6TH APIMEDICA & 5TH APIQUALITY

Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri

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"Essential and omega fatty acid content of beebread and its effect on heart diseases"

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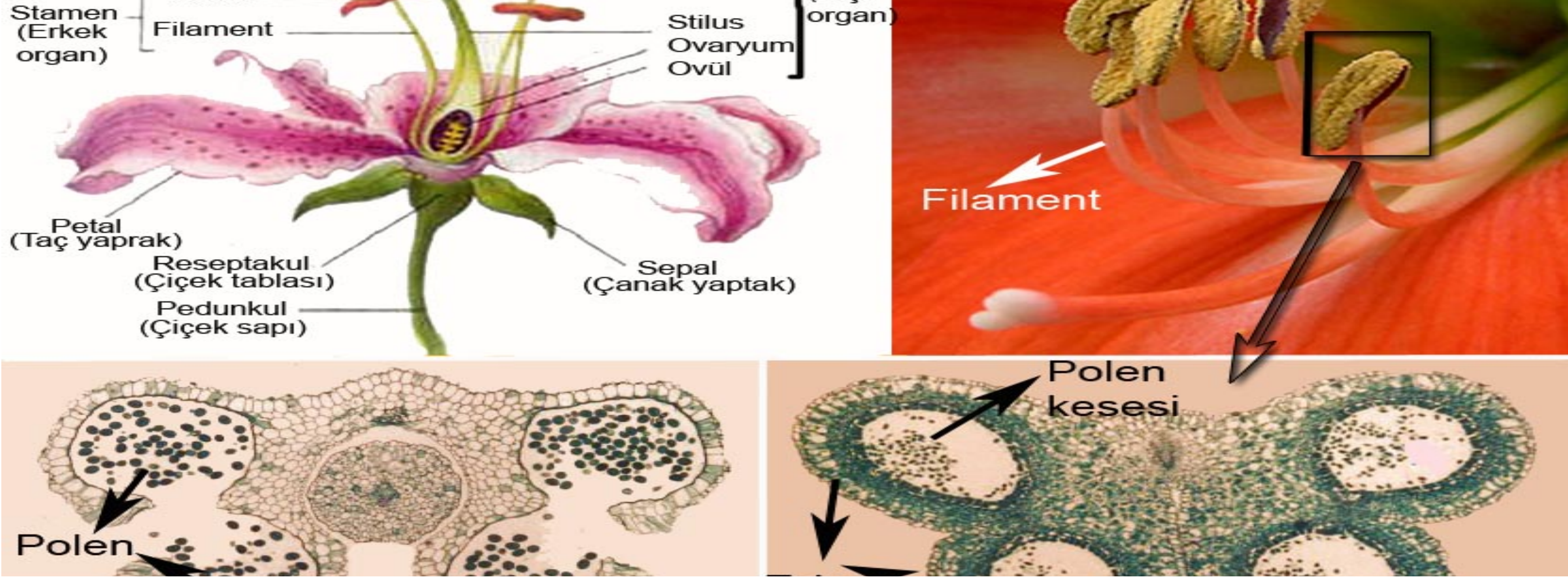


Pollen and nectar are essential components of honeybee, *Apis mellifera* L., diet. Nectar provides carbohydrates for honeybee, while pollen supplies protein, lipid, and vitamins

Pollen collected by foraging worker bees is combined with honeybee secretions

Beebread is processed pollen stored and packed by bees in the hexagonal cells of wax comb, with the addition of various enzymes and nectar or honey, which undergoes lactic acid fermentation

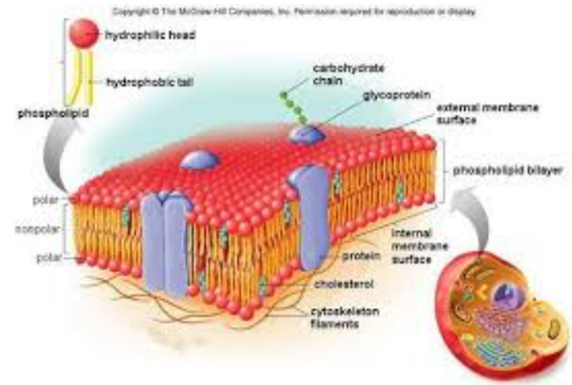
Lipids are important to honeybees primarily as a source of energy with some components of lipids involved in the synthesis of reserve fat and glycogen and the membrane structure of cells

