Turkish Journal of Agriculture - Food Science and Technology, 9(8): 1443-1447, 2021
DOI: https://doi.org/10.24925/turjaf.v9i8.1443-1447.4256

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

# Does the Presence and Absence of Queen Bee in the Production of Royal Jelly Affect the Amount of Soluble Protein and Ratio of 10-Hydroxy-2-Decenoic Acid?

**Aytül Uçak Koç[1,a,*], Mete Karacaoğlu[1,b], Zehra Burcu Bakır[2,c], Burcu Keser[3,d]**

[1]*Animal Science, Faculty of Agriculture, Aydin Adnan Menderes University, Aydın 09100, Turkey*
[2]*Biology Department, Faculty of Arts and Sciences, Aydin Adnan Menderes University, Aydın 09000, Turkey*
[3]*Laboratory Technologies Department, Kocarli Vocational School, Aydin Adnan Menderes University, Aydın 09100, Turkey*
[*]*Corresponding author*

ARTICLE INFO

*Research Article*

Received : 13/02/2021
Accepted : 12/07/2021

*Keywords:*
Honey bee
Starter-finisher colony
Queenless colony
Royal jelly yield
Soluble protein

ABSTRACT

Royal jelly (RJ), is one of the important honey bee products and a functional food item in the regulation of diets and in the cosmetic industry. RJ has a potential towards various human disease treatments. The chemical content of RJ is influenced by some factors. In this study, the effect of the presence or absence of the queen on the amount of 10-hydroxy-2-decenoic acid (10-HDA) and soluble protein in RJ is determined. For this reason, colonies were prepared as queenless, queenright and starter-finisher. RJ yields in colonies queenless, queenright and starter-finisher were determined as 15.2 ± 0.89 g, 12.0 ± 0.90 g and 9.6 ± 0.72 g, respectively. Group queenless was different from the other two groups. While 10-HDA values of the groups were similar (queenless, queenright and starter-finisher, respectively; 2.0 ± 0.06%, 2.1 ± 0.06% and 2.0 ± 0.05%), the soluble protein amounts of the groups (queenless, queenright and starter-finisher, respectively, 9.65 ± 0.179%, 7.68 ± 0.184%, 7.50 ± 0.203%) were found different and significant from each other. As a result, the RJ production colony queenless or queenright affected the amount of soluble protein. The worker bees of queenless colonies secreted RJ containing more soluble protein.

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 9(8): 1443-1447, 2021

# Arı Sütü Üretim Kolonilerinin Anasız ve Ana Arılı Olması Arı Sütündeki 10-HDA ve Çözünür Protein Miktarını Etkiler mi?

MAKALE BİLGİSİ

*Araştırma Makalesi*

Geliş : 13/02/2021
Kabul : 12/07/2021

*Anahtar Kelimeler:*
Bal arısı
Başlatıcı-bitirici koloni
Anasız koloni
Arı sütü verimi
Çözünür protein

ÖZ

Arı sütü, diyetlerin düzenlenmesinde ve kozmetik endüstrisinde de fonksiyonel gıda maddesi olan önemli bal arısı ürünlerinden biridir. Arı sütü, insanlarda çeşitli hastalıkları tedavi edici potansiyele sahiptir ve kimyasal içeriği bazı faktörlerden etkilenir. Bu çalışmada arı sütü üretim kolonilerinde ana arı varlığının ve yokluğunun arı sütündeki 10-hidroksi-2-dekenoik asit (10-HDA) ve çözünür protein miktarı üzerine etkisi belirlenmiştir. Bu nedenle koloniler anasız, ana arılı ve başlatıcı-bitirici olarak hazırlanmıştır. Arı sütü verimleri anasız, ana arılı ve başlangıç-bitirici kolonilerinde sırasıyla; 15,2 ± 0,89 g, 12,0 ± 0,90 g ve 9,6 ± 0,72 g olarak belirlenmiştir. Anasız grup diğer iki gruptan farklı bulunmuştur. Anasız (%2,0 ± 0,06) ana arılı (%2,1 ± 0,06) ve başlatıcı-bitirici (%2,0 ± 0,05) kolonilerin 10-HDA değerleri benzer iken; çözünür protein miktarları ise, anasız (%9,65 ± 0,179), ana arılı (%7,68 ± 0,184) ve başlatıcı-bitirici (%7,50 ± 0,203) koloniler birbirinden farklı ve önemlidir. Sonuç olarak, arı sütü üretim kolonisinin anasız veya ana arılı olarak hazırlanması arı sütü çözünür protein miktarını etkilemiştir.

