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PIGMENTATION OF SOME HONEYBEE, *APIS MELLIFERA* L., PRODUCTS

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ABSTRACT

Some floral pigments (including antioxidants); carotenoids, chlorophylls, anthocyanins and xanthophylls were determined colorimetrically in some honeybee, *Apis mellifera* L., products; citrus, clover and cotton honeys, three types of beeswax; white, brown and dark and propolis; brown, dark brown and dark. Two solvents (acetone or ethanol) were used for extraction and determination of pigments at special wave lengths. Pigment contents were obviously different qualitatively and quantitatively in the two tested solvents for all samples. The obtained results revealed that cotton honey had high pigment content compared to citrus or clover honeys. Pigment contents were proportionally increased with the increasing in darkness of color in the types of beeswax and propolis. Pigments were higher in pollen grains of cotton, clover and citrus than those of their flowers. Meanwhile, pollen spectra in tested honeys indicated that different pollen species were represented in each honey type regardless the main plant source for nectar. The importance of recorded pigments (colorants) is not only contributing as "markers" of the origin of bee products and distinguishing adulteration but also many of them (esp. carotenoids) is more valuable substances as antioxidants.

KEY WORDS: Honeybee products - Color- Floral pigments – Antioxidants - Pollen spectrum - Spectrometric analysis.

INTRODUCTION

Research into the composition of bee products has developed through two main stages since the early studies on the physical and chemical properties of honey. Early workers examined what might be called the extensive properties. The analysis of various honeybee products must have three aims; quality control, purity and identification of adulteration. In order to secure minimum certainty of the quality control, it is wise to know and be able to check some essential criteria **(Pourtaillier & Taliercio, 1972)**. Among them the color of these products which indicates the floral source and geographical origin. Because honeys (or other bee products) vary widely in their aroma, flavor and color these features are often characteristic of particular

floral sources. Thus chemical analysis of the components responsible for these features can be expected to show unique composition that are characteristic of each floral source. These components are potentially useful as' markers' for those floral sources **(Molan,1998)**.

Historically there has been an interest in identifying minor chemical components of honeybee products that would serve as markers for particular floral sources. And because there are few studies on pigment determination in bee products, this work was done to focus on the importance of floral pigments in some Egyptian bee products not only as colorants but also as valuable antioxidants.

MATERIALS AND METHODS

The present work was conducted in the Faculty of Agriculture, Fayoum, Cairo University, through spring and summer seasons of the year 2003. Three honeybee products; honey, beeswax and propolis produced by honeybee colonies headed by hybrid local Carniolan (*A. m. carnica*) queens were used. The experimentation included the following:

A-Sampling:

1-Honey types: Citrus honey (not produced in Fayoum Governorate) was obtained from the apiary of the High Institute for Agricultural Cooperation, Shubra El-Khiema, Qualubia, Egypt on April, 2003. Clover and cotton honeys were obtained from the apiary of the Faculty of Agriculture at Dar El-Ramad region, Fayoum Govern., Egypt at the end of June and July, 2003, respectively.

2-Beeswax and propolis types: Three types (yellowish-white, brown and dark) of beeswax and three types of propolis (brown, dark brown and dark) were obtained from colonies situated in the apiary of the Faculty of Agriculture, Fayoum Govern., during spring season, 2003.

3-Plant materials: Flowers and pollens of citrus (*Citrus* spp.), Egyptian clover (*Trifolium alexandrinum*) and cotton (*Gossypium* sp.) were obtained from each plant species during flowering period in the same year (2003). Pollen grains of the previously mentioned plants were obtained by placing pollen traps at the entrance of Langstroth's bee hives, of the same apiary, at Fayoum Govern. Then collected pollen pellets were separated (depending on color) and then identified microscopically (**Louveaux *et al.* ,1978)**. Freshly flowers and pollens were taken to the lab. to run a rapid determination.

