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DETERMINATION AND IDENTIFICATION OF ORGANIC ACIDS IN THREE TYPES OF ROYAL JELLY

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ABSTRACT

Organic acids in three types of royal jelly (RJ) [Egyptian, Germany and Chinese RJ] were identified and determined with high performance liquid chromatography (HPLC). The results indicate that oxalic, formic, tartaric, and adipic acids were not found in the three experimental RJ types (Egyptian, Germany and Chinese). Six of organic acids were present in Egyptian RJ, while in both of Germany and Chinese RJ there were only four and five organic acids, respectively. Three organic acids were present only in Egyptian RJ (malonic, maleic and succinic acids). Citric acid was not found in Egyptian RJ and present only in both of Germany and Chinese RJ, and the difference between them was significant. There were three organic acids were common in the three experimental RJ (shikimic, propionic and lactic acid) and the difference between them was significant. Butyric acid was present only in Chinese RJ, so it could be used to differentiate between Chinese RJ and the other two RJ (Egyptian and Germany RJ). Citric acid can be used to distinguish Egyptian RJ from the other two RJ (Germany and Chinese).
Keywords: Royal jelly / organic acids / HPLC/Egypt/China/Germany

INTRODUCTION

Royal jelly, or bee's milk, is a creamy products secreted by the hypopharyngeal glands in the head of the young nurse worker bees primarily for developing and maintaining the queen bee. It is a special food serving only the queen bee throughout her life, while other sexually immature females are served royal jelly for only the first 2-3 days. Young worker honey bees (called nurses) use RJ to feed young larvae of workers and drones for less than three days (Chen and Chen, 1995). In contrast, the queen bee is fed with RJ throughout her life and the cast differentiation between queen and worker bees is related strictly to differences in the feeding of the larvae. The queen bee is 50% larger than worker bees and also lives longer, about 4-5 years, compared to worker bees that live only through one season. The nutritional significance of royal jelly has been proven since any larvae from a fertile egg, if given royal jelly throughout its larvae period, develops sexually so that it becomes a perfect queen, otherwise, the larvae develops into a sexually immature worker.

Royal jelly appears as a whitish substance with a gelatinous consistency, often not homogenous due to the presence of undissolved granules of varying size. It has a distinctively sharp odors and taste. It is partially soluble in water and highly acidic (pH 3.4-4.5) with a density of 1.1 g/mL (Lercker, 2003). RJ is characterized by a complex composition: moisture (60–70%), crude protein (12–15%), lipids (3– 6%) most of which are free fatty acids, sugars (6–18%) and minerals (0.8–3%) (Lercker *et al*., 1981;

Lercker *et al*., 1982; Howe *et al*., 1985; Crane, 1990; Chen and Chen, 1995 and Bogdanov, *et al*. 004). It's B Vitamin content is high, and with 17 amino acids, including all 8 essential amino acids, it is a nutritious hormone-rich substance with a wide spectrum of potential benefits. Royal Jelly also contains around 15% aspartic acid, which is important for tissue growth, muscle and cell regeneration. The most characteristic compound of RJ is trans-10-hydroxy-2-decenoic acid (10-HDA). No other natural product has been reported to contain 10-HDA and so, being specific to RJ, 10-HDA represents the main criterion in quality control for the determination of RJ authenticity (Bloodworth *et al*., 1995).

Commercial RJ is produced by transferring (grafting) young larvae (1 day old) into artificial queen cells to induce nurse bees to provision the cell with RJ (Chen *et al*., 2002). The majority of RJ producers harvest three days (72 h) after grafting because at this time the amount of RJ in queen cells reaches its peak (Lercker *et al*., 1985, Piana, 1996). China is the largest producer of RJ with an annual production of around 2000 tons, corresponding to 90% of the world's output (Li *et al*., 2003).

Now a day, there is a lot production of royal jelly whose composition is unknown. Therefore, the aim of this study was to identification and determination of organic acids in Egyptian royal jelly order to perceive how it is different from foreign royal jelly (Chinese RJ and Germany RJ). This work has never been done before.