[a] *aucak@adu.edu.tr* *http://orcid.org/0000-0001-5969-1609*
[b] *mkaracaoglu@adu.edu.tr* *http://orcid.org/0000-0002-1152-0808*
[c] *zbakir@adu.edu.tr* *http://orcid.org/0000-0002-9241-0749*
[d] *bkeser@adu.edu.tr* *http://orcid.org/0000-0002-1828-4617*

## Introduction

Royal jelly (RJ) is secreted from the hypopharyngeal and mandibular glands of young worker bees at the age of 5-15 days. Queen honey bee feeds with RJ throughout her life, also worker and drones feed with RJ at the young larva period in the colony. The most important reason for young worker bee larvae to turn into queen bee is feeding with RJ. It is the caste determination food for honey bees (Evans and Wheeler 1999, 2001; Moritz et al., 2005; Hu et al., 2017).

RJ is yellowish white and creamy liquid appearance. In the structure of its, which has a water-soluble pH of 3.4 - 4.5; water (60-70%), protein (9-18%), fatty acids and lipids (3-8%), carbohydrates (7-18%), ash (0.8-3%), 10-HDA, (>1.4%), small amounts of vitamins (B group complex, vitamin C, vitamin E) (copper, zinc, iron, calcium, manganese, potassium, sodium) are found (Stocker et al., 2005; Sabatini et al., 2009; Isidorov et al., 2012; Kösoğlu et al., 2013). Various bioactive compounds such as B complex vitamins, fatty acids, proteins, peptides, amino acids, acetylcholine, adenosine and trace minerals found in RJ make it among functional foods (Barnutiu, 2011; Wytrychowski et al., 2013; Wang et al., 2015; Xin et al., 2016). RJ shows numerous biological effects such as antioxidan, hormone balance, anti inflammatory effect immunity regulation, anti-ageing, cholesterol-lowering, tumour vascularisation inhibition, wound – healing and anti - biotic and hepatoprotective effects, activities positive effects on bone metabolism in postmenopausal women (Kohno et al., 2004; El-Nekeety et al., 2007; Kanbur et al., 2009; Nakajima, 2009; Morita et al., 2012; Arzi et al., 2015; Fan et al., 2016; Seyyedi et al., 2016; Kocot et al., 2018; Matsushita et al., 2020).

It is widely believed that almost all the positive effects listed above are caused by RJ of 10-HDA and MRJP. While the protein content of RJ varies between 9-18%, the SP rate includes between 83-90% of protein content. Soluble RJ proteins (SRJPs) include the major RJ protein family (MRJP), which contribute to the physiological actions of RJ (Simuth, 2001; Scarselli et al., 2005; Nozaki et al., 2012).

10-HDA, one of the RJ major fatty acids, is an unsaturated fatty acid present only in RJ and its content is considered as one of the authenticities and quality parameters (Sabatini et al., 2009). Until today, only several countries have defined national quality standards or guidance for RJ quality determination (Kanalis et al., 2015). In fatty acids (especially 10-HDA) RJ is antibiotic effective compounds against many bacteria and fungi. Therefore, it has a beneficial effect on strengthening the immune system. 10-HDA exhibits several biological activities, including anticancer activity, and provides protection against acute radiation injury. However, royal jelly is not a standard food because its content is affected by many factors. For example, the content of RJ varies according to the feeding of bees, season and age of larvae and harvest time of RJ, number of queen cell (Liu et al., 2008; Zheng et al., 2011; Kösoğlu et al., 2013; Karacaoğlu et al., 2019; Balkanska, 2018; Araujo et al., 2020). RJ content is affected by many factors, do production colonies queenright or queenless affect soluble protein ratio containing immune proteins? There was no answer to this in previous studies. In this study, we compared the SP and 10-HDA ratios of RJ produced in starter-finisher colonies, which is a classical commercial RJ production method queenless and queenright.

