 <sup>a</sup>
C20:4n6 Arachidonic acid	$\omega$ -6	1.14±0.61 <sup>b</sup>	1.10±0.06 <sup>b</sup>	0.05±0.01 <sup>a</sup>	2.98±0.02 <sup>c</sup>	0.19±0.01 <sup>a</sup>
C22: 2 cis-13,16 Docosadienoic	$\omega$ -6	1.24±0.06 <sup>d</sup>	0.68±0.06 <sup>b</sup>	0.46±0.03 <sup>a</sup>	0.91±0.03 <sup>c</sup>	0.63±0.02 <sup>ab</sup>
C18:3n3 a-Linolenic acid	$\omega$ -3	0.11±0.03 <sup>a</sup>	0.71±0.03 <sup>a</sup>	17.22±0.19 <sup>c</sup>	0.29±0.07 <sup>a</sup>	6.78±0.11 <sup>b</sup>
C20: 3n3 cis-11,14,17-Eicosatrienoic acid	$\omega$ -3	0.52±0.05 <sup>ab</sup>	0.21±0.00 <sup>a</sup>	0.58±0.02 <sup>ab</sup>	1.11±0.07 <sup>b</sup>	0.88±0.02 <sup>b</sup>
C20: 5n3 cis-5,8,11,14,17-Eicosapentaenoic acid	$\omega$ -3	0.52±0.05 <sup>ab</sup>	0.88±0.02 <sup>b</sup>	0.21±0.00 <sup>a</sup>	0.58±0.02 <sup>ab</sup>	1.11±0.07 <sup>b</sup>
C22:6n3 Docosahexaenoic acid	$\omega$ -3	0.07±0.01 <sup>a</sup>	0.11±0.05 <sup>ab</sup>	0.04±0.00 <sup>a</sup>	0.43±0.02 <sup>c</sup>	0.23±0.01 <sup>ab</sup>
<b>Total</b>		<b>56.21</b>	<b>64.27</b>	<b>65.28</b>	<b>69.07</b>	<b>63.95</b>
Unsaturated/saturated		1.28	1.78	1.85	2.23	1.74

The lipid content of pollen is quite variable with values ranging from 0.8-18.9 % [23]. One important component of lipids for development of honeybees is fatty acids [6]. The nutrition aspect of fatty acids to honey bees is well known. Of the seven common fatty acids found in all bee bread examined in our study, palmitic, stearic, and arachidic acids are saturated fatty acids, while oleic, erucic and eicosenoic acids are mono saturated fatty acids (also known as omega 9) and linoleic acid (polyunsaturated, omega 6). In honeybees, pollen stimulates the development of the hypopharyngeal gland that is responsible for producing royal jelly used to feed larva [24]. Several studies were made on fatty acid related to royal jelly and the honeybee body. Royal jelly was found to be dominated by palmitic, oleic, linolenic and myristoleic acids all in amounts greater than 10 % of the total lipid [25]. In addition, they also showed that adult honeybee tissue had large amounts of oleic acid, whilst for 6 days old larvae, oleic and palmitic acids were equally dominant. Manning [26] reported that linoleic acid was present in merged bees and in adult bees, linoleic acid was the second highest fatty acid by concentration.

Studies on fatty acid content of pollen and bee bread are limited. Serra Bonvehi and Escola Jorda [27] stated that bees select pollen with a high level of unsaturated fatty acids, which are more adequate for honey metabolism. For example, oleic acid an important fatty acid honey bees. Some fatty acids such as linoleic, linolenic, myristic and dodecanoic acids are highly antimicrobial [28,29]. Ceksteryte et al. [9] reported oleic and arachidonic were found to be the most abundant unsaturated fatty acids, constituting around 15% of total fatty acids. Moreover, Ceksteryte, & Jansen [10] showed linolenic acid (n-3) was reported to

be the highest within twenty-two fatty acids identified in spring bee bread.

In our study, although having the same botanical origin, the geographical origin affected the quantity of fatty acids particularly. Citrus samples from both Mersin and Adana contained all the following palmitic, stearic, arachidic (saturated), oleic, linoleic, cis Eicosanoic, and erucic (unsaturated) acids at significantly different percentages. Therefore, the differences in the compositions of the samples from these locations are not only related to the intensity of plant populations, but also with the different chemical composition of species of Citrus genus and preferences of honeybees. Szczesna [30] published similar findings to ours for Eucalyptus bee pollen, namely, Australian eucalyptus pollen contained linoleic and linolenic acid, whereas Italian eucalyptus pollen contained a higher proportion of linoleic acid.

The total of unsaturated fatty acids was higher than the sum of saturated fatty acids found in all the samples. The fatty acid content of bee bread is very important because deficiency of polyunsaturated fatty acids on insects' diet may result in slow body development, deformed wings and lower productivity. Also, fatty acids release the required energy on the flight muscles during the flight [31]. However, unsaturated fatty acids are not just essential for bees but also for human nutrition. Unsaturated fatty acids have many beneficial health effects such as reducing the level of serum triglycerides [32]; possessing cardio protective properties through reducing blood cholesterol, triglyceride level and exerting an anti-arrhythmic, anti-thrombotic, anti-inflammatory impact [33]. In addition,

linoleic acid is an essential fatty acid required by humans and external source. It is reported in helping lower the ratio of low-density lipoproteins which carry such lipids as cholesterol from the body via the liver [34].

As a conclusion, the changes of the pollen content, fatty acid composition, and chemical composition of Citrus bee bread samples originated from different geographical origin were determined. The results revealed that Citrus plants preferred or readily available for bees as pollen resource was detected extensively in the bee bread samples. It is clearly seen from the results of current study that the proximate composition and the fatty acid composition of Citrus bee bread samples from different geographical origins varied significantly.

### Arı Ekmeği Üzerine Bir Değerlendirme: Kimyasal ve Pallinolojik Analiz

**Öz:** Arı ekmeği, ilkbaharda bal arıları için önemli bir protein kaynağıdır. Arı ekmeğinin kimyasal bileşimi coğrafik

orijine göre değişebilir. Bu çalışmada, farklı coğrafi orijinlerden elde edilen beş narenciye arı ekmeği örneğinin kimyasal ve yağ asitleri bileşimi belirlenmiştir. Narenciye arı ekmeği örneklerinin nem içeriği % 11.0-16.4, kül % 1.86-2.4, yağ % 7.0-13.4 ve protein % 18.6-21.6 arasında değişmiştir. Toplamda otuz yedi yağ asidi (FA) tanımlanmış ve bunlardan palmitik, stearik, araşidik, oleik, eikosinik, erüsik ve linoleik asitler tüm örneklerde en çok oranda bulunmuştur. Arı ekmeği örneklerinde doymamış/doymuş yağ asitleri oranları, 1.28 ile 2.23 arasında değişmiştir ve bu sonuç, narenciye arı ekmeğinin iyi bir doymamış yağ asidi kaynağı olduğunu göstermiştir. Arı ekmeğinin polen, kimyasal ve yağ asidi bileşimi, coğrafik orijine göre önemli ölçüde değişebilmektedir ve narenciye arı ekmeği iyi bir doymamış yağ asitleri kaynağıdır.

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