EUROPEAN FOOD SCIENCE AND ENGINEERING

European Food Science and Engineering

Eur Food Sci Eng 2020, 1 (1), 9-12

https://dergipark.org.tr/tr/pub/efse

Effect of harvesting period on chemical and bioactive properties of royal jelly from Turkey

Murat Emir*

http://www.aricimdan.com

ARTICLE INFO

Research Article
Article History:
Received: 16 May 2020
Accepted: 16 June 2020
Available Online: 22 June 2020
Keywords:
Royal jelly
Harvesting period
10-hydroxy-2-decenoic acid (10-HDA)
Chemical properties
Bioactive properties

ABSTRACT

Royal jelly is a natural viscous, milky-white product which is secreted from special glands of young worker honey bees (*Apis mellifera* L.) used to feed queen larvae and the queen bee throughout its life. It can be used as a dietary complement because of its composition, and also it contains a large number of bioactive substances such as peptides, flavonoids, fatty acids and also 10-hydroxy-2-decenoic acid (10-HDA). The variation in chemical compounds of royal jelly depends on some factors such as floral sources, queen cup types and harvesting time. In this study, the effect of harvesting period (April, May, June, July and August) on physicochemical properties and 10-HDA content in royal jelly collected from different producers in Turkey. The total solid contents as well as protein contents of royal jelly significantly changed depend on the harvesting period, but no alteration in pH value. The maximum means of total phenolic content and antioxidant activity were found in samples harvested in July. 10-HDA content varied from 0.91% to 2.05%, with a mean of 1.38%. A significant effect of month in 10-HDA content was found, and the highest mean value observed in June.

1. Introduction

Recently, consumer demand for natural products with health-promoting effects has been increased. Among the natural products, royal jelly has been most widely accepted beehive product, which is mainly composed proteins, lipids, carbohydrates, vitamins, and minerals (Balkanska, Marghitas, & Pavel, 2017). It is one of the most attractive bee product due to rich source of bioactive components. It is secreted from the cephalic glandular system (hypopharyngeal and mandibular glands) of young worker bees (*Apis mellifera* L.) and is used to feed the larvae for only 3 days and the queen throughout larval and adult stages (Kolayli et al., 2016; Yavuz & Gürel, 2017). Royal jelly is a strongly acidic (pH 3.1-3.9) and milky-white colored product that is a thick and viscous substance because of the presence of varying sizes and insoluble protein granules (El-Guendouz et al., 2020; Kamyab, Gharachorloo, Honarvar, & Ghavami, 2019).

Studies have shown that royal jelly comprises water (50–60%), proteins (18%), carbohydrates (15%), lipids (3–6%), mineral ash (1.5%) and vitamins, with a large number of bioactive substances such as 10-HDA, fatty acids, peptides and flavonoids (Alu'datt et al., 2015). So that, today royal jelly is widely consumed as a dietary supplement and has been used in foods, pharmaceutical and cosmetic industry (El-Guendouz et al., 2020; Emir 2017; Zhu et al., 2019). It has various beneficial effects, including anti-bacterial, antioxidant, anti-fatigue, anti-allergic, antitumor, anti-hypercholesterolemic, antihypertensive, life-spanelongating, antiinflammatory, DNA-protective, hepatoprotective effects, and insulin-like (Bincoletto, Eberlin, Figueiredo, Luengo, & Queiroz, 2005; El-Nekeety et al., 2007; Emir & Emir, 2017; Inoue, 2003; Kamakura, Mitani, Fukuda, & Fukushim, 2001; Kohno et al., 2004; Okamoto et al., 2003; Park et al., 2019; Vittek, 1995; Zimmermann, 2002).