B-Analysis of pollen in honey (melissopalynology): Pollen spectrum in the tested honeys was evaluated according to the method of **Louveaux *et al.* (1978)**, mentioned by **Nour (1988)** and **Owayss (1996)** as follows :

Honey samples (20g each) were dissolved in 20 ml warm dist. water (40 ºC). The solutions were centrifuged for 15 min. at 3000 rpm. The supernatant was carefully discarded and the sediment was then dispersed in 10 ml dist. water to remove sugars, then centrifuged again for 10 min. The supernatant decanted off and the sediment spread over 2x2 cm glass slide. After drying by slight heating, not above 40 ºC, the sediment was mounted with glycerine-gelatine liquid medium and spread under cover glass. Microscopical examination for pollen grains was made and divided into categories explained as: very frequent (VF); grains more than 45% of the total, frequent (F); 16-45% of the total; rare (R); 3-15%of the total and sporadic (S); less than 3% of the total.

C-Spectrophotometric determinations of pigments: All the tested pigments were colorimetrically determined using spectrophotometer (Spectronic 20, Bausch & Lomb) as follows:

C-1-Anthocyanins: Total anthocyanins was estimated using the method described by **Fuleki and Francis (1968)** as follows: Samples of 3g of each tested product were mixed with ethanol 95% and HCL 1.5N (85:15 v/v), made up to 100ml then filtered. The absorbance of the extracted solution was measured at 535 nm against a blank. Total anthocyanins was calculated (µg/g) by the following equation:

$O.D._{535}$ x D.V. x 100 / F.W. x 1 / 98.2 where; O.D. = optical density (absorbance) of the diluted sample, D.V.= diluted volume (ml) of the extract prepared for O.D. measurements and F.W.= fresh weight (g) of the sample.

C-2-Chlorophylls and xanthophylls: These pigments were determined according to the modification of the spectrocolorimetric procedure given by **Bacot (1954)**: In a porcelain mortar, 3g of each product were well mixed with 30 ml of 80% acetone or ethanol then ground and filtered. The filtrate was made up to 100ml with 80% solvent. The absorbance of the extract was measured against 80% acetone (as a blank) at 663, 645 and 452 nm. Chlorophylls were expressed as µg/g fresh weight of the product according to the equations given by **Arnon (1949)** as the following:

Chlorophyll A = 12.7 x $O.D._{663}$ – 2.69 x $O.D._{645}$

Chlorophyll B = 22.4 x $O.D._{645}$ – 4.68 x $O.D._{663}$

Total chlorophylls = 8.02 x O.D. $_{663}$ + 20.2 x O.D. $_{645}$

Xanthophylls = 2026.1 x O.D. $_{470}$ – 2288.6 x O.D. $_{485}$ + 0.0036 (A)– 0.06518 (B)

C-3-Carotenoids: Furthermore the determination of the previous pigments, carotenoids were also estimated according to the methods of **Britton (1995)**; the amount of each present pigment was calculated from the equation:

X =Ay / ($A^{1\%}_{1cm}$ x 100); where X = the mass of carotenoids (g), y = the volume of solution (ml), A= the measured absorbance, and $A^{1\%}_{1cm}$ = the specific absorption coefficient, that is, the absorbance (nm) of a solution of 1g of that carotenoids in 100ml of solution. Absorptions used are included in Table (1).

Table (1). Light absorption maxima of some carotenoids determined in tested honeybee products.

Carotenoid	λ_{max} (nm)	Solvent *	Carotenoid	λ_{max} (nm)	Solvent *
Actinoerythrol	508	A	Isocryptoxanthin	450	E
Antheraxanthin	444	E	Isozeathanyhin	451	E
8\ -Apo-β-caroten 8\ - al	463	E	Lactucaxanthin	440	E
Astaxanthin	480	A	Lutein	445	E
Canthaxanthin	474	E	Lutin 5,6-epoxide	442	E
α - Carotene	444	E	Lycopene	472	E
	448	A		474	A
β-carotene	450	E	Neoxanthin	439	E
	452	A	Neurosporene	440	E
γ- Carotene	460	E	Violaxanthin	440	E
	461	A	Violerythrin	566	A
ε-Carotene	440	E	β-Zeacarotene	428	E
ξ-Carotene	399	E	Zeaxanthin	450	E
Crocetin	423	E		452	A
β-Cryptoxanthin	450	E			

* Solvents: A= acetone, E= ethanol.