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

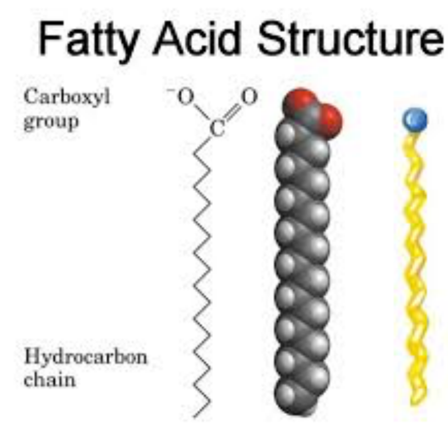
Beebread is about 20% proteins, 24-34% carbohydrates and 1,5 % of lipids. Beebread has a large variety of minerals and has high quantities of iron, cobalt, phosphorus, calcium. It is one of the richest natural foods containing selenium. Beebread is also an excellent source of potassium and B-group vitamins.

Bee-pollen is referred to as the “only perfectly complete food”, as it contains all the essential amino acids needed for the human organism

Fatty acids

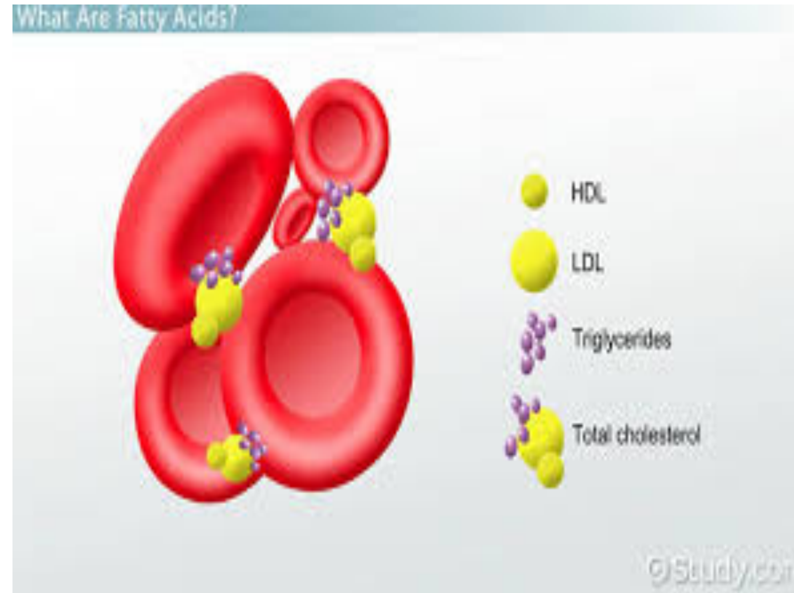


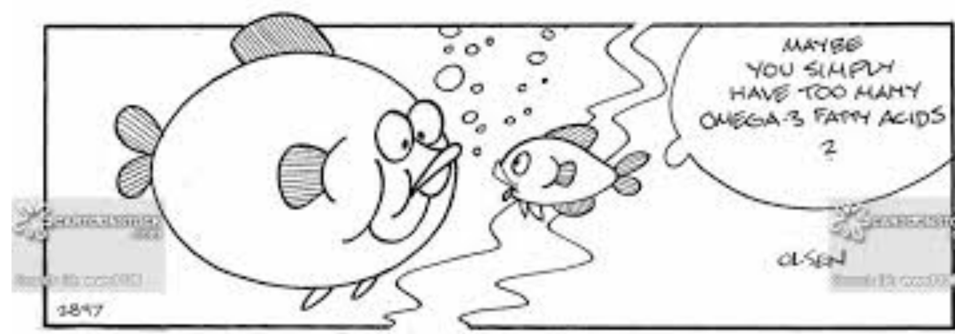
- ❖ Fatty acids are aliphatic carboxylic acids
- ❖ Have the general formula R-(CH2)n-COOH
- ❖ They occur mainly as esters in natural fats and oils but do occur in the unesterified fom as free fatty acids, a transport form found in the plasma
- ❖ Fatty acids that occur in natural fats
- ❖ The chain may be saturated (containing no double bonds) or unsaturated (containing one or more double bonds)



