

ORGANIC ACIDS IN DIFFERENT TYPES OF EGYPTIAN HONEY

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ABSTRACT

Organic acids in 4 types of Egyptian honey were analyzed and compared using high performance liquid chromatography (HPLC). The main types of Egyptian honeys used in this study were: Citrus, Clover, Cotton and artificial honey (or adulteration honey) which collected from colonies feeding on sugar syrup. Organic acids which present in some samples of Egyptian honey were eight types in Citrus honey, seven types in Clover honey, five types in Cotton honey and four types in artificial honey. There were only three organic acids (formic, malonic and maleic acids) common in the four experimental types of Egyptian honey. Citrus and Clover honey contains the same seven organic acids (formic, malonic, tartaric, shikimic, maleic, succinic and proponic acids), the 8th organic acid was citric acid present only in Citrus honey. The organic acids present in Cotton honey were formic, malonic, tartaric, shikimic and maleic acids, while in artificial honey it contains formic, malonic, maleic and succinic acids. Statistical analysis represented that there were a significant difference in formic, malonic, tartaric, shikimic and maleic acid between the four tested honeys. Citric acid was present only in Citrus honey, so it can be used to differentiate between Citrus honey and the other three honeys. Also, succinic acid absent in Cotton honey so it could be used to distinguished Cotton honey from the other three honeys. So, organic acids can be used to distinguish between the main different types of Egyptian honey (Citrus, Clover and Cotton) and artificial honey (sugar syrup honey).

Keywords: Honey, organic acids, Citrus honey, Clover honey, Cotton honey, artificial honey, HPLC analysis.

INTRODUCTION

Honey is the most important primary product of beekeeping quantities, from both a quantitative and an economic point of view. Honey was considered a potential complete food, regarding nutritional standards, being a natural product rich in simple sugars (fructose, glucose), enzymes (invertase, glucose oxidase, catalase, phosphatase), amino and organic acids (proline, gluconic acid, acetic acid), vitamins (ascorbic acid, niacin, riboflavin), volatile oils, phenolic acids and flavonoids, minerals and carotenoid like substances (Sudhanshu, *et al.* 2010). As honey contains naturally organic acids, measurement of individual acids in honey is important because they are probably responsible for the antimicrobial action of honey (Bogdanov, 1997). Although organic acids represent less than 0.5% of honey's constituents, they make important contributions to organoleptic, physical, and chemical properties of honey (Inés, *et al.* 2006). Their concentration varies with a wide range, according to honey origin. From the seventeen organic acids of honey (Crane, 1990), non-aromatic organic acids can also be used as predictors of fermentation, antioxidant activity and as

botanical/geographical markers (Mato, *et al.* 2003). In addition, scientists have gathered evidence that organic acid profiles can help to distinguish between different honeys, according to their botanical and geographical origins, and can be also used to detect honey spoilage. The acidity in honey is caused by the organic acids usually existing in all honeys (tartaric, citric, oxalic, acetic, etc. acids), either from nectar or bees' secretions. It is mainly attributed to maleic acid, although it is not the major organic acid of honey (Root and Root 2005). The average pH of honey is 3.9 (with a typical range of 3.4 to 6.1). The low pH of honey inhibits the presence and growth of microorganisms.

Artificial honeys can be produced from carbohydrate sources that have glucose-fructose composition that are within a close range with that of natural honey. These artificial honeys often have similar taste and physical appearance as natural honeys, but they lack the medicinal and nutritional properties of natural honeys because of the absence of the minor constituents that are present in natural honeys. It has also been observed that the composition of minor constituents of natural honeys varies with location/nectar sources and different climatic conditions.

The aim of this study was to use organic acids to distinguish between different types of Egyptian honey as this work was not done before. These natural honey types were Citrus, Clover, Cotton and artificial honey (sugar syrup).

MATERIAL AND METHODES

The present investigation was carried out in the Beekeeping Research Department, Plant Protection Research Institute, during year 2010 to determine the organic acids in 4 types of Egyptian honeys. Honey samples were collected from different regions from Giza governorates. These types were Citrus honey, (*Citrus* spp.) which was represented by 8 samples, Clover honey (*Trifolium alexandrinum*) which was represented by seven samples, Cotton honey (*Gossypium barbadence*) which was symbolized by five samples and artificial honey (feeding the bees with sugar syrup) which was represented by 4 samples. The determination of organic acids in Egyptian honey samples was done using HPLC. The honey solution was obtained by diluted 1 g. honey with 20ml dist. water then mixed and centrifuged. This mixture filtered at 0.45 µm and directly injected into HPLC.

