DOI: 10.29050/harranziraat.1016909
Harran Tarım ve Gıda Bilimleri Derg. 2022, 26(1): 109-117

Research Article/Araştırma Makalesi

Determination of total protein, trans- 10-Hydroxy-2-Decenoic Acid (10-HDA) and major royal jelly proteins in royal jelly produced at different harvest times in queenless and queenright colonies

Farklı hasat zamanlarında ana arısız ve ana arılı kolonilerde üretilen arı sütlerinde toplam protein, trans 10-HDA ve arı sütü majör proteinlerinin tayini

Aytul UCAK KOC[1*], Mete KARACAOGLU[2], Zehra Burcu BAKIR[3], Kadir KIZILKAYA[4]

[1,2,4]Aydin Adnan Menderes University, Faculty of Agriculture, Department of Animal Science, Aydin/Türkiye
[3]Aydin Adnan Menderes University, Faculty of Agriculture, Department of Agricultural Biotechnology, Aydin/Türkiye

[1]https://orcid.org/0000-0001-5969-1609; [2]https://orcid.org/0000-0002-1152-0808;
[3]https://orcid.org/0000-0002-9241-0749; [4]https://orcid.org/0000-0003-2708-6636

To cite this article:
Koc Ucak, A., Karacaoglu, M., Bakır, Z.B. & Kızılkaya, K. (2022). Determination of total protein, trans- 10-Hydroxy-2-Decenoic Acid (10-HDA) and major royal jelly proteins in royal jelly produced at different harvest times in queenless and queenright colonies. Harran Tarım ve Gıda Bilimleri Dergisi, 26(1): 109-116.
DOI:10.29050/harranziraat.1016909

***Address for Correspondence:**
Aytul Ucak KOC
e-mail:
aucak@adu.edu.tr

Received Date:
01.11.2021
Accepted Date:
20.12.2021

ABSTRACT

Two of the most important ingredients to add functional properties to royal jelly are 10-HDA and major royal jelly proteins (MRJPs). The effect of royal jelly (RJ) production and the effect of royal jelly harvest time (24, 48 and 72 hours) on 10-HDA, the total protein content of RJ, and molecular weights of major proteins in RJ were investigated in queenright and queenless colonies. RJ production colonies were divided into 2 groups as queenless and queenright where the queen was confined with frames. Subgroups were formed within each group (queenless and queenright) according to harvest time (24, 48 and 72 hours). 80 larvae were transferred to each colony. In this study, as the harvest time increased, total protein (TP) ratio decreased ($p<0.05$); TP ratio in RJs harvested at 24, 48 and 72 hours was determined as 18.4±1.24%, 15.2±0.80%, 10.6±0.27%, respectively. As the harvest time increased, 10-HDA decreased. It was determined 10-HDA rates in RJ harvested at 24, 48 and 72 hours respectively; 2.52±0.109%, 2.20±0.110%, 2.00±0.112%. MRJP1, MRJP2, MRJP3 and MRJP5 were found from the MRJP family, and their molecular weights were determined as 53 kDa, 46.5 kDa, 66.8 kDa, 80.9 kDa, respectively. As a result, the early harvested (24 and 48 hours) RJ had higher values in terms of TP and trans 10-HDA than the royal jelly harvested at 72 hours.

Key Words: Honey bee, Early harvest royal jelly, Aegean Ecotype of Anatolian bee, MRJP1, Functional food

ÖZ

Arı sütüne (AS) fonksiyonel özellik katan en önemli içeriklerinden ikisi 10-HDA ve majör arı sütü proteinleridir. Bu çalışmada, hasat zamanı (24, 48 ve 72 saat) ve AS üretim kolonilerinin ana arılı ve ana arısız olmasının arı sütünün 10-HDA, toplam protein (TP) içeriğine etkisi belirlenmiş ve majör arı sütü proteinlerinin (MASP) molekül ağırlıkları saptanmıştır. Bu amaçla AS üretim kolonileri 2 gruba ayrılmış, birinci grup ana arısız, ikinci grup ise ana arılı grubu oluşturmuştur. Birinci grubun ana arıları kovandan alındığı gün, diğer grubunun ana arıları da 2 çerçeve ile birlikte plastik sınırlandırma kafeslerine yerleştirilmiştir. Her bir koloniye 80 adet larva transfer edilmiştir. Her grup içinde (anasız ve ve ana arılı) 2'şer koloni 24, 48 ve 72 saat hasat zamanı alt gruplarını oluşturmuştur. Bu çalışmada hasat zamanı uzadıkça TP oranı azalmış ($P<0.05$); 24, 48 ve 72 saatte hasat edilen arı sütlerinde TP oranı sırasıyla; %18.4±1.24, 15.2±0.80, 10.6±0.27 olarak belirlenmiştir. 10-HDA üzerine hasat zamanın etkisi önemli ($P<0.05$) bulunmuş, hasat zamanı uzadıkça 10-HDA azalmış, 24, 48 ve 72 saatte hasat edilen sütlerde 10-HDA sırasıyla; %2.52±0.109, %2.20±0.110, %2.00±0.112 saptanmış ve 24 saatte hasat edilen arı sütü, 48 ve 72 saatte hasat edilen sütlerden 10-HAD bakımından farklı bulunmuştur ($P<0.05$). Bu çalışmada MASP ailesinden MASP1, MASP2,

