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# **Determination of Organic Acids in Saudian Bee Honey Types**

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## **ABSTRACT**

Eight types of bee honey represented different regions of Saudi Arabia country. Fakhera, Rabie Foyad, El Bashaier, Rabie Kabba, El Saiel Al Kabeer, El Nokhbba, Taba and OmmAzooba were analyzed to determine several organic acid were found as a chemical marker of Saudian bee honey types. It was proved that among the detected acids, formic and Malonic acids were the main acid in the eight honeys, also were detected in the highest amounts as compared with the other organic acids. Oxalic, shikimic, butiric and benzoic acids were not detected in any of the Saudi Arabia tested honeys. Tartaric acid was detected only in Rabie Kabba honey. We can suggested that organic acid profiles can help to distinguish between different honeys, according to their botanical and geographical origins, and can be also used to protect honey spoilage.

Keywords: Bee Honey, organic acids, Saudi Arabia.

## INTRODUCTION

Honey is the most important primary of bee keeping quantities, from both a quantitative and an economic point of view. Honey was considered a potential complete food, regarding nutritional standards, being a nature 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 acid 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 constituent. thev make important contributions to organoleptic, physical and chemical properties of honey (Ines, et al. 2006). Their concentration varies

with a wide range according to honey origin. From the seventeen organic acid 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 distinguish between different honeys, according to their botanical geographical origins, and can be also used to protect 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 aim of this study was to use organic acid to distinguish among different types of Saudian honeys, this work may be was not done before. These honey types were Fakhera, RabieFoyad, El Bashaier, RabieKabba, El Saiel Al

Kabeer, El Nokhbba, Taba and OmmAzooba.

#### MATERIALS AND METHODS

The present investigation was carried out in the Bee keeping Research Department, Plant Production Research Institute, during year 2013 to determine the organic acids in eight types of Saudin honeys. Honey samples were collected from different regions from Saudi Arabia. These types were Fakhera, RabieFoyad, El Bashaier, RabieKabba, El Saiel Al Kabeer, El Nokhbba, Taba and OmmAzooba. The determination of organic acids in Saudian honey samples was done using HPLC. The honey solution was obtained by diluted 1ghoney with 20 ml distilled water then mixed and centrifuged. This mixture filtered at 0.45 um and directly injected into HPLC.

# Determination of organic acids in honey samples

Samples of honeys were analyzed at the Chemical Analysis Laboratory of Honeybee Products at Bee keeping 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 um particle size. A constant flow rate of 0.7 ml / min sulphuric acid in distilled water at PH 2.45 was as mobile phases, the detector set at wavelength 210 nm, which was the for optimum 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).

## RESULTS AND DISCUSSION

As shown in Table (1), twelve organic acids were detected in analyzing the eight honeys under investigation. They were: Oxalic, Formic, Malonic, Tartaric, Shikimic, Maleic, Citrice, Succinic, Proponic, Butiric, Isobutiric and benzoic acids. Only two of them and malonic acids) (formic common and were found in the eight acid tested honeys. Tartaric determined only in RabieKabba honey type.

Fakhera and El Bashaier honeys were the richest honeys contained the six observed organic acids, followed by El Saiel Al Kabeer, Taba and OmmAzoaba honeys which contained only five of them, while RabieFoyad and El Nokhbba honeys were contained only four organic acids.

	Table	e 1:	determination of	f organic a	cids of	Saudi	Arabia	honey types	(mg/100g)
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	Saudian Honey Types									
Acids	Fakhera	Rabie Foyad	El Bashaier	Rabie Kabba	El Saiel Al Kabeer	El nokhbba	Taba	OmmAzoaba		
Oxalic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
Formic	0.16	0.17	0.18	0.001	0.25	0.19	0.21	0.15		
Malonic	0.02	0.01	0.015	0.01	0.02	0.02	0.03	0.02		
Tartaric	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00		
Shikimic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
Maleic	0.001	0.0004	0.001	0.0002	0.001	0.00	0.00	0.002		
Citrice	0.02	0.00	0.05	0.00	0.03	0.004	0.025	0.02		
Succinic	0.00	0.00	0.002	0.001	0.00	0.00	0.00	0.00		
Proponic	0.001	0.02	0.07	0.01	0.06	0.00	0.034	0.03		
Butiric	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
Isobutiric	0.005	0.00	0.00	0.00	0.00	0.01	0.01	0.00		
Benzoic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		

It was also proved that among the detected acids, formic and Malonic acids were the main acid in the eight honeys, also were detected in the highest amounts as compared with the other organic acids. Oxalic, shikimic, butiric and benzoic acids which could be found in some types of honeys were not detected in any of the Saudi Arabia tested honeys. Tartaric acid was detected only in RabieKabba honey.

