Review paper

ROYAL JELLY: CHEMISTRY, STORAGE AND BIOACTIVITIES

Atefe Maghsoudlou[1]*
Alireza Sadeghi Mahoonak[1]
Hossein Mohebodini[2]
Fidel Toldra[3]

[1]Gorgan University of Agricultural Sciences and Natural Resources, Department of Food Science & Technology, Iran
[2]University of Mohaghegh Ardabili, Faculty of Agricultural Science, Ardabil, Iran
[3]Instituto de Agroquímica y Tecnología de Alimentos (CSIC), Avenue Agustín Escardino 7, 46980 Paterna, Valencia, Spain

*corresponding author: atefe.maqsoudlou@gmail.com
Received: 05 June 2018; accepted: 06 February 2019

Abstract

Royal jelly (RJ) has been known for centuries, but in the last 5-6 decades its systematic production and consumption has increased. RJ is secreted by the hypopharyngeal and mandibular glands of worker honeybees (*Apis mellifera*). This thick and milky substance contains water, proteins, carbohydrates, lipids, minerals, vitamins and such bio-active compounds as acetylcholine, peptides, the hormones testosterone, progesterone, prolactin, estradiol, (hydroxydecanoic acid) (HAD), adenosine monophosphate (AMP)-N1Oxide, polyphenols, flavonoids and adenosine. Because of its bioactive compounds, RJ can be considered as a functional and nutraceutical food. The main goal of this review is to summarize and update its physicochemical properties, bio-active ingredients, storage stability and shelf life. The functional properties are antioxidative activity, insulin-like action, improvement against diabetes, liver protection, antitumoral action, neurotrophic action, antibiotic effect, anti-inflammatory action and wound healing, hypotensive effect and blood regulatory actions, anti-aging effect and skin protection, effects on the reproductive system and fertility and also fortifying, tonic action and immunomodulating and anti-alergic activity. RJ may cause allergic reactions, asthma and even fatal anaphylaxis in some humans. Therefore, RJ should be orally ingested as nutreaceutical agent or food-ingredient only after an allergy test.

Keywords: bioactive, functional, nutraceutical, physicochemical, royal jelly, storage

INTRODUCTION

Royal jelly (RJ) is secreted by the hypopharyngeal and mandibular glands of worker honeybees *(Apis mellifera)* (Balkanska, Zhelyazkova, & Ignatova, 2012). This secretion is produced in worker bees' stomach due to the incomplete digestion of honeydew (Moselhy, Fawzy, & Kamel, 2013; Melliou & Chinou, 2014). RJ is part of the diet of honeybee larvae and plays a major role in caste differentiation of this species. The larvae designated to become queens receive RJ for up to three days, while the larvae selected to become workers receive a mixture of honey, pollen and water. Thereafter, a honeybee queen lives for several years, and the worker bees only for a few months (Moselhy, Fawzy, & Kamel, 2013). Even though RJ has been known for centuries, its systematic production and consumption has increased in the last sixty years, the most being in China.

Because of its bioactive compounds, RJ is known as a functional and nutraceutical food. A functional food receives an additional function, often one related to general health promotion, by adding new specific ingredients or by increasing certain existing ingredients. A nutraceutical a food or food product is claimed to provide health and medical benefits to consumers, including the prevention and treatment of certain diseases. RJ not only enhances the overall health of the body but also allegedly cures some diseases (Bogdanov, 2014). Though many studies have already been conducted, this review attempts

to incorporate the latest and most comprehensive information about RJ including its physicochemical properties, bio-active ingredients, storage-stability and shelf-life, its commercial applications, main biological activities and therapeutic effects as well as allergic side effects.

Physicochemical properties

Physical Properties

RJ is a thick and milky substance, partially soluble in water, with a density of 1.1 g/mL (Ramadan & Al-Ghamdi, 2012). Abundant in nutrients, it has a sharp pungent odor and fruity somewhat bitter taste (Shirzad et al., 2013; Moselhy, Fawzy, & Kamel, 2013). Its color is whitish to yellow which increases during storage (Ramadan & Al-Ghamdi, 2012; Barnutiu, 2011; Isidorov et al., 2009). These sensory characteristics are important quality criteria (Pavel, 2011; Popesco, Marghitasl, & Dezmireand, 2008).

Chemical composition

RJ water content is within the range of 60-70%, and water activity (a_w) above 0.92. The remaining dry matter is composed of protein material (27-41%), carbohydrates (near 30%), lipids (8-19%), minerals, trace elements and some vitamins (Sabatini et al., 2009). It has a high acidity of 3.4-4.5 (Barnutiu et al., 2011; Popesco et al., 2008). Wongcha and Ratanavalacha (2002) reported that the moisture and carbohydrate contents were high in the rainy season, the lipid content was in the highest level in the hot season, and protein content scarcely altered, while ash and pH values were constant throughout the year.

Table 1 shows the vitamin and mineral content of RJ. The values of component in RJ obtained by various authors are fairly in agreement, not including the high variability displayed for sugars and lipids. RJ is naturally non-homogeneous, and reported findings refer to many samples, locations, production and methods of sampling and analysis used. The analysis of samples from different geographical origins have shown that environmental conditions do not significantly influence the main components (Sabatini et al., 2009; Popesco et al., 2008; Balkanska, Zhelyazkova, & Ignatova, 2012). As all foods of animal origin, RJ could be contaminated with antibiotic or pesticide residues (Zhang et al., 2012).

Table 1.

Vitamin and mineral content of RJ
Adapted from Sabatini et al. (2009) and Bărnuţiu et al. (2011)

Minerals and Vitamins	RJ (mg/100 g)
Potassium	200-1000
Magnesium	20-100
Iron	1-11
Zink	0.7-8
Copper	0.33-1.6
B_1 (Thiamin)	0.1-1.7
B_2 (Riboflavin)	0.5-2.5
B_3 (Niacin)	4.5-19
B_5 (Pantothenic acid)	3.6-23
B_6 (Pyridoxin)	0.2-5.5
H (Biotin)	0.15-0.55
Folic acid	0.01-0.06

Water content

The water content of RJ ranges between 60-70% (Ramadan & Al-Ghamdi, 2012). Its water activity is over 0.92, but it displays considerable microbial stability (Sabatini et al., 2009). Water content, an important quality criterion and in raw RJ quality control in, is determined through freeze-drying, Karl Fischer titration, vacuum oven, dessication and infrared (Garcia-Amoedo, Bicudo, & Almeida-Muradian, 2002; Barnutiu et al., 2011; Nabas et al., 2014). Sesta and Lusco (2008) used the refractometric method for the evaluation of water content.

Proteins

Proteins with a content ranging from 17 to 45% of dry weight represent the most important portion of RJ dry matter (Ramadan & Al-Ghamdi, 2012) as well as 97-98% of the nitrogenous substances present in RJ. Popescu, Marghitasl and Dezmireand (2008) and Barnutiu (2011) determined the total protein of RJ with the Kjeldahl method, but many studies have used either a gel-based proteomic approach, including sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) or two-dimensional polyacrylamide gel electrophoresis (2-D PAGE), mostly in combination with mass spectrometry (MS), or gel-free proteomics. Nabas et al. (2014) developed a simple method to isolate SRJPs from RJ by using ultra centrifugation, size-exclusion HPLC and SDS–PAGE and 2-DE. RJ contains a high number of native and derivated proteins that are divided into MRJP - a major RJ protein, minor proteins and peptides, and free amino acids, which proves the influence of such honeybee enzymes as endopeptidases and exopeptidases in the final composition of RJ. Honeybees use these enzymes to digest pollen proteins to change them into major proteins (Maqsoudlou et al., 2018). About 80% of the proteins are soluble RJ proteins (SRJPs), called major RJ protein (MRJP). These proteins are analysed through dialysis and centrifugation contributes to the physiological actions of RJ (Nabas et al., 2014). MRJPs are thought to be responsible for the specific physiological role of RJ in queen honeybee development, as they include numerous essential amino acids, similar to ovalbumin and casein (Tamura et al., 2009). The MRJP family consists of nine members MRJPs 1-9 (Drapeau et al., 2006; Schonleben et al., 2007). MRJP1, MRJP2, MRJP3 (that exist in five isomers), MRJP4, and MRJP5 represent about 82% of proteins present in the RJ (Melliou & Chinou, 2014; Xu & Gau, 2013). MRJP1 is a weak acidic glycoprotein accounting for more than 45% of MRJPs (pI 4.9-6.3, 55kDa) and is estimated to be 350 or 420kDa (Kimura et al., 2003). It is classified as albumins and therefore named as apalbumin-α. It is reasonable to suggest that an interaction between apalbumin-α and fatty acids resulted in the formation of the water-insoluble protein fraction of RJ. Apalbumin-α was shown to have formed a basic subunit structure of about 420 kDa built up from the basic 55kDa monomer. Depending on the concentration of apalbumin-α, various regular repeating structures are generated, and the self-assembling structure of apalbumin-α was created as a result of the oligomerization of its subunits (Fig. 1). Other RJ proteins do not have this ability, though they have a high degree of sequential homology with apalbumin-α (Šimúth, 2001). MRJP 2-5 were estimated to be glycoproteins of 49, 60-70, 60 and 80kDa, respectively and mainly in the basic pI range of 8.3 (Schonleben et al., 2007; Tamura et al., 2009). MRJP3 exhibits a size polymorphism as detected using SDS-PAGE. MRJPs include numerous essential amino acids (Kimura et al., 2003). MRJP2 and MRJP3 named apalbumin-β and apalbumin-γ, respectively, do not have the ability to form gel, though they have a high degree of homology with apalbumin-α (Šimúth, 2001).

