# Fatty Acids and B Vitamins Contents in Honey Bee Collected Pollen in Relation to Botanical Origin

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**Received 7 April 2016 - Accepted 9 June 2016**

## ABSTRACT

The study was conducted to determine the fatty acids and B vitamins concentrations of bee-pollen from the major pollen sources in Al-Ahsa, Saudi Arabia. The highest concentration of total lipids was obtained from bee-pollen collected from summer squash. The fatty acids analysis of fresh bee-pollen showed that the tested bee-pollen samples were rich in palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), linolenic ($C_{18:3}$), arachidic ($C_{20:0}$), behenic ($C_{22:0}$), and lignoceric ($C_{24:0}$) acids. The amount of fatty acids was varied and well correlated with the origin of the samples. Among the fatty acids, oleic content showed high value in bee-pollen from alfalfa and date palm. The contents of stearic and linolenic fatty acids were found in high ratio in pollen collected from summer squash. The concentration (mg/100g) of B vitamins ranged from 0.04 to 0.77 ($B_3$), 0.03 to 0.77 ($B_6$), 0.03 to 2.33 ($B_9$) and 0.05 to 2.50 ($B_{12}$) in bee-pollen collected from the five plants sources. Overall, fatty acids composition of pollen containing both the unsaturated and saturated showed considerable variations affected by the plant species from which pollen was collected. It was concluded, that the fatty acids composition and vitamin B of bee-pollen can be correlated with the botanical origin.

**Key Words:** B vitamins, Bee-pollen, Botanical origins, Fatty acids, Lipids.

## INTRODUCTION

Honey is the main source of carbohydrates in bee-food. The other important nutrients namely proteins, lipids, minerals and vitamins are obtained from pollen (Almeida-Muradian *et al*., 2005; Human and Nicolson, 2006; Mărghitaş *et al*., 2009; and Taha, 2015a and b). Furthermore, pollen is the main source of survival, reproduction and the productivity of the colony (Morais *et al*., 2011). Meanwhile, honey bees development, nutrition and reproduction are due to the fatty acids present in pollen (Manning, 2001; and Szczesna, 2006). Two main factors the botanical origin and the environment are greatly influence the pollen composition (Szczesna *et al*., 2002).

The concentration of lipids in collected pollen is varied depended on the plant species. The range of ether-extractable material was ranged from 0.80 % to 18.90 % in dry collected pollen (Roulston and Cane, 2000). Whereas, the lipids contents were low in pollen collected from different plant species and when mixed with bee substances (Campos *et al*., 1996; Abo-Lila *et al*., 1998; and Al-Jabr and Nour, 2001). Besides, pollen is a reliable source of bee nutrition (Dimou and Thrasyvoulou, 2009).

The fatty acids provide $O_2$ to blood for cellular division and hemoglobin synthesis (Youdim *et al*., 2000; and Yehuda *et al*., 2002). Among the various fatty acids, oleic and palmitic are the major components of body fat up to 60 % in worker bees, 40 % in larvae and 58 % in queen pupae (Manning, 2001). Besides, oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) are the main fatty acids along with dominant mineral concentration of potassium, phosphor, calcium and magnesium in the pollen (Bonvehi and Jorda, 1997).

It has been reported that pollen collected by honey bees contains less than 10 % of total lipid contents. Whereas, the fat contents of fresh pollen are higher compared to either stored or honey bee collected pollen (Human and Nicolson, 2006). Many researchers found that lipids composition of pollen depend on the botanical and geographical origin (Muniategui *et al*., 1991; Roulston and Cane, 2000; Szczesna, 2006; and Taha, 2015b). Also, bee-pollen contains high contents of long-chain fatty acids with predominant level of linoleic (18:2n-6), α-linolenic (18:3n-3), and palmitic (16:0) (Szczesna, 2006). Furthermore, the fat composition of pollen is different depended on the geographical origin and some contains high concentration of unsaturated fatty acids (Human and Nicolson, 2006; and Szczesna, 2006). Generally, growth and health of insects depends on the vitamins present in the pollen containing some organic compounds (Ball, 1994; and de Arruda *et al*., 2013).