Determination of organic compounds in honey samples

Samples of honeys were analyzed at the Chemical Analysis Laboratory of Honeybee Products at Beekeeping Research Department, Plant Protection Research Institute. ARC. Giza, Egypt for the following properties:-

Identification of organic compounds of the honey samples was performed by a JASCO HPLC, using a hypersil C₁₈ reversed- phase column (250 X 4.66 mm) with 5 µm particle size. A constant flow rate of 0.7ml/min sulphuric acid in distilled water at pH 2.45 was used as mobile phases; the detector set at wavelength 210 nm, which was the optimum for the

simultaneous determination of the acids. The concentration of individual compound was calculated on the basis of the peak area measurements. All chemicals and solvents used were in HPLC spectral grade (Anna, *et al.* 1994).

The data were statistically analyzed using one way ANOVA. LSD was used to evaluate the significant difference between means (comparison of means) at the level of $P < 0.05$ level.

RESULTS AND DISCUSSION

Data in Table (1) represent the organic acids that can be found in the Egyptian honeys under investigation.

Table (1): Organic acids (mg/100gm) in four types of Egyptian honeys

Honey types	Citrus	Clover	Cotton	Artificial	LSD
Organic acids					
formic	1.5719	1.3743	1.3410	0.5710	0.5387
malonic	0.0747	0.2722	0.7990	0.2027	0.2251
tartaric	0.3776	0.0264	0.0513	0	0.1463
shikimic	0.2330	0.1812	0.6190	0	0.1996
maleic	0.1358	0.1115	0.5680	0.1330	0.1852
citric	0.4950	0	0	0	-
succinic	0.1568	0.1097	0	0.0730	0.0838
propionic	0.0630	0.0399	0	0	n.s

n.s= not significant

As represented in Table (1), eight organic acids were detected in analyzing the four honeys under investigation. They were: formic, malonic, tartaric, shikimic, maleic, citric, succinic and propionic acids. Only three of them (formic, malonic and maleic acids) were common and were found in the four tested honeys. Citrus honey was the richest honey as it contained the eight observed acids followed by Clover honey which contained only seven of them. Citric acid was detected only in Citrus honey. Propionic acid was found only in Citrus and Clover honeys. Cotton honey contained five organic acids named formic, malonic, tartaric, shikimic and maleic acids. Only four organic acids (formic, malonic, maleic and succinic acids) were found in the artificial honey. This may be due to the absence of nectar and pollen grains in the artificial diet (sugary syrup).

Table (1) also proved that among the detected acids, formic acid was the main acid in the four honeys under investigation as it was not only detected in the four honeys, but also was detected in the highest amounts as compared with the other organic acids. The Table also showed that oxalic, butyric, isobutyric and benzoic acids which could be found in some types of honeys was not detected in any of the Egyptian tested honeys.

The results in Table (1) and Fig (1) represented that formic acid was detected in the four tested honeys with no significant difference between the three nature honeys (Citrus, Clover and Cotton honey). Artificial honey had the least concentration which differs significantly from any of the natural

honeys. Capolongo, *et al.* (1996) and Stoya, *et al.* (1986) found that the content of formic acid varies from 5 to 600 mg/kg.