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 3 types of royal jelly Egyptian, Germany and Chinese. Determination of organic acids in these types was done using HPLC. RJ was produced by transferring (grafting) young larvae (1 day old) into artificial queen cells to induce nurse bees to provision the cell with RJ (Chen *et al*., 2002). Egyptian RJ was freshly collected from these cells with larvae three days (72 h) after grafting because at this time the amount of RJ in queen cells reaches its peak according to Lercker *et al*., (1985), while both of Chinese and Germany RJ were provided by importers. All the samples were kept refrigerated at 4 °C before analysis,

Determination of organic compounds in RJ samples

Samples of RJ 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:-

Royal jelly samples were prepared for organic acids determination according to the method described by Anna *et al.*, (1994). RJ solution was obtained by diluted 1 g. RJ with 20ml dist. water then mixed and centrifuged. This mixture filtered at 0.45 µm and directly injected into HPLC. Identification of organic compounds of the RJ samples was performed by a JASCO HPLC, using a hypersil C_{18} reversed- phase column (250 X 4.66 mm) with 5 µm particle size. A constant flow rate of 0.7 ml/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.

Statistical analysis:

The data of organic acids were statistically using one-way ANOVA. LSD was used to evaluate the significant differences between means (comparison of means) at the level of $p<0.05$.

RESULTS AND DISCUSSION

The results in table (1) and fig. (1: 3) represent that oxalic, formic, tartaric, and adipic acids were not found in the three experimental RJ (Egyptian, Germany and Chinese). Six types of organic acids were present in Egyptian RJ, while in both of Germany and Chinese RJ there were only 4 and 5 organic acids, respectively. Three organic acids were found only in Egyptian RJ (malonic, maleic and succinic acids) and not found in Germany and Chinese RJ.

Table (1) Organic acids (mg/100gm) in Egyptian, Chinese and Germany royal jelly

RJ types / Organic acids	Egypt	Chinese	Germany	LSD
shikimic	0.132	0.018	0.0134	0.0062
malonic	1.103	0	0	--
maleic	0.006	0	0	--
citric	0	0.342	1.019	0.0013
succinic	0.188	0	0	--
propionic	0.903	0.052	1.438	0.0026
butyric	0	0.495	0	--
lactic	0.871	1.078	1.065	0.0023

