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BIOCHEMICAL STUDIES ON POLLEN GRAINS OF SOME MEDICINAL AND CLASSICAL PLANTS

Farag, R. S. *, Rashed, M. M.,* Safaa A. E. bdel-Wahab**, Ahmed, A. S.***, and Hala A. habet***

- * Biochemistry Department, Faculty of Agriculture, Cairo University, Egypt
- ** Food Irradiation Department, National Centre for Radiation and Technology, Egyptian Atomic Energy Authority, Giza, Egypt *** Food Technology Research Institute, Agriculture Research Centre, Giza, Egypt

ABSTRACT

A study was conducted to evaluate the nutritional effect of pollens collected from some medicinal (palm, chamomile, coriander) and classical (sunflower) plants. Therefore, the gross chemical composition (ash, protein, lipid, total carbohydrate and fiber contents). fatty acids, amino acids, polyphenol patterns were determined. The results indicated that the gross chemical composition of pollens was largely dependent on their botanical origin. The gas chromatographic analysis of sunflower and palm pollens demonstrated that the most predominant saturated and unsaturated fatty acids were 22:0. 18:3 and 22:0, 18:1, respectively. Chamomile pollens were characterized by the highest concentrations of palmitic (12.73 %) and oleic (23.05%) acids as saturated and unsaturated ones, respectively. The pollens of coriander plants were distinguished by having the acids 16:0, 18:1 and 18:3 as major substances. The present results demonstrated that fatty acids, taken a group, may provide a key for the identification of pollen sources. The fatty acid patterns of pollens elucidated that the atherogenic index of the different pollens was arranged in descending order: chamomile > sunflower > coriander > palm. These findings suggest the use of chamomile pollens as a supplement for elderly people to overcome to some extent the corony heart disease. Seventeen amino acids were identified in all pollens using amino acid analyzer. In general, palm pollens contained the highest amounts of all essential amino acids compared with other pollen sources. The least limiting essential amino acids in all pollens were the sulfur amino acids (methionine + cysteine). According to the chemical score values, the quality of pollen proteins was arranged in the decreasing order: palm > sunflower > chamomile = coriander.

HPLC chromatographic analysis indicated that chlorogenic acid was the most abundant polyphenol compound in pollens. The levels of chlorogenic acid in pollens were ranked in different plants according to the descending order: coriander > sunflower > chamomile > palm.

Keywords: Pollen grain, Medicinal plant, Chemical composition, Fatty acid, Amino acid and Polyphenol patterns.

INTRODUCTION

Bee-collected pollens can be considered as a potential source of energy and nutrients for human consumption (Serra-Bonvehi and Escola- Jorda, 1997). Also, Liebelt and Calcagnetti (1999) reported that bee-pollens of certain commercially available brands contain all the essential nutritional elements to provide normal growth and development of both the laboratory rat and mouse without the occurrence of any toxic or organ damaging effects when it is the sole source of nutrition. Bee-collected pollens contain nutritionallyessentially substances like carbohydrates, proteins, amino acids, lipids, vitamins and minerals (Almeida-Muradian et al., 2005). Also, they contain significant amounts of polyphenol substances mainly flavonoids (Kroyer and Hegedus, 2001 and Leja et al., 2007). Beecollected pollen is recognized as a well balanced food (Gonzàlez-2001). This bee product also has Güerca et al.. pharmacological properties, such as antibiotic, antineoplasic, antidiarrhoeatic and antioxidant agent (Campos et al., 1997 and Ohta et al., 2007).

More specifically, ingestion of bee-pollen by rats has been shown to decrease the level of the lipid oxidation products, malondialdehyde and conjugated dienes, in the erythrocytes (Dudov et al., 1994, Dudov and Starodub 1994 and Kroyer and Hegedus, 2001). Thus, suggesting an antioxidant role for bee-pollen and immunostimulation activity on primary and secondary levels of IgM and IgE in rabbits fed on a bee-pollen containing diet for 1 month. Bone loss with ageing induces osteoporosis, which is widely recognized as a major public health problem. In this respect, the extract of bee-pollen can be used for prevention of osteoporosis (Yamaguchi et al., 2006). In addition, pollens are good source of rutin, a bioflavonoid that helps

to increase the strength and integrity of blood cell walls. The rutin content f pollen helps minimizing bleeding and encourages coagulation. Rutin also helps to strengthen the heart and control high blood pressure by regulating blood flow (Rita Elkins, 1996). The aforementioned data demonstrated the valuable effects of bee-pollens on the health of human beings. The main goal of the present study was to indicate the nutritional compounds of pollens collected from some medicinal (palm, chamomile and coriander) and classical (sunflower) plants.