MASP3 ve MASP5 tespit edilmiş ve aynı sırayla molekül ağırlıkları; 53 kDa, 46.5 kDa, 66.8 kDa, 80.9 kDa olarak belirlenmiştir. Sonuçta erken hasat (24 ve 48 saat) arı sütleri 72 saatte hasat edilen arı sütlerinden TP ve 10-HDA bakımından daha yüksek değerlere sahip olmuştur.

Anahtar Kelimeler: Bal arısı, Erken hasat arı sütü, Anadolu arısı Ege ekotipi, MASP1, fonksiyonel gıda

Introduction

Royal jelly (RJ) is a caste-determining food that plays an important role in the transformation of worker bee larvae into queens, secreted from the mandible and hypopharyngeal glands of worker bees aged 5-15 days. RJ consumption by young larvae affects DNA methylation process (Kucharski et al., 2008) and this results in the development of gyne morphology (Zheng et al., 2011). For this reason, it is defined as a "superfood" today (Knecht and Kaatz, 1990; Li et al., 2010). It is used for feeding the young larval stage of worker and drone bees during the whole life of the queen bee in the colony.

RJ is cream-colored, sticky, and sour in taste. In the structure of RJ; water (60-70%), protein (12-15%), fatty acids and lipids (3-8%), carbohydrates (7-18%), ash (0.8-3%), small amounts of vitamins (group B complex, vitamin C, vitamin E), minerals (Fe, Na, Ca, K, Zn, Mg, Mn and Cu), enzymes, hormones, polyphenols, nucleotides and minor heterocyclic compounds (Boselli et al., 2003; Sabatini et al., 2009; Isidorov et al., 2012; Melliou and Chinou, 2014; Xue et al., 2017) .

RJ, which is among the functional foods, gets this feature mostly from trans 10-HDA, which is the major fatty acid, and major royal jelly proteins. Fatty acids make up 80-85% of the lipid composition of RJ, and trans 10-HDA constitutes the largest part of the fatty acid fraction with 32% (Lercker et al., 1981; Terada et al., 2011). Trans 10-HDA displays various biological and pharmacological activities (Sugiyama et al., 2012; Li et al., 2013; Chen et al., 2016) and is used as a marker of RJ quality and authenticity (Sabatini et al., 2009). Studies have shown that trans 10-HDA has antimicrobial activity against various gram-positive and gram-negative bacteria (mostly human pathogens) (Blum et al., 1959; Yatsunami and Echigo, 1985; Garcia et al., 2013), it has been reported that it is a potent anti-inflammatory (Chen et al., 2018), alleviates lipopolysaccharide (LPS)-induced neuroinflammation, and protects against LPS-induced blood-brain barrier (BBB) damage (You et al., 2019).

Major royal jelly proteins (MRJPs), also known as immune proteins, constitute 82-90% of RJ proteins, one of the important components of RJ (Schmitzova et al., 1998; Santos et al., 2005; Drapeau et al., 2006; Shinkhede and Tembhare, 2009; Mureşan and Buttstedt, 2019). Among the MRJPs, MASP1, which has been studied the most, has longevity, anti-tumor, anti-oxidant effect, immunomodulatory effects, and positive effects of MASP2 on anti-tumor and cell proliferation have been reported (Ramanathan et al., 2018).

The content of RJ is affected by the applications made in the production of RJ. Studies have revealed that the time of harvest, the age of the grafted larva, the number of queen cell, the season, and supplemental feeding affect the trans 10-HDA and protein content of RJ (Liu et al., 2008; Zheng et al., 2011; Kösoğlu et al., 2013; Erdoğan et al., 2017; Ucak Koc et al., 2021a).

In commercial RJ production, RJ is harvested within 72 hours because the amount of RJ in the queen cell cups peaks at this time (Lercker et al., 1985). In recent years, some producers in China produce RJ harvested two days after grafting (48 hours) or one day (24 hours) harvested. Early harvest shortens the production cycle, and it is suggested that early harvest RJ is fresher because they wait less at the hive temperature (35 ^{0}C) (Liu et al., 2008; Zheng et al., 2011; Kösoğlu et al., 2013).