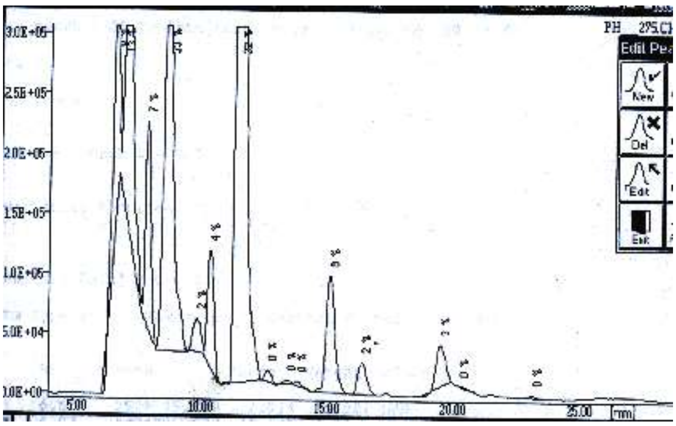

Fig. (1) Organic acids in Egyptian royal jelly

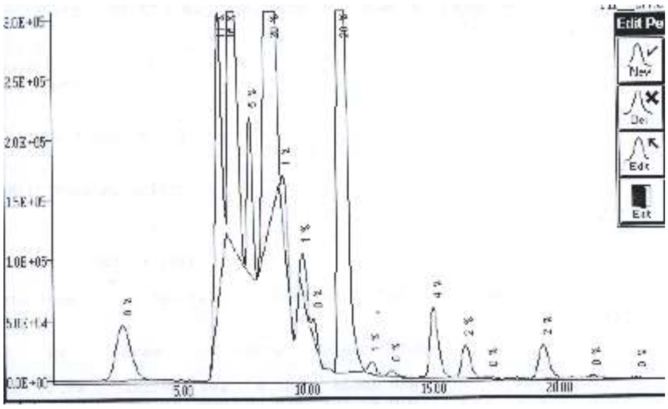

Fig. (2) Organic acids in Chinese royal jelly

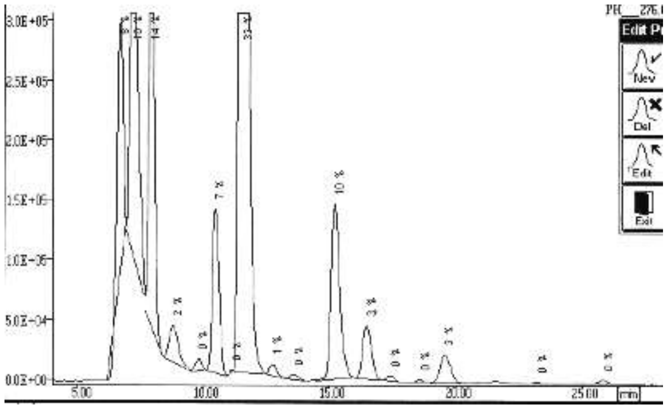

Fig. (3) Organic acids in Germany royal jelly

Data in table (1) represented that there were 3 organic acids (shikimic, propionic and lactic acid) were common in Egyptian, Germany and Chinese RJ, and the difference between them was statistically significant.

The results in fig.(4) indicated that shikimic acid was the greatest concentration in Egyptian RJ, while the lowest concentration was found in Germany RJ. The difference between them was significant. Also, the difference between Chinese and Germany RJ was a significan.

Citric acid was not found in Egyptian RJ and present only in both of Germany and Chinese RJ as in fig. (5). The highest concentration was found in Germany RJ, and the difference was significant. So, citric acid can be used to differentiate between Egyptian RJ and both of Germany and Chinese RJ

