



*Royal Jelly: Proteins and Peptides*  
*Arı Sütü: Proteinleri ve Peptidleri*

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Received/Geliş Tarihi: 24/01/2020, Accepted/ Kabul Tarihi: 30/03/2020

doi: 10.35206/jan.679534

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e-ISSN: 2667-4734

## Abstract

Royal jelly is secreted by the hypopharyngeal and mandibular glands of 5-15 days worker honeybees (*Apis mellifera*). Royal jelly is a thick and milky bee product with high nutritional value. Besides the nutritional functions on queen and worker larvae, it also has a very important role on the specific functions of queen bees. Due to its important biological properties, royal jelly has been used in the pharmaceutical, food and cosmetics industry especially for the last 50-60 years as a functional and nutraceutical food. The studies have shown that royal jelly has antioxidant, antidiabetic, antitumor, neurotrophic, antimicrobial, anti-inflammatory, hypotensive, hepatoprotective, antihypercholesterolemic, hypoglycaemic properties and effects on the reproductive system and fertility. These activities are attributed to the bioactive components it contains such as major royal jelly proteins (MRJPs), jelleins and royalisin peptides and 10-hydroxy-2-decenoic acid (10-HDA). Especially MRJPs are considered as an important factor in the development of queen bees. The aim of this study is to summarize and update physicochemical and bioactive properties of royal jelly, as well as characterization and functions of royal jelly proteins (RJPs) and peptides.

**Keywords:** Royal Jelly, Bioactive Properties, Major Royal Jelly Proteins, Peptides, Characterization, Apitherapy

**Abbreviations:** MRJPs, Major Royal Jelly Proteins; Royal Jelly Proteins (RJPs); 10-Hydroxy-2-Decenoic acid (10-HDA)

## Özet

Arı sütü 5-15 günlük genç işçi arıların üst çene (mandibular) ve boğaz bezlerinden (hipofaringeal) salgılanmaktadır. Arı sütü yüksek besin değeri ile yoğun ve süt kıvamında bir arı ürünüdür. Kraliçe ve işçi larvaları üzerindeki beslenme fonksiyonlarının yanı sıra, kraliçe arıların özel fonksiyonları üzerinde de çok önemli rolü vardır. Özellikle son 50-60 yıldır önemli biyolojik özellikleri nedeniyle arı sütü fonksiyonel ve nutrasötik bir gıda olarak ilaç, gıda ve kozmetik endüstrisinde kullanılmaktadır. Çalışmalar, arı sütünün antioksidan, antidiyabetik, antitümör, nörotrofik, antimikrobiyal, antienflamatuar, hipotansif, hepatoprotektif, antihiperkolesterolemik, hipoglisemik özellikleri ve üreme sistemi ve doğurganlık üzerine etkileri olduğunu göstermektedir. Bu aktiviteler, arı sütünün içerdiği majör arı sütü proteinleri, jellein ve royalisin peptidleri ile 10-hidroksi-2-dekenoik asit (10-HDA) gibi biyoaktif bileşenlere atfedilir. Özellikle majör arı sütü proteinleri (MRJPs) bal kraliçe arıların gelişiminde önemli bir faktör olarak kabul edilir. Bu çalışmanın amacı, arı sütünün fizikokimyasal ve biyoaktif özellikleri ile arı sütü protein ve peptidlerinin karakterizasyon ve fonksiyonlarını özetlemek ve güncellemektir.

**Anahtar kelimeler:** Arı Sütü, Biyoaktif Özellikler, Majör Arı Sütü Proteinleri, Peptidler, Karakterizasyon, Apiterapi

## 1. INTRODUCTION

The importance of royal jelly from bee products was recognized in the 1600s and it was called "Royal Jelly" which means perfect food in English (Akyol & Baran, 2015). However, especially in the last fifty-sixty years its commercial production and consumption has increased. The royal jelly is secreted from the mandibular and hypopharyngeal glands of 5-15 days worker bees (*Apis mellifera* L.) (Balkanska, Zhelyazkova & Ignatova, 2012). This secretion is produced by the ingestion of pollen and nectar in the digestive organs of the worker bee. Royal jelly is not only nutrient for the growth of the honeybee larvae, but it is also necessary for the queen bees to feed and maintain its functions. In addition, royal jelly has great importance in the caste differentiation of these species. All larvae are fed with only royal jelly throughout the first 3 days, while larvae that will be queen are fed with only royal jelly in all larval and adult periods (Balkanska & Kashamov, 2011; Kolayli et al., 2016; Šimúth, 2001). Although having the same genetic structure, larvae fed by royal jelly continuously develop as queen bees; larvae that feed on royal jelly only in the first 3 days of the larval period and then a mixture of honey and pollen as worker bees (Akyol & Baran, 2015). Thereafter, a honeybee queen lives for 4 to 5 years, and a worker bee approximately 6 to 8 weeks (Moselhy Fawzy & Kamel, 2013).