MATERIALS AND METHODS

Sources of bee pollens

The pollen grains under study were obtained from palm, coriander, chamomile and sunflower plants grown at Fayoum governorate and sorted out by colour. The identification and authenticity of the pollen botanical origin were performed using an ordinary optical microscope and against standard pollens with well trained expertise persons. The bee-pollens were collected from traps fitted at the hive entrance. It is worth noting that palm pollens exceptionally were collected by hand. The collected pollens were cleaned from dust, air-dried, finely ground and kept in a deep-freezer until analysis. Table (1) shows the characteristic colours of pollen grains.

Table (1): English, scientific, and family names and colours of the bee-collected pollens:

English name	Scientific name	Family name	Colour		
Palm	<u>Phenoix</u> <u>dacylifera</u>	Palmaceae	White		
Chamomile	<u>Matricaria</u> recutita	Asteraceae	Yellow		
Sunflower	<u>Helianthus</u> <u>anns</u>	Compositae	Reddish		
Coriander	Coriandrum sativum	Umbellifereae	Green		

Gross chemical composition of bee pollens:-

Moisture, ash, crude proteins, crude lipids, and crude fiber contents of pollen grains were determined as outlined in *A.O.A.C.* (2000). Total carbohydrate content was estimated by the method of Kimberley and Taylor (1995).

Fatty acid analysis

a- Extraction of lipids:-

Lipids were extracted from pollen samples using chloroform : methanol ($2:1,\,v/v$). The lipids were dried over anhydrous sodium sulphate (Kates, 1972).

b- Preparation of fatty acid methyl esters (FAME)

The pollen fatty acid methyl esters were prepared using methylating agent consists of concentrated sulphuric acid, anhydrous methanol and toluene as mentioned by Hamilton and Hamilton (1992).

c- Gas chromatographic analysis

The chromatographic separation was performed using hp 6890 gas chromatography instrument equipped with a flame ionizing detector using innowax-coss linked polyethylene glycol fused silica column (30 m long, 0.32 mm i.d.; 0.5 µm film thickness). Oven temperature was programmed from 150°C for 1 min. then elevated to 235°C with a rate of 17 C/min, and then was raised again to 245°C with a rate of 1C min and hold at 245°C for 5 min. Gasses flow rates for N2 as a carrier gas, H2 and air were 1.3, 40 and 400 ml / min., respectively. Flame ionization detector and injection temperatures were 275°C and 260°C, respectively.

Determination of amino acids:

The amino acid content of the pollen samples was determined after acid hydrolysis according to Farag *et al.* (1990).

Acid hydrolysis:-

A known amount of finely pollen sample (0.01-0.05 g) was hydrolyzed with 6N HCl (10 ml) in a sealed test tube at 110 °C for 24 h. After hydrolysis, an aliquot from the hydrolysate (2ml) was evaporated to dryness, dissolved in 2 ml loading citrate buffer (6.2 M., pH 2.2) and filtered before loading into the amino acid analyzer.

Citrate elution buffers and detection reagent:

Three citrate buffer solutions were used to elute 18 amino acids. Sodium citrate buffer 1 (0.2 M, pH 3.2) and citrate buffer 2 (0.2 M, pH 4.25) elute the acidic and neutral amino acids, respectively. Citrate buffer 3 (1.2 M, pH 6.45) elutes the basic amino acids. All buffers and NaOH solutions were LKB ultropac chemicals. Ninhydrin detection reagent was consisted of ultrosolve, (2.5 L) ninhydrin (22.5 g), and hydrinantin (1.8 g).

Apparatus and analytical conditions:-

All analysis were performed on an LKB Alpha plus amino acid analyzer equipped with 200 x 46 mm stainless steel column packed with ultropac 8 (8 μ m \pm 0.5 μ m) cation exchange resin. Stepwise elution with the previously mentioned 3 buffers resolved 18 amino acids. The following programmer was used for the separation and detection of amino acids. Buffer 1 was pumped for 7 min followed by buffer 2 for 13 min and buffer 3 for 17.5 min. The column was regenerated (0.4N NaOH) for 3 min followed by equilibration using buffer 1 for 17 min. The column temperature was started at 56 °C for 17 min then raised to 90 °C for 25.5 min, finally cooled down to 50 °C for the remainder of the analysis cycle (13 min). The cycle time from injection to injection was 59.5 min. The flow rate for ninhydrin was 35 ml/h. The reaction between the amino acids and ninhydrin occurred at 135°C in the reaction coil (0.3 mm I. D.) immersed in silicon oil. Detection was performed at two wavelengths, i.e., 570 nm and 440 nm for amino acid and imino acids, respectively. The qualitative and quantitative determination of each amino acid was performed using LKB recorder integrator.