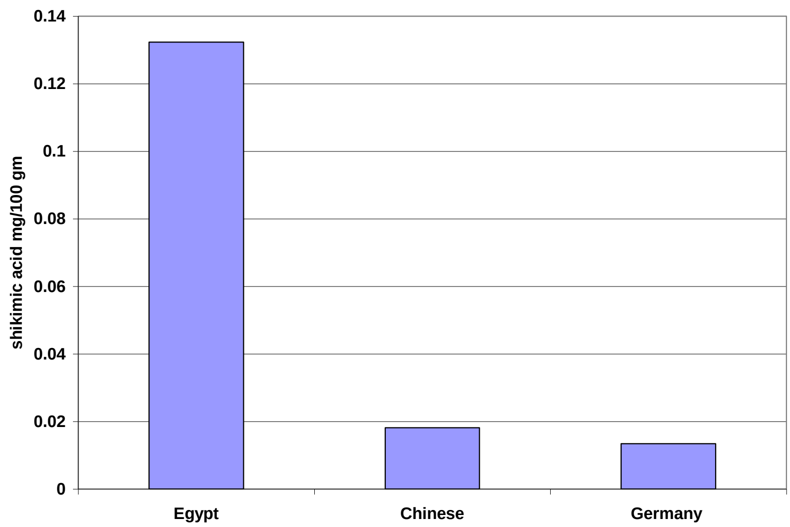

Fig (4) Shikimic acid in three types of RJ

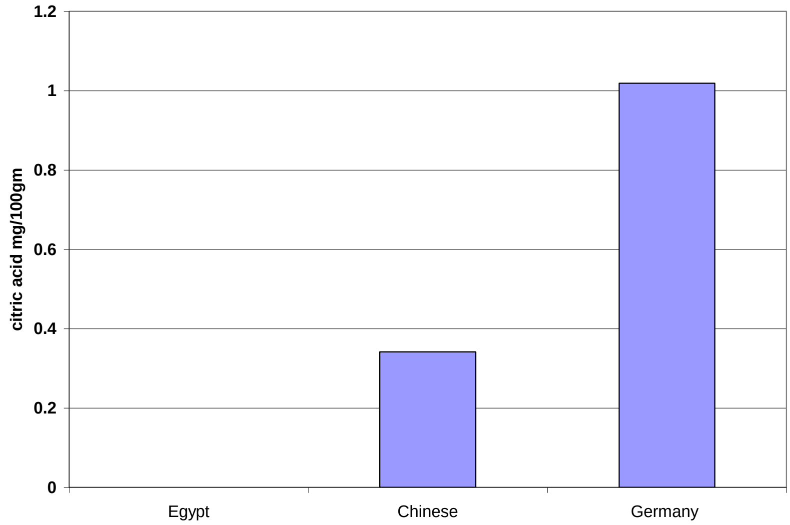

Fig (5) Citric acid in three types of RJ

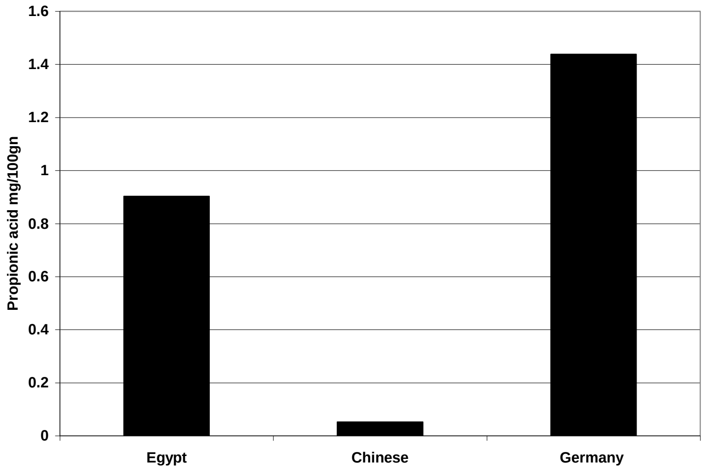

Fig (6) Propionic acid in three types of RJ

Highest concentration of propionic acid was found in Germany RJ, and the lowest concentration was found in Chinese RJ, while Egyptian RJ came in between Fig.(5). Statistical analysis represented that there was a significant difference in propionic acid between Germany RJ and both of Egyptian and Chinese RJ. There was also significant difference between Egyptian and Chinese RJ.

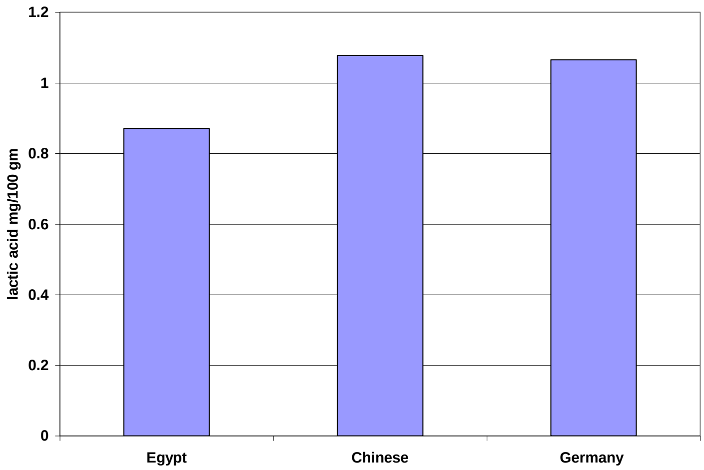

Fig (6) Lactic acid in three types of RJ

Data in fig.(6) represented that there was a significant difference in lactic acid between Chinese RJ and both of Germany and Egyptian RJ. Also, the difference between Chinese and Egyptian RJ was significant. The lowest value was found in Egyptian RJ.

Comparing between the concentrations of organic acids found in the tested RJ Table (1) proved that malonic, maleic and succinic acids not found in both of Germany and Chinese RJ and found only in Egyptian RJ. Accordingly they could be used to distinguish between Egyptian RJ and the other two tested RJ.