Besides the MRJPs, low amounts of several minor proteins including bioactive peptides are present in RJ (Jamnik, Raspor, & Javornik, 2012). MALDI-TOF-MS analysis of RJ showed that the main parts of its peptides are cleavage products of abundant protein families. Such peptides are released from the MRJPs through proteolytic cleavage. Other small peptides are independent from major proteins and therefore show no sequence homology to the MRJPs (Schonleben et al., 2007). Many antioxidative peptides are

isolated from RJ hydrolysate and some have a strong hydroxyl radical scavenging activity (Ramadan & Al-Ghamdi, 2012).

In addition, such peptides as royalisin, jelleines and apisimin with antimicrobial properties have been identified. Royalisin is a peptide isolated from RJ, and its molecular weight is 5523 Da, consisting of fifty-one amino acids, in which six cysteine residues form three intramolecular disulfide linkages resulting in a compact globular structure. Royalisin contains a typical disulfide-rich structure (40 amino acids) with a unique amphipathic α-helix and an amidated carboxyl terminal tail (11 amino acids) (Schonleben et al., 2007). It is stable at low pH and high temperature probably because of the disulfide bonds present in its structure (Barnuţiu et al., 2011). Jelleines are peptides with antimicrobial activity of RJ. Jelleine-I, Jelleine-II, Jelleine-III and Jelleine-IV were purified from RJ by using RP-HPLC and sequenced by applying MS. They are very short peptides, representing hydrophobic sequences and do not display any similarity with any other known antimicrobial peptides (Jamnik, Raspor, & Javornik, 2012; Barnuţiu et al., 2011). Apisimin is a peptide from RJ composed of fifty-four amino acids and 5540 Da stimulates the proliferation of human monocites. It is rich in Val (18.5%), and Ser (16.7%), no Cys, only two basic amino acids, and contains only one aromatic amino acid, Phe. The fifty-four amino acids of apisimin do not include Cys, Met, Pro, Arg, His, Tyr and Trp residues (Fontana et al., 2004).

Although there have been few studies about the mechanism of peptide generation in RJ, according to a report by Maqsoudlou et al. (2018) none of the natural peptides identified in RJ were attributed to honeybees since they originated from the plant species source where the pollen had been collected by them. Therefore it is proposed that the honeybees do not have the gene involved in the production of these peptides. Some of these peptides are naturally found in pollen and the honeybee receives them directly by eating pollen (Schönleben et al., 2007). Possibly some of the pollen proteins are converted into peptides in RJ through honeybee enzymes (Maqsoudlou et al., 2018).

Free RJ amino acids are determined through chromatography (Popescu, Marghitasl, & Dezmireand, 2008; Barnutiu, 2011). Free amino acids represent only 0.6-1.5%, the majority of which are essential. Akamatsu and Mitsuhashi (2013) determined free amino acids in dietary supplements containing RJ by using capillary electrophoresis coupled to tandem mass spectrometry. Liming et al. (2008) determined twenty-six RJ amino acids by the fast method of ultra-performance liquid chromatography. The results showed that the average contents of free amino acids and total amino acid in fresh RJ were 9.21 mg/g and 111.27 mg/g, respectively. The major free amino acids were Pro, Gln, Lys, Glu and the most abundant total amino acids were Asp, Glu, Lys and Leu. The changes in amino acids content may be an effective way to evaluate the quality of RJ. The amino acids present with the highest percentages were Pro, Glu, Ala, Phe, Asp and Ser (Boselli et al., 2003). Free amino acids in RJ include Gly (2.1%), Leu (13.3%), Ala (1.7%), Pro (39.8%), Thr (1.0%), Val (1.7%), Ser (3.5%) and Ile (1.3%) (Akamatsu & Mitsuhashi, 2013; Liming et al., 2009).

Many studies have reported the presence of phosphorylated, glycosylated, methylated and deamided proteins in RJ. These post translational modifications could explain the heterogeneous composition of peptides in the RP-HPLC profile of RJ (Maqsoudlou et al., 2018). It has also been reported that major RJ protein genes have also been reported to be present in a honeybee, as they can make them (Zheng et al., 2012). Schönleben et al. (2007) identified a total of twenty different proteins and demonstrated a very high degree of degradation in the major RJ protein family. They also investigated protein phosphorylation of RJ proteins. Zhang et al. (2012) investigated the position of phosphorylation, methylation and deamidation in RJ proteome using complementary proteomic approaches of 2-DE and shotgun analysis. Their work proved several important post-translational modifications in RJ and some information on their biological roles. Schönleben et al. (2007) applied various methods for the pre-fractionation and separation of RJ proteins in order to

circumvent the short comings of such individual techniques as 2-D PAGE or multidimensional chromatography, which only yield certain sub-populations of a proteome due to the specific bias of each method. In this way, they achieved a high coverage of the RJ proteome and were able to identify twenty different proteins and to demonstrate a high degree of cleavage of different proteins in MRJP. Furthermore, they investigated the protein phosphorylation of RJ proteins, identified and located two phosphorylation sites within venom proteins.

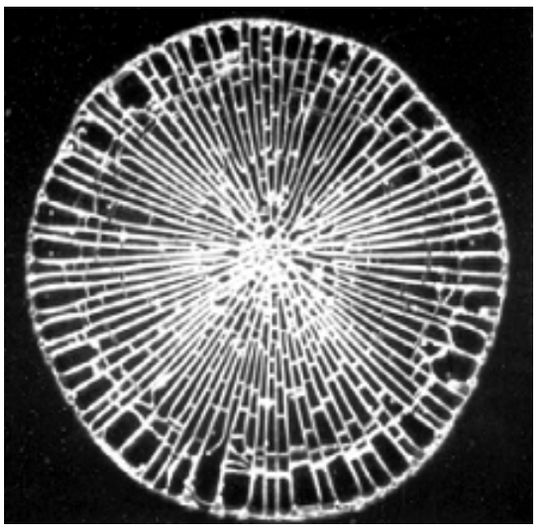

Fig. 1. The self-assembling of regular filamentous structures of apalbumin-α
Adapted from Šimúth (2001)

Carbohydrates

Carbohydrates are mainly composed of fructose, glucose and sucrose. Fructose and glucose are in relatively constant proportion similar to honey (Melliou & Chinou, 2014). Glucose and fructose contents are in the ranges of 3.4-7.7% and 2.3-7.8%, respectively (Daniele & Casabianca, 2012). Major carbohydrates are highly constant in qualitative terms, and considerable variability exists from a quantitative view, so a small quantity of sugar is present which is used as a good marker for the detection of adulteration (Ramadan & Al-Ghamdi, 2012). The minor sugars in RJ are mannitol, erlose, maltotriose, arabinofuranose, arabinitol, galactose, ribitol, methyl α-fructopyranoside, isomaltose, α-sorbofuranose, methyl β-fructofuranoside, pinitol, turanose, α- and β-fructofuranose, inositol, β-fructopyranose, β-glucofuranose, gluconic acid, γ-lactone, maltulose, α- and β-glucopyranose, melezitose, α- and β-galactopyranose, maltose, sorbitol, glucitol, chiro-inositol, gentiobiose, myo-inositol, gluconic acid, 2-(acetamido)-2-deoxygalactopyranose, 2-(acetamido)-2-deoxyglucopyranose, palatinose, α-lactulose, sucrose, trehalose, α-cellobiose, leucrose, α-cellobiose, α-isomaltose, raffinose, 1-kestose (Daniele & Casabianca, 2012; Melliou & Chiou, 2014). The sugar content of RJ is usually determined through gas chromatography or HPLC methods (Bărnuţiu et al., 2011; Nabas et al., 2014).

Lipids

Lipids with 3 to 19% of the RJ dry weight (Boselli et al., 2003) are the second most important bioactive compounds after the proteins. The lipid composition is reported as 80-85% fatty acids, 4-10% phenolic lipids, 5-6% waxes, 3-4% steroids and 0.4-0.8% phospholipids (Kodai et al., 2007). A major part of the organic acids are found as free form and have structure which is rarely encountered in nature consisting of mono and dihydroxy acids and dicarboxylic acids with eight and ten carbon atoms (Melliou & Chinou, 2014). The fatty acids has been reported to contain 32% trans-10-hydroxy-2-decenoic acid, 24% gluconic acid, 22% 10-hydroxydecanoic acid (10-HDA), 5% dicarboxylic acids and several other acids (Ramadan & Al-Ghamdi, 2012). 10-HDA is the main unsaturated acid which is determined through HPLC for the evaluation of RJ genuity (Melliou & Chinou, 2014; Barnutiu et al., 2011). No quantification is available for the other fatty acids which are all saturated mono and dihydroxy, mono and dicarboxylic acids. The content of these compounds has been roughly estimated at about 0.5 to 1g/100g (Xu & Gua, 2013). Short chain (8 up to 12 carbon atoms) hydroxy fatty acids found in RJ are shown in Tab.1 of supplementary data. 10-HDA and the other fatty acids of RJ have antibacterial properties (Moselhy, Fawzy, & Kamel, 2013), and thus they contribute to the relatively low content of bacteria in this product. 10-HDA is present only in RJ and is known for having

various pharmacological effects (Isidorova et al., 2009). Sterols are other fatty compounds of RJ. 24-Methylenecholesterol (49-58% of total sterols) constitutes the most important sterol of RJ. Other sterols present in RJ include β-sitosterol (19-24% of total sterols), isofucosterol (9-16% of total sterols), campesterol (6-7% of total sterols), and desmosterol (0.5-4.5% of total sterols). RJ contains testosterone (11-12 ng/g) (Melliou & Chinou, 2014). The lipid content of RJ is determined as free and total organic acids through gas chromatography or as total lipids through solvent extraction (Barnutiu et al., 2011; Popescu, Marghitasl, & Dezmireand, 2008; Nabas et al., 2014).

