

Determination of Amino Acids in Royal Jelly Produced by Honeybees (*Apis mellifera* L.), Fed on Brewer's Yeast and Yeast Extract Diets

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ABSTRACT

This study were carried out to estimate the artificial supplements effect Prepared with brewer's yeast and yeast extraction amino acid composition of royal jelly (RJ) produced by Carniolian hybrid honeybee colonies. Considering these results, providing supplements for honeybee colonies subjected to royal jelly production can help and strengthen the technological development of the Egyptian beekeeping industry increasing its consumption in the national market. This research presents values analyzed of amino acid composition of RJ a little different from produced honeybee colonies fed on brewer's yeast and yeast extract. While found differences recorded between amino acids Serine (2.517 & 2.755 mg/g), and Threonine (1.441 & 1.629 mg/g) respectively, in RJ all tested samples. This fact shows the important to discuss or change the official method for royal jelly analysis. The characterization of physicochemical parameters is important in order to standardize quality royal jelly produced.

Keywords: Honeybees; royal jelly analysis; Amino acids composition; Brewer's yeast; yeast extract.

INTRODUCTION

Royal jelly (RJ) is a secretion of worker bees, used for feeding bee larvae in the first three days of life and feeding the queen for its entire life. This substance is really a RJ product, because it contained all the chemical compounds that are needed by an organism in order to grow and develop, to gain strength and have a long life (Garcia-Amoedo and Almeida-Muradian, 2007). Honey bees require proteins (amino acids), carbohydrates (sugars), lipids (fatty acids, sterols), vitamins, minerals (salts), and water, and these nutrients must be in the diet in a definite qualitative and quantitative ratio for optimum nutrition (Standifer, 1973). Found different from the honey that produced from flowers nectar or other sugary substances. RJ is solely of glandular secretion product; so, its physicochemical composition isn't affected by the artificial supplementation giving intact characteristics of the final products (Haydak, 1960). Thus, there is no restriction on the colonies' artificial supplementations. In contrast, since international beekeeping and Brazilian products are demanded increasingly, special care is necessary to ensure that final product composition is not affected in use of artificial supplementation thus ensuring both honeybees and customers' health (Brasil, 2007). Due to the RJ high protein content, honeybees need a diet rich in sugars, fatty acids, vitamins, minerals, and all essential amino acids from pollen consumption with a small possible addition of honey (Winston, 1987; Schmidt, et al., 1992; Lercker, et al., 1993). Many authors' evaluate the effect of artificial supplements and pollen substitute diets to the possibility of improving productivity of honeybee colonies' quantity and quality (Zidan; Abd El-Naby, 2012; Abd El-Naby; Zidan, 2014; Abd El-Wahab, et al., 2016). The recent discovery that RJ-proteins may have physiological functions as suppressors of allergic reactions, as well as their established anti-hypertensive and proliferation stimulatory properties, opened a new era in application of RJ and honey (Okamoto, et al., 2003).

(Nafea, 2004) fed honeybee colonies on 4 floral honeys and two medicinal plant extracts to produce royal jelly. He found variations between the amino acids composition of royal jelly. (Modifications that affect the protein and amino acid fractions play a major role in RJ commercial quality evaluation during the product shelf life. (Chen and Chen, 1996).

RJ complex composition consists of amino acids, proteins, organic acids, sugars, sterols, phenols, minerals and other substances unknown (Viuda-Martos et al., 2008). RJ is one of the richest natural products in amino acids. Amino acids are very important for humans and animals. RJ contains at least 17 amino acids, including the eight essential ones, and five non-identified related compounds. The main RJ acid, 10-hydroxy-2-decenoic acid is known to have various pharmacological effects, including antibiotic, antitumoral, (Bărnăuțiu, et al., 2011). Several authors have studied the chemical composition of RJ, but available data is highly variable due to the products' substantial variability, different analytical methods and no reference methods for RJ (Wytrychowski et al., 2013). The aim of this paper is to influence the medical dried yeast given to honeybee colonies by two different ways on RJ qualitative variations between total amino acid composition in royal jelly. In addition, experiment beekeeping products are advising.

