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Correlations of Physicochemical Parameters, Antioxidant Activity and Total Polyphenol Content of Fresh Royal Jelly Samples

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A B S T R A C T

Royal jelly (RJ) is one of the most attractive bee products. RJ has long been used in traditional medicine. Due to various pharmacological properties, including antioxidative, anti-inflammatory and antibacterial properties, RJ has been widely consumed in daily diets in numerous countries. The purpose of the present study is to find out some correlations of physicochemical parameters, antioxidant activity and total polyphenol content of fresh RJ samples. The following parameters were analyzed in fresh RJ samples: water content, proteins, pH, total acidity, electrical conductivity, sugars (glucose, fructose, sucrose), trans-10-hydroxy-2-decenoic acid (10-HDA). The antioxidant activity of RJ was examined by two methods: 1,1-diphenyl-2-picrylhydrazyl (DPPH) and the ferric reducing antioxidant power (FRAP) assay. Total phenolic content was determined according to Folin Ciocalteu procedure. High positive linear correlations between the total antioxidant activity determined by the DPPH method and 10-HDA ($r=0.858$, $p<0.05$) and FRAP method and total polyphenols ($r=0.812$, $p<0.05$) were found.

Introduction

Royal jelly (RJ) a honeybee hypopharyngeal and mandibular secretion of young worker honeybees and exclusive nourishment for bee queen is one of the most attractive bee products.

It has been used since ancient times for care and human health. Now days it is still very important in traditional and folkloristic medicine (Fratini *et al.*, 2016). RJ is composed mainly of proteins, sugars, lipids, free amino acids, vitamins and mineral elements. Fresh RJ contains water (50 – 70%), proteins (9 – 18%), sugars (7 – 18%), lipids (3 – 8%), mineral elements (about 1.5%). RJ has been known for its medicinal and health promoting properties. For this reason, it is widely used in medical products, health foods and cosmetic products (Sabatini *et al.*, 2009; Ramadan and Al-Ghamdi, 2012).

The bioactive compounds and health-promoting properties are described by Ramadan and Al-Ghamdi (2012). It has been reported that RJ has shown different pharmacological activities such as antimicrobial (Bílikova *et al.*, 2015), antiviral (Hashemipour *et al.*, 2014), antitumor (Guo *et*

al., 2007; Premratanachai and Chanchao, 2014), antioxidant activities (Özkök and Silici, 2017). Various possible mechanisms by which RJ may inhibit growth and proliferation of tumors or cancer diseases were reported. The most recent research showed the effect of RJ on the growth of breast cancer in mice.

The results corroborated the efficacy of RJ supplementation in diets and suggested that the antioxidant and immunomodulatory activities of RJ serve an important role on antitumor growth (Zhang *et al.,* 2017). RJ composition is tightly associated to biological activities of the product. Biological activities of royal jelly are mainly attributed to bioactive fatty acids, proteins and phenolic compounds (Jamnik *et al.,* 2012; Ramadan and Al-Ghamdi, 2012).

Liu *et al.,* (2008) evaluated the antioxidant properties of RJ collected from larvae of different ages that were transferred in artificial bee queen cells. Furthermore, the authors reported that the polyphenolic compounds may be the major component for giving the antioxidant activities in RJ. In this respect, the purpose of the present study is to find out some correlations of physicochemical parameters, antioxidant activity and total polyphenol content of fresh RJ samples.

Materials and Methods

A total of 10 RJ samples from Bulgaria were obtained from experimental apiary of Institute of Animal Science, Kostinbrod. The following physicochemical parameters were determined: water content by refractometer; proteins by Folin Ciocalteu reagent; pH by pH meter; total acidity by titration with 0.1 N NaOH according to ON 2576693-84 Fresh and lyophilized royal jelly); electrical conductivity (Bogdanov *et al.,* 1997), sugars (fructose, glucose, sucrose) by High-performance liquid chromatography (HPLC) after Sesta (2006).

Trans-10-hydroxy-2-decenoic acid (10-HDA)

10-HDA contents were determined via HPLC using an Aligent HPLC 1100 Series, equipped with Diode Array Detectors (DAD) G1315B and column Intersil 5 ODS-2 (250mm x 4.6 mm L x I.D., particle size 5 µm). The maximum absorbance of 10-HDA was confirmed to be 215 nm. The mobile phase was mixture of methanol and distillated water (35:65 v/v) and acetic acid (pH 3). The flow rate was 1.0 ml/min. The total run time for each sample was 10 min. A standard *trans*-10-hydroxy-2-decenoic acid (10-HDA) was purchased from Larodan AB, Sweden (Lot no: E013:1). Stock standard solutions were prepared in methanol. All chemicals were of HPLC grade and used without further purification.