Royal jelly is a dense and milky homogeneous substance with a density of 1.1 g / mL and partially soluble in water (Ramadan & Al-

Ghamdi, 2012). It is highly acidic having the pH 3.4–4.5 (Popesco, Marghitasl & Dezmiereand, 2008). It has a sharp phenolic smell and characteristic sour-bitter taste. Its colour is slightly beige and yellowish-whitish and darkens during storage (Shirzad, Kordyazdi, Shahinfard & Nikokar, 2013).

The water content of royal jelly is between 60-70% and the water activity (aw) is above 0.92. Addition, royal jelly consists of proteins (9–18 %), carbohydrates (10–16 %), fats (3–8 %), small amounts of mineral matter, polyphenols and vitamins (Sabatini, Marcazzan, Caboni, Bogdanov & Almeida-Muradian, 2009; Xue, Wu, & Wang, 2017). Table 1 shows the vitamin and mineral content of royal jelly (Benfenati, Sabatini & Nanetti, 1986; Maghsoudlou, Mahoonak, Mohebodini & Toldra, 2019) In addition, the chemical composition of royal jelly varies depending on a lot of factors: seasons and ecological conditions, race and caste of the honey bee, harvest time of royal jelly, methods of sampling and analysis used (Maghsoudlou et al., 2019; Ramanathan, Nair & Sugunan, 2018).

**Table 1.** Vitamin and mineral content of royal jelly

Minerals and Vitamins	Royal Jelly (mg/100 g)
Potassium	200-1000
Magnesium	20-100
Iron	1-11
Zink	0.7-8
Copper	0.33-1.6
B1 (Thiamin)	0.1-1.7
B2 (Riboflavin)	0.5-2.5
B3 (Niacin)	4.5-19
B5 (Pantothenic acid)	3.6-23
B6 (Pyridoxin)	0.2-5.5
H (Biotin)	0.15-0.55
Pantothenic Acid	0.2-0.25
Nicotinic Acid	0.4-0.48
Folic Acid	0.01-0.06
Inositol	0.1-0.12

Royal jelly is one of the most interesting food among functional and nutraceutical foods. The

studies have shown that royal jelly has antioxidant (Guo et al., 2008; Nagai, Sakai, Inoue, Inoue & Suzuki, 2001; Kolayli et al., 2016), antidiabetic (Dania et al., 2008; Maleki et al., 2019; Pourmoradian, Mahdavi, Mobasseri, Faramarzi & Mobasseri, 2014), antitumor (Kimura, 2008; Nakaya et al., 2007; Premratanachai & Chanchao, 2014), neurotrophic (Furakawa, 2008; Hattori, Nomoto, Fukumitsu, Mishima & Furukawa, 2007; Mannoor, Tsukamoto, Watanabe, Yamaguchi, K., & Sato, 2008), antimicrobial (Bărnăuțiu, Mărghițaș, Dezmirean, Mihai & Bobis, 2011; Bílikova, Huang, Lin, Šimůth & Peng, 2015; Coutinho, Karibasappa & Mehta, 2018), anti-inflammatory (Aslan & Aksoy, 2015; Karaca et al. 2012; Kohno et al., 2004), hypotensive (Nagai, Inoue, Suzuki & Nagashima., 2008; Pan et al., 2019; Takaki-Doi, Hashimoto, Yamamura & Kamei, 2009), hepatoprotective (Almeer et al., 2018; Chen, Fang & Wang, 2020; Kanbur et al., 2009; Ghanbari, Nejati & Azadbakht, 2015;), antihypercholesterolemic (Chiu et al., 2017; Guo et al., 2007; Kamakura, Moriyama & Sakaki, 2006), hypoglycaemic (Fujii et al., 1990), and anti-aging activities (Han et al., 2011; Qiu et al., 2019; Pasupuleti, Sammugam, Ramesh & Gan, 2017) and effects on the reproductive system and fertility (Eshtiyaghi, Deldar, Pirsaraei & Shohreh, 2016; Husein & Haddad, 2006; Yang et al., 2012). These activities are mainly attributed to the its bioactive components such as fatty acids, proteins and phenolic compounds (Ramadan & Al-Ghamdi, 2012). One of the major bioactive components is the major royal jelly proteins

(MRJP), which are considered to be an important factor in the development of the honey bee queen (Tamura et al., 2009).