Minerals and trace elements

The total ash content in fresh RJ is about 1% and around 2-3% in the lyophilized RJ which is determined through atomic absorption (Nabas et al., 2014; Melliou & Chinou, 2014; Popescu, Marghitasl, & Dezmireand, 2008; Bărnuţiu et al., 2011). The major elements are K, P, S, Na, Ca, Al, Mg, Zn, Fe, Cu and Mn, but there are trace amounts (0.01-1 mg/100 g) of Ni, Cr, Sn, W, Sb, Ti and Bi (Melliou & Chinou, 2014; Ramadan & Al-Ghamdi, 2012).

Vitamins

RJ is exceptionally rich in vitamins. Riboflavin, thiamin, niacin and folic acid are fairly uniform while pyridoxine, biotin, pantothenic acid and inositol are present in a larger variation (Mohebodini et al., 2018). Traces of vitamin C have also been found in RJ but does not contain vitamins A, D and K (Ramadan & Al-Ghamdi, 2012).

Bioactive compounds

RJ contains bioactive compounds with health-promoting properties. Each of these compounds with their amount and health promoting effects are classified and described in detail in table 2 of supplementary data.

Storage stability and shelf life

Temperature is an important factor affecting the physico-chemical properties of RJ during its maintenance. The physical properties of RJ change twenty hours after harvest if stored at ambient temperature (Boselli et al., 2003). Old RJ improperly stored tends to darken due to a browning reaction and possesses an undesirable taste due to increased titratable acidity (Pavel et al., 2011b; Ramadan & Al-Ghamdi, 2012). These changes might be due to lipid oxidation and Maillard reactions. The carbohydrate and protein content significantly diminished in RJ stored at room temperature due to Maillard reactions (Pavel et al., 2011a). In this regard, Abdelnur et al. (2011) studied the thermostability of RJ samples through electrospray ionization mass spectrometry in order to evaluate the changes in chemical composition because of exposure to heating and storage at room temperature at different time intervals. There were

Table 2.

Shelf life of RJ.
Adapted from Burimistrova et al. (2008) and Balkanska & Kashamov (2011)

	Shelf life	
	4˚C	<-18˚C
Fresh RJ	6 months	2 years
Lyophilized RJ	1 years	> 2 years
Fresh or lyophlised RJ in honey (humidity less than 18 %)	Two years at room temperature (All enzymatic activity is stopped)	
Apilac pills (lactose-glucose- freeze dried RJ)	At 4 to 8 °C for 2 years	

some changes in the chemical profile of RJ and it degraded after heating and storage. The viscosity of RJ increased when stored at temperatures below 5°C. Such increase appears to be related to reduction in soluble nitrogen and free amino acids and increase in water insoluble nitrogenous compounds (Drijfhout, 2005; Pavel et al., 2011b). Upon storage at room temperature, because of proteolytic enzyme activity, Pro and Lys content increased in the first three months and after six to ten months decreased to levels slightly lower than their initial content, while if RJ had been stored at 4°C, in a dark and dry place, no significant changes in free amino acids could be encountered for ten months (Boselli et al., 2003; Ramadan & Al-Ghamdi, 2012; Pavel et al., 2011b). In this regard, Liming et al. (2009) reported that Gln and total Met significantly decreased during storage, and this could be used to predict RJ quality. The remaining free amino acids and total amino acids did not reduce during storage. Also, changes in the physiochemical properties of RJ are apparently due to interaction between the lipid and protein fractions and continued enzymatic activities (Pavel et al., 2011a). The 10-HDA content of RJ significantly diminishes at room temperature during storage (Henrique et al., 2003). The growth of proteolytic and lipolytic molds usually begins on the surface of RJ even after several months of storage in a refrigerator, which is responsible for the alteration process. Even lactic acid bacteria can grow at refrigeration temperature. The pH of RJ is neutral, so the putrefactive bacteria can grow by the gradual release of ammonia (Marghitas, 2008). Only storage in a frozen state prevents the decomposition of bioactive proteins of RJ (Ramadan & Al-Ghamdi, 2012). In this regard Isidorov, Bakier and Grzech (2012) investigated the volatile and extractable compounds of crude RJ through the gas chromatographic-mass spectrometry method. Volatile RJ compounds remained unchanged for ten months at −18°C and 4°C, but phenol contents at room temperature decreased two-fold during the same time. In this, condition aliphatic acids increased 2.8 times due to the presence of both acetic and butyric acids. As seen in Tab. 2, the storage capability of RJ can be improved through the use of freez drying (Nascimento, 2015). Ramadan & Al-Ghamdi (2012) reported that deterioration can be prevented by storing RJ in Argon after harvesting. Various criteria used to determine the freshness of RJ include the amounts of glucose oxidase enzyme, furosine, 10-HAD and RJ proteins (Tab. 3 of supplementary data).

Commercial applications

RJ has been widely used in commercial medical products, health foods, and cosmetics in many countries for over thirty-five years (Viuda-Martos et al., 2008). It can be directly ingested (Munstedt et al., 2010) or used in a raw form (in combination with honey or alone), in a lyophilised form or as pills (Kamakura et al., 2001). In Russia, freeze-dried RJ is often available in Apilac pills containing lactose-glucose (Burimistrova, 2008). If RJ is mixed with honey in order to prevent spoilage from fermentation, the honey should have a low humidity, <16% water (Bogdanov, 2014). Similarly, RJ was a common ingredient in health foods, beverages and dietary supplements (Salem, 2013; Nabas et al., 2013). One of the approaches for application of the RJ is encapsulation of lyophilized RJ (Bogdanov, 2014). Munstedt, Bargello and Hauenschild (2009) and Munstedt et al. (2010) found that capsules containing RJ could enhance the efficacy of RJ to improve glucose metabolism in humans.

Functional properties

RJ is assumed to bring balance in the human body, probably due to the adequate balance and proportion between its components (Dinkov & Stoyanchev, 2014). The functional properties of RJ, how it affects health and the results of recent research are classified and described in the next sections.

Antioxidative activity

Recently RJ and enzymatic hydrolysates of RJ have been tested for antioxidant properties. The antioxidant properties have been proven in various experimental models attributed to proteins and peptides, which are potentially associated with the recovery after fatigue (Moriyama, Bagchi, & Bagchi, 2017; Pavel et al.,

2011a). Considering that RJ is produced after the digestion of bee pollen by natural enzymes in honeybee, and that all pollen phenolic compounds are also found in RJ, the antioxidant activity of RJ can be concluded to be related to phenolic compounds, proteins and peptides (Maqsoudlou et al., 2018).

Some studies have been exploring the biological effects of RJ with the use of whole-animal experimental systems in *in vitro* conditions. For instance Nagai et al. (2001) and Maqsoudlou et al. (2018) examined the antioxidative effect of RJ by measuring the scavenging abilities of the superoxide and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical and ferric reducing power. Based on the result of these studies, RJ showed high antioxidant activity. Silici et al. (2010) protected the DNA tissue of mice from oxidative damage by feeding RJ to them and which reduced the oxidative stress marker levels (8-hydroxy-2-deoxyguanosine) in kidney DNA and serum. RJ decreased intracellular oxidation in a dose dependent manner. RJ in the cell acts as a scavenger of reactive oxygen species and affects protein expression (Cemek et al., 2010). Mannoor et al. (2009) reported that water and alkaline extracts of and enzymatic hydrolysates of RJ show antioxidative properties. Nabas et al. (2014a) proved that it modulates oxidative stress and apoptosis in liver and kidneys, and Guo et al., (2009) showed that it reduced fumonisin-induced oxidative stress and decreased the toxic effects of chemical agents in a high antioxidant and free radical scavenging capacity (Guo et al., 2009). Pavel et al. (2014) declared peptides derived from RJ protein exhibit antioxidant activity. Jamnik, Raspor and Javornik (2012) used yeast as a model organism to investigate the antioxidative action of RJ in the cell by measuring intracellular oxidation, cell energy metabolic activity, and analysed the protein profile of yeast cell extract. The results showed that it decreased the intracellular oxidation in a dose dependent manner. Additionally, it affected growth and cell energy metabolic activity in a growth phase dependent manner. Protein profile analysis showed that RJ in the cell acts as a scavenger of reactive oxygen species.

Antidiabetic effect

RJ is antidiabetic and prevents insulin resistance and hypercholesterolemia in diabetic patients (Zamami et al., 2008). Insulin-like peptides in RJ resemble mammals' insulin (Kanbur et al., 2009). Insulin-like peptides, chromium, sulphur, vitamins B_3 in RJ participate in the oxidation of glucose to obtain energy and to sustain the optimal blood sugar level (Ashry & Elkady, 2014). In addition, RJ reduces alloxan induced diabetes which destroys the insulin structure. In the liver, it acts as a potent free radicals scavenger which prevents or ameliorates the toxic effects of drugs (Li et al., 2011).

There are many reports about the protective effect of RJ in the liver. Pourmoradian et al. (2012) reported that it modulated oxidative stress and apoptosis in liver and kidneys. Galaly et al. (2014) reported that RJ repaired alcoholic liver damage. Kanbur et al. (2009) managed diabetic patients' weight by supplementing their diet with RJ. Ahmed et al. (2014) showed that RJ prevents genotoxicity and nephrotoxicity induced by valproic acid in mice and improves liver exposed to sodium fluoride. RJ could also reduce azathioprine-induced hepatotoxicity exhibited by the pathological changes in the liver.