Formic acid was detected in the eight tested honeys with no highest difference between the six Saudi Arabia honeys (Fakhera, Rabie Faved. El Bashaier, El Nokhbba, Taba and OmmAzooba) was 0.16, 0.17, 0.18, 0.19, 0.21 and 0.15 mg / 100g respectively, while El Sail Al Kabeer honey contained 0.25 mg / 100g it was highest all of them. RabieKabba honey had the concentration 0.001 mg / 100g. malonic acid was found in the eight tested Saudian honeys the content varies from 0.01 to 0.03 mg / 100gm . Stoya, et al. (1986) and Capolongo, et al (1996) found that the content of formic acid varies from 5 to 600 mg / kg. El Mohandes (2011) found in four the Egyptian honeys formic acid from 0.5719 mg / 100 gm.

Succinic acid was found in El Bashaier and RabieKabba honeys 0.002, 0.001 mg / 100g respectively, and not detected in the others tested honeys, but the proponic acid it's found in six tested honeys Rang 0.001 to 0.07 mg / 100 g but it's not found in El Nokhbba honey.

These results disagreed with Stinson; et al (1960) found that the acids from clover honey were butyric, acetic, formic, lactic succinic, pyroglutomic, maloic, citric and gluconic, while oxalic acid was tentatively identified when isolated by ion-exchange adsorption. Zhu, et al. (2010) determined 5 organic acids (1- malic acid, medicine acid, succinic acid, citric acid and d-malic acid) in honey by solid-phase extraction

and high performance liquid chromatography (SPE- HPLC). Also, (Suarez-Lugue, et al, 2002) identified malic, maleic, citiric, succinic and fumaric acids in honeys they used a rapid high-performance liquid chromatographic method. In this study Formic acid and Malonic acid were present in all tested of Saudi Arabia honeys and this result was disagreed with that mentioned by El Mohandes, (2011) she found that Formic acid and Malonic acid were present in all Egyptian floral honevs.

In conclusion, types and percentage of organic acid in Saudian honeys 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. Organic acids may be used to distinguish among the main types of Saudian honeys.

## REFERENCES

Anna, c.; Lorenz, s.; Carlo, T. and Paolo,c (1994): Solid-phase extraction and high-performance liquid chromatographic determination of organic acids in honey. Journal of chromatography a.669:59-64.

Bogdanov, s.; martin, p. and lullmann, c. (1997): Harmonised methods of the European honey commission. Apidologie, APIDGB5 (extra issue), 1-59

Capolongo, F.; Baggio, A.; Piro, R.; Schivo, A.; Mutinelli, F.; Sabatani A.G.; Colombo, R.; Marcazzan, G.L.; Massi, S. and Nanetti, A. (1996): trattamentodellavarroasi con acidoformico: accumulonelmiele e influenza sulle sue caratteristiche, l'Ape nostra Amica, 18, 4-11.

Crane, E. (1990): Bees and Beekeeping. Science, Practice and World Resources. Heinemann; Oxford, UK. 614 pp.

Elmohandes, S.S. (2011) organic acids in different types of Egyptian honey. J. of plant protection and pathology Mansoura University, Egypt V-2 no. (10): 865-872.

Ines, M.; Jose H.F; Juses, s. and Teresa, S.M. (2006): Analytical methods for the

- Determination of Organic Acids in Honey. Critical Reviews In Analytical Chemistry Pulish date: January 1, 2006.
- Mato, I; Huidobro, J.F; Simal-Lozano, J.; Sancho, M.T. (2003): Significance of nonaromatic organic acids in honey. Journal of Food Protection, 66(12):2371-6.
- Nelson, E.K. and Mottern, H.H. (1931): some organic acids in honey. Industrial and engineering chemistry 23(3):335-336.
- Root, A. I. and Root, E. R. (2005): The ABC and XYZ of Bee Culture. Kessinger Publishing; whitefish, MT. 740 pp.
- Stinson, E. E.; Subers, M. H.; Petty, J. and white, J.W. Jr. (1960): The Composition of Honey. V. Separation and Identification of the organic acids. Archives of Biochemistry and Biophysics Vol., Issue 1:6-12.