Unlike other studies, in this study, RJ was produced in queenless colonies and queen-restricted colonies instead of the starter-finisher colonies commonly used in commercial RJ production. The effect of harvesting time (24, 48 and 72 hours) and royal jelly production colonies

(queenright and queensless) on 10-HDA and total proteins were investigated, and the molecular weights of major royal jelly proteins were determined.

Materials and methods

This study was carried out at Aydin Adnan Menderes University, Faculty of Agriculture, "Honey Bee and Silkworm Research and Application Unit". A total of 12 Anatolian bee Aegean Ecotype colonies were divided into 2 groups, the queens of 6 colonies of the first group (queenless) were removed. That is, these colonies were left without a queen. The queens of the colonies forming the second group (queenright) were found and confined to the confinement cage with 2 frames. Two days after this procedure (the day of the larva transfer), all colonies were arranged to be 12 frames of adult bees (approximately 2.5 kg), equalized in terms of honey and pollen stocks, young brood frames were removed, and queen cells were degraded. By reducing the number of frames of the hive to 7 frames, the bees were provided to form clusters on and under the frame. During the experiment, colonies were fed with sugar syrup. One-day old 80 larvae were transferred to queen cell cups made of beeswax to each colony. The larvae transfer was carried out in a room with 60-70 % humidity at a temperature of 25-30 °C. During the experiment, a total of 7360 larvae were transferred for both groups. In the study, 24, 48 and 72-hours RJ production was carried out in a rotation in the colonies in both groups, respectively. RJ production was carried out between 14 April-1 May 2021, and all analyzes were carried out in July 2021.

Chemical analysis

All samples (78 pieces) were kept at -18 ^{0}C and protected from light until analysis about 2 months). The 10-HDA determination was done as previously done by Ucak Koc et al. (2021a). HPLC Agilent 1260 Infinity series (UV-DAD) Luna C18 (150 mm x 4.6 mm x 5 mm) column was used [Mobile phase: Methanol: Water: Phosphoric Acid (55: 45: 5), flow rate 1 mL/min, column temperature 30^{0}C, injection amount 20 mL, analysis time 15 minutes, DAD detector 215 nm]. By weighing 0.01 g of 10-HDA analytical standards to dissolve 50 mL (final density 200 mg/mL) in water: methanol (50:50) and diluting from this solution to 5, 10, 20, 50, 100, 200 (ppm) mg/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 mL were injected into HPLC (Caparica et al., 2007; Kim & Lee, 2010).

Bradford's method was used to measure total protein concentration in RJ (Bradford, 1976) using bovine serum albumin as the standard. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (10 %), was used to check the molecular weight of the major royal jelly proteins by the method of Laemmli (1970). Protein staining was carried out using Coomassie brilliant blue G-250.

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. The statistical model is given below.

$$yijkl = \mu + ai + bj + (ab)ij + eijk$$

μ = mean
ai = group (i = queenless and queenright)
bj = harvest time (j = 24, 48 and 72 hours)
$(ab)ij$ = group $*$ harvest time interaction effect
$eijk$ = error

Results and Discussion

In this study, the effect of groups (queenless and queenright) and harvest time on RJ yield is significant ($p<0.01$). As the RJ harvest time increased (24, 48 and 72 h), RJ yield values

decreased (3.67 g, 12 g and 17.5 g). While 12.9±0.65 g RJ was obtained in the group queenless, it was determined as 9.3±0.67 g in the queenright group. The difference between the two groups is significant (p< 0.05) (Table 1).

Table 1. Royal jelly yield (g) according to royal jelly harvest times (h) and groups

Groups	Time of harvest (after the larval transfer)			Overall mean
	24 h	48 h	72 h	
Queenless	4.3±1.27	14.0±1.07	20.3±1.00	12.9±0.65[a]
Queenright	3.0±1.27	10.0±1.00	14.7±1.60	9.3±0.67[b]
Overall mean	3.67±0.515[a]	12.0 ±0.762[b]	17.5±1.092[c]	

a,b,c; p<0.05

In Table 2, 10-HDA ratios in queenless and queenright colonies are given according to harvest time. As the harvest time increased, 10-HDA decreased, RJ harvested in 24 hours and RJ harvested in 72 hours were different and significant (p<0.01).

Table 2. Trans-10-hydroxy-2-decenoic acid rates (%) according to royal jelly harvest times (h) and group

Groups	Time of harvest (after the larval transfer)			Overall mean
	24 h	48 h	72 h	
Queenless	3.16±0.186	2.72±0.157	2.27±0.152	2.71±0.095
Queenright	3.12±0.170	2.62±0.152	2.25±0.170	2.66±0.094
Overall mean	3.14±0.126[A]	2.67 ±0.109[AB]	2.26 ±0.114[B]	

A, B; P<0.01

In similar studies, 10-HDA ratios in RJ harvested in 24, 48 and 72 hours, in the same order; 1.97±0.07%, 2.05±0.04%, 1.60±0.04% (Liu et al., 2008) Zheng et al. (2011) reported it as 2.5% ± 0.4%, 2.0 ± 0.3%, 2.1 ± 0.2%. The 10-HDA ratios obtained in this study are higher than the values found by (Liu et al., 2008) Zheng et al. (2011) and lower than Ucak Koc et al. (2021a) and Kösoğlu et al. (2013).