Antitumoral action

RJ modulates the immune system and shows a positive effect on it, and strengthening the immune system prevents or reduces the growth of cancer cells. The therapeutic effects of RJ remain in a cancer patient's body for a long time (Shirzad et al., 2013). Its effects on the immune system and antitumor activity requires time and because of the small size of tumors in the early days, it is not possible to comment how it affects tumor growth, but due to effects of RJ, some days tumor growth decreases (Shirzad et al., 2013). Most immunomodulatory and antitumoral effects have been ascribed to the 10-HDA and such protein components as MRJP3 and apalabumin1 (Feng & Li, 2010; Kohno et al., 2004).

The antitumor activity of these compounds has

different mechanisms. For instance, 10-HDA promotes the growth of interleukin-2 and T-lymphocyte subsets which possess anticancer activities and immunoregulation (Izuta et al., 2009). Apalbumin-1 and apalbumin-2, two major proteins in RJ, stimulate macrophages to release TNF-α (tumor necrosis factor). The RJP30 protein fraction (including 76, 82, and 88 k Da proteins, obtained by precipitation of crude protein extract of RJ with 30% ammonium sulfate) was found to play an antitumoral role (Salazar-Olivo & Paz-Gonza´lez, 2005; Dzopalic et al., 2011).

There are many reports about the antitumor effect of RJ. In the patients with breast cancer, it stimulates immunoglobuline production by lymphocytes and increases IgM and IgG (the anti-cancer factors). It also shows antiestrogenic activity by inhibiting the effect of bisphenol A or BPA (an environmental estrogen which stimulates the proliferation of human breast cancer MCF-7 cells). RJ prevents myelosuppression induced by tumor evolution (immunostimulating effect) (Kamakura et al., 2007), and it has an important effect in the regression of fibrosarcoma cells and it shows a delayed effect in the control of fibrosarcoma. Shirzad et al. (2013) found that it inhibits the growth-promoting effect of BPA on MCF-7 cells, and Nakaya et al. (2007) reported an anti-environmental estrogen activity. They reported that RJ inhibits the growth-promoting effect of BPA on MCF-7 cells, even though it cannot affect the proliferation of cells in the absence of BPA.

Neurotrophic action

RJ stimulates and activates the central nervous system through a glial cell-derived neurotrophic factor and increases the differentiation of brain cells from neural stem cells. 10-HDA increases the generation of neurons (Pyrzanowska et al., 2012). Furukawa (2008) showed that RJ play neurotrophic and neuroprotective roles on the hippocampus of the adult mouse brain. Ito et al. (2011) found that RJ simplifies the differentiation of all types of brain cells: neurons, oligodendrocytes and astrocytes (Acetylcholine in RJ is a neurotransmitter in the peripheral and central nervous systems and is only a neuromodulator used in the motor division of the somatic nervous system (Mannoor et al., 2008). AMP N1-oxide stimulates both the formation of different brain cells and the expression of protein to mature neurons (neurofilaments-M). AMP N1-oxide stops the proliferation of PC 12 cells. RJ was found to reduce neuronal death and neurogenesis in Alzheimer's and Parkinson's diseases; it improves the spatial memory and acts as an inhibitor of stress-related immunodepression (Mohamed et al., 2015; Pasupuleti et al., 2017). Pyrzanowska et al. (2014) showed that the administration of RJ protects rat pup brain tissue functioning and structure against the ruinous effects of tartrazine. It improves the memory of rats by reducing the serotonin and dopamine in the prefrontal cortex. Aslan et al. (2012) showed that it ensures a better quality of life in old age and that it reduces secondary neuronal damage after experimental spinal cord injury in rabbits by decreasing the apoptotic cell number. Teixeira et al. (2017) investigated the corticosterone levels and the antioxidant defense system in the cerebellum and brain, as well as in the isolated regions of stressed rats supplemented with RJ. Rats supplemented with RJ showed decreased corticosterone, maintained glycemia and decreased lipid peroxidation in the brain, cerebellum, as well as striatum and hippocampus, as well as improved the glutathione defense system in the cerebral cortex and striatum. Therefore, they suggested the antistress and neuroprotective effect of RJ under stress conditions.

Antibiotic effect

There are different results concerning the antimicrobial properties of RJ. It affects the growth of fluconazole-resistant fungal strains of *Candida* spp. and has a strong antibacterial *in vitro* action against Gram-positive bacteria but not against Gram-negative bacteria (Koc et al., 2011). A high RJ concentration affects *Pseudomonas aeruginosa* and low concentrations affects *Escherichia coli*. Garcia, Finola and Marioli (2010) showed that RJ inhibits the parasitic mite, *Varroa destructor.* Such RJ com-

positions as 10-HDA, Royalisin and Jeleines I, II, III showed an antibiotic effect against microorganisms (Moselhy, Fawzy, & Kamel, 2013). 10-HDA showed an antibiotic effect against such fungi and bacteria as *Neurospora sitophila, Micrococcus pyogenes, E. coli*. *Staphylococcus aureus, Streptomyces griseus,* and three unclassified strains of *Streptomyces, Bacillus subtilis (*Abd-Alla et al., 2008; Fratini et al., 2016). Royalisin, an antibacterial peptide, has an antibiotic effect against the following Gram-positive and Gram-negative bacteria and fungi: *Lactobacilus helveticus, Clostridium, Corynebacterium, Leucnostoc*, *Staphylococcus, Streptococcus*, *E. coli, S. aureus, S. griseus,* as well as three unclassified strains of *Streptomyces, Paenibacillus larvae*, a honeybee pathogen that causes American foulbrood, a lethal disease in honeybee larvae. The antimicrobial activity of Royalisin-D (an extra stretch of eleven amino acid residues at the C-terminus of royalisin) is similar to that of royalisin (Fratini et al., 2016; Bílikova et al., 2015). Jeleines I, II, III are active against yeast, Gram positive and Gram negative bacteria (Boukraâ et al., 2009; Fratini et al., 2016). Water-soluble components (proteins and peptides such as Apalbumin) showed an antibiotic effect against Gram positive bacteria and fungi (Boukraâ et al., 2008). Such secondary metabolites as phenols, flavonoids, glycosides, terpenoids, sterols, lignin and saponins are active against both Gram positive and Gram negative bacteria (Henshaw, Twigg, & McLennan, 2014). A mixture of RJ and starch showed an antibiotic effect against *S. aureus* and *E. coli* (Shen et al., 2010), while a mixture of RJ and honey showed an antibiotic effect against *S. aureus* and diabetic foot infection (Romanelli et al., 2011; Pasupuleti et al., 2017).

The antimicrobial action of a RJ component can be explained through influencing the membrane structure of microorganisms. In fact, as a result of the lipophilic character of such RJ components as 10-HAD, they preferentially partition from aqueous phase into membrane structures. This results in membrane expansion, increases membrane fluidity and permeability, disturbs membrane-embedded proteins, inhibits respiration and alters ion transport processes (Trombetta et al., 2005). Generally, the first sign of increased cytoplasmic membrane permeability and autolysis in microorganisms is provided by the leakage of intracellular solute potassium (Cushnie & Lamb, 2005; Parveen & Rao, 2012). By increasing membrane permeability, bacteria lose their capacity for ATP synthesis, membrane transport and motility potential (Siavash et al., 2011). The activity of RNA polymerase of microorganisms is inhibited through increased leakage of intracellular solute potassium (Moselhy, Fawzy, & Kamel, 2013). The active substances of RJ are destroyed during digestion and do not exhibit antibiotic and anti-inflammatory effects. Therefore, the sublingual application of RJ is recommended in order to achieve a direct transmission into the blood and to avoid eventual decomposition of such active substances as proteins in the digestion tract (Bogdanov, 2014).

Anti-inflammatory action and wound healing effect

The fatty acids and proteins of RJ and its antimicrobial properties make it appropriate for wound healing (Majtan et al., 2010). It improves regeneration of skin after wounds, and MRJP3 suppresses the secretion of pro-inflammatory cytokines and activates keratinocytes involved in wound healing (Tatsuhiko, Naoko, & Yuko, 2011). RJ reduces inflammation by hormone-like effects (Abdelatif, Yakoot, & Etmaan, 2008). 10-hydroxy-2-decenoic acid (10H2DA) and 10HDA increase collagen production (Siavash et al., 2011). RJ shortens the healing period of desquamated skin lesions, and by decreasing exudation, it increases the wound healing capacity and collagen formation in granulation tissue formation (Galaly et al., 2014). It enables wound healing by inhibiting capillary permeability and may effectively treat diabetic foot ulcers besides the standard treatments (Mannoor et al., 2009). Yang et al. (2010) administered RJ orally to reduce oral mucositis. Because of its vasodilation effects around the affected wound, it can help to dilate the blood vessels to enhance blood flow (Siavash et al., 2015; Pasupuleti et al., 2017). 10H2DA has a therapeutic value

in inhibiting joint destruction in rheumatoid arthritis. It was found that RJ has anti-inflammatory and cell regeneration effect in the colon of rats with acetic acid induced colitis (Karaca et al., 2012). El-Gayar et al. (2016) reported that RJ could eradicate hospital acquired infections due to Methicillin resistant *S. aureus* (MRSA) and promote wound healing.