## Materials and Methods

This study was carried out at the "Honey Bee and Silkworm Research Unit of Adnan Menderes University Faculty of Agriculture" in Aydın. The colonies of Aegean Ecotype of Anatolian honey bee were used. Three (3) trial groups were carried out in this study. The first group is "queenless colonies", the second group is "queenright colonies" (the group in which the movement of the queen is restricted, and the third group is the "starter - finisher" group. Four (4) harvests (between 24 April-4 May 2018) were made in each trial colony. The groups are described in detail below.

### *Group Queenless*

In this application, it was used 6 colonies for RJ production. Each of them had pollen-honey stock and 3 kg adult worker bee. It was transferred to each colony 120 larvae and harvested RJ after 72 hours.

### *Group Queenright*

The queen was trapped in a cage made of queen excluder with 2 frames. In this application, it was used 6 colonies. Each of them had pollen-honey stock and 3 kg adult worker bee. It was transferred to each colony 120 larvae and harvested RJ after 72 hours.

### *Group Starter-Finisher*

This application is widely used in commercial RJ production. It was added to the trial for comparison. In this application, it was prepared 4 starter and 8 finisher colonies. The queen of the starter colonies was taken (It was prepared starter colonies as group queenless colonies. These colonies had 3 kg of adult worker bees, sufficient stocks of pollen and honey. It was transferred 120 larvae to each colony. After 24 hours, the larvae were separated into 2 finisher colonies. The finisher colonies were double-decked (They had honey supers). The queen was restrained with a queen bee excluder to deep hive body (brood). The larvae were placed to the second floor (honey super). Larvae were kept for 48 hours in the finisher colonies. Each finisher colony fed approximately 60 larvae.

After 72 h grafting, RJ yield was determined. To know the number of larvae that feeding of colonies the larvae acceptance rate was determined. RJ samples were collected from each production colony. The collected RJ samples were kept at -20°C until further analysis. Soluble protein and 10-HDA analyzes were performed in the RJ.

### *Chemical Analysis*

For 10-HDA analysis in RJ, HPLC Agilent 1260 Infinity series (UV-DAD) Luna C18 (150mm×4.6mm×5 µm) column was used (Mobile phase: Methanol: Water: Phosphoric Acid (55: 45: 5), flow rate 1 mL / min, column temperature 30°C, injection amount 20 µl, analysis time 15 minutes, DAD detector 215 nm). Weigh 0.01 g of 10-HDA analytical standards to dissolve 50 mL (final density 200

µg/mL) in water: methanol (50:50) and dilute from this solution to 5, 10, 20, 50, 100, 200 (ppm) µg / mL. calibration curve was created. Then 0.05 g of RJ was weighed into 50 mL cap tubes and shaken by placing 12.5 mL methanol on it. Then 12.5 mL of water was added to this solution and mixed by closing the lid. After the mixture was kept in ultrasonic water bath for 30 minutes, the tubes were centrifuged at 6000 rpm for 5 minutes and filtered through black band filter paper and 20 µl were injected into HPLC (Caparica et al., 2007; Kim and Lee, 2010).

Bradford's method was used to measure the protein concentration and bovine serum albumin was used as the standard for total and soluble protein determination (Bradford, 1976). RJ samples were weighed to 100 mg / mL and dissolved in ultrapure water. Samples were sonicated in an ice bath for 1 min at 40 % of the maximum power (BANDELIN SONOPULS-HD2200, Germany). These homogenized samples were used for total protein determination. Samples were then centrifuged for 10 min at 4°C and 15 000 × g. The supernatant was separated and reserved for the measurement of the soluble protein assay.

***Statistical Analysis***

All data were analyzed using the general linear model procedure available from SAS (1999) package program and the differences between the groups were determined according to Tukey ($P<0.05$) multiple comparison test.