FUNCTIONS OF FATTY ACIDS

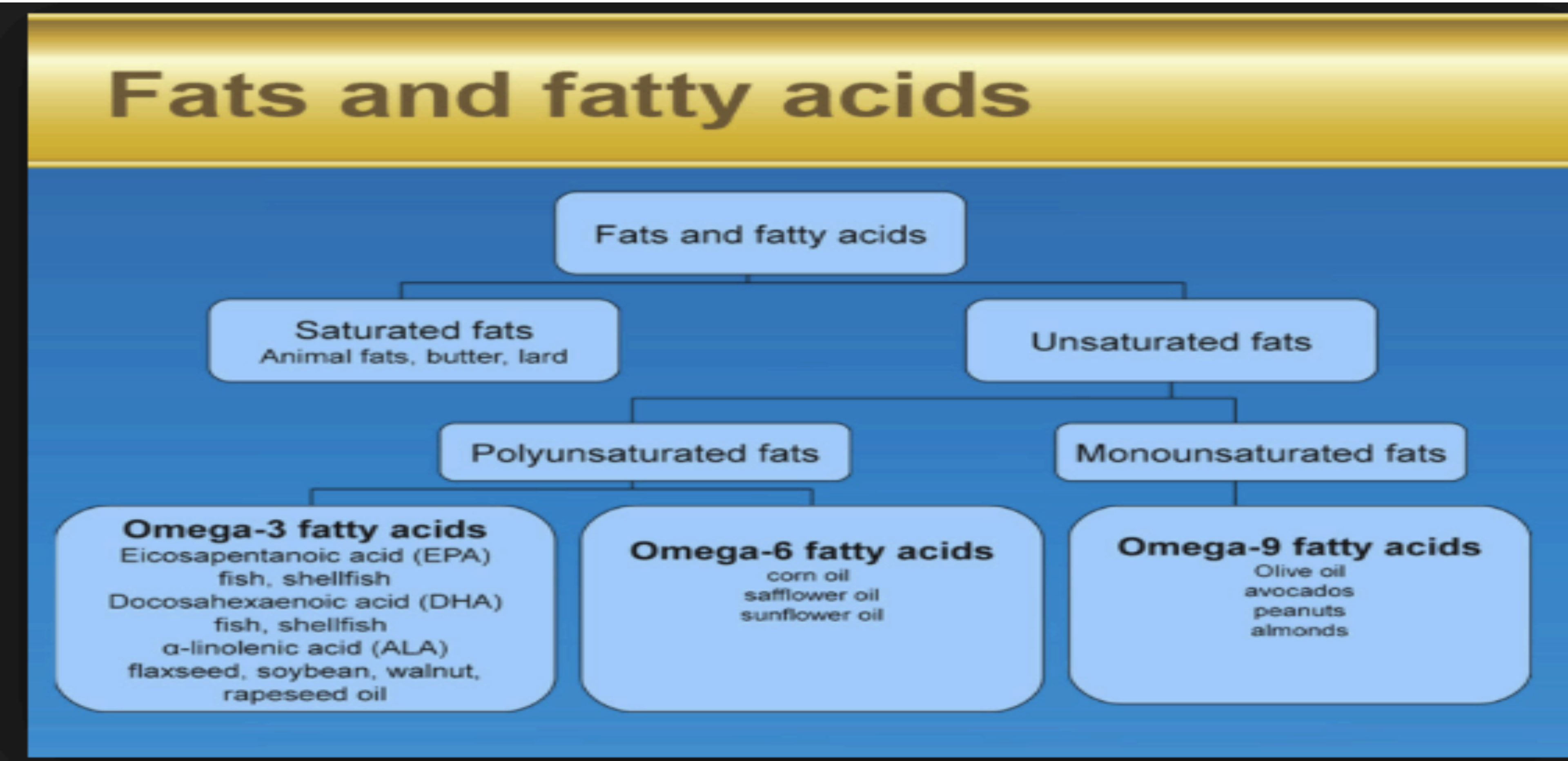
- ✧ Useful in the treatment of atherosclerosis by transport of blood cholesterol, triglycerides and lowering it
- ✧ Hormones are synthesized from them
- ✧ Enter in structure of all cellular and subcellular membranes and transporting plasma phospholipids
- ✧ Essential for skin integrity, normal growth and reproduction
- ✧ Important role in blood clotting (intrinsic factor)
- ✧ Important role in health of the retina and vision
- ✧ They can be oxidized for energy production