Hypotensive and blood regulatory actions

RJ improves the quality and number of red blood cells. It has hypo-cholesterolemia and hepato-protective action, and controls and lowers cholesterol and triglycerides by attaching to the phytosterols like biosterol in the intestinal tract, which increases HDL levels and number of blood cells, lowers plasma fibrinogen levels and thrombosis and prevents myocarditis (Takaki-Doi et al., 2009). This could result from the adjustment of squalene epoxidase enzyme (SQLE) and the low-density lipoprotein receptors (LDLR) which incorporate cholesterol in the liver (Kanbur et al., 2009). RJ showes antihypertenisive, hypotensive and vasodilatative effects, and dilates the vascular system in lower limbs and facilitates blood flow in the vascular bed (Nagai et al., 2008). RJ and its peptides were found to reduce high blood pressure due to trans-2-octenoic acid, the hydroxydecanoic acid and angiotensin I-converting enzyme inhibitory activities. RJ increases the oxygen flow to the liver and promotes hepatocytes growth and liver health (Nagai et al., 2008). Furthermore, it prevents nicotine-1 inducing by increasing the cholesterol levels. The immunomodulatory effect of RJ treatment represses the deleterious effects of hypercholesterolaemia (Viuda-Martos et al., 2008). RJ acts against stenocardia and heart infarct (Ibrahim, 2014).

Anti-aging effect and skin protection

Since ancient times, RJ has been believed to prolong life by protecting the DNA and lowering oxidative stress (Pasupuleti et al., 2017). RJ increases respiration and oxidative phosphorylation and causes increased body abilities (Han et al., 2011). It impacts on the estrogen and gonadotropin effects in cells (Suzuki et al., 2008). RJ reduces such eye problems as conjunctivitis, corneal burn and blepharitis high eye blood pressure in old people (Park et al., 2012). It promotes the building of collagen in cell cultures and prevents the development of such skin lesions as atopic dermatitis and itching (Oribe et al., 2012; Kim et al., 2010). It prevents wrinkles and spots through the moisturizing of skin and reduces melanine synthesis (Yamaura et al., 2013; Tatsuhiko, Naoko, & Yuko, 2011). 10H2DA, a major fatty acid component of RJ, enhances collagen production, and therefore protects against UV B–induced photo aging in human skin fibroblasts (Kamakura et al., 2001; Park et al., 2010). RJ protects fibroblast against deleterious lipid peroxidation by-products (Bonte et al., 2013).

Effects on the reproductive system and fertility

RJ increases the fertility of women and men because it is an important source of para-aminobenzoic. RJ exhibits a low estrogenic activity and affects osteoporosis and perimenopausal symptoms, and improves the hormonal equilibrium and fertility by increasing sperm and ovules quality (Al-Masri, 2011; Karacal & Aral, 2008). RJ improves intestinal absorption of calcium in ovariectomized females (Abdelnur et al., 2011) and the functioning of the hypothalamic-pituitary-ovarian axis in postmenopausal females (Salem, 2013). Kazu-Michi et al. (2008) demonstrated that RJ or its components affect gene expression to mimic the effects of estrogen and improve menopausal symptoms. The oral intake of RJ could improve the bone quality of ovariectomized rats by modulating the post-translational modification of type I collagen and collagen cross-linking in bones (Gimenez-Diaz et al., 2012). RJ improves the reproductive response of sheep and ewes and increases its first service conception rate (Kaku et al., 2014). RJ administration to heat-stressed male rabbits can improve their physiological status and resolve their "summer infertility" (Silici et al., 2009). RJ was found to decreases progesterone concentrations and increase the pregnancy rate in sheep (Moghaddam et al., 2013). RJ prevents cisplatin-

induced spermiotoxicity through its antioxidative effect (Taavoni et al., 2014), and reduces the severity of premenstrual syndrome in women (Yang et al., 2012). The use of RJ might be a simple and reasonably effective method of treating asthenozoospermia. The amino acid and 10H2DA content of RJ enhance acrosome reaction and sperm motility and quality and thus improve fertilization (Elnagar, 2010; Shahzad et al., 2016). RJ as an antioxidant source improves the sperm parameters of ram semen (Moradi et al., 2013). Seyyedi, Rafiean-Kopaei and Miraj (2016) reported the postmenopausal treatment effect of RJ in humans. Abdel-Hafez, Rifaai and Abdelzaher (2017) studied how RJ possibly protects male albino rats against cyclophosphamide-induced prostatic damage. Results showed that RJ provides histo-pathological and biochemical improvement in cyclophosphamide-induced prostatic tissue toxicity. This improvement was associated with a decrease in the tissue oxidative damage and apoptosis. Eshtiyaghi et al. (2017) studied how RJ improved glucose metabolism and redox state of ovine oocytes and fertilization. They reported that RJ caused oocyte maturation and high intracellular glutathione level and capacity to develop the blastocyst stage. RJ also increased the fertilization and blastocyst formation due to increased activity of the glycolysis and the pentose phosphate pathway in cumulus cells and antioxidant enzymes activity in both oocyte and cumulus cells. Alcay et al. (2016) evaluated different concentrations of RJ supplemented extenders for post-thaw quality and incubation resilience of goat spermatozoa. Alcay et al. (2016) showed that RJ supplemented extenders benefit post-thaw and after incubation of goat sperm motility rates, acrosome, plasma membrane and DNA integrity. RJ extends the survivability and longevity of spermatozoa. Therefore, RJ supplementation increases the fertilizing possibility of oocytes during artificial insemination.

Fortifying and tonic action

RJ is an ideal food additive for newborn infants. It improves appetite and general conditions, and increases weight, hemoglobin and red blood cells in premature babies or babies with nutritional deficiencies. RJ increases energy levels, vital capacity, respiratory function and muscular effort capacity. It improved appetite, and body mass and strength during malnutrition. The pantothenic acid (B_5) in RJ is converted to coenzyme A (helps the body to metabolize lipids) which improves stress response capacity (Kamakura et al., 2001).

In heart disease patients, RJ increases euphoria, strength and appetite. In patients with chronic fatigue syndrome, it increases the feeling of being energetic. RJ improves the physical performance of humans and the general condition of old people. It benefits energy metabolism and pancreatic lipase activity. RJ supplementation has positive effects on body composition (Joksimović, Stanković & Joksimović, 2009).

Immunomodulatory and anti-alergic activity

RJ works against respiratory problems and asthma. RJ suppresses type-I allergic reactions through the restoration of macrophage function and Th1/Th2[1] cytokine responses. Unsaturated fatty acids, amino and gamma globulin, enzymes, proteins and vitamin E and A help the immune system fight infections, cancer, allergy and inflammation. Most of these compounds are found in RJ, so it can boost the immune system (Mohebodini et al., 2018). The effect of RJ on interferon-alpha inhibition of human colon cancer cells proliferation inhibits the formation of metastases (Mannoor et al., 2008). 10HDA and 3-10-dihydroxydecanoic acid stimulate the proliferation of T cells, but in high concentrations, they inhibit it, decrease Interleukin-2 production and increase Interleukin-10. MRJP1 stimulates the proliferation of monocytes. The MRJP3 stimulates the growth of lymphocyte cells in serum deprived mediums. Water extract possesses the most potent immunomodulatory activity (Mannoor et al., 2009).

The oral administration of RJ (1g/kg) inhibits

[1] Helper T cells (The two helper T-cell classes also differ by the type of immune response they produce. While Th1 cells tend to generate responses against such intracellular parasites such as bacteria and viruses, and Th2 cells produce immune responses against helminths and other extracellular parasites)

histamine discharge, significantly decreases the serum levels of specific Immunoglobulin E, lowers the macrophage production of Prostaglandin E2 and improves the Th1/Th2 ration in favor of Th1. RJ increases the number of leucocytes, lymphocytes and antibodies production in chicks (Simsek, Karadeniz, & Bayraktaroglu, 2009). It inhibits auto-immunity, by dropping the anti-erythrocyte antibodies and anti-DNA antibodies, and reduces the splenic autoreactive B lymphocytes. In autoimmune hypothyroidism, RJ plays an immunomodulatory role. It produces the proliferation of healthy lymphocytes and increases the blood cells and secretion of gamma-interferon, and decreases the production of other cytokines. RJ reduces the levels of antibodies against the thyroid stimulating hormone receptor (Ac-TSHR) and inhibits macrophages to generate the proinflammatory cytokine. A mixture of RJ and honey as a chemopreventive agent can reduce the genotoxic side effects of the anticancer drug cyclophosphamide (Fahmy et al., 2015).

Allergic side effects

RJ may cause allergic reactions, asthma and even fatal anaphylaxis in humans, so it remains unavailable in most countries (Dinkov & Stoyanchev, 2014). Oral administration may cause such less-severe allergic reactions as atopies, light gastrointestinal problems and such more severe as asthma, anaphylactic shock, intestinal bleeding and even death (Pavel et al., 2011). Therefore, only after an allergy test, RJ should be orally ingested as a medicine or food-ingredient. El-Aidyet al. (2015) evaluated RJ in the improvement of lung inflammation and peripheral blood leukocytes in a mouse conalbumin-induced asthma model. The incidence of asthma cascade cases has been reported to increase in sensitised and RJ-treated groups because of the immunostimulatory and vasodilatory effects of RJ, which were antagonistic to bronchial asthma cases, and increased inflammatory cells. RJ is not recommended for patients with bronchial asthma during an attack. RJ causes stronger allergies than other such bee products as pollen, honey, venom (Dinkov & Stoyanchev, 2014). People with bee products allergy such as honey, pollen, venom should not intake RJ orally (Majtan et al., 2006). Skin rashes and eczemas may be caused by pure RJ or applied ointments. More attention should be given in pregnant and lactating women and children (Bogdanov, 2014).