RESULTS AND DISCUSSION

Data presented in Tables (2, 3, 4 &5) show the pigment contents in the tested honeybee products and in floral materials as follows:

A-Honeybee products; 1-Honey:

It has been observed that pigment contents varied according to types of tested products and solvents (acetone or ethanol). Some pigments were not detected in the acetone extract compared to ethanolic one *e.g.* β-cryptoxanthin, isocryptoxanthin, lactucaxanthin, lutein, lutein-5,6-epoxide, neoxanthin, neurosprene, violaxanthin, β-zeacarotene, anthraxanthin, 8\-apo-β-caroten 8\-al, canthaxanthin, ε-carotene, ξ-carotene, citraxanthin and crocetin. On the other hand, the amounts of pigments were greater in acetone extract than those extracted by ethanol.

In the case of acetone extract (Table, 2), the tested honey types (citrus, clover and cotton honeys) showed different pigment contents. The highest values; 20.85, 20.24& 19.44 µg/g were reported for total chlorophylls (TC) followed by xanthophylls (13.85, 13.45& 12.60 µg/g) then anthocyanins (10.50, 9.89& 14.35 µg/g) for citrus, clover and cotton honeys, respectively. While the lowest values (2.33, 5.69& 4.21 µg/g) recorded for violerythrin in citrus, clover and cotton honeys, respectively. Meanwhile, some carotenoid pigments were present in the ethanolic extract but, were not detected in the acetone extract (Table, 3). The highest content (3.67, 5.00,& 5.00 µg/g) were obtained for ξ-Carotene followed by (4.00, 2.87& 3.98 µg/g) for crocetin and 3.33, 3.94& 4.69 µg/g for α-carotene, while the lowest values (2.33, 1.46& 2.58 µg/g) were for astaxanthin. The other pigment contents were in between, but in general, cotton honey has the highest content compared to the other two types.

The color of honey is often used as an indicator of its flavor; generally the lighter the color (citrus and clover honeys in this study) the milder the honey. The most familiar honey colors are the yellowish, the yellowish-gold (cotton honey) and light ambers, but honey may vary from an almost colorless to dark, and red-brown amber **(White, 1978; Hassan, 1985; USDA, 1985; Nour, 1988; Abd-Elbary and Mishref, 1993 & Owayss, 1996)**. The color results from the unequal absorption of different wave-lengths of light by substances within the honey. These substances include pigments, tannins and/or colloidal matter **(Fell, 1978)**.

Table (2). Some pigments (µg/g) in acetone extract of the tested honeybee products.

No.	Pigments	Types of honey			Types of beeswax			Types of propolis		
		Citrus	Clover	Cotton	White	Brown	Dark	Brown	D. B.	Dark
1	α-Carotene	6.80	5.30	6.78	3.80	4.80	6.40	6.41	11.20	16.40
2	β-carotene	6.33	4.75	6.70	3.60	4.60	5.80	6.84	9.20	12.80
3	γ-Carotene	5.90	4.61	6.42	3.40	4.40	5.20	5.98	10.00	13.20
4	Lycopene	4.33	4.26	6.29	3.00	4.20	4.80	4.70	9.60	12.00
5	Violerythrin	2.33	5.69	4.21	0.60	0.70	1.20	2.14	2.40	5.60
6	Zeaxanthin	5.67	6.21	6.48	3.60	4.60	5.80	6.84	10.80	13.20
7	Astaxanthin	4.33	4.25	6.12	2.00	3.20	3.60	3.42	7.60	9.20
8	Actinoerythrol	3.00	4.63	6.09	1.90	3.10	3.60	3.51	7.20	9.70
9	Anthocyanins	10.50	9.89	14.35	4.60	5.20	6.80	10.97	29.95	34.65
10	Xanthophylls	13.85	13.45	12.60	1.43	1.75	2.10	54.73	23.05	4.70
11	Chlorophyll(A)	7.67	6.61	6.76	0.81	1.05	1.22	57.84	2.20	0.60
12	Chlorophyll(B)	12.50	13.48	12.50	1.42	1.87	2.13	39.33	3.90	1.06
13	Total Chlorophylls	20.85	20.24	19.44	2.28	2.99	3.42	98.69	6.22	1.70