## Results

Table 1 shows RJ yield per colony of groups. RJ yields, the differences between groups were significant ($P<0.05$). The highest RJ yield was obtained in the second harvest ($20.0 \pm 2.19$ g) in the queenless group. The lowest RJ yield was obtained in the 4th harvest ($8.0 \pm 1.18$) in the queenless group. After the first harvest, RJ yields increased in both groups, while yields decreased in the 3rd and 4th harvests (Table 1).

RJ yields ranged from 7.5 to 20 g. Average RJ obtained in four harvests (Overall mean), queenless group produced more RJ than the queenright and starter-finisher group. It was obtained mostly RJ in colonies queenless ($15.2 \pm 0.89$), then in the queenright group ($12.0 \pm 0.90$) and least in the starter-finisher colonies ($9.6 \pm 0.72$ g).

Table 2 shows soluble protein, 10-HDA and average RJ yield of groups. In our study, the 10-HDA amounts of the groups are similar. The queenless group with the highest soluble protein ($9.65 \pm 0.179$%) is different from the queenright and starter-finisher groups ($7.68 \pm 0.184$ % and $7.50 \pm 0.203$ %).

Table 1. Royal Jelly yield (per colony) of groups

| Harvests | Queenless (Mean±SE) | Queenright (Mean±SE) | Starter-Finisher (Mean±SE) |
|---|---|---|---|
| 1 | $18.7 \pm 2.22$ | $13.6 \pm 1.99$ | $9.1 \pm 1.43$ |
| 2 | $20.0 \pm 2.19$ | $14.9 \pm 1.96$ | $11.8 \pm 2.03$ |
| 3 | $14.2 \pm 0.77$ | $9.9 \pm 0.69$ | $10.0 \pm 0.68$ |
| 4 | $8.0 \pm 1.18$ | $9.6 \pm 1.06$ | $7.5 \pm 0.85$ |
| Overall mean | $15.2 \pm 0.89^{a}$ | $12.0 \pm 0.90^{b}$ | $9.6 \pm 0.72^{b}$ |

$P<0.05$: a, b, c

Table 2. Soluble protein, 10-HDA and royal jelly yield of the groups

| Groups | Soluble Protein (%) | | 10-HDA (%) | | Royal jelly Yield (g) | |
|---|---|---|---|---|---|---|
| | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE |
| Queenless | 18 | $9.65 \pm 0.179^{a}$ | 24 | $2.0 \pm 0.06$ | 6 | $15.2 \pm 0.89^{a}$ |
| Queenright | 17 | $7.68 \pm 0.184^{b}$ | 19 | $2.1 \pm 0.06$ | 6 | $12.0 \pm 0.90^{b}$ |
| Starter-Finisher | 14 | $7.50 \pm 0.203^{b}$ | 38 | $2.0 \pm 0.05$ | 8 | $9.60 \pm 0.72^{b}$ |

$P<0.05$: a, b

## Discussion and Conclusion

In this study, we tried to determine how the amount of 10-HDA and protein which are important contents of RJ, are affected by the applications made in RJ production. Or, from another point of view, how much are these contents affected by the presence and absence of the queen bee? For this purpose, we analyzed RJ produced in starter-finisher colonies applied by commercial RJ producing enterprises and in colonies without queen bee where small amounts of RJ are produced. Apart from these known applications, we used the cage application (queenright group), which is unknown and not used in RJ production before our study.

In our study, the absence of queen bees in RJ production affected the SP ratio in RJ, but not the 10-HDA ratio. Namely, SP content of RJ produced by queenless colonies was determined more than queenright and starter-finisher colonies in our experiment. At the same time, the amount of RJ produced by these colonies was also high. Studies on the applications in RJ production colonies are limited. In these limited studies, that factors such as the age of the grafted larva, harvest time and the number of queen cell cup are observed to have affected the content of RJ (Liu et al., 2008; Zheng et al., 2011; Kösoğlu et al., 2013; Karacaoğlu et al., 2019). No study has been found on the RJ content of RJ producing colonies with or without a queen (or we could not reach). It has been reported that queens reared in queenless colonies had higher weight at emergence (Cengiz et al., 2009). In this study, we can say that queenless colonies feed their larvae with more and more qualified RJ. Because the absence of the queen makes the worker bees more helpless and warrant. For this reason, worker bees feed more larvae with higher quantity and quality of RJ. In this study, queenright colonies were able to feed 60 larvae, while colonies queenless were able to feed 80 larvae from 120 larva transfers. These results were found to be compatible with the literature (Sahinler and Kaftanoglu, 2005; Karacaoğlu et al., 2004). The larvae that