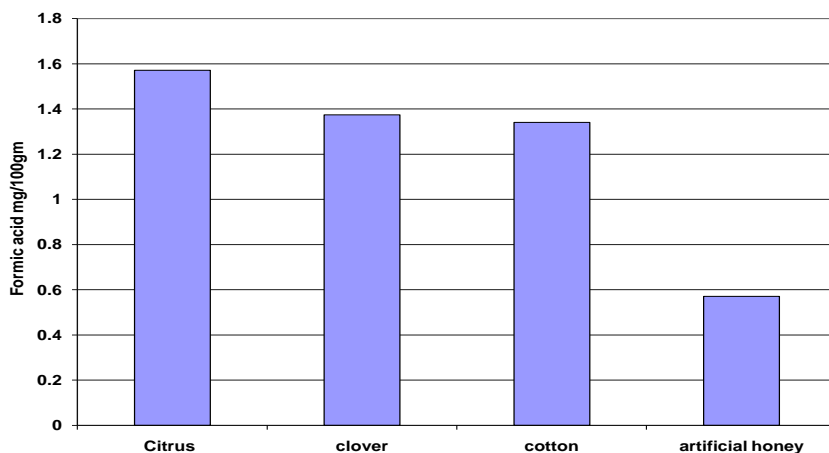


Fig.(1) Formic acid in four types of Egyptian honeys

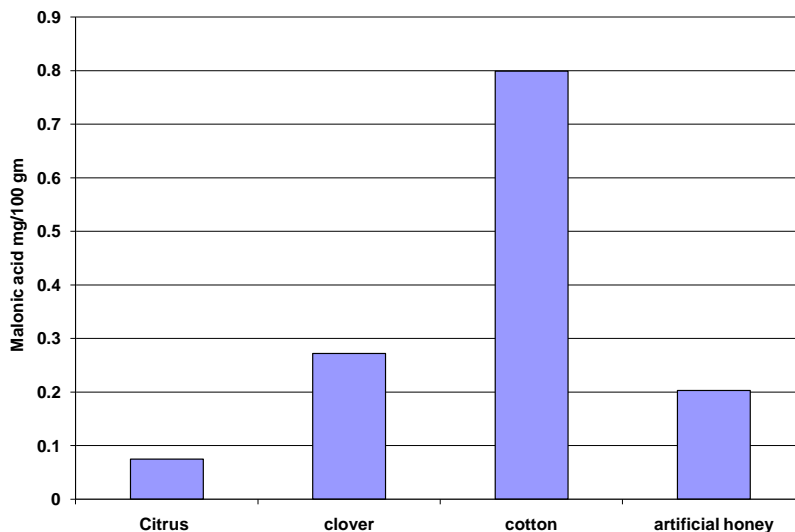


Fig.(2) Malonic acid in four types of Egyptian honeys

Data in Fig. (2) indicated that malonic acid was found in the four honeys under investigation with the highest concentration in the Cotton honey. Citrus honey contained the least concentration, while Clover and artificial honey came in between. The difference in Cotton honey was significantly compared to the three other honeys, while the difference between Clover honey and artificial honey was non-significant. Also, the difference in Citrus honey was significantly and the other three honeys.

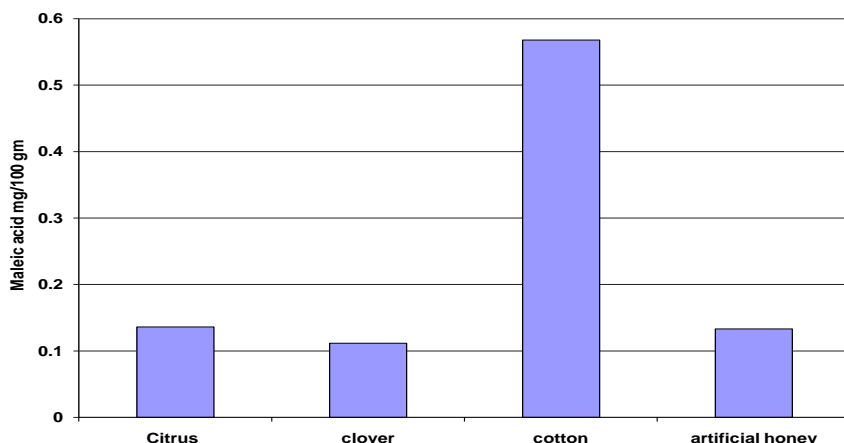


Fig.(3) Maleic acid in four types of Egyptian honeys

The results indicated that maleic acid was found in the four tested Egyptian honeys (in table (1) and Fig.3). Highest concentration was detected in the Cotton honey which differs significantly than the other honeys followed by the artificial honey. Clover honey contained the least quantity.