Chemical score:-

The nutrition assessment of the tested pollens was based on their constituents of essential amino acids. The term chemical score is one of the parameters used to assess the quality of proteins. The chemical score was calculated by assignment the most deficient (limiting) essential amino acid. This value was achieved by dividing the amount of each essential amino acid in the sample by the same essential amino acid in reference protein. The lowest value was then multiplied by 100 to obtain the chemical score (FAO/WHO, 1989).

Determination of polyphenols

a- Separation of total phenolic compounds:

The pollen was first defatted with hexane and filtered. The sample was then recovered from the filter and extracted with 50% ethanol. The ethanol was evaporated under vacuum at 35°C and the remaining water was extracted with ethyl acetate (1:1) to separate the phenolic substances. The phenolic fractions were stored in the dark at 4°C until analysis (Campos et al., 2003).

b- Colourimetric determination of polyphenols

The concentration of total polyphenols of pollens was colourimetricly estimated with Folin-Ciocalteau reagent (Gutfinger, 1981).

c- Qualitative and quantitative determination of polyphenols

A Hewlett-Packard Series 1,100 liquid chromatographic system (Waldbronn, Germany) loop 20 µl equipped with a diode array detector and a lichrosorb RP 15 column (4.0 mm i.d.x 250 mm; particle size 5 mm) (Merck, Darmstadt) was used. Elution was performed at a flow rate of 1.0 ml / min with mobile phase of water / acetic acid (98 : 2 v/v, solvent A) and methanol / acetonitril (50 : 50, v/v solvent B), starting with 5 % B and increasing B to levels of 30% at 25 min, 40% at 35 min, 52% at 40 min, 70% at 50min, 100% at 55 min, and kept at this stage for 5 min. A re-equilibration time of 15 min was then required. Quantitation was achieved at 280 nm by internal standard method (Evangelisti, et al., 1997).

RESULTS AND DISCUSSION

1. Proximate chemical composition of pollens

Table (2) presents the chemical composition of sunflower, palm, chamomile and coriander pollens. Palm pollens are characterized by the highest concentrations of ash (14.3%) and crude proteins (39.5%), being about 7.15, 6.22, 3.97 and 2.18, 1.50, 1.16 times as high as that of chamomile, sunflower and coriander pollens, respectively. Coriander pollens had the highest level of crude lipids (13.7%), being more than 2.14, 1.53 and 1.75 times as great as that in palm, chamomile and sunflower pollens, respectively. Chamomile pollens were distinguished by the highest contents of fibres (13.70%), approximately being about 2.12, 3.77 and 2.58 times as high as that in palm, sunflower and coriander pollens, respectively. Chamomile pollens had the highest content of carbohydrates (60.1%), being about 1.79, 1.04 and 1.38 times as high as that in palm, chamomile and coriander pollens, respectively. These findings indicate that the chemical composition of pollens is largely depending upon the botanical origin. Similar results were outlined by Youssef et al. (1978).

Parameter	Sunflower	Palm	Chamomile	Coriander		
Ash	2.3	14.3	2	3.6		
Crude proteins	26.3	39.5	18.1	34.1		
Crude lipids	7.8	6.4	8.9	13.7		
Crude fibers	3.5	6.2	13.20	5.1		

336

57.8

43.5

60.1

Table (2): Proximate chemical composition (%) of studied pollen grains based on dry weight

2. Fatty acid patterns of pollens

Total Carbohydrates

Table (3) shows the fatty acid composition of sunflower, palm chamomile and coriander pollens under investigation. For simplicity, the fatty acid constituents of pollens were divided into three main groups, i.e., trace (<1%), minor (<10% - >1%) and major (>10%) components. The fatty acid pattern of sunflower pollens indicates the presence of 24:0 as a minor substance. On the other hand, the acids 16:0, 18:1, 18:2, 18:3 and 22:0 were present as major constituents. The most predominant saturated and unsaturated acids were 22:0 and 18:3, respectively. Palm pollens contained 13:0, 16:1, 18:0, 18:2, 18:3, 20:0, 23:0 and 24:0 acids as minor substances whereas, 16:0, 18:1 and 22:0 acids were present as major constituents. The most predominant saturated and unsaturated fatty acids were 22:0 (38.79%) and 18:1 (13.99%), respectively. Chamomile pollens contained 20:0, 23:0 and 24:0 as minor substances. The acids 10:0, 16:0, 18:1, 18:2 and 18:3 were present as major constituents. Chamomile pollens were characterized by the highest concentration of palmitic (12.73 %) and oleic (23.05%) acids as saturated and unsaturated ones, respectively.