were fed in starter colonies for the first 24 hours were fed in finisher colonies for 48 hours. We think that the pheromones of the queen bee (trapped deep super by the queen excluder) in finisher colonies are felt inside the hive, which affects the content of RJ. These results showed us that workers without queen bees take no risk and feed more larvae and more protein.

The presence of 10-HDA has been regarded as a marker to differentiate RJ from other products. Its concentration has been used as a parameter of RJ quality. The minimum concentration of 10-HDA is 1.4% for pure RJ (Hu et al. 2017). In this study, the amount of 10-HDA was similar in all three groups. Kamyab et al. (2020) reported that 10-HDA content varied significantly in different climatic regions, and that 10-HDA amounts in RJ produced in hot and dry regions were higher (2.443 ± 0.011%). Karacaoğlu et al. (2019) determined that 10-HDA amounts vary significantly according to harvest time, with the highest 10-HDA amounts in RJ harvested in 24 hours. Liu et al. (2008) have been determined age of larvae grafted and harvest time to affect 10-HDA content. Dietary supplementation with mineral Fe affected the protein content and number of proteins in the experimental period (Araujo, 2020). Balkanska (2018) found the 10-HDA ratio (2.13 ± 0.27%) of RJ produced by colonies fed with baker's yeast to be different and significant compared to the control group (1.89 ± 0.22%) the group fed with A, D3 and E vitamins.

RJ's functionality is directly related to its major RJ proteins (MRJPs) and 10-HDA (Kamakura et al. 2001; Okamoto et al. 2003; Kohno et al. 2004; Fratini et al. 2016). RJ has been on the agenda more frequently along with other bee products and natural supplements due to its beneficial and pharmaceutical value during the pandemic due to Covid-19. RJ has hypotensive and blood pressure regulatory, antitumor, antiviral, anti-inflammatory and antioxidant functions (Ramadana and Ghamdi, 2012; Wytrychowski et al., 2013; Wang et al., 2015; Martinez-Dominguez, et al., 2016; Xin et al., 2016; Chen et al., 2017; Hu et al., 2017), but studies on the effects of environmental factors related to its production on the content are limited, so studies on this subject will provide benefits in obtaining quality RJ. In this study, RJ production colonies queenright or queenless affected the amount of soluble protein. For this reason, applications in production colonies should be considered. Because it will contribute to the revision of the RJ standard, which is a therapeutic food. We suggest further research on the relationships between the MRJPs family and RJ production. There are many unknowns about RJ still. However, with further research on this and similar issues, the unknowns about RJ will be resolved.

## Acknowledgements

We thank the Scientific Research Projects Commission of Aydin Adnan Menderes University (BAP-ZRF 18001) for its financial support and thank TARBIYOMER for its laboratory service support.

## References

Araujo WLP, Negrao AF, Vieira JC, S Bittarello AC, Padilha PM, Orsi RO. 2020. Biological Trace Element Research, 195:648–657.

Arzi A, Olapour S, Yaghooti H, Karampour NS. 2015. Effect of royal jelly on formalin induced-inflammation in rat Hind Paw. Jundishapur J Nat Pharm Prod. February; 10(1): e22466.

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(4): 3738-3743.

Barnutiu LI, Dezmirean DS, Mihai CM, Bobis O. 2011. Chemical composition, and antimicrobial activity of royal jelly–review. Journal of Animal Science and Biotechnologies, 44 (2): 67-72.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72, 248–254.

Caparica C, Marcucci S, Marcucci MC. 2007. Quantitative determination of trans- 10-Hydroxy-2-Decenoic Acid (10-HDA) in Brazilian royal jelly and commercial products containing royal jelly. Journal of Apicultural Research and Bee World, 46(3): 149–153.