CONCLUSION

RJ is a natural bee product with a great potential for use in medical products, health foods. It has numerous precious therapeutic properties used from ancient times until today. In conclusion, RJ attracted scientific interest since 1852 when the first scientific studies for RJ were published and it still remains "hot" natural product. Numerous extremely interesting scientific results that appear in the international literature every year from scientific groups all over the world show that it will be kept under investigation for many more years in the future.

REFERENCES

Abdel-Hafez, S. M. N., Rifaai, R. A., & Abdelzaher, W. Y. (2017). Possible protective effect of royal jelly against cyclophosphamide induced prostatic damage in male albino rats; a biochemical, histological and immuno-histo-chemical study. *Biomedicine & Pharmacotherapy, 90*, 15-23. DOI: 10.1016/j.biopha.2017.03.020

Abdelatif, M., Yakoot, M., & Etmaan, M. (2008). Safety and efficacy of a new honey ointment on diabetic foot ulcers: a prospective pilot study. *Journal of Wound Care*, *1*(3), 108-110. DOI: 10.12968/jowc.2008.17.3.28667

Abdelnur, P. V., Abe, S., Cunha, I. B. S., Lima-Pallone, J. A., Godoy, T., Eberlin, M. N., Catharino, R. R. (2011). Metabolic fingerprinting of royal jelly: characterization and proof of authenticity. *Quality Assurance and Safety of Crops & Foods*, *3*(4), 185-190. DOI: org/10.1111/j.1757-837X.2011.00112.x

Alcay, S., Toker, M. B., Onder, N. T., & Gokce, E. (2017). Royal jelly supplemented soybean lecithin-based extenders improve post-thaw quality and incubation

resilience of goat spermatozoa. *Cryobiology*, *74*, 81-85. DOI: org/10.1016/j.cryobiol.2016.11.011

Al-Masri, A. (2011). The royal jelly, honeybee kingdom and its derivation. In Bartolome, J A A L F M P (ed.) Arabic Book House Publishers. 291-300.

Ashry, K., & Elkady, A. A. (2014). Royal Jelly Modulates Hepatotoxic Effect of Rats Treated with Cisplatin. *Arab Journal of Nuclear Science and Applications*, *47*(1), 172-180.

Bărnuţiu, L. I., Mărghitaş, L. A., Dezmirean, D. S., Mihai, C. M., Bobi, O. (2011). Chemical composition and antimicrobial activity of Royal Jelly. *Animal Science and Biotechnologies, 44*(2), 109-117.

Balkanska, R., Zhelyazkova, I., & Ignatova, M. (2012). Physico-chemical quality characteristics of royal jelly from three regions of Bulgaria. *Agricultural Science and Technology*, *4*(3), 302-305.

Balkanska, R., & Kashamov, B. (2011). Composition and physico-chemical properties of lyophilized Royal jelly. *Bee Science*, *11*(4), 114-117.

Batchelder, T. (2002). Royal Jelly: A novel mechanism of liver enhancement from a traditional bee product. *Townsend Letter for Doctors and Patients*, *233*, 46-48.

Bogdanov, S. (2014). Royal Jelly, Bee Brood: Composition, Health, Medicine. *Bee Product Science*. Available from: http://www.bee-hexagon.net/royal-jelly/harvest-composition-quality.

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. DOI: 10.1051/apido:2003011

Boukraâ, L., Meslem, A., Benhanifia, M., & Hammoudi, S.M. (2009). Synergistic effect of starch and Royal Jelly against *Staphylococcus aureus* and *Escherichia coli*. *Journal of Alternative and Complementary Medicine*, *15*(7), 755-757. DOI: 10.1089/acm.2008.0483

Boukraâ, L., Niar, A., Benbarek, H., & Benhanifia, M. (2008). Additive action of royal jelly and honey against *Staphylococcus aureus*. *Journal of Medicinal Food*, *11*(1), 190-192. DOI: 10.1089/jmf.2007.567.

Burimistrova, L., Agafonova, A., Budnikova, N., & Haritonova, M. (2008). Methods for the stabilisation of biologically active components royal jelly (Russian). *Apitherapy today, Ribnoe*. 175-182.

Carvalho, V., Silveira, V., Do-Prado, R., & Carvalho, Y. (2011). Effect of estrogen therapy, soy isoflavones, and the combination therapy on the submandibular gland of ovariectomized rats. *Pathology - Research and Practice*, *207*(5), 300-305. DOI: 10.1016/j.prp.2011.01.002. Epub 2011 Apr 21.

Cemek, M., Aymelek, F., Buyukokuroglu, M. E., Karaca, T., Buyukben, A., Yilmaz, F. (2010). Protective potential of royal jelly against carbon tetrachloride induced toxicity and changes in the serum sialic acid levels. *Food and Chemical Toxicology*, *48*(10), 2827-2832. DOI: 10.1016/j.fct.2010.07.013. Epub 2010 Jul 15.

Chan, Q.W.T., Melathopoulos, A.P., Pernal, S.F., & Foster, L. J. (2009). The innate immune and systemic response in honeybees to a bacterial pathogen, *Paenibacillus larvae*. *BMC Genomics, 10*(387), 175-188. DOI: org/10.1186/1471-2164-10-387.

Cushnie, T. P. T., & Lamb, A. J. (2005). Detection of galangin-induced cytoplasmic membrane damage *in Staphylococcus aureus* by measuring potassium loss. *Journal of Ethnopharmacology, 101*, 243-248.

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 honeybees. *Genome Research*, *16*, 1385-1394. DOI: 10.1101/gr.5012006.

Dinkov, D., & Stoyanchev, T. (2014). Antibacterial effect of Royal gelly, mix from Royal jelly and rape honey (1:100), rape and oak honeydew honeys against *Escherichia coli* (ATCC 25922). *African Journal of Pharmacy and Pharmacology*, *8*(11), 321-326. DOI: 10.5897/AJPP2012.601

Drijfhout, F. P., Kochansky, J., Lin, S., & Calderone,

N. (2005). Composition of honeybee royal jelly as detterents of the parasiticv *arroa* Mite, *Varroa destructor. Journal of Chemical Ecology*, *31*(8), 1747-64. DOI: 10.1007/s10886-005-5925-6

Dzopalic, T., Vucevic, D., Tomic, S., Djokic, J., Chinou, I., Colic, M. (2011). 3-10-Dihydroxy-decanoic acid, isolated from royal jelly, stimulates Th1 polarising capability of human monocyte-derived dendritic cells. *Food Chemistry*, *126*(3), 1211-1217. DOI: org/10.1016/j.foodchem. 2010.12.004

El-Gayar, M. H., Aboshanab, K. M., Aboulwafa, M. M., & Hassouna, N. A. (2016). Antivirulence and wound healing effects of royal jelly and garlic extract for the control of MRSA skin infections. *Wound Medicine*, *13*, 18-27. DOI: org/10.1016/j.wndm.2016.05.004

Eshtiyaghi, M., Deldar, H., Ansari, Z., & Shohreh, B. (2016). Royal jelly may improve the metabolism of glucose and redox state of ovine oocytes matured in vitro, and embryonic development following in vitro fertilization. *Theriogenology, 86*(9), 2210-2221. DOI: 10.1016/j.theriogenology.2016.07.019

Fratini, F., Cilia, C., Mancini, S., & Felicioli, A. (2016). Royal Jelly: An ancient remedy with remarkable antibacterial properties. *Microbiological Research*, *192*, 130-141. https://doi.org/10.1016/j.micres.2016.06.007

Fontana, R., Maria, A., Mendesb, B., Monson de Souza, K., Konno, Lílian Mari M. César, Osmar, M., Palma, M.S. (2004). Jelleines: a family of antimicrobial peptides from the Royal Jelly of honeybees (*Apis mellifera*). *Peptides*, *25*, 919-928.

Furukawa, S. (2008). Stimulatory effects of royal jelly on the generation of neuronal and glial cells-expectation of protection against some neurological disorders. *Foods and Food Ingredients Journal of Japan*, *213*(7), 620-626.

Galaly, S. R., Abdella, E. M., Mohammed, H. M., & Khadrawy, S. M. (2014). Effects of royal jelly on genotoxicity and nephrotoxicity induced by valproic acid in albino mice. *Beni-Suef University Journal of Basic and Applied Sciences*, *3*, 1-15. DOI: org/10.1016/j.bjbas.2014.02.001

García, M. C., Finola, M. S., & Marioli, J. M. (2010). Antibacterial activity of royal jelly against bacteria capable of infecting cutaneous wounds. *Journal of ApiProduct and ApiMedical Science*, *2*(3), 93-99. DOI: 10.3896/IBRA.4.02.3.02

Garcia-Amoedo, L.H., Bicudo, L., & Almeida-Muradian, L.B. (2002). Comparacao de metodologias para a determinacao de umidade em geleia real. *Quim Nova*, *25*, 676-679. DOI: org/10.1590/S0100-40422002000400024

Gimenez-Diaz, C., Emsen, B., Emsen, E., Kutluca, M., Koycegiz, F. (2012). Improved reproductive response of sheep in intrauterine insemination program with the use of royal jelly. *African Journal of Biotechnology, 11*(61), 12518-12521. DOI: org/10.5897/AJB12.200

Hattori, N., Nomoto, H., Fukumitsu, H., Mishima, S., Fukurawa, S. (2010). AMP N(1)-oxide, a unique compound of royal jelly, induces neurite outgrowth from PC12 cells via signaling by protein kinase A independent of that by mitogen-activated protein kinase. *Evidence-based complementary and alternative medicine*, *7*(1), 63-68. DOI: 10.1093/ecam/nem146

Hattori, N., Ohta, S., Sakamoto, T., Mishima, S., Furukawa, S. (2009). Royal jelly facilitates restoration of the cognitive ability in trimethyltin-intoxicated mice. *Evidence-Based Complementary and Alternative Medicine*, *63*(1), 57-64. DOI: 10.1093/ecam/nep029

Henrique, L., Amodeo, G., Bicudo, L., & Muradian, A. (2003). Determination of trans-10-hydroxy-2-Decenoic acid (10-HDA) in royal jelly from Sao Paulo State, Brazil. *Ciência e Tecnologia de Alimentos*, *23*, 62-65.