Antioxidant activity and total polyphenols

In the recent years, several methods have been applied to evaluate antioxidant activity of RJ. The antioxidant activity of RJ was estimated by determination of DPPH (2,2-diphenyl-1-picrylhydrazyl), according to the procedure of Brand-Williams *et al.,* (1995) and modified after Liu *et al.,* (2008). FRAP assay (Ferric Reducing Antioxidant Power), (Benzie and Strain, 1996) was performed as described in Mohammadzadeh *et al.,* (2007) with modification of sample preparation. For both methods, 1 mM ascorbic acid was used as positive control (DPPH – 98.21% inhibition, FRAP – 14.77 mM Fe^{2+}/g).

Total polyphenol content was determined by Folin – Ciocalteu colorimetric method (Slinkard and Singleton, 1977) using gallic acid as a calibration standard as described in Liu *et al.,* (2008) with measuring the absorbance at 760 nm. The analyses were carried out in triplicate.

Statistical analyses

Statistical analyses of data were performed using IBM SPSS Statistics version 21 for Windows. Data was expressed as means ± standard deviations (SD). Correlation analyses between the antioxidant activity and physicochemical parameters were done. Level of statistical significance was defined as $p<0.05$.

Results and Discussion

RJ is mainly composed of water (60 – 70%). The results for water content are presented in Table 1 as means ± standard deviation, minimal and maximal values. The data for water content was comparable with the results presented by Sabatini *et al.,* (2009) and Wytrychowski *et al.,* (2013).

Up to 15% of RJ is composed of proteins, the most abundant have been termed as major royal jelly proteins (MRJPs) (Buttstedt *et al.,* 2014).·Several peptides present in RJ are known for their antioxidant activity. The mean protein content of fresh RJ (14.96%) with minimal and maximal values are in accordance with data previously published by Pavel *et al.,* (2014) and Sabatini *et al.,* (2009). No correlation between protein content and antioxidant activity was found for all analyzed RJ samples.

In general, RJ is relatively acidic with a high buffering capacity (pH 3.20 – 4.01) and total acidity varies between 2.48 – 4.66 ml 0.1 N NaOH/g (Table 1). These results agree with those reported by Nabas *et al.,* (2014), Pavel *et al.,* (2014), Mureşan *et al.,* (2016). Water content, proteins, pH, total acidity and electrical conductivity are ones of common the criteria used for RJ quality analysis.

The most abundant sugars in RJ are fructose, glucose and sucrose (Ramadan and Al-Ghamdi, 2012; Wytrychowski *et al.,* 2012). The contents of fructose, glucose, sucrose and the sum of them are comprised in the established confidence intervals for fresh samples. The average value for the sum of the three sugars (fructose, glucose, sucrose) was 11.76±1.39%. RJ is mostly consisted of fructose and glucose. Fructose is prevalent. In many cases fructose and glucose together account for over 90% of the total sugars. Sucrose is always present but often in highly variable concentrations. The results for the sugar content in the RJ samples are presented on Figure 1. The fructose content ranges from 3.64 to 6.74%, glucose content from 2.55 to 5.87%, sucrose content from 1.94 to 5.08%.

The major fatty acid in RJ is trans-10-hydroxy-2-decenoic acid. The fatty acids in RJ are known for their many medicinal properties. In this study, the content of 10-HDA content in 10 RJ samples was determined with HPLC method. The linearity of the method was estimated in concentration range from 0.01 mg/ml to 0.12 mg/ml (Figure 2). A direct relationship was estimated between the peak areas and the amount of 10-HDA standard. The correlation equation of this relationship was calculated as $y=128164x+731.64$ ($R^2=0.9997$).

The results for 10-HDA in all analysed RJ samples are presented on Figure 3. 10-HDA content in collected RJ samples ranged between 1.71% and 2.99% and according to the recommendations given by Sabatini *et al.,* (2009) and Kanelis *et al.,* (2015) the samples are fresh and authentic. The mean value and standard deviation is 2.34±0.43%.

The results for DPPH, FRAP assays and total polyphenols are presented in Table 2. A high positive linear correlation between the antioxidant activity determined by DPPH method and 10-HDA content (Figure 4A) for all RJ samples was observed.