Cengiz M, Emsen B, Dodologlu A. 2009. Some characteristics of queenbees (Apis mellifera L.) rearing in queenright and queenless colonies. Journal of Animal and Veterinary Advances, 8 (6): 1083-1085.

Chen D, Liu F, Wan JB, Lai CQ, Shen LR. 2017. Effect of major royal jelly proteins on spatial memory in aged rats: Metabolomics analysis in urine. Journal of Agricultural and Food Chemistry, 65(15): 3151–3159.

El-Nekeety AA, El-Kholy W, Abbas NF, Ebaid A, Amra HA, Mosaad AV. 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.

Evans JD, Wheeler DE. 1999. Differential gene expression between developing queens and workers in the honeybee, Apis mellifera. Proceedings of the National Academy of Sciences of the United States of America. 96 (10): 5575–5580.

Evans JD, Wheeler DE. 2001. Expression profiles during honeybee caste determination. Genome Biology, 2 (1): 1-6.

Fan P, Han B, Feng M, Fang Y, Zhang L, Hu H, Hao Y, Qi Y, Zhang X, Li J. 2016. Functional and proteomic investigations reveal major royal jelly protein 1 associated with antihypertension activity in mouse vascular smooth muscle cells. Scientific Reports | 6:30230 | DOI: 10.1038/srep30230.

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

Hu FL, Bilikova K, Casabianca H, Daniele G, Espindola SF. et al. 2017. Standard methods for *Apis mellifera* royal jelly research. Journal of Apicultural Research, 58: 1–69.

Isidorov VA, Bakier S, Grzech I. 2012. Gas chromatographic-mass spectrometric investigation of volatile and extractable compounds of crude royal jelly. Journal of Chromatography B, (885–886): 109–116.

Kamakura M, Fukuda T, Fukushima M, Yonekura M. 2001. Storage-dependent degradation of 57-kDa protein in royal jelly: A possible marker for freshness. Bioscience, Biotechnology, and Biochemistry, 65, 277–284.

Kamyab S, Gharachorloo M, Honarvar M, Ghavami M. 2020. Quantitative analysis of bioactive compounds present in Iranian royal jelly. Journal of Apicultural Research, 59(1), 42–52.

Kanbur M, Eraslan G, Beyaz L, Silici S, Liman, BC, Altınordulu Ş, Atasever A. 2009. The effects of royal jelly on liver damage induced by paracetamol in mice. Experimental and Toxicologic Pathology, 61(2):123–132.

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

Karacaoğlu M, Kösoğlu M, Uçak Koç A. 2004. Farklı yöntemlerin Ege ekotipi (*A. m. anatolica*) ve Kafkas (*A. m. caucasica*) x Ege melezi bal arılarının arı sütü verimleri üzerine etkileri. ADÜ Ziraat Fakültesi Dergisi, 1(1): 29-33.

Karacaoğlu M, Uçak Koç A, Bakır BZ, Metin K, Keser B, Birincioglu B. 2019. The effect of harvest time and number of queen cell on 10-HDA and total protein content in royal jelly. 11. International Animal Science Conference, Cappadocia, Turkey, 20-22 October, p:380-384.

Kim J, Lee J. 2010. Quantitative analysis of trans-10hydroxy-2-decenoic acid in royal jelly products purchased in USA by high-performance liquid chromatography. Journal of Apicultural Science, 54(1), 77–85.

Kocot J, Kiełczykowska M, Luchowska-Kocot D, Kurzepa J, Musik I. 2018. Antioxidant potential of propolis, bee pollen, and royal jelly: possible medical application. Hindawi Oxidative Medicine and Cellular Longevity, Article ID 7074209, 29 pages https://doi.org/10.1155/2018/7074209.

Kohno K, Okamoto I, Sano O. 2004. Royal jelly inhibits the production of proinflammatory cytokines by activated macrophages. Bioscience, Biotechnollogy and Biochemistry, 68:138–145.

Kösoğlu M, Yücel B, Gökbulut C, Konak R, Bircan C. 2013. The effect of harvesting time on some biochemical and trace element compositions of royal jelly. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 19(2), 233-237.