Very few data are available upon the fatty acids content in Saudi bee-pollen. The aim of this study is to determine fatty acids and B vitamins composition of pollen collected from the major sources of Al-hasa Oasis, eastern region of Saudi Arabia.

## MATERIALS AND METHODS

The study was carried out at the Agricultural and Veterinary Training and Research Station, King Faisal University, Al-hasa province (Saudi Arabia). The geographical location of the site is at latitude 25° 25′ 46″ N and longitude 49° 37’ 19” E and 121 m above sea level. Five colonies (each of nine combs) of *Apis mellifera jemenitica* having a set of equal strength (brood, stored food, and bee population) were used for pollen collection throughout the year.

### Pollen collection

Bee-pollen was collected twice a week from a trap at the entrance of hives from January to December in 2014. The fresh pollen was dried at 40°C for 2 hours. The bee-pollen was hand sorted by appearance and color. The floral origin of each fraction was identified by microscopical examinations (Vorwhol, 1981).We identified the pollen types according to their size and shape by comparison with pollen reference slides made by (Taha, 2015a). Pollen samples containing mainly monofloral pollen loads from summer squash (*Cucurbita pepo* Thunb), date palm (*Phoenix dactylifera* L.), sunflower (*Helianthus annuus* L.), rape (*Brassica napus* L.), and alfalfa (*Medicago sativa* L.) were selected as major sources (Taha, 2015 a,b), and stored at -21°C until analyzed.

### Analysis of pollen

Pollen samples were analyzed for different parameters by following the appropriate methods. A sample of 2.00 g was used for determination of the lipids using a Soxhlet extractor with diethyl ether as the solvent (Folch *et al*., 1957). Fatty acids and their fractions with the methods of Bligh and Dyer (1959); Szczesna (2006) by utilization of a Hewlett-Packard 5890 gas chromatograph. Overall, the concentration of different fatty acids was calculated on dry weight basis.

### Composition of different vitamins

Bee-pollen contains various types of B vitamins which were determined by using HPLC following the standard analytical procedures. For example: Vitamin $B_3$ and $B_6$ were determined by following the method of Szczesna and Rybak-Chmielewska (1998), Vitamin $B_9$ was determined by the procedure described by Stanley and Linskens (1974), and Vitamin $B_{12}$ was determined by methods described by Bogdanov (2004) and Antakli *et al*. (2014).

### Statistical analysis

Experimental data were analyzed by analysis of variance (ANOVA) according to SAS PROC GLM ver. 9.1 (2003). Duncan’s Multiple Range Test was applied for comparing means using 0.05 significance level, (Duncan, 1955). The following model was used;

$Y_{ij}=\mu+B_i+e_{ij}$

where $y_{ij}$ is the dependent variable

u is the overall mean

$B_i$ is the Plant source (summer squash, date palm, sunflower, rape, and alfalfa).

$e_{ij}$ is the random error assumed to be normally distributed with zero mean and variance $\sigma^2$e.

Due to missing observations, data of behenic acid were analyzed by T-test. Meanwhile, data of the lignoceric acid were not analyzed because it was detected in bee-pollen from date palm only.

## RESULTS AND DISCUSSION

### Lipids and Fatty Acids

The content of lipids found in present study ranged from 2.20 to 6.13%. Similar results were obtained by (Rogala and Szymaś, 2004) for Polish pollen, (Taha, 2015a and b) for Saudi bee-pollen, but lower than the values obtained by Singh *et al*. (1999), Carpes *et al*. (2009), and Martins *et al*. (2011) Mărgăoan *et al*. (2012). Furthermore, analysis of the lipid content of bee-pollen indicated that the highest amount (6.13%) of extracted lipids was found in summer squash pollens, while the lowest value (2.20%) was obtained from date palm pollen (Table 1).