Table.1 Physicochemical parameters of fresh RJ samples (n=10)

Parameters	Mean±SD	Min	Max
Water content, %	63.15±1.94	59.70	65.80
Proteins, %	14.96±2.11	12.46	18.53
pH	3.72±0.23	3.20	4.01
Total acidity, ml 0.1 N NaOH/g	3.93±0.56	2.48	4.66
Electrical conductivity, µS/cm	189±16	173	223

*SD – standard deviation

Table.2 Antioxidant activity expressed by DPPH and FRAP assays and total polyphenols in fresh RJ samples (n=10)

Samples	DPPH (% inhibition)	FRAP (mM Fe^{2+}/g)	Total polyphenols (mg GAE/g)
1RJ	16.17	8.49	17.11
2RJ	15.07	7.27	17.15
3RJ	26.14	8.03	17.33
4RJ	35.59	2.23	13.67
5RJ	26.17	9.75	19.39
6RJ	26.12	9.70	24.54
7RJ	18.76	4.62	15.85
8RJ	29.39	2.36	11.82
9RJ	29.20	8.91	17.80
10RJ	26.37	9.71	26.07
Mean±SD	24.90±6.41	7.11±2.96	18.07±4.39
Ranges (Min – Max)	15.07 – 35.59	2.23 – 9.75	11.82 – 26.07

Fig.1 Average values and standard deviation of sugars (fructose, glucose, sucrose) in fresh RJ samples, (n=10)