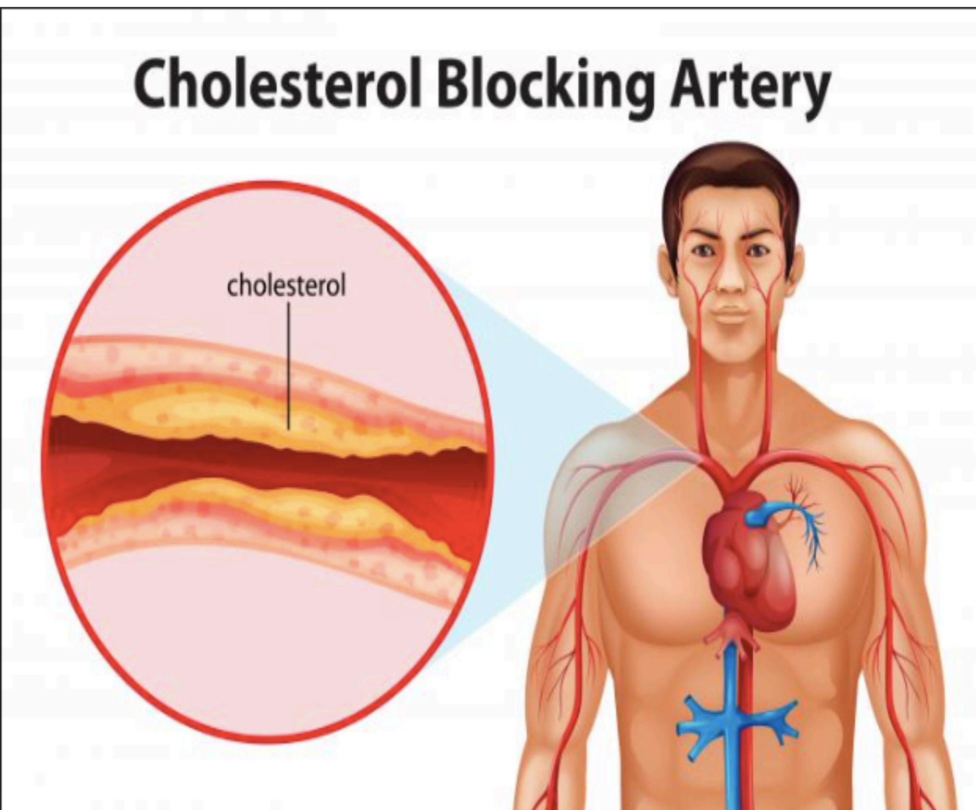


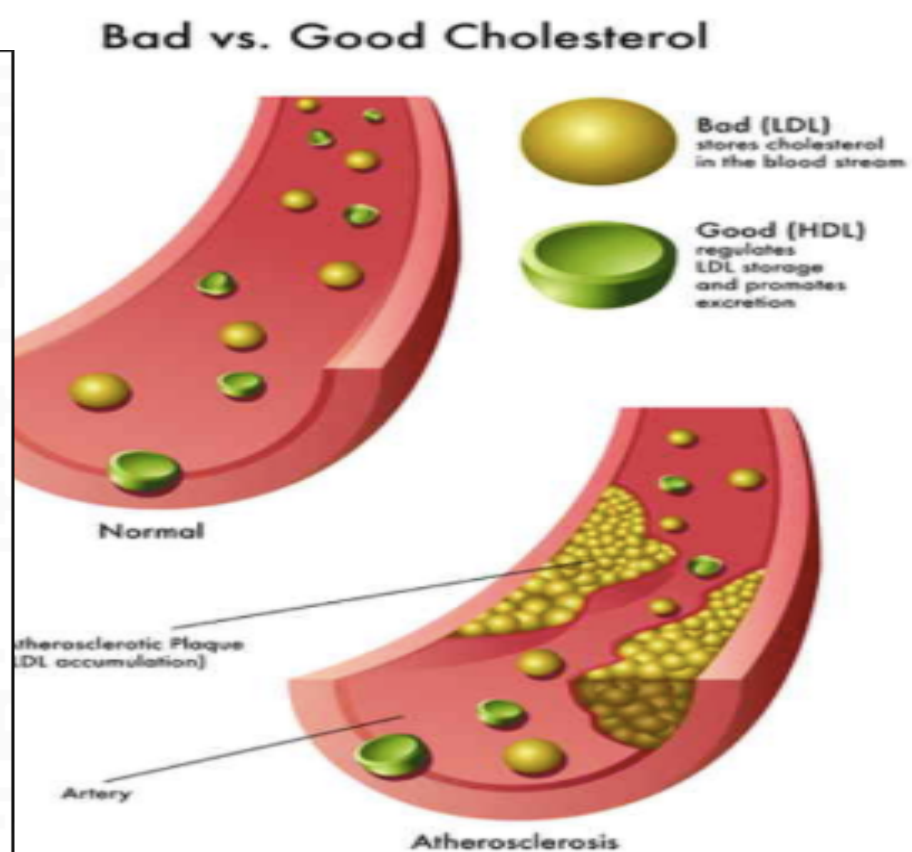
It is known that two types of fatty acids are considered essential. Omega-3 and omega-6 fatty acids cannot be synthesized in the human body, and must be obtained from the diet

These fatty acids are major structural components of membrane phospholipids, act as precursors to the biologically active eicosanoids, and influence membrane fluidity and ion transport across cell membranes



The omega-6 fatty acids are distributed evenly in most mammalian tissues, while the omega-3 fatty acids are concentrated in a few tissues. While the Omega-3 fatty acids