Table 1. Saturated, unsaturated and essential fatty acids of bee-pollen from selected botanical origins.

| Botanical origin | Lipid content (%) | Saturated fatty acids (%) | Unsaturated fatty acids (%) | Unsaturated/ Saturated ratio | Essential fatty acids (%) |
|---|---|---|---|---|---|
| Alfalfa | 3.23[c] | 33.83[e] | 66.15[a] | 1.96[a] | 17.07[c] |
| Date palm | 2.20[d] | 48.98[b] | 51.00[d] | 1.04[d] | 11.54[d] |
| Rape | 4.37[b] | 35.99[d] | 63.99[b] | 1.78[b] | 36.16[a] |
| Summer squash | 6.06[a] | 52.23[a] | 47.74[e] | 0.91[e] | 33.50[b] |
| Sunflower | 4.96[b] | 40.87[c] | 59.10[c] | 1.45[c] | 36.19[a] |
| Significant | ** | ** | ** | ** | ** |

Different letters in same column indicate significant difference. ** indicate $p<0.01$.

The high lipid levels in sunflower pollen were confirmed by Nicolson and Human (2013) and Taha (2015a and b). The present investigation showed significant differences in concentrations among fatty acids in various bee pollen (Table 1). The maximum concentration of saturated fatty acids (SFAs) was 52.23% in summer squash, 48.98% in date palm; followed by sunflower (40.87%) and 35.99% in rape; whereas the least value 33.83% was registed in alfalfa. On the other hand, the maximum concentration of unsaturated fatty acids (USFAs) was 66.15% in alfalfa, 63.99% in rape; followed by 59.10 % in sunflower and 51.00% in date palm; whereas the lowest value 47.74% was remarked in summer squash (Table 1). Data in Table 1 showed that the ratios of USFAs/ SFAs differed significantly among different botanical origins. The highest ratio was 1.96 in alfalfa, 1.78 in rape; followed by sunflower with 1.45 and 1.04% in date palm; whereas 0.91 was the lowest ratio in summer squash. Similar views were expressed by Bonvehi and Jorda (1997); Szczesna (2006); and Mărgăoan *et al*., (2014) who reported a maximum ratio of 1.82 with a mean value of 1.43. Similar views were expressed by many researchers who reported that different levels of various fatty acids depend on the pollen source, geographical origin, age and nutritional status of plants required for the development and brood of honey bees (Manning, 2001; and Morais *et al*., 2011). The highest concentration (36.19%) of essential fatty acids was found in sunflower, whereas the least value (11.54%) was obtained from date palm.

Mean concentration of all the fatty acids was significantly ($p<0.01$) different in all the bee-pollen samples (Table 2).

Table 2. Fatty acid content (%) of pollen from selected botanical origins.

| Fatty acid | Concentration (%) | | | | | Significant |
|---|---|---|---|---|---|---|
| | Alfalfa | Date palm | Rape | Summer squash | Sunflower | |
| Palmitic ($C_{16:0}$) | 27.49[b] | 13.34[d] | 17.38[c] | 11.23[e] | 30.10[a] | ** |
| Stearic ($C_{18:0}$) | 6.34[d] | 4.72[e] | 14.97[b] | 29.28[a] | 7.77[c] | ** |
| Oleic ($C_{18:1}$) | 49.08[a] | 39.46[b] | 27.83[c] | 14.24[e] | 22.91[d] | ** |
| Linoleic ($C_{18:2}$) | 7.62[b] | 4.92[c] | 7.14[b] | 3.68[d] | 27.40[a] | ** |
| Linolenic ($C_{18:3}$) | 9.45[b] | 6.62[d] | 29.02[a] | 29.82[a] | 8.79[c] | ** |
| Arachidic ($C_{20:0}$) | ND | 3.22[c] | 3.64[b] | 9.28[a] | 3.00[d] | ** |
| Behenic ($C_{22:0}$) | ND | 18.34[a] | ND | 2.44[b] | ND | ** |
| Lignoceric ($C_{24:0}$) | ND | 9.36 | ND | ND | ND | – |
| Sum of $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ | 72.49[c] | 55.72[e] | 78.96[a] | 77.02[b] | 66.87[d] | ** |

The means of each row followed by a different letter are significantly different. ** indicate p<0.01. ND= non-detected.