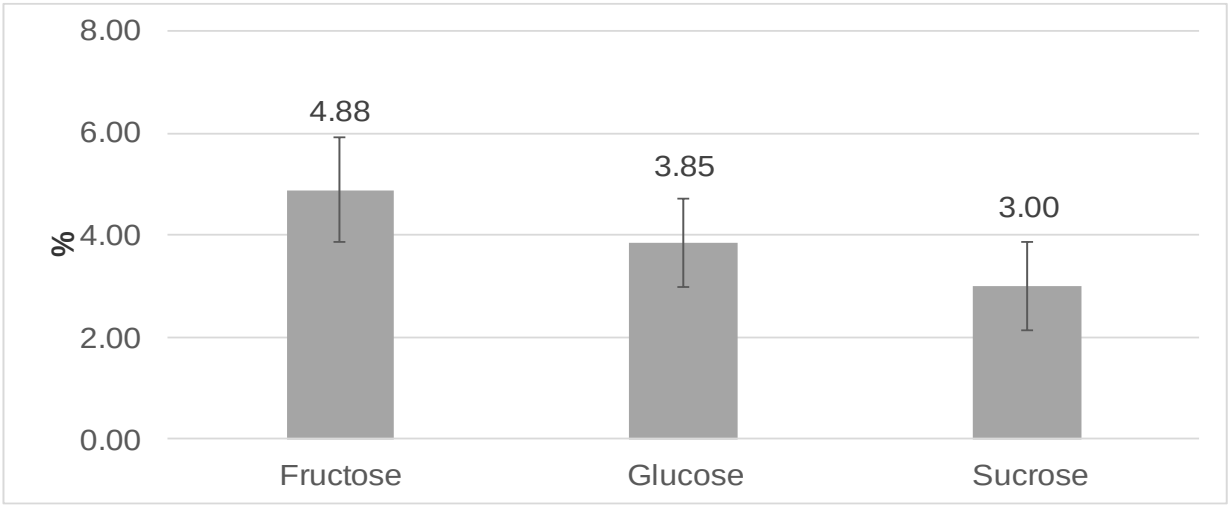


Fig.2 Standard curve for 10-HDA standard, mg/ml

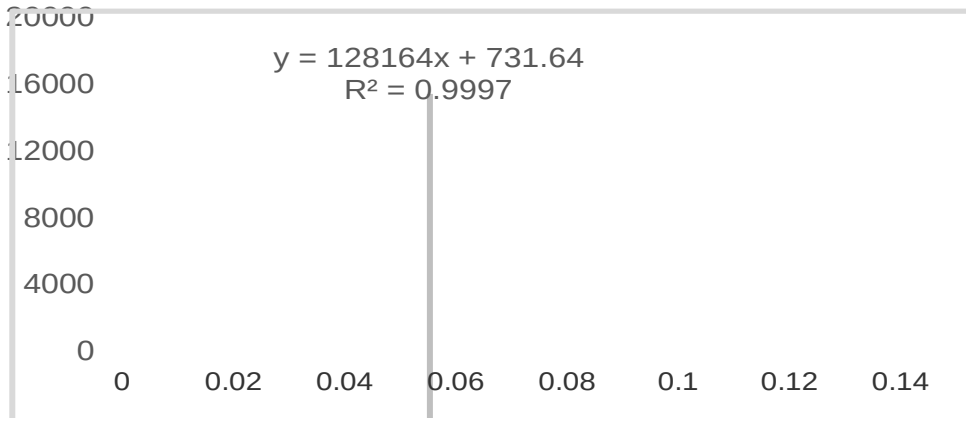


Fig.3 Box plot diagram of 10-HDA. Minimal, maximal and median value are shown

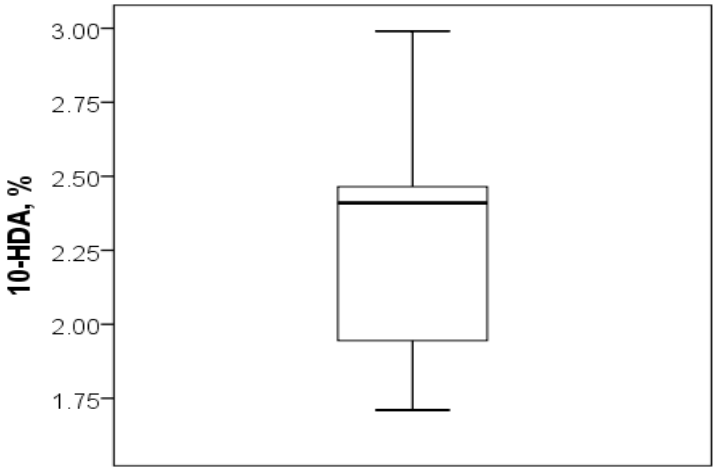
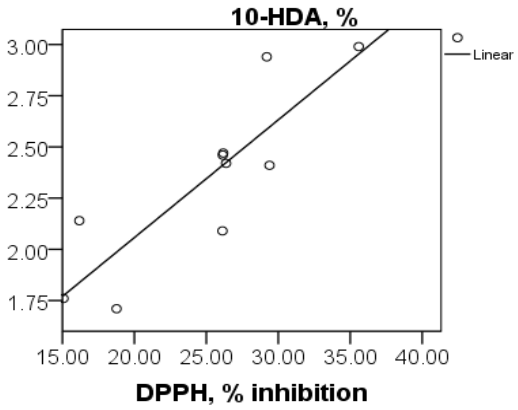
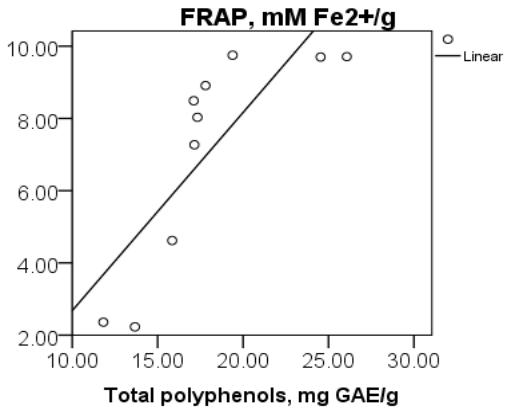


Fig.4 Linear correlations between DPPH method and 10-HDA, R^2=0.737, p<0.05 (A) and between FRAP assay and total polyphenols, R^2=0.660, p<0.05 (B)



A)



B)

The correlation coefficient (r=0.858, p<0.05) indicates that the 10-HDA is responsible for the antioxidant behaviour of RJ. This result suggests that 74% of the antioxidant capacity of the RJ samples accessions results from the contribution of 10-HDA. Also, a high positive correlation (r=0.812, p<0.05) was found between the antioxidant activity determined by FRAP method and total polyphenols for all tested samples (Figure 4B). The results showed that 66% of the antioxidant activity analysed by FRAP method depends on 10-HDA content in fresh RJ samples.

High positive linear correlations between the total antioxidant activity determined by the DPPH method and 10-HDA (r=0.858, p<0.05) and FRAP method and total polyphenols (r=0.812, p<0.05) were found.

RJ may render it a good source of antioxidants thus increasing its potential therapeutic activity. In addition, estimation of antioxidant activities of RJ and total polyphenol content may also be used as good parameters for the assessment of RJ quality.

References

Benzie, I. F., and Strain, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry, 239, 70–76.

Bílikova, K., S. C. Huang, I. P. Lin, J. Šimuth and C. C. Peng. 2015. Structure and antimicrobial activity relationship of royalisin, an antimicrobial peptide from royal jelly of *Apis mellifera*. Peptides, 68, 190-196.

Bogdanov, S., P. Martin and Lüllmann, C. 1997. Harmonised methods of the European Honey Commission. Apidologie, extra issue, 14–16.