Table (3) .Carotenoids (µg/g) in ethanolic extract of the tested honeybee products.

No.	Pigments	Types of honey			Types of beeswax			Types of propolis		
		Citrus	Clover	Cotton	White	Brown	Dark	Brown	D. B.	Dark
1	β-cryptoxanthin	3.50	3.42	3.41	2.69	3.40	4.10	6.20	7.62	10.60
2	Isocryptoxanthin	4.67	2.54	3.63	2.36	3.00	3.40	4.91	6.60	10.60
3	Isozeaxanthin	3.30	2.44	3.45	2.48	3.10	3.40	4.32	6.40	9.80
4	Lactucaxanthin	3.50	3.00	3.98	2.80	3.50	4.10	3.89	7.2	11.60
5	Lutein	3.00	2.64	3.84	2.30	2.90	3.50	4.40	5.80	10.40
6	Lutein-5,6-epoxide	3.67	2.55	3.71	2.10	2.60	3.00	3.19	6.00	10.80
7	Lycopene	2.33	2.15	3.00	1.70	2.10	2.50	2.84	5.80	9.40
8	Neoxanthin	3.50	3.71	3.92	2.50	3.10	3.70	2.88	7.40	11.20
9	Neurosporene	3.00	2.48	4.09	2.90	3.60	4.30	5.74	5.80	10.80
10	Violaxanthin	3.50	2.64	4.21	2.80	3.40	4.10	5.01	6.00	9.80
11	β-zeaxanthin	3.20	1.99	4.69	3.00	3.70	4.40	4.00	7.80	12.60
12	Zeaxanthin	2.75	2.14	3.52	2.35	2.90	3.70	3.73	5.60	10.40
13	Antheraxanthin	3.00	2.00	3.88	2.50	3.00	3.60	4.20	7.00	12.40
14	8\-Apo-β-caroten 8\-al	2.67	1.88	3.39	2.25	2.80	3.40	4.81	6.60	10.20
15	Astaxanthin	2.33	1.46	2.58	1.60	2.00	2.50	3.13	5.60	8.80
16	Canthaxanthin	1.95	2.00	2.74	1.70	2.10	2.60	2.69	4.80	8.40
17	α –carotene	3.33	3.94	4.69	5.70	6.60	7.90	4.11	8.00	12.40
18	β-carotene	2.67	4.05	4.78	6.80	8.00	9.70	2.93	9.20	12.60
19	γ-Carotene	2.50	3.00	3.93	4.00	4.90	6.20	4.70	6.40	10.00
20	ε-Carotene	3.00	2.95	4.21	5.50	6.80	8.10	4.62	7.60	11.20
21	ξ-Carotene	3.67	5.00	5.00	7.00	8.60	10.30	4.21	9.00	12.80
22	Citranxanthin	2.50	1.98	3.07	2.00	2.40	2.80	2.90	5.40	9.60
23	Crocetin	4.00	2.87	3.98	4.35	5.30	6.40	2.61	5.40	8.80

D.B. = dark brown

In this respect, **White (1975)** reported that the minor components in honey include plant pigments; carotenes, chlorophylls and xanthophylls. On the other hand, **Molan (1998)** reviewed that no honey produced by bees flying free is likely to be entirely unifloral. The 'unifloral honey' is used to describe honey in which the major part of the nectar has been derived from a single plant species. He added that for a honey to be called unifloral the pollen of the nominal species generally should be at least 45% of the total pollen count in the honey. But this percentage does not apply when a floral source gives nectar which had a higher or lower number of pollen grains than the average. A further relationship was found between the darkness of honey and the ash content.