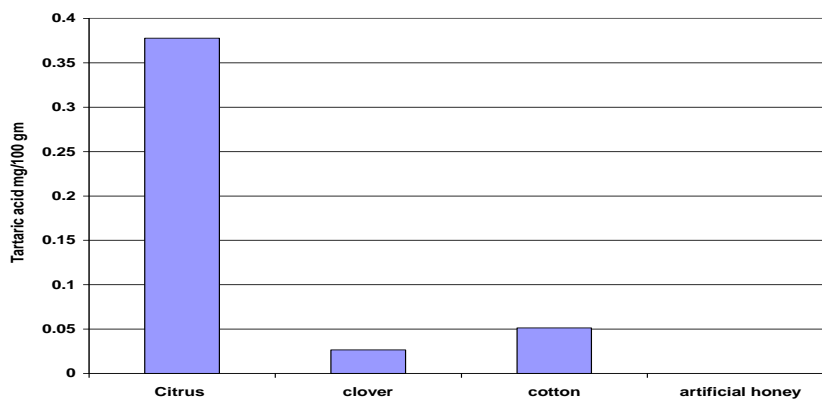


Fig.(4) Tartaric acid in four types of Egyptian honeys

Tartaric acid was found in all the floral honeys (Citrus, Clover and Cotton honeys) but not detected in the artificial honey (Fig. 4). The highest concentration of tartaric acid was detected in Citrus honey which differed significantly than the other two honeys. The lowest concentration was detected in Clover honey and the difference was not significant with the Cotton honey. As tartaric acid was not found in artificial honey it could be used to differentiate between the artificial honey and the three other natural honeys (Citrus, Clover and Cotton).

The results in Table (1) and Fig.(5) showed that shikimic acid was not found in the artificial honey. Cotton honey had the greatest amount of

shikimic acid while it was found in moderate amounts in Citrus and Clover honeys with no significant differences between them. As shikimic acid was not found in artificial honey, it could be used to differentiate between the artificial honey and the other natural honeys.

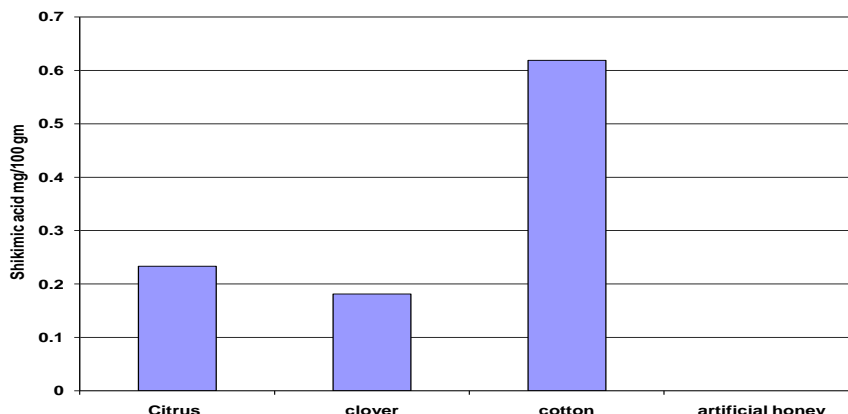


Fig.(5) Shikimic acid in four types of Egyptian honeys

Comparing between the concentrations of organic acids found in the tested honeys Table (1) proved that succinic and propionic acids were found in the lowest quantities. Succinic acid ranged between 0.1563 and 0.073 mg/100 g honey while propionic acid ranged between 0.063 and 0.0339 mg/100 g honey. As shown in Fig. (6) succinic acid was not found in cotton honey accordingly it could be used to distinguish between the Cotton honey and the other three types of honey.

Types and percentages of organic acids in Egyptian honey were differing from that represented in other honey types and this difference may be due to the differences in location and botanical origin of honey. The results represented that Clover honey contained seven organic acids formic, malonic, tartaric, shikimic, maleic, succinic and propionic acids and this results disagreed with Stinson, *et al.* (1960) who mentioned that the acids from Clover honey were butyric, acetic, formic, lactic, succinic, pyroglutamic, malic, citric, and gluconic, while oxalic acid was tentatively identified when isolated by ion-exchange adsorption. Zhu, *et al.* (2010) determined 5 organic acids (L-malic acid, maleic acid, succinic acid, citric acid and D-malic acid) in honey by solid-phase extraction and high performance liquid chromatography (SPE-HPLC). Also, Suárez-Luque, *et al.* (2002) identified malic, maleic, citric, succinic and fumaric acids in honey they used a rapid high-performance liquid chromatographic method. In this study citric acid was present only in Citrus honey and this result was disagreed with that mentioned by Nelson and Mottern (1931) who found that citric acid were present in all floral honeys.