The pollens of coriander plants were distinguished by having the acids 16:0, 18:1 and 18:3 as major substances. Whereas, 14:0, 18:0, 18:2, 20:0, 23:0 and 24:0 occurred as minor components.

Coriander pollens had the highest 16:0 content and were approximately 1.26, 1.25 and 1.38 and times as high as that of sunflower, palm and chamomile, respectively. In addition, the fatty

acid chromatogram of coriander pollens indicates the presence of 14:0 and not found in any other pollens sources under study. Also, 18:3 content of coriander pollens was about 1.88, 5.68 and 2.26 times as great as that in sunflower, palm and chamomile, respectively.

Chamomile pollens had the highest levels of 18:1 and 18:2 (38.84%), being about 1.54, 1.77, and 1.39 times as high as that in sunflower, palm, and coriander, respectively.

Table (3): Fatty acid composition (area %) of sunflower, palm, chamomile, and coriander pollens

Fatty acid	Sunflower	Palm	Chamomile	Coriander
C10:0	ND	ND	10.11	ND
C13:0	ND	5.47	ND	ND
C14:0	ND	ND	ND	6.87
C16:0	13.96	14.12	12.73	17.62
C16:1	ND	1.17	ND	ND
C18:0	ND	1.33	ND	2.22
C18:1	14.14	13.99	23.05	20.95
C18:2	11.14	8.12	15.79	6.99
C18:3	20.86	6.92	17.38	39.34
C20:0	ND	5.13	6.32	2.73
C22:0	31.72	38.79	ND	ND
C23:0	ND	3.31	7.15	2.08
C24:0	8.18	1.65	7.47	1.2

The ratio of 18:3 / 18:2 of different pollen sources can be arranged in the descending order: palm > chamomile > sunflower > coriander. Fatty acid ratios shown in Table (4) indicate qualitative and quantitative differences between the fatty acids of different pollen sources. The present results demonstrate that fatty acids, taken a group, may provide a key for the identification of pollen sources. In other words, the fatty acids of pollens indicate that their patterns are largely dependent on its botanical origin.

In general, the pollens under study are considered as rich sources in essential fatty acids of interest for human consumption. Similar results were reported by Saa-Otero *et al.* (2000). It is of interest to note that, the fatty acids present in highest concentrations were: palmitic, linoleic and linolenic. This finding is in line with results of Diaz-Losada *et al.* (1996).

The Food and Agriculture Organization promotes increasing the ratio of polyunsaturated to saturated fatty acids in the human diet to prevent atherosclerosis and coronary heart disease (Department of Health, UK, 1994). Also, dietary supplementation with ω -3 PUFS was shown to decrease plasma triglycerides (Boberg *et al.*, 1986). The fatty acids of pollens were rich in C18:2 ω -6 and C18:3 ω -3PUFA.

The results of the previously mentioned authors indicate that there is a relationship between the content of saturated fatty acids and coronary heart disease. Hence, the atherogenic index for the pollens under investigation was calculated according to De Lorenzo *et al.* (2001). The atherogenic index of the different pollens was arranged in descending order: chamomile > sunflower > coriander > palm. These findings suggest the use of chamomile pollens as a supplement for elderly people to overcome to some extent the corony heart disease.

Table (4): Fatty acid ratios and atherogenic index ratios of pollens

Parameter	Sunflower	Palm	Chamomile	Coriander	
C18:3/ C18:2 ratio	1.87	0.85	1.10	5.63	
Total PUFA ω-6	11.14	8.12	15.79	6.99	
Total PUFA ω-3	20.86	6.92	17.38	39.33	
Total PUFA ω-6/Total PUFA ω-3	1.87	0.85	1.1	5.63	
Atherogenic index	0.303	0.468	0.226	0.364	

SFA, MUFA and PUFA refer to total saturated, monounsaturated and polyunsaturated fatty acids, respectively.

The atherogenic index is calculated by the following equation (De Lorenzo et al. (2001):

 $AI = (12:0+14:0+16:0)/(\omega-3PUFA+\omega-6PUFA+MUFA).$

3. Amino acid profiles of pollens

The amino acid patterns of sunflower, palm, chamomile and coriander pollens are shown in Table (5). In general, palm pollens contained the highest amounts of all essential amino acids compared with other pollen sources. In this respect, the total essential amino acids of palm was approximately 2.1, 2.78, and 1.59 times as great as

that in sunflower, chamomile and coriander pollens, respectively. Proline was about 2.32, 2.86 and 2.04 times as high in coriander pollens as in palm, chamomile and sunflower pollens, respectively. The present results are in line with the findings of Yuan *et al.* (1988), Diaz-Losada *et al.* (1996) and Szczêsna (2006) who mentioned that all the essential amino acids were detected in pollens of buckwheat, rape, sunflower, maize, and *Codonopsis pilosula*, collected by honey bees. The amino acids occur in highest concentration were glutamic acid, aspartic acid and proline in the pollens under study. Similar results were reported by Diaz-Losada *et al.* (1996) and Szczêsna, (2006).