In a former study, **Owayss (1996)** found that cotton honey had higher ash content than clover honey. Other observations was given by **Molan (1998)** stated that the carotenoids were largely responsible for the color of light honey. He added that much of the coloring matter of dark honeys appeared to be water-soluble and this could be related to the ash and amino acid/sugar explanations of honey colors. Another report described the coloring matter of honey as carotenoids and

anthocyanins **(Thawley, 1969)**. Also **Tan *et al*. (1988)** showed that the analysis of organic substances in honey could assist in the identification of its floral origins. He found that carotenoids occur in some honeys with concentrations between 100-180 μg/g. He added that dark-colored honeys seem to contain more antioxidants than do lighter varieties.

In addition, many honeys provide small amount of vitamin antioxidants such as vitamin C, vitamin E vitamin B2, β-carotene, lutein, lycopene coenzyme Q10 and cysteine which are bioavailable and convey antioxidant protection to healthy human subjects by helping in eliminate free radicals, which are reactive compounds in the body. Free radicals are created through the normal process of metabolism and are believed to contribute many serious diseases **(Berenbaum, 1998; Yanoski, 2001; Yanoski and Cardetti, 2001 and Schramm and Keen, 2002)**.

2- Beeswax:

Data presented in Table (2) show the pigment contents in the tested beeswax types in acetone extract. In general, the pigment content proportionally increased as a function of the increasing in darkness of wax type increased. Anthocyanins (4.60, 5.20 & 6.80 μg/g) had the highest value followed by α-carotene (3.80, 4.80 & 6 40 μg/g) and zeaxanthin (3.60, 4.60 & 5.80 μg/g), while the lowest contents (0.60, 0.70 & 1.20 μg/g) were for violerythrin in white, brown and dark beeswax, respectively. The same trend was observed in ethanolic extract (Table, 3), but ξ-carotene (7.00, 8.60 & 10.30 μg/g) had the highest values followed by β-carotene (6.80, 8.00 & 9.70 μg/g) and α-carotene (5.70, 6.60 & 7.90 μg/g), while the lowest values (1.70, 2.10 & 2.50 μg/g) were reported in lycopene for white, brown and dark beeswaxes, respectively. However, the pigment contents in ethanolic extract were lower than those in acetone extract as recorded in honey types.

3-Propolis:

Concerning the acetone extract (Table, 2), data show that the tested propolis types contained, generally, higher pigment contents than honey or beeswax types and that may be due to the composition of propolis and its floral origin (plant resins and pollen). In this respect, **Nikolaev (1978)** reported that propolis contains about 55% resins and balsams; 30% waxes, 10% etheric oils and 5% pollen. The obtained results explained that the darkness the propolis, the increasing in pigment content except for chlorophyll A, B, TC. Xanthophylls had the highest contents (54.73, 23.05 & 4.70

μg/g) followed by anthocyanins (10.97, 29.95 & 34.65 μg/g), while the lowest values (2.14, 2.40 & 5.60 μg/g) were reported in violerythrin for brown, dark brown and dark propolis types, respectively. The contents of chlorophyll A, B & TC were relatively high in brown propolis; 57.84, 39.33 & 98.69 μg/g, respectively compared to the other propolis types.

In ethanolic extract, data in Table (3) revealed that the same trend was observed although recorded amounts were relatively lower than those in acetone extract. β-Cryptoxanthin; 6.20, 7.62 & 10.60 μg/g represented the highest value, while canthaxanthin; 2.69, 2.69 & 4.80 μg/g recorded most low contents for brown, dark brown and dark propolis types, respectively. In general, the other pigment contents were in between. It is also noticed that tested propolis types contained more pigments in ethanolic extract as compared to honey or beeswax types.