increase bleeding time; decrease platelet aggregation, blood viscosity, and fibrinogen; and increase erythrocyte deformability, decrease low-density-lipoprotein (LDL) cholesterol, omega-6 fatty acids are known to be essential for growth and reproduction.





Current literature suggest that pollen and beebread are a good source of polyunsaturated fatty acids (PUFAs) that are essential for human nutrition and cannot be synthesized by the body

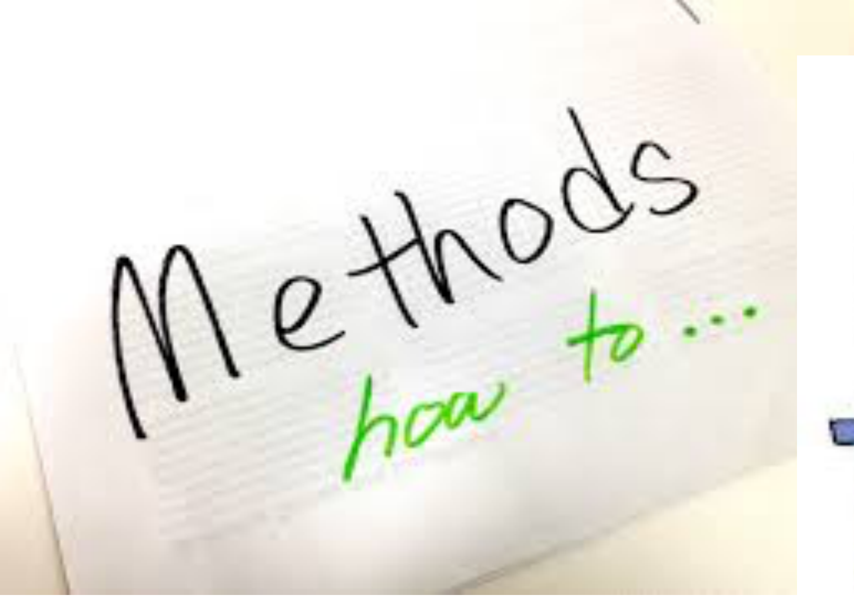
However, in particular, scientific research exploring various properties of beebread is scarce and additional research into this topic is highly required