MATERIALS AND METHODS

The present investigation was carried out at Apiculture Department, Plant Protection Research Institute, Dokki, Giza, during year 2016. Twelve healthy honeybee colonies from Carniolian hybrid were conducted for this study. These colonies divided into two groups, each of about equal strength containing at least eight combs covered with bees.

Feeding Honeybee Colonies:

(Group 1) fed of each colony A cake of 100 gm food supplement consisted of one part brewer's yeast mixed with nine parts powder sugar (w/w) according to Zidan, 2009.

(Group2)fed of each colony yeast extract adding sugar syrupy at a ratio of 1:9.

Yeast extract was prepared from brewer's yeast (*Saccharomyces cerevisiae*), dissolved in water followed by adding sugar at a ratio of 1:1 and kept 24 hours in a warm place for reproduction according to the methods of Morsiet al., 2008.

All groups of the tested honeybee colonies were fed for 2 month/ two once weekly consecutively, throughout the experimental periods, from first February until end of March 2016.

The Royal jelly collected from all groups of the tested honeybee colonies into 500 mg capsules to be stored in the freezer at -20°C till the study began.

Laboratory analysis:

Determination of Amino acids content.

Separation of Amino acids by GLC: Amino acids were transported to micro reaction vial and derivatization. Procedure were carried out according to Landault and Guiochen 1964, using nbutanol and trifluoroacetic anhydride. A Varian Model 3700 GC equipped with FID, was used. The Separation were accomplished with the following conditions: Column: 39, OV-225 an (Chromosorb-W-HP, 80-100 mesh) glass. Program: 4 min. At 80°C and increased to 200°C at rate of 40°C/min. then stable for a min. Inject and detect: Temp. 250°C. Carrier gas (N₂, 20 ml/min, H₂ 20 ml/min and air 200 ml/min) At ten: 16x10⁻¹ AMPS.MV.

Reagent:

Iron-Acetic acid solution. A weight of 0.22 g. of FeCh - 6H₂O in 0.5 ml distilled water and then completed up to one liter with glacial acetic acid.

Accurately 0.5 g of royal jelly were mixed with 10 ml HCL at 100°C overnight, then filtered and the remaining HCL was removed by heating till dryness.

Statistical analysis:

Amino acids content data for all analyzed RJ samples to analysis of variance (ANOVA) through SPSS computer program. Means were comparing using Duncan's Multiple Range tests, and defined as p<0.05.

RESULTS AND DISCUSSION

Although freshly harvested and commercial RJ composition has been studied extensively (Marghitas et al., 2010; Pavel et al., 2014), amino acids content in RJ have received little research attention. In the present study, compare between results of amino acids composition in fresh RJ produced by honeybees fed on brewer's yeast and yeast extract diets, are present in Table 1. The most abundant amino acids in RJ were aspartic acid (average 9.67 & 10.26 mg/g). There was no significant difference between the content of aspartic acid for both group of tested samples. The next most abundant amino acids were Lucien (average 4.055 & 4.463 mg/g). The lowest amino acids concentration of all groups with Methionine (average 0.597 & 0.584 mg/g) respectively. The data previous according to (Balkanska and Zhelyazkova, 2015) determined high concentration of free aspartic acid has the highest concentration and the lowest was with Met of all amino acid found in commercial and fresh RJ.

The concentration of Lysine, Valise, Proline, Glutamic acid, Isoleucine, Glycine, Histidine, Phenylalanine, Tyrosine, Alanine is no significant differences between RJ samples was found for amino acids the limit of recorded ranged to 3.001 - 1.217 mg/g. brewer's yeast and yeast extract.

Table 1. Estimate of amino acids in the royal jelly produced by honeybees (*Apis mellifera* L.) fed on brewer's yeast and yeast extract diets.