Natural royal jelly is produced by the transformation of nectars and pollens collected in the environment of the hives during the foraging activities of the honeybees (Wytrychowski et al., 2013). Therefore, the chemical composition of royal jelly is affected by several factors such as floral variety and geographical and environmental conditions as well as harvesting time, which has been proven some studies (Jie et al., 2016; Kolayli et al., 2016; Ramadan & Al-Ghamdi, 2012; Zheng, Hu, & Dietemann, 2011). However, there is limited number of studies focusing the effect of different harvesting period or season on the chemical composition of royal jelly. Therefore, the aim of this work was to evaluate the effect of different harvesting periods (April, May, June, July, and August) on the physicochemical and bioactive properties of royal jelly samples collected from Turkey.

2. Materials and Methods

2.1. Materials

Fresh royal jelly samples (20 g) produced from different regions of Turkey, in particularly Aydın, Balıkesir, Bursa, Çanakkale, Gaziantep, Yalova and Zonguldak, were collected from beekeepers. Royal jelly samples were collected in two replicates for each period. The samples had been collected in the middle of production months since April to August 2016. A total of 39 royal jelly samples were obtained from beekeepers between April and August. The samples were freshly harvested on the 15th of every month and transported to the laboratory in an icebox (approximately 4 ^{o}C) placed into 25 mL glass bottle just harvesting.

2.2. Chemical analysis

The total solid contents of royal jelly samples were determined by oven drying at temperature of 105 ^{o}C until a constant weight was obtained. The total protein contents of royal jelly samples were determined using the Kjeldahl method and the quantity of crude protein was calculated using the factor of 6.25 for conversion to protein content. The pH values of royal jelly samples were read directly from the calibrated pH meter (Eutech Cyberscan pH 2700, Ayer Rajah Crescent, Singapore). The pH of samples was measured in solution of 1 g royal jelly in 10 mL of milli-Q water.

2.3. 10-Hydroxy-2-decenoic acid (10-HDA) analysis

Determination of 10-HDA was performed using high-performance

*Corresponding author
E-mail address: mrtemir@gmail.com

liquid chromatography (HPLC) (Agilent 1260 infinity, CA, USA) system coupled with a UV detector set at 225 nm. The separation of 10-HDA was carried out using a Shimadzu column (150 mm x 4.0 mm x 5 µm) adjusted to 35 ºC as column temperature. The elution solvent was composed of 45% methanol in water with 0.1% phosphoric acid (pH 2.5). The flow rate was set at 0.5 mL/min and the injection volume was 20 µL. Comparing a retention time obtained by 10-HDA standard (Sigma-Aldrich) was identified the sample peak. Seven standard solutions in a concentration range of 10 - 1000 µg/mL were used for the preparation of the calibration curve. The regression coefficient of the calibration curve was 0.9999.

To extraction of 10-HDA, approximately 1 g of royal jelly sample was dissolved by sonication at room temperature for 30 min in 50 mL of solvent (methanol and ultrapure water, 50:50, v/v) adjusted at pH 2.5 with phosphoric acid. After the sonication, the sample was filtered through 0.45 µm Millex syringe filter (Millipore, Carrigtwohill, Co. Cork, Ireland) and the filtrate was transferred to a vial for analysis (Antinellia et al., 2003).

2.4. Bioactive properties

Extraction was carried out as the procedure described by Kolayli et al. (2016) with some modification. Approximately 1 g of royal jelly sample was mixed with 9 mL of 80 % methanol solution and vortexed (MX-S model, Dragon lab, Beijing, China) for 1 min. The resulting solution was centrifuged at 6800 g for 10 min at 4 °C and supernatant was collected. The extracts were stored at 4 °C for total phenolic content and antioxidant capacity analysis.

Total phenolic content (TPC)

The TPC was determined by Folin-Ciocalteu colorimetric method described by Singleton and Rossi (1965). The extract (0.5 mL) was mixed with 2.5 mL of Folin Ciocelteau's phenol reagent (0.2N) and 2 mL of sodium carbonate (7.5%) and incubated at dark conditions in room temperature. After 1 hour of the incubation, the absorbance was measured at 760 nm using a UV/VIS spectrophotometer (Agilent Technologies, Cary 60 Model, Victoria, Australia). The results were expressed as µg gallic acid equivalents (GAE) per gram of sample (calibration curve linearity range: $R^2 = 0.997$).