Henshaw, F. R., Twigg, S. M., & McLennan, S. (2014). What's the buzz: bee products and their potential value in diabetic wound healing. *The Journal of Diabetic Foot Complications, 6*(2), 24-39.

Ibrahim, A.A. El-M. (2014). Immunomodulatory effects of royal jelly on aorta CD3, CD68 and eNOS

expression in hypercholesterolaemic rats. *The Journal of Basic & Applied Zoology, 67*(4), 140-148. https://doi.org/10.1016/j.jobaz.2014.08.006

Isidorova, V. A., Czyzewska, U., Isidorovab, A. G., & Bakier, S. (2009). Gas chromatographic and mass spectrometric characterization of the organic acids extracted from some preparations containing lyophilized royal jelly. *Journal of Chromatography B, 877*, 3776-3780. DOI: org/10.1016/j.jchromb.2009.09.016

Isidorov, V. A., Czyzewska, U., Jankowska, E., & Bakier, S. (2011). Determination of royal jelly acids in honey. *Food Chemistry, 124*, 387-391. DOI: org/10.1016/j.foodchem.2010.06.044

Isidorov, V. A., 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. DOI: 10.1016/j.jchromb.2011.12.025

Ito, S., Nitta, J., Fukumitsu, H., Soumiya, H., Ikeno, K., Nakamura, T., Furukawa, S. (2011). Antidepressant-like activity of 10-hydroxy-trans-2-decenoic acid, a unique unsaturated fatty acid of royal jelly, in stress-inducible depression-like mouse model. *Evidence-based complementary and alternative medicine*, Article ID 139140, 1-6. DOI: org/10.1155/2012/139140

Izuta, H., Narahara, Y., Shimazawa, M., Mishima, S., Kondo, S., Hara, H. (2009). 1, 1-Diphenyl-2-picrylhydrazyl Radical Scavenging Activity of Bee Products and Their Constituents Determined by ESR. *Biological and Pharmaceutical Bulletin, 32*(12), 1947-1951.

Jamnik, P., Raspor, P., & Javornik, B. (2012). A Proteomic Approach for Investigation of Bee Products: Royal Jelly, Propolis and Honey. *Food Technology and Biotechnology, 50*(3), 270-274.

Joksimović, A., Stanković, D., & Joksimović, I. (2009). Royal jelly as a supplement for young football players. *Sport Science, 1*, 62-67.

Kanbur, M., Erasian, G., Beyaz, L., Silici, S., Liman, B.C., Altinordulu, S., Atasever, A. (2009). The effects of royal jelly on liver damage induced by paracetamol in mice. *Experimental and Toxicologic Pathology, 61*(2), 123-132. DOI: 10.1016/j.etp.2008.06.003

Kamakura, M., Mitani, N., Fukuda, T., & Fukushima, M. (2001). Antifatigue effect of fresh royal jelly in mice. *Journal of Nutritional Science and Vitaminology, 47*(6), 394-401. PMID: 11922114.

Karadeniz, A., Simsek, N., Karakus, E., Yildirim, S., Kara, A., Can, I., Kisa, F., Emre, H., Turkeli, M. (2011). Royal Jelly Modulates Oxidative Stress and Apoptosis in Liver and Kidneys of Rats Treated with Cisplatin. *Oxidative Medicine and Cellular Longevity*, Article ID 981793, 1-10. DOI: org/10.1155/2011/981793

Kazu-Michi, S., Yoichiro, I., Hiroe, M., Yayoi, Y., Yukio, N., Shozo, O., Yoko, A., Takeshi, M., Satoshi, M. (2008). Estrogenic Activities of Fatty Acids and a Sterol Isolated from Royal Jelly. *Evidence-Based Complementary and Alternative Medicine, 5*(3), 295-302. DOI: 10.1093/ecam/nem036

Kim, J., Kim, Y., Yun, H., Park, H., Kim, S.Y., Lee, K. G., Han, S.M., Cho, Y. (2010). Royal jelly enhances migration of human dermal fibroblasts and alters the levels of cholesterol and sphinganine in an in vitro wound healing model. *Nutrition Research and Practice, 4*(5), 362-368. DOI: 10.4162/nrp.2010.4.5.362

Kimura, M., Kimura, Y., Tsumura, K., Okihara, K., Sugimoto, H., Yamada, H. (2003). 350-kDa royal jelly glycoprotein (apisin), which stimulates proliferation of human monocytes, bears the beta1–3galactosylated N-glycan: Analysis of the N-glycosylation site. *Bioscience, Biotechnology, and Biochemistry, 67*, 2055–2058. DOI: 10.1271/bbb.67.2055

Kodai, T., Umebayashi, K., Nakatani, T., Ishiyama, K., Noda, N. (2007). Compositions of royal jelly II. Organic acid glycosides and sterols of the royal jelly of honeybees (*Apis mellifera*). *Chemical & Pharmaceutical Bulletin, 55*(10), 1528-1531. PMID: 17917301.

Koc, A. N., Silici, S., Kasap, F., Hörmet-O, H.T., Mavus-Buldu, H., Ercal, B. D. (2011). Antifungal activity of the honeybee products against *Candida* spp. and *Trichosporon* spp. *Journal of Medicinal Food*, *14*(1-2), 128-34. DOI :org/10.1089/jmf.2009.0296

Li, C., Mannoor, M. K., Toma, N., Taniguchi, T., Inafuku, M., Yamaguchi, K., Sato, Y., Watanabe, H. (2011). The efficacy of Royal Jelly in the restoration of alcoholic liver injury in mouse model. *Biomedical Research, 22*(1), 1-8.

Majtan, J., Kumar, P., Majtan, T., Walls, A. F., Klaudiny, J. (2010). Effect of honey and its major royal jelly protein 1 on cytokine and MMP-9 mRNA transcripts in human keratinocytes. *Experimental Dermatology*, *19*(8), 73-79. DOI: 10.1111/j.1600-0625.2009.00994.x

Majtan, J., Kovacova, E., Bilikova, K., & Simuth, J. (2006). The immunostimulatory effect of the recombinant apalbumin 1 - major honeybee royal jelly protein - on TNF alpha release. *International immunopharmacology*, *6*(2), 269-278. DOI: 10.1016/j.intimp.2005.08.014

Mannoor, M. K., Tsukamoto, M., Watanabe, H., Yamaguchi, K., Sato, Y. (2008). The efficacy of royal jelly in the restoration of stress-induced disturbance of lymphocytes and granulocytes. *Biomedical Research India*, *19*(2), 69-77.

Mannoor, K. M., Shimabukuro, I., Tsukamotoa, M., Watanabe, H., Yamaguchi, K., Sato, Y. (2009). Honeybee royal jelly inhibits autoimmunity in SLE-prone NZB×NZW F1 mice. *Lupus SAGE Publications Los Angeles, London, New Delhi and Singapore*, *18*, 44-52. DOI: 10.1177/0961203308094765

Mărghitaş, L.A. (2008). Produsele apicole şi principalele lor însuşiri terapeutice. In: Albinele şi produsele lor. L.A. Mărghitaş LA. Second edition. Bucharest: Ceres. pp. 280-378.

Matsubara, T., Sugimoto, H., & Aida, M. (2008). A theoretical insight into the interaction of fatty acids involved in royal jelly with the human estrogen receptor beta. *Bulletin of the Chemical Society of Japan*, *81*(10), 1258-1266. DOI: org/10.1246/bcsj.81.1258

Maqsoudlou, A., Sadeghi Mahoonak, A., Mora, L., Mohebodini, H., Toldrá, F., Ghorbani, M. (2018). Peptide identification in alcalase hydrolysated pollen and comparison of its bioactivity with royal jelly. *Food Research International, 116*, 905-915. DOI: 10.1016/j.foodres.2018.09.027

Melliou, E., & Chinou, I. (2014). Chemistry and bioactivities of royal jelly. *Studies in Natural Products Chemistry, 43*, 261-290. DOI:org/10.1016/B978-0-444-63430-6.00008-4

Miki, K., Takeshi, T., & Yoshiyuki, K. (2001). Anti-obesity action of royal jelly (RJ) on mice fed a high-fat diet. *Japan Science and Technology Agency, 63*, 78-83.

Mohebodini, H., Maqsoudlou, A., Dastar, B., & Tahmasebi, G. (2018). Royal jelly production, amount of royal jelly thiamine and hypopharyngeal glands development in Iranian honeybee (*Apis mellifera meda*) colonies fed with different levels of thiamine. *Journal of Animal Researches, 31, 152-159.*

Moriyama, H., Bagchi, M., & Bagchi, D. (2017). Royal Jelly in Medicinal to Functional Energy Drinks. *Sustained Energy for Enhanced Human Functions and Activity, Academic press,* 281-298. DOI: org/10.1016/B978-0-12-805413-0.00018-1

Moutsatsou, P., Papoutsi, Z., Kassi, E., Heldring, N., Zhaohao, C., Tsiapara, A., ... Dahlman-Wright, K. (2010). Fatty acids derived from royal jelly are modulators of estrogen receptor functions. *Plos One*, 5, 15594. DOI : org/10.1371/journal.pone.0015594

Moselhy, W. A., Fawzy, A. M., & Kamel, A. A. (2013). An evaluation of the potent antimicrobial effects and unsaponifiable matter analysis of the royal jelly. *Life Science Journal, 10*(2), 290-296.