Brand-Williams, W., M. E. Cuvelier and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. LWT – Food Science and Technology, 28: 25–30.

Buttstedt, A, R. F. A. Moritz and Erler, S. 2014. Origin and function of the major. Royal jelly proteins of the honeybee (*Apis mellifera*) as members of the *yellow* gene family. Biol Rev, 89(2): 255–269.

Fratini, F., G. Cilia, S. Mancini and Felicioli, A. 2016. Royal jelly: An ancient remedy with remarkable antibacterial properties. Microbiological Research, 192, 130–141.

Guo, H., A. A. Saiga, M. Sato, I. Miyazawa, M. Shibata and Takahata Y. 2007. Royal jelly supplementation improves lipoprotein metabolism in humans. J Nutr Sci Vitaminol (Tokyo), 53: 345–348.

Hashemipour, M.A., Z. Tavakolineghad, S. A. Arabzadeh, Z. Iranmanesh and Nassab, S. A. 2014. Antiviral activities of honey, royal jelly, and acyclovir against HSV-1. Wounds, 26(2):47–54.

Jamnik, P., P. Raspor and Javornik, B. 2012. A proteomic approach for investigation of bee products: royal jelly, propolis and honey. Food Technology and Biotechnology, 50: 270–274.

Kanelis, D., C. Tananaki, V. Liolios, M. Dimou, G. Goras, M. A. Rodopoulou, E. Karazafiris and Thrasyvoulou, A. 2015. A suggestion for royal jelly specifications. Arch. Hig. Rada Toksikol. 66, 275–284.

Liu, J. R., Y. C. Yang, L. S. Shi and Peng C.C. 2008. Antioxidant properties of royal jelly associated with larval age and time of harvest. Journal of Agricultural and Food Chemistry, 56: 11447–11452.

Mohammadzadeh, S., M. Sharriatpanahi, M. Hamedi, Y. Amanzadeh, S. E. S. Ebrahimi and Ostad, S. E. 2007. Antioxidant power of Iranian propolis

extract. Food Chemistry, 103(3): 729–733.

Mureşan, C. I, L. Al. Mărghitaş, D. S. Dezmirean, O. Bobiş, V. Bonta, I. Zacharias, R. Mărgãoan and Paşca, C. 2016. Quality parameters for commercialized royal jelly. Bulletin UASVM Animal Science and Biotechnologies, 73(1), 1–7.

Nabas, Z., M. S. Y. Haddadin, J. Haddadin and Nazer, I. K. 2014. Chemical composition of royal jelly and effects on symbiotic with two different locally isolated probiotic strains on antioxidant activities. Pol J Food Nutr Sci 64: 171–180.

Özkök, D. and Silici, S. 2017. Antioxidant activities of honeybee products and their mixtures. Food Science and Biotechnology, 26, 1, 201–206.

Pavel, C., L. Al. Marghitas, D. Dezmirean, L. Tomos, V. Bonta, A. Sapcaliu and Buttstedt, A. 2014. Comparison between local and commercial royal jelly – use of antioxidant activity and 10-hydroxydecenoic acid as quality parameter. Journal of Apicultural Research, 53(1): 116–123.

Premratanachai, P. and Chanchao, C. 2014. Review of the anticancer activities of bee products. Asian Pac J Trop Biomed., 4(5): 337–344.

Ramadan, M. F., and Al-Ghamdi, A. 2012. Bioactive compounds and health promoting properties of royal jelly: A review. Journal of Functional Foods 4:39–52.

Sabatini, A. G., G. Marcazzan, M. F. Caboni, S. Bogdanov and Almeida Muradian, L. B. 2009. Quality and standardisation of royal jelly. Journal of Api Product and ApiMedical Science, 1: 1–6.

Sesta, G. 2006. Determination of sugars in royal jelly by HPLC. Apidologie 37: 84–90.

Slinkard, K., and Singleton, V.L. 1977. Total phenol analysis – automation and comparison with manual methods. American Journal of Enology and Viticulture 28: 49–55.

Wytrychowski, M., S. Chenavas, G. Daniele, H. Casabianca, M. Batteau, S. Guibert and Brion, B. 2013. Physicochemical characterisation of French royal jelly: Comparison with commercial royal jellies and royal jellies produced through artificial bee-feeding. J. Food Compos. Anal. 29: 126–133.

Zhang, S., Q. Shao, H. Geng and Su, S. 2017. The effect of royal jelly on the growth of breast cancer in mice. Oncology Letters, 14, 6, 7615–7621.

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