Antiradical scavenging activity

2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of the extracts was determined according to the procedure of Silici, Sagdic, and Ekici (2010) with some modification. An aliquot of 0.1 mL of extract was mixed with 4.9 mL of DPPH solution (0.1 mM in methanol) and vortexed. The mixture was incubated for 1 h at dark conditions in room temperature and then the absorbance was measured at 517 nm by a spectrophotometer (Agilent Technologies, Cary 60 Model, Victoria, Australia). Methanol was used as a control instead of extract. Antiradical activity (%) was calculated using the equation shown below:

$$Antiradical\ activity\ (\%) = \left(\frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control}\right) \times 100$$

2.5. Statistical analysis

Data analysis was performed with the software IBM SPSS Statistics 21 (IBM SPSS, USA). All the results obtained in this study were expressed as mean ± standard deviation (SD). ANOVA and Duncan's multiple range test was applied to finding significant differences among the means of normally distributed samples at a significance level of 0.05. Bivariate correlations were used between total phenolic content and antioxidant capacity, determined by Pearson's correlation coefficient.

3. Results and Discussion

3.1. Chemical properties

The chemical properties of royal jelly samples collected at different harvesting periods were presented in Table 1. The total solid contents of samples were determined between 28.84% and 34.67% and there was no statistical difference between the results obtained different harvesting periods (P>0.05). The mean total solid contents of samples was found as 31.98% and our results were in accordance with the findings of Kolayli et al. (2016) and Yavuz and Gürel (2017). Anna Gloria Sabatini, Marcazzan, Caboni, Bogdanov, and de Almeida-Muradian (2009) stated that proteins (27-41%), carbohydrates (near 30%) and lipids (8-19%) represent the important portion of the total solid contents of royal jelly. The total solid contents of royal jelly samples harvested in April and May were higher than other samples harvested in June, July and August. Similar result obtained by Wongchai and Ratanavalacha (2002), who reported that the carbohydrate content maximally increased in rainy season and also lipid and protein contents of royal jelly slightly changed depending on the season.

The royal jelly under investigation had a protein content between 11.25% and 14.56%, in accordance with limit (ranging from 9 to 18%) allowed by ISO 12842 (ISO, 2016). This results show similarity with Yavuz and Gürel (2017), who are reported crude protein in royal jelly samples ranged from 9.76-12.57%. Similarly, Kolayli et al. (2016) found 11.4% and 15.1%, with a mean protein value of 14.1% of protein in royal jelly samples from Turkey. In another study, Kamyab et al. (2019) found a total protein content in Iranian royal jelly between 11.5-14.5%. The proteins in royal jelly are mostly soluble in water (80%) and eight major protein components of royal jelly have been characterized until now: named as apalbumine, and apart from these proteins, royalactin, royalisin, jelleines, apimisin, glucose oxidase and apalipophorin III like proteins (Çakır, Şirin, & Kolaylı, 2019; Kolayli et al., 2016). The protein content changed only slightly depending on the harvesting period. Samples harvested in May presented the highest amount of total protein content (mean value as 13.54%), while samples harvested in July showed the lowest amount (12.33%). These finding are in agreement with Wongchai and Ratanavalacha (2002), who stated that the protein contents of royal jelly slowly decreased during verging into the hot season and it increased to the former level in the rainy season.

The pH values of royal jelly samples were ranged from 3.71 to 4.01 and harvesting period had a little effect on the pH values of royal jelly (P>0.05). This is in agreement with previous studies in the literature, with values between 3.4 and 4.5 (Anna Gloria Sabatini et al., 2009; Kolayli et al., 2016; Saricaoglu, Cinar, Demircan, & Oral, 2019; Yavuz & Gürel, 2017). The pH values of samples harvested after June was increased and the highest pH value (3.94) was determined for August samples. Wongchai and Ratanavalacha (2002) reported that the pH values of royal jelly (between 3.40-3.60) were constant throughout the year.