- Stoya, W.; Wachendorfer G.; Kary I.; siebentritt P,; Jaiser E. (1986): Ameisensaure Als Therapeuticumgegan Varroatose und ihrAuswirkungen auf den Honig. Deutsche Lebensmittel-Runshau 82:217-221.
- Suarez-Luque, S.; Mato, I.; Huidobro, J.F.; Simal-Lozano, J. and Sancho M.T. (2002): Rapid determination of minority organic acids in honey by high-performance liquid chromatograndaphy. J. chromatography A.Vol. 955. Issue 2, 207-214.
- Sudhanshu, S.; satyendra, G. and Sarun, S. (2010): Physical, biochemical and antioxidant properties of some Indian honeys. Food Chem. 118: 391-397.
- Zhu, X.L.; Ye, F, Yang, J.; Xiau, X:Wen, H and Liu, R. (2010): Determination of Organic acids in honey by solid Phase extraction-high performance liquid chromatography., Sepu, 28(10), 945-949

## **ARABIC SUMMARY**

## تقدير بعض الأحماض العضوية في الاعسال السعودية

عماد الدين احمد عبد الحميد نافع  $^1$  إيهاب وفيق زيدان $^1$ اسماء محمد فوزى $^2$  ابراهيم عبدالرزاق شحاته  $^1$  عماد الحيزة  $^2$  معهد بحوث وقاية النباتات  $^2$  قسم بحوث النحل  $^2$  الجيزة  $^2$  مرع  $^2$  كلية الزراعه جامعة الازهر القاهرة

أجرى هذا البحث في قسم بحوث النحل – الدقي عام 2013 لمعرفة الاحماض في الأنواع المختلفة من الاعسال السعودية التي تم تجميعها من ثماني مناطق مختلفة أنتجت فيها و هي :

فخيرة وربيع قويد والبشير وربيع كابا والسيل الكبير والنخبة وطأبا وأم أزوبا. وقد تم تحليل عينات العسل في معمل تحليل العسل بقسم بحوث النحل – معهد بحوث وقاية النباتات – الجيزة باستخدام جهاز ( HPLC).

 $\dot{x}$  المناطق تحتوي على عدد من الأحماض العضوية وجود أن جميع أعسال تلك المناطق تحتوي على عدد من الأحماض العضوية تتراوح ما بين 4 – 6 أحماض حيث تشترك جميع أعسال تلك المناطق في وجود حمضين الأحماض الفور ميك وحمض المالونيك و لا يوجد في جميعها أربعة أحماض من عدد الاحماض المختبرة و هي حمض الأوكساليك وحمض الشيكيميك وحمض البيوتريك وحمض البيز ويك ويتميز عسل فخيرة والبشير و ربيع كابا بوجود أعلى عدد من الأحماض المختبرة حيث بلغت 6 أحماض حيث تشترك في وجود 4 أحماض بدرجات مختلفة ، فعسل فخيرة و البشير يشتركا في وجود حمض السيتريك بينما ينفرد عسل فخيرة وكذلك يتميز عسل ربيع وينفرد عسل البشير و ربيع كابا بوجود حمض السيكسينيكعن جميع الأعسال المختبرة وكذلك يتميز عسل ربيع كابا بوجود حمض الطرطريك عن جميع الأعسال المختبرة. ويوجد في عسل السيل الكبير وطابا وأم أز وبا عدد 5 أحماض من عدد الأحماض المختبرة ، تشترك هذه الأعسال في وجود 4 أحماض ويوجد حمض الماليك في السيل الكبير وأم از وبا وينفرد عسل طابا عنهما بوجود حمض الأيزو بيوتريك ويوجد في عسل ربيع فويد والنخبة عدد 4 احماض من الأحماض العضوية المختبرة يجتمعا في وجود حمض الفور ميكوالمالونيك حيث يوجدا بدرجة اعلى قليلا في عسل النخبة ويختلفا في وجود الحمضين الأخرين حيث يوجد في عسل ربيع فويد حمض الماليك والبر وبونيك بينما يوجد في عسل النخبة حمض السيتريكوالأيز وبيوتريك.

يتضرح من النتائج المتحصل عليها إمكانية استخدام الاحماض العضوية في التمييز بين الأعسال السعودية في تلك المناطق .