The order of abundance of various fatty acids in the bee-pollen was oleic acid ($C_{18:1}$) from (14.24% to 49.08%) followed in descending by palmitic acid ($C_{16:0}$) from 11.23% to 30.10% > linolenic acid ($C_{18:3}$) from 08.79% to 29.82% > stearic acid ($C_{18:0}$) from 04.72% to 29.28% > linoleic acid ($C_{18:2}$) from 3.86% to 27.40%. Small and miniscule amounts of behenic ($C_{22:0}$) from 02.44% to 18.34%, arachidic ($C_{20:0}$) from 03.00% to 09.28% and lignoceric ($C_{24:0}$) (09.36%). Total of fatty acids were also significantly ($p < 0.01$) different among botanical origins. Rape (78.96%) and summer squash (7.02%) had the highest fatty acids concentrations, followed by alfalfa (72.49%) and sunflower (66.87%), while date palm came lastly (55.72%) (Table 2). The dominant fatty acids in pollen collected from different sources in this study were palmitic ($C_{16:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), and α-linolenic ($C_{18:3}$) acids which agree with findings of (Szczesna 2006; and Mărgăoan *et al*., 2014).

In the present study, oleic and lignoceric acids were significantly different in the bee-pollen which are similar to the results of Szczesna, (2006), and Barissova *et al*., (2010). In another study, the dominant fatty acid in pollen from Brazil was arachidic acid (Bastos *et al*., 2004). On the other hand, the concentration of long-chain saturated fatty acid found in this study was less than 1.5 % which similar with the findings of Szczesna (2006).

Previously many investigations reported that the concentration of different fatty acids vary with the location and sources of pollen (Roulston and Cane, 2000; Manning, 2001; Human and Nicolson, 2006; Szczesna, 2006; Barissova *et al*., 2010, and Mărgăoan *et al*., 2014).

**B Vitamins**

Data in Table 3 show the concentration range of vitamin $B_3$ (niacin) from 0.04 to 0.77 mg/100g, vitamin $B_6$ (pyridoxine) from 0.03 to 0.77 mg/100g, vitamin $B_9$ (folic acid) from 0.03 to 2.33 mg/100 g and vitamin $B_{12}$ (cobalamin) from 0.05 to 2.50 mg/100 g in bee-pollen collected from different sources. Folic acid ($B_9$) was found to be the most abundant estimated vitamin and represented by 90.90, 60.30 and 75.16% of total determined B vitamins for date palm, rape and alfalfa, respectively. Furthermore, cobalamin ($B_{12}$) was found to be the predominant vitamin in bee-pollen from summer squash which estimated as 83.06%

of total determined B vitamins. Moreover, sunflower was characterized with high level (44.76%) of $B_3$ (niacin) and $B_6$ (pyridoxine). These results agreed with those of Stanley and Linskens (1974), Szczesna and Rybak-Chmielewska (1998), Campos *et al*., (2008), and de Arruda *et al*., (2013) who reported varying concentrations of all forms of vitamins useful for human consumption. In addition, they observed that concentration variation among different vitamins may be due to soil type, location, plant species, climate and handling procedures. These results bring new information on fatty acids and B vitamins compositions of pollen as well as the botanical sources visited by pollen-collecting bees. The data will help regulatory bodies establish parameters of quality control for pollen produced in Saudi Arabia. Further studies are necessary to discriminate the effects of botanical origin versus processing and storage conditions on pollen unsaturated/saturated fatty acid ratios.