3.2. 10-Hydroxy-2-decenoic acid (10-HDA)

10-HDA is the most important fatty acid in royal jelly, because it has only been found in royal jelly, and so it is an important quality marker for assessing the royal jelly adulteration (Yang et al., 2019). As shown in Table 2, 10-HDA levels determined in royal jelly samples varied between 0.91% and 2.05%. These values showed similarity with the other authors' findings. El-Guendouz et al. (2020) found the range of 10-HDA content from the Mediterranean area to be 0.9% to 1.2%. Balkanska (2018) described that the content of 10-HDA in the royal jelly samples from Bulgaria ranged from 1.64% to 2.73%, with a mean value of 1.97%. Also, previous studies have reported that the amount of 10-HDA in royal jelly samples obtained from Tukey shows great variability (0.57-3.11%) (Kolayli et al., 2016; Yavuz & Gürel, 2017).

The amount of 10-HDA in pure royal jelly samples significantly varies depending on the harvesting period (P<0.05). The 10-HDA content of royal jelly markedly increased the transition from April to June and then decreased during the hot season. The highest and lowest mean value of royal jelly harvested in June and April was determined as 1.84% and 1.03%, respectively. It is related with the lipid content of royal jelly and Wongchai and Ratanavalacha (2002) reported that the lipid content increased during the transition from cool season to hot season. Kamyab et al. (2019) found that the royal jelly obtained from Mashhad (hot and dry) and Ardebil (cold and dry) had better quality than Amol (moderate and wet) in terms of 10-HDA content.

Table 1. Chemical properties of royal jelly samples harvested in April (n=6), May (n=16), June (n=6), July (n=6) and August (n=5)

Months	Total solid (%)		Protein (%)		pH	
	Min-max	Mean ± SD	Min-max	Mean ± SD	Min-max	Mean ± SD
April	30.6 – 33.99	32.32 ± 1.27^{a}	12.55 – 13.94	13.25 ± 0.52^{a}	3.79 – 3.93	3.85 ± 0.04^{b}
May	30.85 – 34.67	32.25 ± 0.87^{a}	12.96 – 14.56	13.54 ± 0.37^{a}	3.71 – 3.99	3.84 ± 0.06^{b}
June	29.93 – 32.53	31.39 ± 0.74^{a}	11.97 – 13.01	12.56 ± 0.29^{b}	3.76 – 3.86	3.81 ± 0.03^{b}
July	28.84 – 33.63	31.62 ± 1.7^{a}	11.25 – 13.12	12.33 ± 0.66^{b}	3.82 – 3.97	3.89 ± 0.05^{ab}
August	30.98 – 33.36	31.79 ± 0.81^{a}	12.08 – 13.01	12.4 ± 0.31^{b}	3.9 – 4.01	3.94 ± 0.04^{a}
Total	28.84 – 34.67	31.98 ± 1.11	11.25 – 14.56	13.02 ± 0.67	3.71 – 4.01	3.86 ± 0.06

Different letters within a column denote significant differences (P<0.05) between means according to Duncan's multiple range test.

Table 2. Bioactive properties of royal jelly samples harvested in April (n=6), May (n=16), June (n=6), July (n=6) and August (n=5)

Months	10-HDA (%)		TPC (mg GAE/100 g)		DPPH (% inhibition)	
	Min-max	Mean ± SD	Min-max	Mean ± SD	Min-max	Mean ± SD
April	0.91 -1.21	1.03 ± 0.1^{d}	28.42 – 32.64	29.96 ± 1.33^{c}	3.28 – 6.47	5.44 ± 0.46^{a}
May	0.91 – 1.66	1.28 ± 0.19^{c}	27.1 – 31.12	29.95 ± 0.94^{c}	4.1 – 6.83	5.61 ± 0.45^{a}
June	1.56 – 2.05	1.84 ± 0.17^{a}	27.63 – 32.5	30.07 ± 1.57^{c}	4.33 – 6.93	5.13 ± 0.39^{a}
July	1.39 – 1.97	1.67 ± 0.18^{b}	29.96 – 36.48	34.87 ± 2.28^{a}	4.35 – 6.8	5.69 ± 0.46^{a}
August	0.93 – 1.69	1.21 ± 0.26^{c}	30.14 – 33.39	31.64 ± 1.13^{b}	3.96 – 4.84	4.43 ± 0.37^{b}
Total	0.91 – 2.05	1.38 ± 0.33	27.09 – 36.48	30.53 ± 2.46	3.96 – 6.93	5.29 ± 1.06