Generally speaking, the content of the total essential amino acids of pollens can be arranged in the decreasing order: palm > coriander > sunflower > chamomile. In case of total non-essential amino acids the concentration order of pollens was coriander > palm > sunflower > chamomile. According to the above mentioned results, one can use the amino acid patterns of pollens under investigation to characterize its botanical origin. In this respect, Szczêsna (2006) mentioned that the concentration of amino acids and crude protein content was depended on the floral origin of plants.

The quality of a protein is largely depended upon its content of essential amino acids and also the comparison between these levels and the levels of the corresponding essential amino acids of a reference protein. FAO/WHO suggested that a deficit of any essential amino acid would be limit protein synthesis to a comparable degree. In order to compare the protein quality of the pollens under study, chemical score values were calculated by assigning the least deficint amino acid (present in lowest value than that of the reference protein) then this value was multiplied by 100. It is worth mentioning that the least limiting essential amino acids in all pollens were the sulfur amino acids (methionine + cysteine). It is worth mentioning that tryptophan is destroyed by acid hydrolysis. Therefore, pollen proteins were acid hydrolysed and this amino acid is not recorded among the present data. Rayner and Langridge (1985) reported that tryptophan is the first limiting amino acid in Australian pollens for honeybees. Therefore, this result is not in agreement with this finding and the data of the present work. According to the chemical score values, the quality of protein pollens under study can be arranged in the decreasing order: palm>sunflower> chamomile = coriander (Table 6).

Table (5): Amino acid compositions (mg/g protein of different Pollens) based on dry weight.

Essential Amino acid	Sunflower	Palm	Chamomile	Coriander	
Threonine	2.6	5.4	2.0	4.2	
Cysteine	0.2	1.0	0.2	0.2	
Isoleucine	1.8	4.0	1.2	2.4	
Methionine	0.2	1.0	0.0	0.0	
Valine	2.6	5.0	1.8	3.2	
Leucine	4.2	8.8	3.0	5.6	
Tyrosine	1.4	4.0	1.0	1.8	
Phenylalanine	2.4	5.2	1.6	3.0	
Histidine	2.0	3.0	1.8	2.0	
Lysine	4.6	8.8	4.0	6.6	
Total essential amino acids	22.0	46.2	16.6	29.0	
Aspartic acid	6.8	11.0	5.0	9.4	
Glutamic acid	7.0	15.4	4.8	11.0	
Serine	3.6	6.4	2.8	5.8	
Proline	36.4	32.0	26.0	74.4	
Glycine	3.0	6.2	2.4	4.0	
Alanine	3.0	7.4	3.0	4.0	
Arginine	3.0	7.0	2.0	3.8	
Total non-essential amino acids	62.8	85.4	46.0	112.4	

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Essential amino acid	Reference protein (A)	Su	Su / (A)	Pa	Pa/(A)	Ch	Ch/ (A)	Co	Co/ (A)
Methionine + Cysteine	35	0.4	0.011	2.0	0.057	0.2	0.006	0.2	0.006
Isoleucine	40	1.8	0.045	4.0	0.100	1.2	0.030	2.4	0.060
Valine	50	2.6	0.052	5.0	0.100	1.8	0.036	3.2	0.064
Histidine	26	2.0	0.077	3.0	0.115	1.8	0.069	2.0	0.077
Leucine	70	4.2	0.060	8.8	0.126	3.0	0.043	5.6	0.080
Threonine	40	2.6	0.065	5.4	0.135	2.0	0.050	4.2	0.105
Tyrosine + Phenylalanine	60	3.8	0.063	9.2	0.153	2.6	0.043	4.8	0.080
Lysine	55	4.6	0.084	8.8	0.160	4.0	0.073	6.8	0.120
Chemical score		1.1		5.7		0.6		0.6	

Table (6): Chemical scores of sunflower (Su), palm (Pa), chamomile (Ch) and coriander (Co) pollen grains.

Chemical score = the least limiting amino acid x100

From the nutrition standpoint of view one would suggest to add palm pollens as a supplement to foods of elderly subjects.