## **2. ROYAL JELLY PROTEINS AND PEPTIDES**

Major Royal Jelly Proteins (MRJPs), also named as apalbumins, constitute 82–90% of royal jelly proteins. In the royal jelly protein (RJP) family, there are nine different members, MRJP1, MRJP2, MRJP3, MRJP4, MRJP5, MRJP6, MRJP7, MRJP8 and MRJP9, which are encoded by nine different genes (Buttstedt, Moritz & Erler, 2014; Nozaki, Tamura, Ito, Moriyama, Yamaguchi & Kono, 2012; Schmitzova et al., 1998). Among the RJP, MRJP 1-5 are the most abundant proteins typifying 90% of RJP, and have essential nutritional function. The remaining content of RJP composes of small proteins, peptides, free amino acids, enzymes such as endopeptidase and exopeptidase (Maqsoudlou et al., 2018). The members of the MRJP family comprise high amounts of essential amino acids necessary to feed both queen bees and all larvae. It is thought to be responsible for the specific physiological roles of royal jelly especially in the development of queen honey bees (Ahmad, Campos, Fratini, Altaye & Li, 2020). Arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine are most common essential amino acids in MRJPs. The essential amino acid contents of MRJPs are given in Table 2 (Ramanathan et al., 2018).

**Table 2.** Essential amino acid contents of MRJPs

MRJPs	Amino Acid Contents
MRJP1	48 %
MRJP2	47 %
MRJP3	39.3 %
MRJP4	44.5 %
MRJP5	51.4 %
MRJP6	42 %
MRJP7	48.3 %
MRJP8	49.5 %
MRJP9	47.3 %

MRJPs contain 400-578 amino acids. Their theoretical molecular weights are from 45 to 68 kDa, isoelectric points change from 4.85 to 6.50 (Table 3) (Buttstedt et al., 2014).

**Table 3.** Molecular characteristics of honeybee MRJPs

MRJPs	Amino Acids	Molecular Weights (kDa)	Isoelectric Points (pI)
1	413	46.86	5.03
2	435	49.15	6.65
3	524	59.49	6.50
4	444	50.67	5.74
5	578	68.13	5.95
6	417	47.58	6.01
7	426	48.66	4.85
8	400	45.06	5.81
9	403	46.27	8.62

However, the studies indicate that molecular weight and isoelectric points of RJP, which are used as very significant parameters in identifying proteins, depend on the types and genetic diversity of honey bees, post translational changes such as glycosylation and phosphorylation, and proteolysis reactions that occur during storage of royal jelly (Ohashi et al., 1997; Qu et al., 2008; Santos et al., 2005; Schmitzova et al., 1998; Zhang et al., 2012).

## 2.1. MRJP1

Among the major royal jelly proteins, MRJP1, the first identified protein, is the most abundant glycoprotein (Tian et al., 2018). It is also known by different names such as apalbumin and royalactin (Ramanathan et al., 2018). MRJP1 exists in different forms, including monomers, oligomers and water-soluble forms. The monomeric forms of MRJP1 have an important role in the queen determiner (Foret et al., 2012; Kamakura, 2011). Oligomeric forms of MRJP1 combine with fatty acids to demonstrate the ability to form spontaneously in aqueous solutions (Šimúth, 2001). Studies have determined that MRJP1 is a weak acidic glycoprotein and its isoelectric point is ranged from 4.23 to 6.3 (Cruz et al., 2011; Santos et al., 2005; Tamura et al., 2009). The molecular sizes of MRJP1 monomers were changed from 48.81 to 57 kDa (Kamakura, 2011; Kamakura, Suenobu & Fukushima, 2001; Majtán, Kováčová, Bíliková & Šimúth, 2006; Santos et al., 2005; Schmitzova et al., 1998). The molecular sizes of MRJP1 oligomers were found to be between 280-450 kDa including apisimin molecule (Bilikova et al., 2002; Kamakura, 2011; Mandacaru et al., 2017; Nozaki et al., 2012; Ramadan ve Al-Ghamdi, 2012). Table 4 shows the molecular sizes of MRJPs and protein purification and characterization methods used by various researchers.