Therefore, the aim of this study is to obtain and compare data on proximate composition, pollen composition and the fatty acid content of beebread samples harvested in Turkey


A total of eight beebread samples were collected from apiaries located in different monofloral honey production regions in Turkey between June and October of 2014

Beebread samples were hand collected from honeycombs and stored in a deep-freezer at -20 °C before analyses

The type of flora and the 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



Chemical analysis of beebread (ash, crude fat and crude protein) was carried out using standard analytical procedures, Association of Official Analytical Chemists 920.153, 991.36, and 960.52 respectively

Determination of oil content in beebread samples was performed using the standard method of ISO 659 The ISO 12966-2 standard method was used for the determination of fatty acid methyl esters (FAMEs) in oils of beebread samples

Table 1. Results of Palynological Analysis of Beebread Samples from Different Botanical Origins

Sample	Geographical origin	Botanical origin	pollen %	Other important pollen 3-15 %
Clover	Urfa	*Trifolium pratense, T. repens*	86.2	Fabacea
Clover	Adıyaman	*Trifolium pratense, T. repens*	85.6	Fabaceae
Cotton	Adana	*Gossypium hirsutum*	65.6	Fabaceae, Lamiaceae
Cotton	Urfa	*Gossypium hirsutum*	66.2	Fabaceae, Asteraceae Lamiaceae
Chestnut	Zonguldak	*Castanea sativa*	94.4	Fabacea
Chestnut	Adana	*Citrus* spp.	54.4	Fabaceae, Brassicaceae, Lamiaceae, Rhamnaceae, Rosacece
Citrus	Mersin	*Citrus* spp.	61.4	Fabaceae, Brassicaceae, Lamiaceae, Rhamnaceae, Myrtaceae
Sunflower	Edirne	*Helianthus annuus*	45.4	Fabaceae, Rosaceae, Apiaceae

Palynological spectrum, proximate and fatty acids composition of eight beebread samples obtained from different botanical origins were determined

They were all identified as monofloral with *Castanea sativa* (94.4 %), *Trifolium* spp. (85.6 %), *Gossypium hirsutum* (66.2 %), *Citrus* spp. (61.4 %) and *Helianthus annuus* (45.4 %)

Beebread moisture contents varied between 11.4-15.9 %, ash 1.9-2.54 %, fat 5.9-11.5 %, and protein between 14.8-24.3 %.

To our knowledge, this is the first study investigating fatty acids (FAs) composition of the selected beebreads

Fatty Acid Composition of Beebread Samples

	Saturated fatty acid	w (fatty acid) (%)							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
C4:0	Butanoic	(0.75±0.01)[c]	(0.65±0.01)[b]	(1.29±0.46)[c]	(0.37±0.01)[a]	(1.16±0.01)[c]	(1.03±0.04)[d]	(1.06±0.02)[d]	(1.30±0.41)[c]
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)[c]	-	(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)[c]	(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)[c]
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)[c]	(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)[c]	(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)[c]
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)[c]
C21:0	Heneicosanoic	(0.04±0.00)[a]	(1.17±0.04)[d]	(0.05±0.00)[a]	(1.70±0.04)[c]	(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)[c]	(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)[c]	(0.06±0.01)[d]	-	-
	***W* (total)%**	**37.28**	**32.48**	**51.59**	**35.25**	**34.51**	**41.97**	**29.37**	**38.67**

Fatty Acid Composition of Beebread Samples

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}	-
C18:1n9	(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}
C18:1n9	(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)^{c}	(13.43±0.23)^{d}
C20:1n9	(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}
C18:2n6	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}
C18:2n6	(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}
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)^{c}	(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 fatty acids)%**	**62.74**	**67.54**	**48.26**	**64.68**	**65.35**	**57.76**	**70.29**	**61.34**
	W (Unsaturated fatty acids)/*w*	1.68	2.08	0.94	1.83	1.89	1.38	2.39	1.59