4. Polyphenols

Several chemical, biochemical and microbiological studies were carried out with a wide variety of compounds from pollens. Recently, the attention has focused on special group mainly the phenolic compounds (Campos *et al.*, 2002 and 2003). Active oxygen free radicals have been implicated as causative agents in certain disease such as cancer, atherosclerosis, cerebral and cardiac ischemia, Parkinson's disease, gastrointestinal disturbances and ageing (Ames *et al.*, 1993). These free oxidative radicals can be produced both by normal metabolism and by external influences, e.g. UV light and carcinogens. The production of these radicals in quantities that overload the body's natural antioxidant and repair defense system, they can bring about a breakdown of vital cellular components such as coenzymes, neurotransmitters and macromolecules.

The total polyphenolic compounds were determined in sunflower, palm, chamomile and coriander pollens and the results

indicate that the pollens under study were rich in polyphenolic compounds. Sunflower pollens are characterized by the highest levels of the total polyphenolic substances (285.7 ppm as caffeic acid), being approximately 1.28, 1.43 and 1.44 times as great as that of palm, chamomile and coriander pollens, respectively.

HPLC chromatographic analysis indicated that chlorogenic acid is the most abundant polyphenols in pollens. Coriander pollen had the higher content of chlorogenic acid and it was approximately, 1.01, 1.02 and 1.06 times as high as that of sunflower, chamomile and palm, respectively. Hence, the content of chlorogenic acid of different pollens was in the decreasing order: coriander > sunflower > chamomile > palm.

Several authors mentioned the valuable beneficial effects of chlorogenic acid. For instance, Bouayed *et al.* (2007) reported that chlorogenic acid has anxiolytic effects coupled with antioxidant activity. Reduced risk for cardiovascular diseases (CVD) is often attributed to phytochemicals lowering excessive serum glucose, cholesterol, and/or triglycerides concentrations (Howard and Kritchevsky, 1997). Chlorogenic acid, have been claimed to modulate the activity of glucose-6-phosphatase involved in reducing the risk of CVD by decreasing oxidation of low density lipoproteins, cholesterol and total cholesterol (Nardini *et al.*, 1995, and 1997).

In addition, Yonathan *et al.* (2006) pointed out that chlorogenic acid possesses strong anti-inflammatory and anti-nociceptive activities. Chlorogenic acid present in pollens may provide health promoting advantages to consumers. These results demonstrated the importance of bee pollen for the health of human being.

REFERENCES

- Almeida-Muradian, L. B.; Pamplona, L. C.; Coimbra, S.; Barth, O. M. (2005). Chemical composition and botanical evaluation of dried bee pollen pellets. J. Food Compos. Anal. *18*: 105-111.
- Ames, B. N.; Shigenaga, M. K. and Hagen, T. M. (1993). Oxidants, antioxidants, and the degenerative diseases of ageing. Proceedings of the National Academy of Sciences USA 90, 7915–7922.
- A.O.A.C., (2000) Official Methods of Analysis of the Association of Official Analytical Chemists, 17th edn (edited by W. Horwitz). Washington, DC.

- Boberg, M.; Vessby, B and Selinus, I (1986). Effect of dietary supplementation with ω-6 and ω-3 long chain polyunsaturated fatty acids on serum lipoproteins and platelet function in hyperglyceridaemic patients. Acta Medical Scandinavia, 220: 153-160.
- Bouayed, J.; Rammal, H.; Dicko, A.; Younos, C. and Soulimani, R. (2007). Chlorogenic acid, a polyphenol from Prunus domestica (Mirabelle), with coupled anxiolytic and antioxidant effects. J. Neu. Sci. 262: 77–84.
- Campos, M. G.; Markham, K. R. and Proença da Cunha, A. (1997). Quality assessment of bee-pollens using flavonoid/phenolic profiles. Polyphen. Comm. *96*: 53-54.
- Campos, M.G.; Webby, R.E.; Markham, K.R. (2002). The unique occurrence of the flavone aglycone tricetin in myrtaceae pollen. Zeitschrift fur Naturforschung C *57*: 944–946.
- Campos, M.G., Webby, R.F., Markham, K.R., Mitchell, K.A., Da Cunha, A.P., (2003). Age-induced diminution of free radical scavenging capacity in bee pollens and the contribution of constituent flavonoids. J. Agr. Food Chem. *51*: 742–745.
- De Lorenzo, A., Petroni, M. L., De Luca, P. P., Andreoli, A., Morini, P., Iacopino, L., (2001). Use of quality control indices in moderately hypocaloric Mediterranean diet for treatment of obesity. Diabetes, Nutr. Metab. *14*: 181–188.
- Department of Health, UK, (1994). Nutritional aspects of cardiovascular disease. Report on health and social subjects No. 41, HMSO, London.
- Diaz-Losada, E.; Fernandez-Gomez, E.; Alvarez-Carro, C. and Saa-Otero, P. (1996). Contribution to the understanding of the floral origin and chemical composition of bee-collected pollen in Galicia, Spain. Boletin de la Real Sociedad Espanola de Historia Natural, Seccion Biologica *92*: 195-202.
- Dudov, I. A.; Morenets, A. A.; Artinkh, V. P.; Starodub, N. F. (1994). Immunomodulatory effect of honeybee flower pollen load. Ukr. Biokhim. Zh. 66(6) 91-93.
- Dudov, I. A. and Starodub, N. F. (1994). Antioxidant system of rat erythrocytes under condition of prolonged intake of honeybee flower pollen load. Ibid. 66: 94-96.
- Evangelisti, F.; Zunin, P.; Tisconia, E.; Petacchi, R.; Drava, G. and Lanteri, S. (1997). Stability to oxidation of virgin olive oils as