In this study, 10-HDA was determined as 2.71% in colonies queenless and 2.66% in queenright colonies, and the difference was found to be statistically insignificant. Ucak Koc et al. (2021b) found 10-HDA ratios in RJ produced in queenless and queenright colonies to be similar to each other.

Table 3 shows total protein (TP) rates according to harvest times and groups. The highest TP content was obtained in RJ harvested 24 hours at queenright group (18.7±1.28%). When the TP content of RJ was examined in terms of harvest time (Table 3), the RJ harvested at 24 h, 48 h and 72 h were different and important than each other (p <0.05).

In some studies, the TP amount of RJ decreased as the harvest time extended. These results are compatible with the literature (Liu et al., 2008; Zheng et al., 2011; Ucak Koc et al., 2021a). Liu et al. (2008), the TP ratios in RJ harvested at 24, 48 and 72 hours at harvest, respectively; 16.5±0.2%, 10.1±0.3%, 9.8±0.2%; Zheng et al. (2011) reported crude protein ratios as 19.6 ± 1.4%, 16.2% ± 1.5, 15.0 ± 1.0% in RJ harvested at 24, 48 and 72 hours according to the harvest time, Al-Kahtani and Taha (2020) reported crude protein ratios (crude protein 17.71 ± 0.08 18.75 ± 0.13 19.58 ± 0.08) in RJ harvested at 24, 48 and 72 hours.

Table 3. Total protein rates (%) according to royal jelly harvest times (h) and groups

Groups	Time of harvest (after the larval transfer)			Overall mean
	24 h	48 h	72 h	
Queenless	18.1±1.28	15.8±1.08	11.0±1.02	14.9±0.65
Queenright	18.7±1.28	14.7±1.01	10.3±1.17	14.6±0.67
Overall mean	18.4±1.24[a]	15.2±0.80[b]	10.6±0.27[c]	

a, b, c; p<0.05

A discriminant analysis was conducted to predict harvest time of a sample by using 10-HDA and total protein as predictor variables and significant differences among harvest times were observed for the predictor variable of total protein ($p<0.001$) but not 10-HDA ($p>0.280$). The discriminate function revealed a significant association among groups and all predictors, accounting for 37.1 % of among group variability, although closer analysis of the structure matrix revealed only one significant predictor, namely total protein (0.996). The classification results from discriminant analysis indicated that 35%, 31% and 88.9% of harvest time 24 h, 48 h and 72 h were correctly classified, respectively. However, 27.6% and 41.4% of harvest time 48 h were classified as harvest time 24 h and 72 h. Classification results and Figure 1 revealed that harvest time 48 h is not discriminated from harvest time 24 h and 72 h and is located between harvest time 24 h and 72 h.