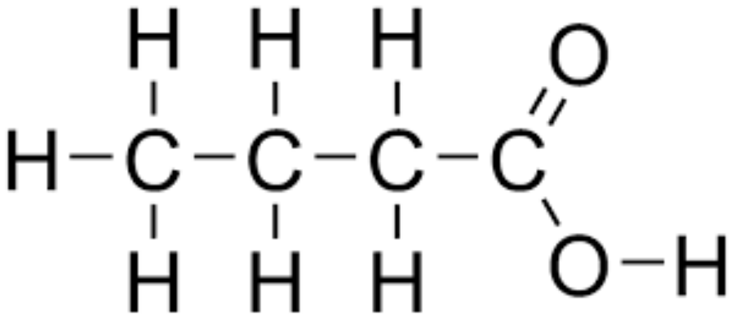
A of total 37 fatty acids including 20 saturated, and 17 unsaturated were identified in the beebread samples obtained from different botanical origins

The results of fatty acid determination embrace both free acids and products of glycerides hydrolysis

Thirty-one of the total fatty acids identified were common to all eight samples

Only six of them were detected in one or more of the samples

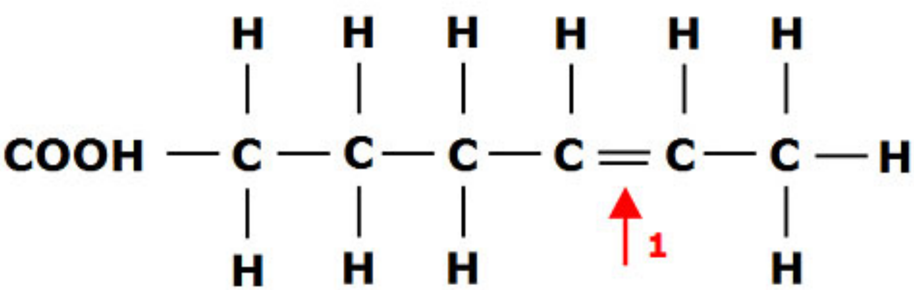
There were statistically significant differences in amounts of those 34 fatty acids determined in the samples



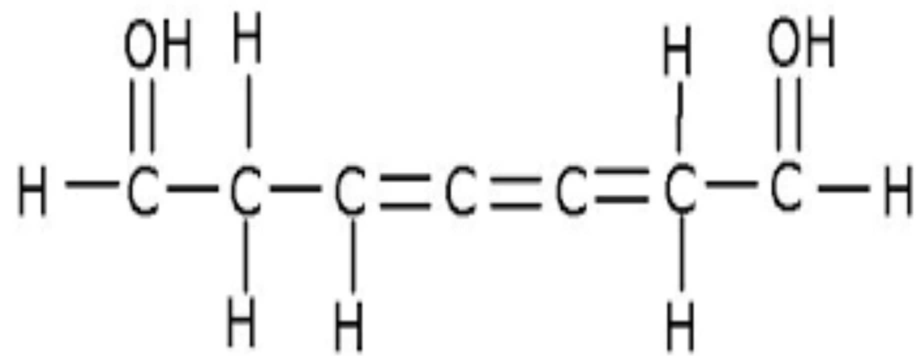
PUFA

The beebread samples contained quite high level of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFAs)

On average, the major saturated fatty acids in the samples in decreasing order of abundance were hexadecanoic acid, octadecanoic acid and icosanoic acid