10-HDA: 10-Hydroxy-2-decenoic acid; TPC: Total phenolic content.
Different letters within a column denote significant differences (P<0.05) between means according to Duncan's multiple range test.

Based on the standards of the ISO and Turkish, the amount of 10-HDA should not be at less than 1.4% for pure royal jelly to attend quality control parameters (ISO, 2016; Turkish-Standard, 2000). The comparison of the values obtained for the content of 10-HDA against the limit value showed that the 10-HDA contents in 22 royal jelly samples were lower than the limit of standard. Yavuz and Gürel (2017) reported that 6 out of 13 royal jelly samples have lower 10-HDA level than the limit of 1.4%. The 10-HDA contents of royal jelly harvested in April were not found in accordance with the standard, while the amounts of 10-HDA for samples harvested in June were higher than the limit value.

3.3. Bioactive properties

Folin-Ciocalteu method was used to determine the total phenolic content (mg GAE/100 g of royal jelly) of the royal jelly samples. The results obtained showed that the total phenolic content varied greatly among the royal jelly samples, as was shown from Table 2. The royal jelly samples contained from 27.09 to 36.48 mg GAE/100 g of phenolic compounds, with the mean value being 30.53 mg GAE/100 g. The values obtained are higher than those reported by Kolayli et al. (2016), who found the total phenolic contents of royal jelly samples obtained from between 91 and 301 mg GAE/kg. Ozkok and Silici (2017) reported higher amount of total phenolic in royal jelly samples from Turkey (59.16 mg GAE/100 g). Other authors found higher amount of total phenols in royal jelly samples from the Mediterranean Area (14.6-39.9 mg GAE/g) (El-Guendouz et al., 2020), Bulgaria (11.66-36.73 mg GAE/g) (Balkanska et al., 2017) and Romania (10.7 mg GAE/g) (Čeksteryté et al., 2016). This difference may result from the methods used for extraction because several authors determined total phenolic content in methanol, ethanol or water extracts of royal jelly. Royal jelly exhibits much lower phenolic content than other bee products (like honey, propolis and pollen) because it is secreted by young worker bees, and phenolic contents in royal jelly can only derive from young bees mixing small quantities of other bee products in with royal jelly (Kolayli et al., 2016).

For determination of the antioxidant capacity, we used the 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay due to an easy, precise, and accurate method that is widely used for antioxidant activity in many different samples, including bee products (Balkanska et al., 2017; Čeksteryté et al., 2016; Kolayli et al., 2016). According to the Amarowicz, Pegg, Rahimi-Moghaddam, Barl, and Weil (2004), the DPPH has the advantage of being unaffected by certain side reactions, such as enzyme inhibition, metal ion chelation. Results of the determination of antiradical activity of royal jelly samples were presented in Table 2. The antiradical activity of royal jelly samples varied from 3.96% to 6.93%, with the mean of 5.29%. All the tested samples possessed the lowest radical scavenging activity due to less than 50% DPPH. These findings are in agreement with those has mentioned by Ozkok and Silici (2017), showing free radical scavenging activity 5.72% for royal jelly samples. On the contrary, Balkanska et al. (2017) and Pavel et al. (2015) found the higher free radical scavenging activity for Bulgarian and Romanian royal jelly samples with the means of 24.23% and 37.23%, respectively.

The harvesting period had a significant affect on the total phenolic contents of samples (P<0.05). The highest mean value was obtained from the samples harvested in July while the lowest mean value was obtained in August samples. Antioxidant capacity of the samples unchanged transition from April to July (P>0.05), but then significantly decreased (P<0.05).