It has been determined that MRJP1 and its degradation products ensue during storage, especially at storage temperatures above 4 °C,

can be used to evaluate the freshness of royal jelly (Kamakura, Fukuda, Fukushima & Yonekura, 2011; Shen et al., 2015).

## **2.2. MRJP2**

MRJP2 is a basic protein with molecular weights between 49-72 kDa (Bilikova et al., 2002; Imjongjirak, Klinbunga & Sittipraneed, 2005; Nozaki et al., 2012; Scarselli et al., 2005; Schmitzova et al., 1998; Šimuth, Bíliková & Kováčová, 2003) and isoelectric points ranging from 4.92-8.3 (Bilikova et al., 2002; Santos et al., 2005).

## **2.3. MRJP3**

MRJP3, unlike other MRJPs, has 14-30 pentapeptides repeating in its structure. Isoelectric points vary between 7.05 and 8.04 and molecular weights range from 55 to 87 kDa (Kubo et al., 1996; Šimuth et al., 2003; Santos et al., 2005; Scarselli et al., 2005).

## **2.4. MRJP4**

MRJP4 that provides nutrient components to royal jelly is determined to be approximately 60 kDa (Schmitzova et al., 1998; Sano et al., 2004; Li, Wang, Zhang & Pan, 2007). Isoelectric point of MRJP4 has been reported to be between 6.28 and 6.48 (Li et al., 2007).

## **2.5. MRJP5**

The most important feature of MRJP5 is that there is a wide repeating region of 174 amino acids between 367 and 540 amino acid residues (Qu et al., 2008). Li et al. (2007) and Santos et al. (2005) have been reported that the molecular

weights of MRJP5 vary between 74.89-79.87 and 79.075-79.471, respectively. In addition, isoelectric points were determined between 6.34 and 6.80 by Santos et al. (2005). MRJPs 2-5 contain glycoproteins of size 49, 60-70, 60 and 80 kDa, respectively (Schonleben et al., 2007; Tamura et al., 2009). Studies show that the isoelectric points vary between 4.93 and 8.3 due to post-translational changes in MRJP2-MRJP5 (Li et al., 2007; Schönleben, Sickmann, Mueller & Reinders, 2007; Tamura et al., 2009). MRJPs 6-9 do not have nutritional function on honey bees.

## **2.6. Peptides**

In addition to MRJPs, royal jelly comprises a low variety of small proteins, including bioactive peptides (Jamnik, Raspor & Javornik, 2012). Most of the peptides in royal jelly are formed by proteolytic hydrolysis from major royal jelly proteins (Schönleben et al., 2007). A lot of these peptides show antioxidant activity in different levels (Ramadan & Al-Ghamdi, 2012). In addition, peptides having antimicrobial properties such as royalisin, jelleins and apisimin have also been identified. Royalisin and jelleins secreted into royal jelly by worker bees provide multiple microbial protections (Fontana et al., 2004).

Royalisin is an antimicrobial peptide isolated from royal jelly and has a molecular weight of 5523 Da. It is stable at low pH and high temperature due to its disulfide bonds in its structure (Barnuțiu et al., 2011). Royalisin prevents microbial contamination in royal jelly for Gram-positive bacteria, especially (Fontana et al., 2004).



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Jelleins are short chain peptides of royal jelly with antimicrobial activity. The jelleins consist of 8-9 amino acid residues. A number of peptides called Jellein-I, Jellein-II, Jellein-III and Jellein-IV have been identified (Ramanathan et al., 2018). Fontana et al. (2004) reported that Jelleins-I and III are effective against Gram-positive and Gram-negative bacteria and yeast. However, Jellein-IV has no antimicrobial activity.

### 2.7. Isolation and Characterization of Proteins

In researches on royal jelly proteins, for isolation and characterization of proteins have used chromatographic methods, particularly gel filtration chromatography, ion exchange

chromatography and high pressure liquid chromatography (HPLC). In chromatographic methods, separation is based on the adsorption, dispersion (partition), ion exchange, affinity or differences in molecular weights of molecules. High performance liquid chromatography (HPLC) is a column chromatography method widely used for the purification, identification, qualitative and quantitative analysis of compounds. The chromatographic methods can be used alone or in combination with other methods depending on the properties of the proteins (Coskun, 2016; Sugecti, Imalı & Kocer, 2018).