In this concern, **Ghisalberti (1979)** showed that the chemical composition of propolis as well as its color and aroma are changed according to the geographical zones. Its color varies from yellowish-green to dark brown depending on its source and age.

B-Floral pigments:

According to data in Table (4), it is obvious that carotenoids, anthocyanins and xanthophylls contents were higher in each plant pollen grains than those in the same plant flower in acetone extract. And that may be due to the higher pigment content in pollen grains than that in flower because the latter contains more water content. Meanwhile, TC, chlorophyll A and chlorophyll B contents were higher in flower than pollen for each plant, except for cotton. The highest value (112.84 μg/g), was recorded for astaxanthin in cotton pollen followed by lycopene (112.45 μg/g), actinoerythrol (112.07 μg/g), while citrus flower contained the lowest content of violerythrin (0.67 μg/g). In ethanol extract (Table 5), the same trend was observed and, generally, cotton flower and pollen contained more pigments followed by citrus and then clover.

Table (4). Some pigments (µg/g) in acetone extract of the tested flowers and pollens.

No.	Plant / Pigments	Citrus		Clover		Cotton	
		Flower	Pollen	Flower	Pollen	Flower	Pollen
1	α-Carotene	2.33	96.15	12.24	30.46	36.43	108.03
2	β-carotene	2.00	98.10	16.59	31.93	33.84	100.51
3	γ-Carotene	2.67	102.51	9.62	29.85	31.96	104.67
4	Lycopene	0.50	96.48	5.44	30.64	38.87	112.45
5	Violerythrin	0.67	2.63	3.61	2.95	30.03	108.00
6	Zeaxanthin	2.00	100.00	6.43	29.74	35.54	100.15
7	Astaxanthin	1.80	58.75	5.24	26.50	31.19	112.84
8	Actinoerythrol	1.50	58.30	4.06	26.39	38.00	112.07
9	Anthocyanin	3.30	46.84	7.15	24.15	29.57	50.00
10	Xanthophyll	2.50	31.52	6.30	20.90	31.85	46.53
11	Chlorophyll(A)	53.75	4.63	6.48	1.08	15.11	22.68
12	Chlorophyll(B)	37.33	6.99	4.27	1.62	32.60	47.01
13	Total Chlorophylls	94.15	11.71	10.91	2.74	47.77	69.80

The color of pollen grains, which ranges from yellowish white to deep blue according to species, is mainly due to two classes of plant pigments, flavones and carotenoids (carotenes and xanthophylls). Carotenes predominate in the pollen of entomophilous species. This may be related to the fact that carotene is an important source of vitamin A for insects, but an exception with *Zea mays*, for example, however, it contains high amount of β-carotene, while the entomophilous *Begonia* has pollen low in carotene.

The carotenoids usually found in bee-collected pollens are α-carotene and β-carotene and, in small amounts, lutein esters, cryptoxanthin, xanthophylls and flavoxanthin **(Muniategui *et al.*, 1990)**. They also reviewed 50–150 µg/g of carotene and 140-400 µg/g of xanthophylls in four samples of fresh bee-collected pollen. Carotenoid content (expressed as β-carotene equivalents) makes up 0.05-0.08% of dry weight (mean 0.06%). They also found that total carotene content varied widely from 0.8 to 315 mg β-carotene/100g lipids.

Table(5). Carotenoids (µg/g) in ethanolic extract of the tested flowers and pollens.