Pearson correlations were performed between the phenolic and antioxidant capacity from both all and groups of royal jelly samples. When considering all the examples, total phenolic was negatively correlated with antioxidant capacity, but this was not of statistical significance (r=0.427), indicated that antioxidant capacity of samples was not due to total polyphenols exclusively. These findings are in agreement with those has mentioned by Kolayli et al. (2016) reported that a negative correlation is observed between royal jelly DPPH radical scavenging capacity values and total polyphenol contents (R^2=0.41, P>0.05). Balkanska et al. (2017) stated that antioxidant activity might also result from the presence of other antioxidant secondary metabolites from pollen grains in royal jelly and the antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. On the other hand, significant linear correlations were found between the total phenolic content and antioxidant capacity of samples harvested in June (r = 0.67, P<0.01) and in August (r = 0.887, P<0.01). Literature data confirm the existence of such relationships (Pavel et al., 2015).

4. Conclusion

The results obtained in this study indicate that the harvesting periods (April, May, June July, August) of royal jelly provided from Turkey caused a change in chemical composition as well as bioactive compounds like 10-HDA. The total solid contents with the amount of protein were the highest in the rainy harvesting period. However, pH values of samples were constant throughout the harvesting periods. Although the total phenolic contents of royal jelly samples harvested until July increased, but antioxidant activity was not affected by harvesting period except August. The maximum mean value of 10-HDA content were found in samples harvested in June, and in terms of 10-HDA, all samples harvested in June and July meet the limit value permitted by Turkish royal jelly standards.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with

respect to the research, authorship, and/or publication of this article.

Acknowledgements

The authors would like to thank to the Association of Honey Promotion for financially supported and also to the Turkish Beekeepers for providing the royal jelly used for in this study.

References

Alu'datt, M. H., Rababah, T., Obaidat, M. M., Ereifej, K., Alhamad, M. N., Mhaidat, N., . . . Ayadi, W. (2015). Probiotics in Milk as Functional Food: Characterization and Nutraceutical Properties of Extracted Phenolics and Peptides from Fermented Skimmed Milk Inoculated with Royal Jelly. *Journal of Food Safety, 35*(4), 509-522. doi:10.1111/jfs.12201

Amarowicz, R., Pegg, R. B., Rahimi-Moghaddam, P., Barl, B., & Weil, J. A. (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chemistry, 84*(4), 551-562. doi:10.1016/s0308-8146(03)00278-4

Anna Gloria Sabatini, A. G., Marcazzan, L. G., Caboni, M. F., Bogdanov, S., & de Almeida-Muradian, L. B. (2009). Quality and standardisation of Royal Jelly. *Journal of ApiProduct and ApiMedical Science, 1*(1), 1-6. doi:10.3896/IBRA.4.1.01.04

Antinellia, J. F., Zeggane, S., Davico, R., Rognone, C., Faucon, J. P., & Lizzani, L. (2003). Evaluation of (E)-10-hydroxydec-2-enoic acid as a freshness parameter for royal jelly. *Food Chemistry, 80*, 85-89.

Balkanska, R. (2018). Determination of Trans-10-Hydroxy-2-Decenoic Acid in Royal Jelly by High Performance Liquid Chromatography after Different Bee Feeding. *International Journal of Current Microbiology and Applied Sciences, 7*(04), 3738-3743. doi:10.20546/ijcmas.2018.704.420

Balkanska, R., Marghitas, L.-A., & Pavel, C. I. (2017). Antioxidant Activity and Total Polyphenol Content of Royal Jelly from Bulgaria. *International Journal of Current Microbiology and Applied Sciences, 6*(10), 578-585. doi:10.20546/ijcmas.2017.610.071

Bincoletto, C., Eberlin, S., Figueiredo, C. A., Luengo, M. B., & Queiroz, M. L. (2005). Effects produced by Royal Jelly on haematopoiesis: relation with host resistance against Ehrlich ascites tumour challenge. *Int Immunopharmacol, 5*(4), 679-688. doi:10.1016/j.intimp.2004.11.015