Among electrophoretic methods, sodium dodecyl sulphate-polyacrylamide gel (SDS-PAGE) method is the most widely used gel electrophoretic method for protein characterization. SDS-PAGE separates proteins based on their rate of movement to molecular weights in an applied electric field (Hu et al., 2017). Another method is two-dimensional polyacrylamide gel (2D-PAGE) electrophoresis method which is developed in addition to SDS-PAGE method. It provides separation based on not only molecular weights, but also isoelectric points (Issaq & Veenstra, 2008). These methods are used in combination with mass spectrophotometry (MS) or gel-free proteomics for identification purposes (Maghsoudlou et al., 2019).

Blotting (western blot, dot blot, northern blot) methods are based on absorbing into the membrane of a targeted protein in a complex

protein separated or not separated by electrophoresis and immunochemical determination of the protein or proteins in the membrane (Burgess, 2009).

In addition for the protein purification and characterization advanced molecular methods such as circular dichroism, N-terminal amino acid sequence, MALDI-TOF methods are also used.

Table 4. Molecular weights for MRJP1-MRJP9 with protein characterization methods

	Molecular Weights (kDa)	Methods	Reference
MRJP 1	55	Affinity Chromatography, SDS-PAGE Electrophoresis, Western Blotting, N-Terminal Amino Acid Sequence	Majtán et al. (2006)
MRJP 1	57	Native-PAGE and SDS-PAGE Electrophoresis, Diethylaminoethyl (DEAE)-Colon Chromatography, Gel Filtration Colon Chromatography	Kamakura et al. (2001)
MRJP1 oligomer	290 (55 kDa MRJP1 monomer and 5 kDa Apisimin)	Size-Exclusion HPLC, Two Dimensional(2-DE), SDS-PAGE and Native-PAGE Electrophoresis, MALDI-TOF MS Analysis	Tamura et al. (2009)
MRJP1 oligomer and monomer	420 55	Ultracentrifugation, SDS-PAGE Electrophoresis, Size-Exclusion Colon Chromatography, Immunoblotting, N-Terminal Amino Acid Sequence	Šimúth (2001)
MRJP 2	49	DEAE-Cellulose Colon Chromatography, SDS-PAGE Electrophoresis, N-Terminal Amino Acid Sequence, Dot blot, Northern blot	Schmitzova et al. (1998)
MRJP 2	52	Ultracentrifugation, Size-Exclusion HPLC, Two Dimensional(2-DE) and SDS-PAGE Electrophoresis	Nozaki et al. (2012)
MRJP 3	55	Two Dimensional (2-DE) Electrophoresis, MALDI-TOF MS Analyses	Scarselli et al. (2005)
MRJP 3	64	SDS-PAGE Electrophoresis, Reverse Phase HPLC, Immunoblotting	Kubo et al. (1996)
MRJP 4	60.71–61.73	Two Dimensional (2-DE) Electrophoresis, MALDI-TOF MS Analyses	Li et al. (2007)
MRJP 5	74.89–79.87	DEAE-Cellulose Colon Chromatography, SDS-PAGE Electrophoresis, N-Terminal Amino Acid Sequence, Dot blot, Northern blot	Schmitzova et al. (1998)
MRJP 6	47.5		Buttstedt, Moriz & Erler (2014)
MRJP 7	48.06		Buttstedt, Moriz & Erler (2014)
MRJP 8	45.06		Buttstedt, Moriz & Erler (2014)
MRJP 9	46.27		Buttstedt, Moriz & Erler (2014)

### 3. CONCLUSION

Royal jelly is a natural bee product with bioactive compounds. It has wide variety of therapeutic properties used from ancient times until today. In addition to being nutritious for bees, it has different functions such as growing and reproduction, caste differentiation, providing antimicrobial protection and extending the life of the queen bee, regulating physiological and temporal mechanisms. Major royal jelly proteins, the main proteins of royal jelly, are thought to be responsible for this multiple functions of royal

jelly. In recent years, royal jelly is one of the most drawing commercial bee products in various industry such as food, medicine, nutraceutical and cosmetic industries. However, present studies are not sufficient, because there is great potential for applications in the neurotherapeutic and food sciences for this product.

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