No.	Plant / Pigments	Citrus		Clover		Cotton	
		Flower	Pollen	Flower	Pollen	Flower	Pollen
1	β-cryptoxanthin	2.33	69.90	3.36	34.13	29.51	90.52
2	Isocryptoxanthin	1.67	50.12	2.97	33.52	31.41	94.42
3	Isozeaxanthin	1.50	47.48	3.10	34.19	29.51	91.68
4	Lactucaxanthin	1.33	49.51	3.49	31.62	30.63	91.89
5	Lutein	1.50	51.63	2.88	32.66	29.49	92.03
6	Lutein-5,6-epoxide	1.30	48.40	2.67	31.71	29.09	90.33
7	Lycopene	1.20	46.67	2.12	38.44	35.62	107.14
8	Neoxanthin	2.00	58.46	3.17	29.54	27.36	88.96
9	Neurosporene	1.50	53.63	3.66	29.77	25.98	81.08
10	Violaxanthin	1.33	39.92	3.50	31.69	30.11	90.62
11	β-zeaxanthin	1.67	47.70	3.77	26.48	29.67	91.44
12	Zeaxanthin	1.50	45.33	2.93	34.43	32.29	96.83
13	Antheraxanthin	1.33	33.61	3.14	32.26	29.17	95.01
14	8$^{\backslash}$-Apo-β-caroten 8$^{\backslash}$-al	1.50	50.03	2.82	39.97	37.05	111.48
15	Astaxanthin	0.97	43.21	1.96	33.34	31.85	94.69
16	Canthaxanthin	1.50	46.73	2.15	35.49	33.34	100.16
17	α–carotene	1.67	45.44	7.18	33.33	39.88	121.10
18	β-carotene	2.67	61.63	9.76	39.61	48.14	120.00
19	γ-Carotene	1.20	60.97	4.95	37.74	43.67	118.52
20	ε-Carotene	1.33	64.85	6.84	29.84	34.15	103.24
21	ξ-Carotene	1.90	65.54	9.65	34.55	35.36	106.99
22	Citranxanthin	1.20	50.55	2.50	36.63	39.15	110.11
23	Crocetin	1.67	49.44	5.44	26.41	29.51	96.03

On the other hand, **Shapira *et al*. (1980)** found that the amount of flavonols, anthocyanins, phenolic acids and carotenoids were higher in some of the pollens than in others. **Czeczuga (1982)** determined the total content of carotenoids in 12 insect species. He found the highest concentration; 3.2µg/g fresh weight in *A. m.* (workers). β-Carotene was one of the main carotenoids present in 7 species, but in *A. mellifera*, zeaxanthin and lutein epoxide were dominant. The content of α-doradexanthin ranged from 3.5 to 18.8% of the total carotenoids; for *A. m.* was 10.7%.

Also the same author **(1985)** compared the carotenoids present in worker bees with those present in the flowers being visited by the bees. Ten of the carotenoids were identified in both the bees and all 7 species of flowers examined. A further 4 were found only in flowers and another 6 only in the bees.

In this concern, **Owayss (1996)** found 1.01 & 1.52, 1.03 & 0.55, 1.24 & 3.21 and 1.24 & 3.20 mg/100g anthocyanin, carotenoids, total chlorophylls and xanthophylls for clover and cotton honeys, respectively.

Regarding pollen spectra in the tested honey types (melissopalynology), data in Table (6) indicated that more than one pollen species was represented in each

honey type. Citrus or clover honeys were represented by each main plant species (*Citrus* spp. or *T. alexandrinum*, respectively) as very frequent (VF), while maize, *Z. mays*, pollen was the most pollen species (VF) in cotton honey and that may due to the noticeable dropping of cotton pollen gathered by bees **(Owayss, 1996).**

The obtained results agree with those reviewed by **Molan (1998)** who showed that the cotton honey is produced from secretions of extrafloral nectarines and the honey from cotton, *G. hirsutum*, contained no pollen. According to **Free (1970)**, 6% only from the field bees foraging on flowering cotton, visit the flowers and 94% visit the extrafloral nectarines. So, the cotton pollen grains are present in very low numbers in the cotton honey.