Čeksteryté, V., Kurtinaitienė, B., Venskutonis, P. R., Pukalskas, A., Kazernavičiūtė, R., & Balžekas, J. (2016). Evaluation of antioxidant activity and flavonoid composition in differently preserved bee products. *Czech Journal of Food Sciences, 34*(No. 2), 133-142. doi:10.17221/312/2015-cjfs

Çakır, H. E., Şirin, Y., & Kolaylı, S. (2019). Royal Jelly and Its SDS-PAGE Electrophoresis Profiles. *Journal of Apitherapy and Nature*, 17-20. doi:10.35206/jan.638063

El-Guendouz, S., Machado, A. M., Aazza, S., Lyoussi, B., Miguel, M. G., Mateus, M. C., & Figueiredo, A. C. (2020). Chemical Characterization and Biological Properties of Royal Jelly Samples From the Mediterranean Area. *Natural Product Communications, 15*(2). doi:10.1177/1934578x20908080

El-Nekeety, A. A., El-Kholy, W., Abbas, N. F., Ebaid, A., Amra, H. A., & Abdel-Wahhab, M. A. (2007). Efficacy of royal jelly against the oxidative stress of fumonisin in rats. *Toxicon, 50*(2), 256-269. doi:10.1016/j.toxicon.2007.03.017

Emir, D., & Emir, M. (2017). Evaluation of Royal Jelly Production in Turkey and First Detection of Probiotics. . *4th International Halal and Healthy Food Congress, 3-5 November, Ankara, TURKEY*.

Emir , M. (2017). Royal jelly in Turkey: Production, cost and marketing. *45th APIMONDIA International Apicultural Congress, 29 September–4 October, Istanbul, TURKEY*.

Inoue, S. (2003). Royal Jelly prolongs the life span of C3H/HeJ mice: correlation with reduced DNA damage. *Experimental Gerontology, 38*(9), 965-969. doi:10.1016/s0531-5565(03)00165-7

ISO. (2016). International Standard. Royal jelly specifications *ISO:12824:2016 (E)*, p. 35.

Jie, H., Li, P. M., Zhao, G. J., Feng, X. L., Zeng, D. J., Zhang, C. L., . . . Chen, Q. (2016). Amino acid composition of royal jelly harvested at different times after larval transfer. *Genet Mol Res, 15*(3). doi:10.4238/gmr.15038306

Kamakura, M., Mitani, N., Fukuda, T., & Fukushim, M. (2001). Antifatigue effect of fresh royal jelly in mice. *Journal of Nutritional Science and Vitaminology, 47*, 394-401.

Kamyab, S., Gharachorloo, M., Honarvar, M., & Ghavami, M. (2019). Quantitative analysis of bioactive compounds present in Iranian royal jelly. *Journal of Apicultural Research, 59*(1), 42-52. doi:10.1080/00218839.2019.1673964

Kohno, K., Okamoto, I., Sano, O., Arai, N., Iwaki, K., Ikeda, M., & Kurimoto, M. (2004). Royal jelly inhibits the production of proinflammatory cytokines by activated macrophages. *Biosci Biotechnol Biochem, 68*(1), 138-145. doi:10.1271/bbb.68.138

Kolayli, S., Sahin, H., Can, Z., Yildiz, O., Malkoc, M., & Asadov, A. (2016). A Member of Complementary Medicinal Food: Anatolian Royal Jellies, Their Chemical Compositions, and Antioxidant Properties. *J Evid Based Complementary Altern Med, 21*(4), NP43-48. doi:10.1177/2156587215618832

Okamoto, I., Taniguchi, Y., Kunikata, T., Kohno, K., Iwaki, K., Ikeda, M., & Kurimoto, M. (2003). Major royal jelly protein 3 modulates immune responses in vitro and in vivo. *Life Sciences, 73*(16), 2029-2045. doi:10.1016/s0024-3205(03)00562-9