Table 3. B Vitamins (mg/100g) contents of bee-pollen in relation to botanical origin.

| Botanical origin | $B_3$ (Niacin) | $B_6$ (Pyridoxine) | $B_9$ (Folic acid) | $B_{12}$ (Cobalamin) |
|---|---|---|---|---|
| Sunflower | 0.77±0.005[a] | 0.77±0.001[a] | 0.13±0.001[d] | 0.05±0.001[d] |
| Summer squash | 0.04±0.001[e] | 0.44±0.001[c] | 0.03±0.001[e] | 2.50±0.002[a] |
| Date palm | 0.05±0.001[d] | 0.03±0.001[e] | 1.50±0.010[c] | 0.07±0.001[c] |
| Rape | 0.17±0.001[b] | 0.33±0.001[d] | 1.90±0.020[b] | 0.75±0.001[b] |
| Alfalfa | 0.14±0.001[c] | 0.65±0.002[b] | 2.33±0.010[a] | 0.06±0.001[cd] |
| Significant | ** | ** | ** | ** |

The means of each column followed by a different letter are significantly different. ** indicate $p<0.01$.

## ACKNOWLEDGEMENTS

This work was supported by the Deanship of Scientific Research, King Faisal University, Al-hasa under Project No. 130275.

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# علاقة محتوى الأحماض الدهنية ومجموعة فيتامين (ب) في حبوب لقاح نحل العسل بالأصل النباتي

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**استلام 7 أبريل 2016م - قبول 9 يونيه 2016م**

**الملخص**

أجريت هذه الدراسة بغرض تقدير تركيزات الأحماض الدهنية ومجموعة فيتامين (ب) في حبوب لقاح من نحل العسل المجموعة من المصادر النباتية الرئيسية لحبوب اللقاح في الأحساء، المملكة العربية السعودية. أشارت نتائج التجربة إلى أن أعلى تركيز من الدهون الكلية تم الحصول عليه من حبوب اللقاح المجموعة من نبات الكوسة. أظهرت نتائج تحليل الأحماض الدهنية في حبوب اللقاح أنها كانت غنية بالأحماض الدهنية البالميتيك، واستياريك، والأولييك، واللينولييك، واللينولينيك، والأراكيديك، وبيهينك، وليجنوسيرك. وقد اختلفت كمية الأحماض الدهنية بشكل ملحوظ متأثرة بالمصدر النباتي. وأوضحت الدراسة أن محتوى حامض الأولييك كان مرتفعًا في حبوب اللقاح المجموعة من البرسيم الحجازي ونخيل البلح، ووجدت الأحماض الدهنية الستياريك، واللينولييك بمحتوى عالٍ في حبوب اللقاح المجموعة من الكوسة. كذلك أظهرت الدراسة أن تركيز (مجم/ 100جم) مجموعة فيتامين (ب) في حبوب اللقاح المختبرة تراوحت بين 0.04 – 0.77 (ب$_{3}$)، و 0.03 – 0.77 (ب$_{6}$)، و0.03 – 2.33 (ب$_{9}$)، و 0.05 – 2.50 (ب$_{12}$).

وعموماً فإن تركيب الأحماض الدهنية في حبوب اللقاح أظهرت تباينًا في محتوى الأحماض الدهنية المشبعة وغير المشبعة متأثرة بالنوع النباتي الذي جمع النحل منه حبوب اللقاح. ويمكن استنتاج أن تركيب الأحماض الدهنية ومجموعة فيتامين (ب) في حبوب اللقاح قد يكون مرتبطًا بالأصل النباتي.

**الكلمات المفتاحية:** الأحماض الدهنية، الأصل النباتي، حبوب اللقاح، الدهون، فيتامين ب.