It was shown that seasonal variations had a moderating influence on the chemical composition of royal jelly, especially on carbohydrate and lipid contents, causing slight changes in protein and moisture contents but no alteration in ash content and pH value. Zheng *et al.*, (2010) mentioned that the harvesting time should be considered when defining new quality standards of RJ. Kodai *et al*., (2007) were isolated two organic acid glycosides (1, 2) and 16 sterols from the royal jelly of honeybees (*Apis mellifera*). The former two were monoglucosides of 10-hydroxy-2E-decenoic and 10-hydroxydecanoic acids. They were the first examples of glycosides isolated from royal jelly. With regard to the constituents of the royal jelly, it contains remarkably high amounts of organic acids in the total lipid fraction.1) Many organic acids mainly consisting of 8 to 11 carbon atoms such as 10-hydroxy-2-decenoic2,3) and 10-hydroxydecanoic acids have been isolated (Apitherapy News, 2007). Tourn, *et al*., (1980) determined quantitatively glucose, fructose, sucrose, malic and citric acid in samples of honeydew, honey and royal jelly by means of enzymic UV tests. The major amino acids in French royal jelly analysis by HPLC were: proline, lysine, arginine and glutamic acid.

As a conclusion, malonic and succinic acids present only in Egyptian RJ, so they can be used to differentiate between it and the other two RJ (Chinese and Germany RJ). Citric acid was absent in Egyptian RJ, so it could be used to distinguish Egyptian RJ from the other two RJ (Germany and Chinese). Also, butyric acid was found only in Chinese RJ, so it could be used to differentiate between Chinese RJ and the both of Egyptian and Germany RJ. This work was not done before, we can use organic acids to distinguish between local and imported royal jelly.

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الاحماض العضوية فى ثلأثة انواع من الغذاء الملكى تقدير وتعريف

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اجرى هذا البحث فى قسم بحوث النحل- الدقى عام 2010 لتقدير الأحماض العضوية فى 3 أنواع مختلفة من الغذاء الملكى وهى: الغذاء الملكى المصرى، و الصينى والألمانى وذلك للتفرقة بينهما حيث أن الأسواق المصري تحتوى على أنواع مختلفة من الغذاء الملكى مجهولة المصدر. وقد تم تحليل عينات الغذاء الملكى فى معمل تحليل منتجات النحل فى قسم بحوث النحل- معهد بحوث وقاية النباتات- الجيزة باستخدام جهاز HPLC .

أظهر التحليل أن عدد الأحماض العضوية التى و جدت فى الغذاء الملكى المصرى 6 أحماض عضوية ، والصينى 5 أحماض عضوية والألمانى 4 أحماض عضوية.

وقد تميز الغذاء الملكى المصرى بوجود 3 احماض عضوية وهم: حمض المالونيك و حمض الماليك وكذلك حمض السكسينيك وغير موجودين فى كل من الغذاء الملكى الصينى والألمانى. وقد أظهرت الدراسة ايضا وجود 3 أحماض عضوية وهم: حمض الشيكيميك، حمض البروبيونك و حمض اللاكتيك فى الأنواع الثلاثة من الغذاء الملكى وكان الفرق بينهم معنويا.

وقد ظهر من التحليل ايضا أن حمض البيوتيرك موجود فقط فى الغذاء الملكى الصينى ولذلك ممكن ان نستخدمة فى تفرقة الغذاء الملكى الصينى عن كل من الغذاء الملكى المصرى والغذاء الملكى الألمانى. وكذلك حمض الستريك لايوجد فى الغذاء الملكى المصربولكنه موجود فى كلا من الغذاء الملكى الألمانى والصينى وكان الفرق بينهما معنويا. ولذلك يمكن أستخدام حمض الستريك فى التفرقة بين الغذاء الملكى المصرى وكلا من الغذاء الملكى الألمانى والصينى.

ومن هذه الدراسة وجد انه يمكن أستخدام الأحماض العضوية فى التفرقة بين الغذاء الملكى المصرى واى نوع آخر موجود فى السوق المصرى حيث أن السوق المصرى يحتوى على انواع مختلفة من الغذاء الملكى.

قام بتحكيم البحث

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