Ozkok, D., & Silici, S. (2017). Antioxidant activities of honeybee products and their mixtures. *Food Sci Biotechnol, 26*(1), 201-206. doi:10.1007/s10068-017-0027-0

Park, H. G., Kim, B. Y., Park, M. J., Deng, Y., Choi, Y. S., Lee, K. S., & Jin, B. R. (2019). Antibacterial activity of major royal jelly proteins of the honeybee (Apis mellifera) royal jelly. *Journal of Asia-Pacific Entomology, 22*(3), 737-741. doi:10.1016/j.aspen.2019.06.005

Pavel, C. I., Mărghitaş, L. A., Dezmirean, D. S., Tomoş, L. I., Bonta, V., Şapcaliu, A., & Buttstedt, A. (2015). Comparison between local and commercial royal jelly—use of antioxidant activity and 10-hydroxy-2-decenoic acid as quality parameter. *Journal of Apicultural Research, 53*(1), 116-123. doi:10.3896/ibra.1.53.1.12

Ramadan, M. F., & Al-Ghamdi, A. (2012). Bioactive compounds and health-promoting properties of royal jelly: A review. *Journal of Functional Foods, 4*(1), 39-52. doi:10.1016/j.jff.2011.12.007

Saricaoglu, F. T., Cinar, A., Demircan, H., & Oral, R. A. (2019). Rheological and microstructural characterization of royal jelly at different temperatures. *Journal of Food Process Engineering, 42*(8). doi:10.1111/jfpe.13285

Silici, S., Sagdic, O., & Ekici, L. (2010). Total phenolic content, antiradical, antioxidant and antimicrobial activities of Rhododendron honeys. *Food Chemistry, 121*(1), 238-243. doi:10.1016/j.foodchem.2009.11.078

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144-158.

Turkish-Standard. (2000). Royal Jelly Standard. *ICS 65.140; 67.230. TS 6666.*

Vittek, V. (1995). Effect of Royal Jelly on serum lipids in experimental animals and humans with atherosclerosis. *Experientia, 51*, 927-935.

Wongchai, V., & Ratanavalacha, T. (2002). Seasonal Variation of Chemical Composition of Royal Jelly Produced in Thailand. *Thammasat International Journal of Science and Technology, 7*(2), 1-8.

Wytrychowski, M., Chenavas, S., Daniele, G., Casabianca, H., Batteau, M., Guibert, S., & Brion, B. (2013). Physicochemical characterisation of French royal jelly: Comparison with commercial royal jellies and royal jellies produced through artificial bee-feeding. *Journal of Food Composition and Analysis, 29*(2), 126-133. doi:10.1016/j.jfca.2012.12.002

Yang, X., Li, Y., Wang, L., Li, L., Guo, L., Huang, F., & Zhao, H. (2019). Determination of 10-Hydroxy-2-Decenoic Acid of Royal Jelly Using Near-Infrared Spectroscopy Combined with Chemometrics. *J Food Sci, 84*(9), 2458-2466. doi:10.1111/1750-3841.14748

Yavuz, İ., & Gürel, F. (2017). Chemical properties of the royal jellies in Turkish markets. *Mediterranean Agricultural Sciences, 30*(3), 281-285. doi:10.29136/mediterranean.360013

Zheng, H. Q., Hu, F. L., & Dietemann, V. (2011). Changes in composition of royal jelly harvested at different times: consequences for quality standards. *Apidologie, 42*(1), 39-47. doi:10.1051/apido/2010033

Zhu, Z., Zhang, Y., Wang, J., Li, X., Wang, W., & Huang, Z. (2019). Characterization of sugar composition in Chinese royal jelly by ion chromatography with pulsed amperometric detection. *Journal of Food Composition and Analysis, 78*, 101-107. doi:10.1016/j.jfca.2019.01.003

Zimmermann, A. (2002). Liver regeneration: the emergence of new pathways. *Medical Science Monitor, 8*(3), 53-63.