Monounsaturated Fat

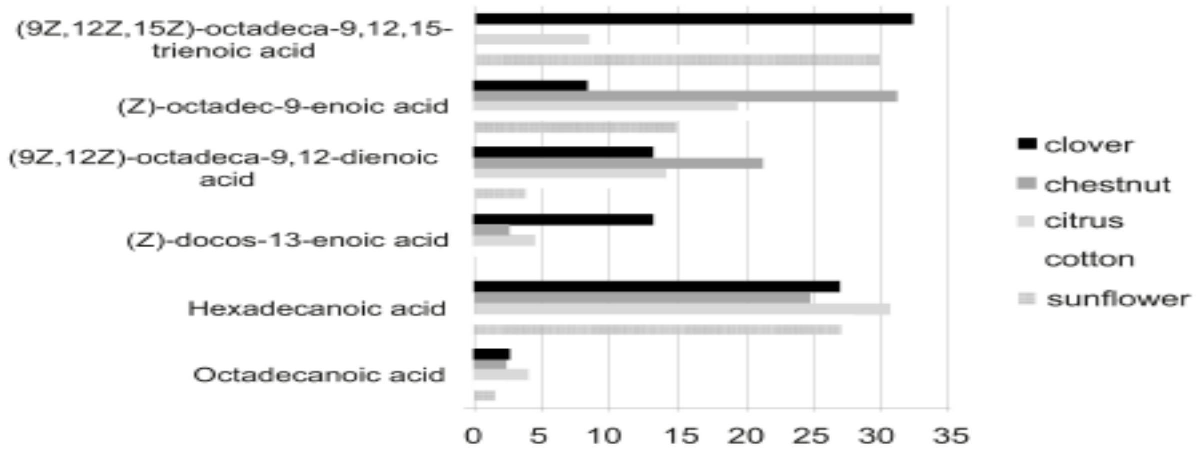


Polyunsaturated fatty acid

Cotton beebread. A total of 35 and 32 fatty acids were identified in beebread samples from Adana and Urfa (cotton, *Gossypium hirsutum* L.), respectively. Undecanoic and tridecanoic acids were not detected in cotton beebreads, while hexanoic acid, octanoic acid and pentadecanoic acids were only present in GA (Adana-cotton) sample

Clover beebread. Each of the clover beebread samples obtained from Urfa and Adana provinces contained 31 fatty acids that are mostly unsaturated. Fatty acid profile of the samples was slightly different from one another. Undecanoic acid and heptadecanoic acid were merely detected in CU (Urfa-clover) sample while two of the saturated fatty acids octanoic acid and tricosanoic acid were found only in CA (Adana-clover) sample

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



A total of thirty-seven FAs were identified

The most abundant fatty acids detected in all the samples were (9Z, 12Z, 15Z)-octadeca-9, 12, 15-trienoic acid, (9Z, 12Z)-octadeca-9, 12-dienoic acid, hexadecanoic acid, (Z)-octadec-9-enoic acid, (Z)-icos-11-enoic acid and octadecanoic acid

Cotton beebread contained the highest level of ω-3 FAs, 41.3 %

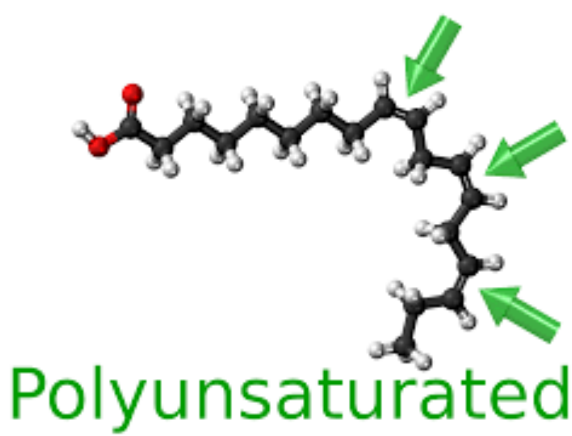
Unsaturated/saturated FAs ratios ranged between 1.38 and 2.39 indicating that beebread is a good source of unsaturated FAs

The pollen, proximate and FAs composition of beebread samples of different botanical origins varied significantly

In this study, the total of unsaturated fatty acids was higher than the sum of saturated fatty acids found in all the samples except citrus sample from Adana.

The fatty acid content of beebread is very important for honeybees and PUFAs are essential for healthy body development and productivity.

However, unsaturated fatty acids are not just essential for bees but also for human nutrition



In conclusion, the results obtained this study could be used as a reference for research into bee and also human health.

The present data could also provide a scientific basis for nutritional value assessment of beebread and contribute to a food composition database. Finally, the results of current study showed that the beebread is a good source of unsaturated FAs.

Thank you