Treatment	Dry yeast	Yeast extract	F	P < 0.05	LS D 0.05
Amino acids (mg/g)					
Histidine	1.549	1.295	1.433	0.1059	0.342 ^{ns}
Lysine	2.959	3.001	0.032	0.8662	0.62 ^{ns}
Lucien	4.055	4.463	3.219	0.147	0.634 ^{ns}
Isoleucine	2.004	1.977	0.106	0.761	0.227 ^{ns}
Valise	2.812	2.649	1.038	0.366	0.436 ^{ns}
Methionine	0.597	0.584	0.143	0.725	0.122 ^{ns}
Phenylalanine	1.500	1.403	1.925	0.2375	0.206 ^{ns}
Proline	2.726	2.813	0.615	0.477	0.307 ^{ns}
Threonine	1.441b	1.629a	36.41	0.008	0.102**
Serine	2.517b	2.755a	10.10	0.034	0.206*
Tyrosine	1.420	1.357	1.176	0.3392	0.16 ^{ns}
Aspartic acid	9.67	10.26	2.04	0.228	1.17 ^{ns}
Alanine	1.239	1.217	0.161	0.7086	0.16 ^{ns}
Glycine	2.041	1.926	1.445	0.296	0.270 ^{ns}
Glutamic acid	2.667	2.779	5.33	0.082	0.136 ^{ns}
Total AA	39.197	40.108	-	-	-

In our study, significant differences recorded between amino acids Serine (2.517 & 2.755 mg/g.), the F tested were 10.10 and (P<0.05). While highest significant were Threonine (1.441 & 1.629 mg/g.), the F tested were 36.41 mg/g. and (P<0.05), respectively in all RJ tested samples. In mind that there are variable factors

affected the yeast extract composition solvent in water conduct absorbent profile easy. (Balkanska and Zhelyazkova 2015) reported that honey and pollen are previous of RJ Honeybees forage different plants. Thus, honeybee and pollen are always mixture of many sources. Bee pollen and honey amino acids profile

can affect the RJ amino acids composition in some directions. All Bulgarian RJ samples were harvested from May to September during different plants flowering season.

Finally, non-significant produced from honeybee colonies fed dry yeast and yeast extract, the total amino acid content of RJ samples determined were 39.197 and 40.108 mg/g, respectively. (Sereia and Toledo 2013) found that royal jelly composition did not affect by artificial supplementation produced by the different treatments, thus the supplements provision to Africanized honeybee colonies viewed to royal jelly production. In addition, the previous authors of literature (Chen and Chen, 1996; Nafea, 2004; Wytrychowski et al., 2013).

CONCLUSION

As conclusion, the artificial supplementation did not affect the most royal jelly composition produced with the different treatments, and thus the provision of supplements considered as a first approach to characterize RJ based on its amino acid content total amino acids was analyzed. Thus, the provision of supplements to honeybee colonies subjected to royal jelly production can help and strengthen the technological development of the Egyptian beekeeping industry improving its national market.

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تقدير الأحماض الأمينية في الغذاء الملكي المنتج من تغذية طوائف نحل العسل بالخميرة الطبية الجافة والمستخلصه

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أجريت هذه التجربة لمقارنة تأثير تغذية طوائف نحل العسل بكل من الخميرة الجافة ومستخلص الخميرة علي جودة الغذاء الملكي المنتج من تلك الطوائف عن طريق تقدير الأحماض الأمينية. وعند النظر في هذه النتائج، وجد أن كلا النوعين من التغذية لطوائف نحل العسل تساعد علي جودة الغذاء الملكي مما يعزز التطور التكنولوجي لصناعة تربية نحل العسل وجودة منتجاته في السوق المحلية. حيث كانت قيم تحليل معظم الأحماض الأمينية في كلا نوعي الغذاء الملكي عالية والاختلافات قليلة ولم تسجل أي فروق معنوية، بينما كان هناك فرقا معنويا مع الحمض الأميني السيرين (2.517، 2.755 مجم/جم)، وفرقا معنويا عاليا مع الثريونين حيث سجل (1.441، 1.629 مجم/جم) في الغذاء الملكي عند تغذية طوائف نحل العسل علي كل من الخميرة الجافة ومستخلص الخميرة علي التوالي. وتشير هذه النتائج الي أهمية الصفات الكيميائية في تحديد جودة المنتج .