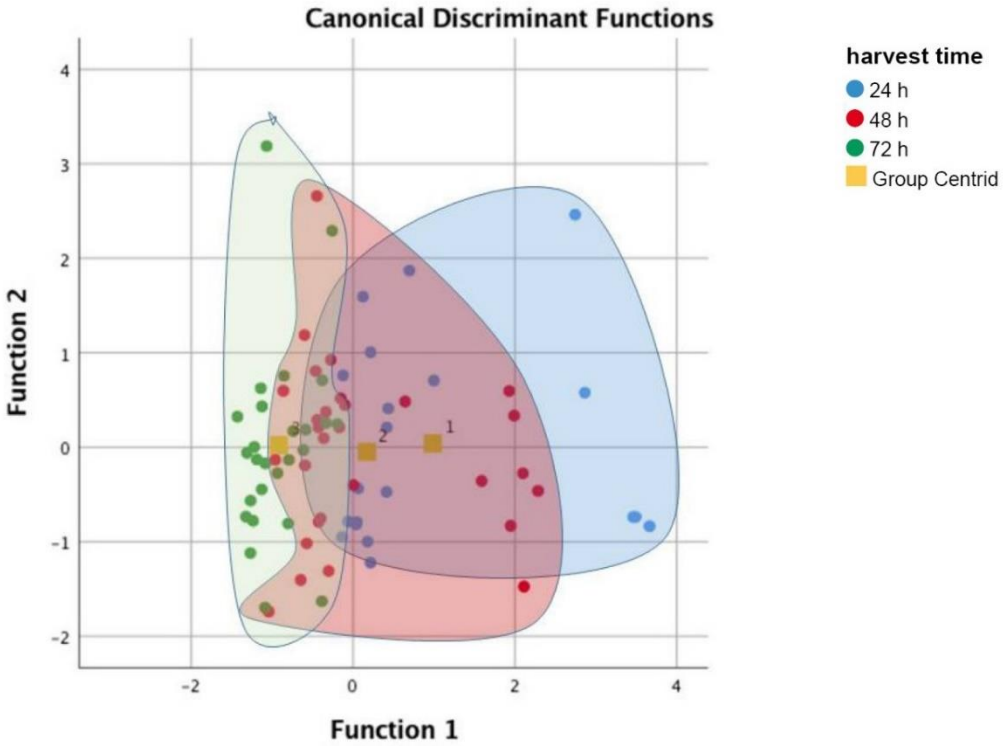

Figure 1. Discriminant analysis of the RJ sampled 24, 48 and 72 h after grafting

In this study, molecular weight determination of MRJPs was made in 3 RJ samples selected randomly from the queenless group according to the harvest time (24, 48, 72 hours) (Table 4). Molecular weights of MRJPs are very similar according to harvest time (Table 4 and Figure 2).

Table 4. Molecular weights of Major Royal Jelly Proteins determined by SDS-PAGE

Lane A Marker (kDa)	Lane B (24h)	Lane C (48h)	Lane D (72h)
250	83.2	83.2	84.2
150	72.1	73.5	73.5
100	56.1	56.9	57.4
75	51.1	52.9	52.9
50			
37			
25			
20			
15			

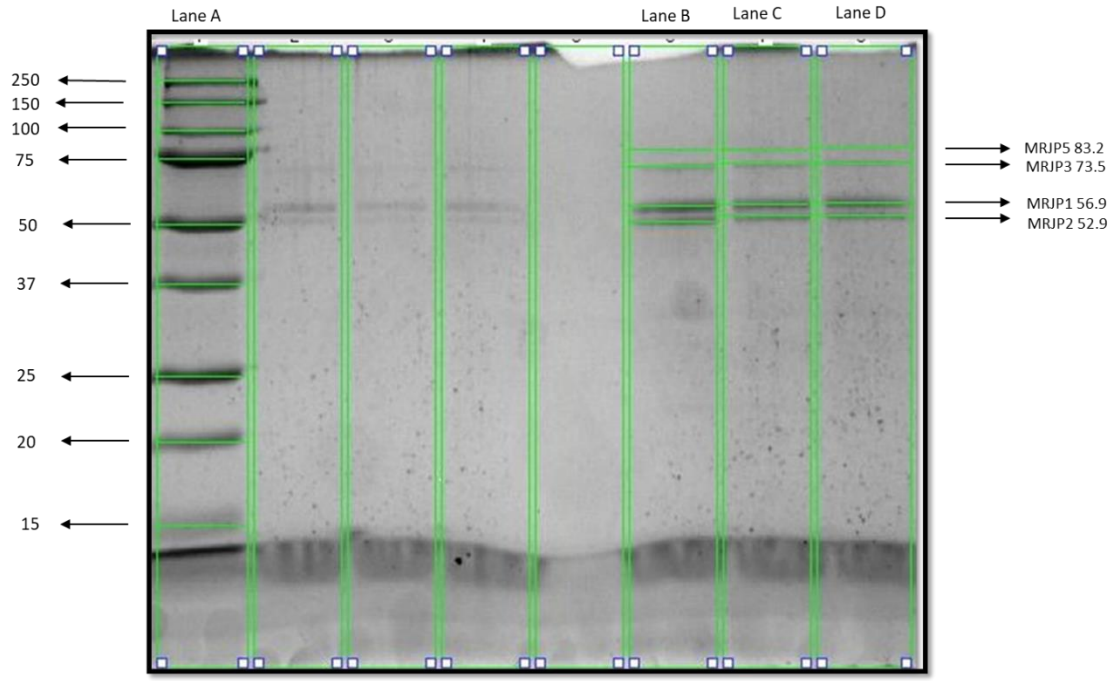

Figure 2. Molecular weights of major royal jelly proteins

In this study, the molecular weight (56.9 kDa) determined for MRJP1 was similar to the values (between 55-57 kDa) detected by Malecova et al. (2003) and Peixoto et al. (2009). Different molecular weights have been determined for MRJP1 in different studies. For example, studies using the HPLC method have determined the molecular size of the MRJP1 oligomer to be 280 kDa (Kamakura, 2001; Ramadan and Al-Ghamdi, 2012), 350 (Simuth, 2001), or 420 kDa (Tamura et al., 2009). According to simple PAGE analysis, the MRJP1 oligomer was reported to be split into two small proteins, 55 kDa (MRJP1 monomer) and 5 kDa (apisimin) (Mandacaru et al., 2017). In another study, Bilikova et al. (2002) reported that apalbumin (MRJP1) is a 420 kDa protein that combines with an apisimin oligomer (5.5 kDa) to form a stable MRJP1 protein complex of approximately 450 kDa (Kamakura et al., 2001; Kimura et al., 2003; Furusawa et al., 2016).