The present results (Table, 6) also showed that pollen of maize was also frequent (F) in clover honey. Other pollens such as eucalyptus; *Eucalyptus* sp., broad bean, *Vicia faba*, Umberilliceae and date palm, *Phoenix dactylifera* were represented in citrus and clover honeys in rare (R) or sporadic (S) amounts.

Also, **Nour (1988)** reported pollen of clover, eucalyptus, citrus, date palm, maize (the most dominant pollen species in Fayoum region; **Ghoniemy, 1984**), sunflower and broad bean as the main pollen sources for Egyptian honeys.

In this respect, **Molan (1998)** showed that the pollen grains in honey reveal the types of plants that were around when the bees produced the honey. He added that pollen spectrum excludes from consideration pollens from sources that do not supply nectar. Also bees may forage selectively from pollen-producing or pollen-free flowers of the same species of plants, which would give further variability in the pollen content of the nectar collected into different hives from the same floral source; bees from adjacent hives have been shown to harvest different sources of pollen.

Table (6). Pollen categories* in tested honeys.

Honey / Pollen	Citrus	Clover	Cotton
Trifolium alexandrinum	-	VF	-
Zea mays	-	F	VF
Citrus spp.	VF	-	-
Eucalyptus sp.	S	R	-
Vicia faba	S	-	-
Umberilliceae	F	S	-
Phoenix dactylifera	S	S	-

*Categories: VF = 45%, F = 16 – 45%, R = 3 – 15% and S = < 3%.
Where; VF = very frequent, F = frequent, R = rare and S = sporadic.

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الملخص العربـــــــــى

المحتوى الصبغى فى بعض منتجات نحـــــل العســـل

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قـــدرت الصـــبغات النباتيـــة؛ الكاروتينويـــدات، والكلـــوروفيلات، والأنثوســـيانينات، والزانثوفيلات ،والتى تضمنت بعض مضادات الأكسدة وذلك فى بعض منتجات نحـــل العســـل مختلفة الألوان والتى تمثلت فى ثلاثة أنواع من العسل(الموالح والبرسيم والقطن) وثلاثة مـــن شمع النحل (أبيض و بنى وبنى غامق) وثلاثة أخرى من صمغ النحل أو البروبـــوليس (بنـــى وبنى غامق وبنى مسود) وذلك بطريقة التقدير اللونى (الامتصاص الضوئى). وقـــد اســـتخدم مذيبان هما الأسيتون والإيثانول لاستخلاص الصبغات الموجودة عند أطوال موجيـــة مختلفـــة. وقد أوضحت النتائج المتحصل عليها اختلاف المحتوى الصبغى كما ونوعا بواسطة المـــذيبين لكل العينات المختبرة. وقد وجد أن عسل القطن كان عاليا فى محتواه الصبغى مقارنة بكل من عسل الموالح وعسل البرسيم. واتضح أيضا أن المحتوى الصبغى يزداد بزيادة دكانة اللون فى عينات الشمع والبروبوليس محل الدراسة. وتبين أن المحتوى الصبغى كان عاليا فـــى حبـــوب لقاح نباتات الموالح والبرسيم والقطن عن مثيله فى أزهار نفس النباتـــات. وبفحـــص حبـــوب اللقاح فى الأعسال المختبرة ،وجد أن هناك أكثر من نوع من حبوب اللقاح لنوع العسل الواحد بغض النظر عن تسمية ذلك العسل باسم المحصول الرئيسى المنسوب إليـــه. وترجـــع أهميـــة إجراء هذه الدراسة ليس فقط فى المساهمة فى تعريف منتجات النحل بتقدير المكونات الدقيقـــة ومن بينها المكونات الصبغية المسئولة عن ألوانها وتحديد المصادر النباتية والجغرافية المؤثرة فى تركيبها ولكن أيضا فإن كثيرا من هذه الصبغات وخاصة الكاروتينات لهـــا أهميـــة كبيـــرة كمضادات للأكسدة مما يزيد من فوائد منتجات النحل فى العلاج والتداوى.