As a conclusion, organic acids may be used to distinguish between the main types of Egyptian honeys (Citrus, Clover and Cotton) and artificial honey (adulteration honey).

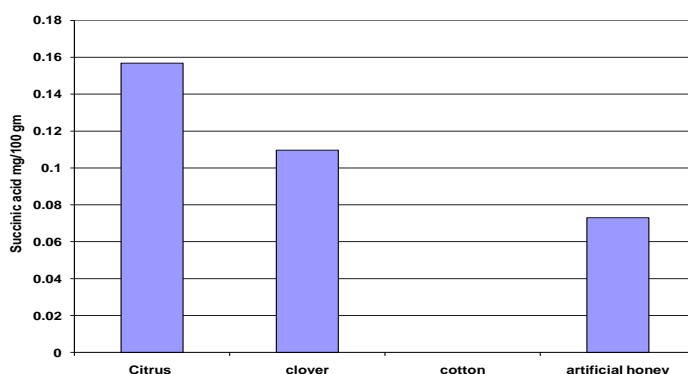


Fig.(6) Succinic acid in four types of Egyptian honeys

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الاحماض العضوية فى الأنواع المختلفة من الأعسال المصرية

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اجرى هذا البحث فى قسم بحوث النحل- الدقى عام ٢٠١٠ لتقدير الأحماض العضوية فى أنواع مختلفة من الأعسال المصرية التى تم تجميعها من أماكن مختلفة فى محافظة الجيزة وهذه الأنواع هى عسل الموالح، عسل البرسيم، وعسل القطن وكذلك العسل الناتج من التغذية السكرية . وقد تم تحليل عينات العسل فى معمل تحليل العسل فى قسم بحوث النحل- معهد بحوث وقاية النباتات- الجيزة باستخدام جهاز HPLC. حيث وجد أن عدد الأحماض العضوية فى عسل الموالح ٨ ، وفى عسل البرسيم ٧ ، وفى عسل القطن ٥ بينما فى عسل التغذية السكرية ٤ . وقد ظهر من التحليل عدم وجود بعض الأحماض العضوية فى الأعسال المصرية وهى اوكساليك، بيوتيريك، ايزوبيوتيريك وكذلك حمض البنزويك . ومن التحليل أيضا وجدت ٣ أحماض عضوية مشتركة فى كل الأعسال المصرية وهى: حمض الفورميك، حمض المالنوك، وكذلك حمض المالك موجود فى كل من عسل الموالح وعسل البرسيم وعسل القطن وكذلك عسل التغذية وكانت الفروق بينهما معنوية. أظهرت الدراسة أن عسل الموالح وعسل البرسيم يحتويا على نفس الأحماض العضوية السبعة وهى: حمض الفورميك، حمض المالنوك، حمض الطرطريك، حمض الشيكيميك، حمض المالك، حمض السكسينيك ، وحمض البروبيونك. بينما حمض الستريك وجد فقط فى عسل الموالح وهو يعتبر مميز لعسل الموالح . الأحماض العضوية الموجودة فى عسل القطن هى: حمض الفورميك، حمض المالنوك، حمض الشيكيميك و حمض المالك؛ بينما الأحماض العضوية الموجودة فى عسل التغذية هى: : حمض الفورميك، حمض المالنوك، حمض المالك وحمض السكسينيك . وقد أظهر التحليل الإحصائى وجود فروق معنوية فى الأحماض العضوية الأتية : حمض الفورميك حمض المالنوك حمض الشيكيميك حمض المالك فى الأعسال المصرية الأتية: عسل الموالح ، وفى عسل البرسيم ، عسل التغذية السكرية. من النتائج المتحصل عليها يتضح أنه يمكن استخدام الأحماض العضوية فى التفرقة بين الأعسال المصرية المختلفة الطبيعية وكذلك الناتجة عن التغذية السكرية.

قام بتحكيم البحث

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