In this study, while the molecular weight of MRJP2 (51.1-52.9 kDa) was similar to Santos et al., (2005) (between 50.6 and 59.9 kDa), Imjongjirak et al. (2005) and Schmitzova et al. (1998) determined for MRJP2 is different from the molecular weight (72 kDa).

It was determined that the molecular weight for MRJP3 (72.1-73.5 kDa) is lower than that of Santos et al., (2005) (80.6 Da-87.0 Da) for MRJP3. The molecular weight determined for MRJP5 in this study is 83.2-84.2 kDa, which is consistent with the value determined by Santos et al. (2005).

The variation in MRJP molecular weights is high in the studies. Practices in RJ production and other environmental and genetic factors have shown that the amount of protein changes (Imjongjirak et al., 2005; Tamura et al., 2009). For this reason, the first studies on the MRJP family started in the 1990s, with some techniques developed depending on technology, studies on this subject have increased in recent years, but studies on this subject are not sufficient.

Conclusion

Proteins constitute more than 50% of the dry weight of RJ and are the most abundant components. MRJPs constitute 80-90% of the total protein content in RJ (Furusawa et al., 2008; Buttstedt et al., 2014). According to the information obtained to date, one of the most important components that add functionality to RJ is MRJP and the other is 10-HDA. This study showed that RJ, which is a valuable bee product, is richer in total protein when harvested early (24-48 hours).

In addition, RJ production in queenless and queenright colonies can be an alternative to the

starter-finisher system used in businesses producing commercial RJ. In small scale family farm, in the apiary, the queen bee of the colonies with old or unproductive queens can be killed and RJ can be produced in these colonies. In this type of colonies, RJ can be produced for 15 days with the supplement of some adult bees and sealed (almost adult) brood workers. With a few queen cells to be left at the end of the 15th day, the queens of the colonies are renewed.

On the other hand, queen confinement cage can be used in colonies with young and fertile queens in the apiary. When the queen is taken to the confinement cage, if a raised frame is provided for the queen to lay eggs, it will be easy to obtain larvae at a suitable age for larva transfer. Finally, when both colony productivity and protein content are evaluated together, it can be recommended to harvest RJ within 48 hours. Nevertheless, in our country where the amount of RJ production is still insufficient, this recommendation may be premature in terms of timing. Because, first and foremost, it is necessary to increase RJ production, and for this, more beekeepers should be encouraged to produce RJ (Özbakır Özmen et al., 2016). RJ production is a demanding job that requires team, organization, discipline, and colony management knowledge and skills. For this reason, most of the beekeepers do not dare to produce RJ. However, with the implementation of the above suggestions, it can provide beekeepers with the skill to produce RJ in smaller quantities, even if it is not at the size of a commercial enterprise.

Acknowledgements

We thank the The Scientific and Technological Research Council of Turkey, (Tubitak-219O413) for its financial support and thank TARBIYOMER for its laboratory service support.

Conflict of Interest: The article authors declare that there is no conflict of interest between them.

Author Contribution: The authors contributed equally to the study.

References

Al-Kahtani, S. & Taha, El-K.A. (2020). Effect of harvest time on royal jelly yield and chemical composition. *Journal of the Kansas Entomological Society*, 93(2):132-139.

Bilikova, K., Hanes, J., Nordhoff, E., Saenger, W., Klaundny, J., Simuth, J. (2002). Apisimin, a new serine-valine-rich peptide from honeybee (Apis mellifera L.) royal jelly: purification and molecular characterization. *FEBS Letters*, 528:125-129.

Blum, M.S., Novak, A.F. & Taber, S. (1959). 10-hydroxy-Δ 2 -decenoic acid, an antibiotic found in royal jelly. *Science*, 130: 452-453.

Boselli, E., Caboni, M.F., Sabatini, A.G., Marcazzan, G.L., Lercker, G. (2003). Determination and changes of free amino acids in royal jelly during storage. *Apidologie* 34 :129–137.

Bradford, M.M. (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.

Buttstedt, A., Moritz, R.F. & Erler, S. (2014). Origin and function of the major royal jelly proteins of the honeybee (*Apis mellifera*) as members of the yellow gene family. *Biological Reviews*, 89:255-269.

Caparica, C., Marcucci S. & Marcucci, M.C. (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.

Chen, Y.F., Wang, K., Zhang, Y.Z., Zheng, Y.F. & Hu, F.L. (2016). In vitro anti-inflammatory effects of three fatty acids from royal jelly. Mediators Inflamm. 2, 2016.