- related to olive conditions: Study of polar compounds by chemometric methods. J. Am. Oil Chem. Soc., 74: 1017-1022.
- FAO/WHO (1989). Protein quality evaluation. Report of the Joint FAO/WHO Expert consultation 4-8 December. Bethesda, MD., USA
- Farag, R. S.; Shaban, O. A.; Ragad, A. A. and Abd El–Aziz, N. M. (1990). Chemical evaluation of macadamia nuts and pritcharadia seeds. Grasas y Aceites, *41*: 313-319.
- González-Güerca, M. C.; Almaraz-Abarcaz, N.; Ávila-Reyes, J. A.; Herrera- Corral, J. and Naranjo-Jiménez, N. (2001). Polen apícola: una alternative alimenticia y terapéuica. Apitec 28: 19-23.
- Gutfinger, T. (1981). Polyphenols in olive oils. J. Am. Oil Chem. Soc. 58:966-968.
- Hamilton, R. J. and Hamilton, S. (1992). Lipid Analysis, A practical Approach 1st ed. Oxford University Press, Inc. Walton Street O X2 6DP, New York p.49.
- Howard, B. and Kritchevsky, D. (1997). Phytochemicals and cardiovascular disease, a statement for healthcare professionals from the American Heart Association. Circulation 95: 2591–2593.
- Kates, M. (1972). Techniques of lipidology, isolation, analysis and identification of lipids, North Holland Pub, Co., Amsterdam, P. 349.
- Kimberley, A. C. and Taylor, C. (1995). A modification of the phenol/sulfuric acid assay for total carbohydrates giving more comparable absorbances. Appl. Biochem. Biotech. *53*: 207-215.
- Kroyer, G. and Hegedus, N. (2001). Evaluation of bioactive properties of pollen extracts as functional dietary food supplement. Innov. Food Sci. Emerging Technol. 2: 171-174.
- Leja, M., Mareczek, A.; Wyzgolik, G.; Klepacz-Baniak, J.; Czekonska, K. (2007). Antioxidative properties of bee pollen in selected plant species. Food Chem. *100*: 237-240.
- Liebelt, R. A. and Calcagnetti, D. (1999). Effect of a bee pollen diet on the growth of the laboratory rat. Am. Bee J. *139*: 390-395.
- Nardini, M.; D'Aquino, M.; Tomassi, G.; Gentili, V.; Di-Felice, M. and Scaccini, C. (1995). Inhibition of human low-density lipoprotein oxidation by caffeic acid and other hydroxycinnamic acid derivatives. Free Radical Bio. Med. *19*:541–552.
- Nardini, M.; Natella, F.; Gentili, V.; Di-Felice, M. and Scaccini, C. (1997). Effect of caffeic acid dietary supplementation on the