Liu JR, Yang YC, Shi LS, Peng CC. 2008. Antioxidant Properties of Royal Jelly Associated with Larval Age and Time of Harvest. Journal of Agricultural Food Chemistry, 56, 11447–11452.

Martinez-Dominguez G, Romero-Gonzalez R, Garrido Frenich A. 2016. Multi-class methodology to determine pesticides and mycotoxins in green tea and royal jelly supplements by liquid chromatography coupled to Orbitrap high resolution mass spectrometry. Food Chemistry, 197: 907-915.

Matsushita H, Shimizu S, Morita N, Watanabe K, Wakatsuki A. 2020. Effects of royal jelly on bone metabolism in postmenopausal women: a randomized, controlled study. Climacteric https://doi.org/10.1080/13697137.2020.1806815

Morita H, Ikeda T, Kajita K.et al. 2012. Effect of royal jelly ingestion for six months on healthy volunteers. Nutrition Journal, 11(77) :1-7.

Moritz RF, Lattorff HM, Neumann P, Kraus FB, Radloff SE, Hepburn HR. 2005. Rare royal families in honeybees, *Apis mellifera*. Naturwissenschaften, 92 (10): 488–491.

Nakajima Y, Tsuruma K, Shimazawa M, Mishima S, Hara H. 2009. Comparison of bee products based on assays of antioxidant capacities. BMC Complementary and Alternative Medicine, 9(4):1-9.

Nozaki R, Tamura S, Ito A, Moriyama T, Yamaguchi K, Kono T. 2012. A rapid method to isolate soluble royal jelly proteins. Food Chemistry, 134, 2332–2337.

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, 2029–2045.

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

Sabatini AG, Marcazzan GL, Caboni MF, Bogdanov S, De Almeida-Muradian LB. 2009. Quality, and standardisation of royal jelly. Journal of ApiProduct and ApiMedical Science, 1(1): 1–6.

Sahinler N, Kaftanoglu O. 2005. The effects of season and honeybee (*Apis mellifera* L.) genotype on acceptance rates and royal jelly production. Turkish Journal of Veterinary and Animal Sciences, 29:499-503.

SAS. 1999. Statistical Analsis System for Windows (Relase 8.2). SAS Institute

Inc.Raleigh, Caroline, USA.

Scarselli R, Donadio E, Giuffrida MG, Fortunato D, Conti A, Balestreri E, Felicioli R, Pinzauti M, Sabatini AG, Felicioli A. 2005. Towards royal jelly proteome. Proteomics, 5, 769–776.

Seyyedi F, Rafiean-Kopaei M, Miraj S. 2016. Comparison of the effects of vaginal royal jelly and vaginal estrogen on quality of life, sexual and urinary function in postmenopausal women. Journal of Clinical and Diagnostic Research, 10(5): QC01-QC05

Simuth J. 2001. Some properties of the main protein of honey bee (Apis mellifera) royal jelly. Apidologie, 32, 69–80.

Stocker A, Schramel P, Kettrup A, Bengsch E. 2005. Trace and mineral elements in royal jelly and homeostatic effects. Journal of Trace Elements in Medicine and Biology, 19 (23): 183–189.

Xin XX, Chen Y, Chen D, Xiao F, Parnell LD, Zhao J. Shen LR. 2016. Supplementation with major royal-jelly proteins increases lifespan, feeding, and fecundity in drosophila. Journal of Agricultural and Food Chemistry, 64(29), 5803-5812.

Wang X, Cook LF, Grasso LM, Cao M, Dong Y. 2015. Royal jelly-mediated prolongevity and stress resistance in caenorhabditis elegans is possibly modulated by the interplays of DAF-16, SIR-2.1, HCF-1, and 14-3-3 proteins. Journals Gerontology Series A: Biological Sciences and Medical Sciences, 70 (7): 827-838.

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.

Zheng HQ, Hu FL, Dietemann, V. 2011. Changes in composition of royal jelly harvested at different times: consequences for quality standards. Apidologie, 42:39–47.