Chen., Y.F, You, M.M., Liua, Y.C., Shia, Y.Z., Wang, K., Lua, Y.Y. & Hua, F.L. (2018). Potential protective effect ofTrans-10-hydroxy-2-decenoic acid on theinflammation induced by Lipoteichoic acid. *Journal of Functional Foods*, 45: 491-498.

Drapeau, M.D., Albert, S., Kucharski, R., Prusko, C., Maleszka, R. (2006). Evolution of the Yellow/Major Royal Jelly Protein family and the emergence of social behavior in honey bees. *Genome Research*, 16:1385-1394.

Erdoğan, A., Uçak Koç, A., Karacaoğlu, M. (2017). Anadolu arısı Ege ekotipi (*Apis mellifera anatoliaca*) ve İtalyan (*Apis mellifera ligustica*) X Ege melezi bal arılarının ve farklı yüksük sayılarının arı sütü verimleri üzerine etkileri. *Harran Tarım ve Gıda Bilimleri Dergisi*, 21(1): 91-98.

Furusawa, T., Rakwal, R., Nam, H.W., Shibato, J., Agrawal, G.K., Kim, Y.S., Ogawa, Y., Yoshida, Y., Kouzuma, Y. & Masuo, Y. (2008). Comprehensive royal jelly (RJ) proteomics using one-and two-dimensional proteomics platforms reveals novel RJ proteins and potential phospho/glycoproteins. *J. Proteome Res.,* 7, 3194-3229.

Furusawa, T., Arai, Y., Kato, K. & Ichihara, K. (2016). Quantitative Analysis of Apisin, a Major Protein Unique to Royal Jelly, Hindawi Publishing Corporation. *Evidence-Based Complementary and Alternative Medicine*, 2016:1-9.

Garcia, M.C., Finola, M.S. & Marioli, J.M. (2013). Bioassay direct identification of royal jelly's active compounds against the growth of bacteria capable of infecting

cutaneous wounds. *Adv. Microbiol*., 3: 138–144.

Imjongjirak, C., Klinbunga, S. & Sittipraneed, S. (2005). Cloning, expression and genomic organization of genes encoding major royal jelly protein 1 and 2 of the honey bee (Apis cerana). *BMB Reports*, 38: 49-57.

Isidorov, V.A., Bakier, S. & Grzech, I. (2012). Gas chromatographicmass spectrometric investigation of volatile and extractable compounds of crude royal jelly. Journal of Chromatography B, (885–886): 109–116.

Kamakura, M., Suenobu, N. & Fukushima, M. (2001). Fifty-seven-kDa protein in royal jelly enhances proliferation of primary cultured rat hepatocytes and increases albumin production in the absence of serum. *Biochemical and Biophysical Research Communications*, 282(4):865-874.

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.

Kimura, M., Kimura, Y., Tsumura, K., Okihara, K., Sugimoto, H., Yamada, H., Yonekura, M. (2003). 350-kDa royal jelly glycoprotein (apisin), which stimulates proliferation of human monocytes, bears the β1-3galactosylated N-glycan: Analysis of the N-gly-cosylation site. *Bioscience, Biotechnology and Biochemistry*, 67: 2055-2058.

Knecht, D. & Kaatz, H. (1990). Patterns of larval food production by hypopharyngeal glands in adult worker honey bees. *Apidologie*, 21: 457-468.

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.

Kucharski, R., Maleszka J. & Maleszka R. (2008) Nutritional control of reproductive status in honeybees via DNA methylation, *Science,* 319:1827-1830.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.

Lercker, G., Capella, P., Conte, L.S., Ruini, F. & Giordani, G. (1981). Components of royal jelly: I. Identification of the organic acids. *Lipids*, 16: 912–919.

Lercker, G., Caboni M., Vecchi M., Sabatini A., Nanetti A., Piana L. (1985) Composizione della frazione glucidica della gelatina reale e della gelatina delle api operaie in relazione all'età larvale, *Apicoltura*, 8, 27–37.

Li, J.K., Feng, M., Begna, D., Fang, Y. & Zheng, A.J. (2010). Proteome comparison of hypopharyngeal gland development between Italian and royal jelly-producing worker honeybees (*Apis mellifera* L). *J. Proteome Res*., 9: 6578-6594.

Li, X., Huang, C. & Xue, Y. (2013). Contribution of lipids in honeybee (Apis mellifera) royal jelly to health. *J. Med. Food,* 16, 96-102.

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

Malecova, B., Ramser, J., O'Brien, J.K., Janitz, M., Judova, J., Lehrach, H. & Simuth, J. (2003). Honey bee (Apis mellifera L.) mrjp gene family: Computational analysis of putative promoters and genomic structure of mrjp1, the gene coding for the most abundant protein of larval food. *Gene* 303: 165-175.