- antioxidant defense system in rat: an in vivo study. Arch. Biochem. Biophys. *342*:157–160.
- Ohta, S., Fujimaki, T.; Uy, M. M.; Yanai, M.; Yukiyoshi, A.; Hirata, T. (2007). Antioxidant hydroxycinnamic acid derivatives isolated from Brazilian bee pollen. Nat. Prod. Res. *21*:726-32.
- Rayner, C. J. and Langridge, D. F. (1985). Amino acids in bee-collected pollens from Australian indigenous and exotic plants. Aust. J. Exp. Agr. 25:722-726.
- Rita Elkins, M. A. (1996). Bee Pollen, Royal Jelly, Propolis and Honey: An Extraordinary Energy and Health Promototing Ensemble. Woodland Publishing, Pleasant Grove, UT 84062.
- Saa-Otero, M. P.; Diaz-Losada, E. and Fernandez-Gomez, E (2000). Analysis of fatty acids, proteins and ethereal extract in honeybee pollen. Grana. *39*: 175-181.
- Serra-Bonvehi, S. J. and Escola-Jordá, E. R. (1997). Nutrient composition and microbiological quality of honeybee-collected pollen in Spain. J. Agr. Food Chem. 45: 725-732.
- Szczêsna, T. (2006). Protein content and amino acids composition of bee-collected pollen originating from Poland, South Korea and China. J. Apicult. Sci. *50*: 91-99.
- Yamaguchi, M.; Hamamoto, R.; Uchiyama, S.; Ishiyama, K. and Hashimoto, K.(2006). Anabolic effects of bee pollen *Cistus ladaniferus* extract on bone components in the femoral-diaphyseal and metaphyseal tissues of rats in vitro and in vivo. J. Health Sci. 52(1): 43-49.
- Yonathan, M.; Asres, K.; Assefa, A. and Bucar, F. (2006). In vivo anti-inflammatory and anti-nociceptive activities of Cheilanthes farinose. J. Ethnopharmacol. *108*: 462–470.
- Youssef, A. M.; Farag, R. S.; Ewis, M. A.; and El- shakaa, S. M. A. (1978). Chemical studies on pollen collected by honeybees in Giza Region. J. Apic. Res. *17*:110-113.
- Yuan, H.; Zhang, C. and Du, J. (1988). Studies on the enzymes and amino acids of bee-collected pollen. Beijing Daxue Xuebao, Ziran Kexueban. 24: 483-488.

دراسات كيماوية حيوية على حبوب اللقاح لبعض النباتات الطبية و العادية

رضوان صدقى فرج * محمد مجدى راشد * صفاء عبد الوهاب عبد الوهاب ** على ابراهيم سيد احمد ** هاله على ثابت ***

* قسم الكيمياء الحيوية-كلية الزراعة - جامعة القاهره.

** قسم تشعيع الأغذية – المركز القومي لبحوث و تكنولوجيا الإشعاع – الهيئة المصرية للطاقة الذرية. *** معهد بحوث تكنولوجيا الأغذية – مركز البحوث الزراعية- وزارة الزراعة.

اجرى البحث بهدف معرفة القيمة الغذائية لحبوب اللقاح المجمعة من عباد الشمس ونخيل البلح و الكاموميل و الكزبرة. تم تقدير التركيب الكيماوى الذى يشمل على الرماد- البروتين- الدهون- الكربوهيدرات الكلية – الألياف وكذلك المحتوى من الأحماض الدهنية و الأمينية بالإضافة الى المركبات الفينولية العديدة. ودلت النتائج على ما يلى:-

- 1- يعتمد التركيب الكيماوى العام لحبوب اللقاح على المصدر النباتي الذي جمعت منه حبوب اللقاح.
- 2- أوضح التحليل الكروماتوجرافي الغازى ان الأحماض الدهنية المشبعة وغير المشبعة شائعة الإنتشار لحبوب اللقاح من عباد الشمس و النخيل هي 22:0 , 18:1 و 18:1 على التوالى. تحتوى حبوب اللقاح من الكاموميل على اعلى تركيز من حمض البالميتيك كحمض مشبع (12.73%) و الأوليك كحمض دهني غير مشبع (23.05%). واوضحت الدراسة انه عن طريق بصمة الأحماض الدهنية يمكن التعرف على المصدر النباتي لحبوب اللقاح. ومن دراسة الأحماض الدهنية تم استنتاج دليل خفض الإصابة بامراض القلب Atherogenic Index و في هذا السياق امكن ترتيب حبوب اللقاح تحت الدراسة على النحو التالى: كاموميل > عباد الشمس > الكزبرة > نخيل البلح.
- 3- تم التعرف على 17 حمض امينى فى كل انواع حبوب اللقاح تحت الدراسة . تحتوى حبوب لقاح النخيل على اعلى محتوى من الأحماض الأمينية الأساسية. وجد ان فى جميع الأنواع تحت الدراسة ان الأحماض الكبريتية هى العامل المحدد لحساب المقياس الكيميائى (Chemical Score). وتبعا للمقياس الكيميائى والذى يدل على نوعية البروتين امكن ترتيب حبوب اللقاح تحت الدراسة الى ما يلى :-
 - نخيل البلح > عباد الشمس > الكاموميل = الكزبرة.
- 4- أوضح التحليل الكروماتوجرافي السائلي (HPLC) ان حمض الكلوروجينيك هو المركب الفينولي العديد السائد في حبوب اللقاح تحت الدراسة. وامكن ترتيب حبوب اللقاح في النباتات المختلفة طبقا لمحتواها من حمض الكلوروجينيك الى ما يلى : الكزبرة > عباد الشمس > الكاموميل > نخيل البلح.

وبصفة عامة تظهر هذه الدراسة اهمية حبوب اللقاح من ناحية القيمة الغذائية.