Mandacaru, S.C., do Vole, L.H.F., Vahidi, S., Xiao, Y., Skinner, O.S., Ricart, C.A.O., Kelleher, N.L., de Souso, M.V. & Konermann, L. (2017). Characterizing the structure and oligomerization of major royal jelly protein 1 (MRJP1) by mass spectrometry and complementary biophysical tools. *Biochemistry*, 56: 1645-1655.

Melliou, E. & Chinou, I. (2014). Chemistry and bioactivities of royal jelly. *In Studies in Natural Products Chemistry*, 43: 261–290.

Mureşan, C.I. &Buttstedt, A. (2019). pH-dependent stability of honey bee (Apis mellifera) major royal jelly proteins. *Nature*, Scientific Reports 9:9014.

Özbakır Özmen, G., Doğan, Z., Öztokmak, A. (2016). Adıyaman İli Arıcılık Faaliyetlerinin İncelenmesi. *Harran Tarım ve Gıda Bilimleri Dergisi* (2016) 20(2): 119-126.

Peixoto, L., G, Calabria, L.K., Garcia, L., Capparelli, F.E., Goulart, L.R., de Sousa MV, & Espindola, F.S. (2009). Identification of major royal jelly proteins in the brain of the honey bee Apis mellifera. *Journal of Insect Physiology*, 55: 671-677.

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

Ramanathan, A.N.K.G., Nair A.J., Sagunan, V.S. (2018). A review on Royal Jelly proteins and peptides. *Journal of Functional Foods,* 44: 255-264.

Sabatini, A.G., Marcazzan, G.L., Caboni, M.F., Bogdanov, S. & Almeida-Muradian, L.B. (2009). Quality and standardisation of royal jelly. *Journal of ApiProduction ApiMedical Science*, 1, 1–6.

Santos, K.S., dos Santos, L.D., Mendes, M.A., de Souza, B.M., Malaspina, O. & Palma, M.S. (2005). Profiling the proteome complement of the secretion from hypopharyngeal gland of Africanized nurse-honey bees (Apis mellifera L.). *Insect Biochemistry and Molecular Biology,* 35: 85-91.

SAS. 1999. Statistical Analsis System for Windows (Relase 8.2). SAS Institute Inc.Raleigh, Caroline, USA.

Schmitzova, J., Klaudiny, J., Albert, S., Schroder, W., Schreckengost, W., Hanes, J., Judova, J. & Simuth, J. (1998). A family of major royal jelly proteins of the honeybee Apis mellifera L. *Cellular and Molecular Life Sciences*, 54: 1020-1030.

Shinkhede, M.M. & Tembhare, D.B. (2009). Royal jelly protein and lipid composition in Apis cerana indica F. *International Journal of Industrial Entomology*, 18: 139-142.

Simuth, J. (2001). Some properties of the main protein of honeybee (Apis mellifera) royal jelly. *Apidologie*, 32: 69-80.

Sugiyama, T., Takahashi, K. & Mori, H. (2012). Royal jelly acid, 10-hydroxy-trans-2-decenoic acid, as a modulator of the innate immune responses. *Endocr. Metab. Immune Disord. Drug Targets*, 12: 368–376.

Tamura, S., Amano, S., Kono, T., Kondoh, J., Yamaguchi, K., Kobayashi, S., Ayabe, T., & Moriyama, T. (2009). Molecular characteristics and physiological functions of major royal jelly protein 1 oligomer. *Proteomics*, 9:

5534-5543.

Terada, Y., Narukawa, M., & Watanabe, T. (2011). Specific Hydroxy Fatty Acids in Royal Jelly Activate TRPA1. |J. Agric. Food Chem., 59:2627–2635.

Ucak Koc, A. Karacaoğlu, M., Uygun, M., Bakır, Z.B. & Keser, B. (2021a). Effect of harvesting time and the number of queen cell cups on royal jelly composition. *Journal of Apicultural Research* (underpress).

https://doi.org/10.1080/00218839.2021.1930956

Ucak Koc, A. Karacaoğlu, M., Bakır, Z.B. & Keser, B. (2021b). 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? *Turkish Journal of Agriculture - Food Science and Technology*, 9(8): 1443-1447.

Xue, X., Wu, L. & Wang, K. (2017). Bee Products-Chemical and Biological Properties; Alvarez-Suarez, J.M., Ed.; Springer: Berlin/Heidelberg, Germany, pp. 181–190.

Yatsunami, K. & Echigo, T. (1985). Antibacterial action of royal jelly. Bull. Fac. Agr. Tamagawa Univ., 25, 13–22.

You, M., Pan, Y., Liu, Y., Chen, Y. W., Si, J., Wang, K. & Hu, F. (2019). Royal jelly alleviates cognitive deficits and β-Amyloid accumulation in APP/PS1 mouse model via activation of the cAMP/PKA/CREB/BDNF pathway and inhibition of neuronal apoptosis. *Frontiers in Aging Neuroscience,* 10(428):1-12.

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