Munstedt, K., Bargello, M., & Hauenschild, A. (2009). Royal jelly reduces the serum glucose levels in healthy subjects. *Journal of Medicinal Food, 12*, 1170-1172. DOI: org/10.1089/jmf.2008.0289

Münstedt, K., Böhme, M., Hrgovic, I., & Hauenschild, A. (2010). An approach to the application royal jelly: encapsulation of lyophilized royal jelly and its effect on glucose metabolism in humans. *Journal of ApiProduct and ApiMedical Science, 2*(1), 29-30. DOI: 10.3896/IBRA.4.02.1.03

Nabas, Z., Haddadin, M. S. Y., Haddadin, J., & Nazer, I. K. (2014 a). Chemical composition of royal jelly and effects of synbiotic with two different locally isolated probiotic strains on antioxidant activities. *Polish Journal of Food And Nutrition Sciences, 64*(3), 171-180. DOI: org/10.2478/pjfns-2013-0015

Nabas, Z., Haddadin, M. S. Y., Haddadin, J., & Nazer, I. K. (2014 b). The influence of royal jelly addition on the growth and production of short chain fatty acids of two different bacterial species isolated from infants in Jordan. *Pakistan Journal of Nutrition*, *13*, 43-49.

Nagai, T., Inoue, R., Suzuki, N., & Nagashima, T. (2008). Angiotensin I-converting enzyme inhibitory activities of enzymatic hydrolysates from royal jelly imported from China. *Journal of Food, Agriculture & Environment*, *6*(3&4), 129-132.

Nagai, T., Sakai, M., Inoue, R., & Suzuki, N. (2001). Antioxidative activities of some commercially honeys, royal jelly, and propolis. *Food Chemistry*, *75*(2), 237-240. DOI: org/10.1016/S0308-8146(01)00193-5

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. DOI: org/10.1186/1472-6882-9-4

Nakaya, M., Onda, H., Sasaki, K., Yukiyoshi, A., Tachibana, H., Yamada, K. (2007). Effect of royal jelly on bisphenol A-induced proliferation of human breast cancer cells. *Bioscience, Biotechnology, and Biochemistry, 71*(1), 253-255.

Oribe, E., Arioka, T., Fukuda, K., Tatefuji, T., Hashimoto, K., Maeda, K. (2012). Moisturizing effect of royal jelly extract and its mechanism on skin. *Journal of Dermatology*, *39*(1), 53-280.

Park, H. M., Cho, M. H., CHO, Y., & Kim, S. Y. (2012). Royal jelly increases collagen production in rat skin after ovariectomy. *Journal of Medicinal Food*, *15*(6), 568-575. DOI: org/10.1089/jmf.2011.1888

Park, H. M., Hwang, E., Lee, K. G., Han, S., M., Cho, Y., Kim, S. Y. (2010). Royal Jelly protects against ultraviolet b-induced photoaging in human skin fibroblasts via enhancing collagen production. *Journal of Medicinal Food, 14*(9), 1-8. DOI: org/10.1089/jmf.2010.1363

Parveen, A., & Rao, S. (2012). Antibacterial activity and phytochemical analysis of leaf extracts of *Aritolochia bracteolate*. *Asian Journal of Experimental Biological Sciences, 3*(3), 577-581.

Pasupuleti, V. R., Sammugam, L., Ramesh, N., & Gan, S. H. (2017). Honey, propolis, and royal jelly: a comprehensive review of their biological actions and health benefits. *Hindawi Oxidative Medicine and Cellular Longevity,* 21 pages. DOI: org/10.1155/2017/1259510

Pavel, I., Marghitash, L. A., Dezmirena, D. S., Bobis, O., Barnuliu, L., Şapcaliu, A. (2011a). Changes in royal jelly composition during storage and possible freshness parameters. *Bulletin UASVM Animal Science and Biotechnologies*, 68(1-2), 245-248.

Pavel, C. I., Mărghitaş, L. A., Bobiş, O., Dezmirean, D. S., Şapcaliu, A., Radoi, I., Mădaş, M. N. (2011b). Biological activities of royal jelly. *Animal Science and Biotechnologies*, *44*(2), 108-118.

Popescu, O., Marghitasl, A., & Dezmireand, L. (2008). A study about physicochemical composition of fresh and lyophilized royal jelly. *Zootehnie şi Biotehnologii, 41*(2), 328-332.

Pourmoradian, S., Mahdavi, R., Mobasseri, M., Faramarzi, E., Mobasseri, M. (2012). Effects of royal jelly supplementation on body weight and dietary intake in type 2 diabetic females. *Health Promotion Perspectives*, *2*(2), 231-235. DOI: 10.5681/hpp.2012.028

Pyrzanowska, J., Piechal, A., Blecharz-klin, K., Graikou, K., Widy-Tyszkiewicz, E., Chiinou, I. (2012). Chemical

analysis of Greek royal jelly - Its influence of the long-term administration on spatial memory in aged rats. *Planta Medica Journal, 78*(11), 1248-1253. DOI: 10.1055/s-0032-1321154

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

Romanelli, A., Moggio, L., Montella, R. C., Campiglia, P., Iannaccone, M., Capuano, F., Pedone, C., Capparelli, R. (2011). Peptides from royal jelly: studies on the antimicrobial activity of jelleins, jelleins analogs and synergy with temporins. *Journal of Peptide Science, 17*(5), 348-352. DOI: 10.1002/psc.1316

Sabatini, A. G., Marcazzan, G. L., Caboni, M. F., Bogdanov, S., Almeida-Muradian, L. B. DE. (2009). Quality and stadarisation of royal jelly. *Journal of ApiProducts and ApiMedical Sciences*, *1*(1), 16-21. DOI: 10.3896/IBRA.4.01.1.04

Sabatini, A. G., Marcazzan, G., Caboni, M. F., Bogdanov, S., Almedia-Muradian, L. B. (2009). Quality and standardisation of royal jelly. *Journal of Analytical Atomic Spectrometry, 1,* 1-6. DOI: 10.3896/IBRA.4.1.01.04

Salazar-Olivo, L. & Paz-González, V. (2005). Screening of biological activities present in honeybee (*Apis mellifera*) royal jelly. *Toxicology in Vitro, 19*, 645–651.

Salem, F. (2013). Modulatory effects of *Trifolium pratense* extract and royal jelly on the function of hypothalamic-pituitary-ovarianaxis in ovariectomized rates. *International Journal of Pharmacy and Pharmaceutical*, *5*(3), 593-597.

Schonleben, S., Sickmann, A., Mueller, M. J., & Reinders, J. (2007). Proteome analysis of *Apis mellifera* royal jelly. *Analytical and Bioanalytical Chemistry*, *389*, 1087-1093. DOI: 10.1007/s00216-007-1498-2

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), 10-16. DOI: 10.7860/JCDR/2016/17844.7715

Sesta, G. & Lusco, L. (2009). Refractometric determination of water content in royal Jelly. *Apidologie, 39*, 225-232.

Shahzad, Q., Mehmoodb, M. U., Khan, H., Husna, A., Qadeerd, S., Azam, A., Naseere, A., Ahmad, E., Safdar, M., Ahmad, M. (2016). Royal jelly supplementation in semen extender enhances post-thaw quality and fertility of Nili-Ravi buffalo bull sperm. *Animal Reproduction Science*, *167*, 83-88. DOI: 10.1016/j.anireprosci.2016.02.010

Shen, L.R., Ding, M.H., Zhang, L.W., Jin, F., Zhang, W.G., Li, D. (2010). Expression of Acc-Royalisin gene from royal jelly of Chinese honeybee in *Escherichia coli* and its antibacterial activity. *Journal of Agricultural and Food Chemistry*, *58*(4), 2266-2273.

Shirzad, M., Kordyazdi, R., Shahinfard, N., & Nikokar M. (2013). Does Royal jelly affect tumor cells? *Journal of Herb Med Pharmacology, 2*(2), 45-48. DOI: 10.1021/jf902574t

Siavash, M., Shokri, S., Haghighi, S., Mohammadi, S., Shahtalebi, M. A., Farajzadehgan, Z. (2011). The efficacy of topical RoyalJelly on diabetic foot ulcers healing: A case series. *International Journal of Research in Medical Sciences*, *16*(7), 904-909. PMID: 22279458.

Siavash, M., Shokri, S., Haghighi, S., Mohammadi, S., Shahtalebi, M. A., Farajzadehgan, Z. (2015). The efficacy of topical royal jelly on healing of diabetic foot ulcers: a double-blind placebo-controlled clinical trial. *International Wound Journal*, *12*(2), 137–142. DOI: 10.1111/iwj.12063

Silici, S., Ekmekcioglu, O., Kanbur, M., & Deniz, K. (2010). The protective effect of royal jelly against cisplatin-induced renal oxidative stress in rats. *World Journal of Urology*, *29*(1), 127–132. DOI: 10.1007/s00345-010-0543-5

Simsek, N., Karadeniz, A., & Bayraktaroglu, A. G. (2009). Effects of L-carnitine, royal jelly and

pomegranate seed on peripheral blood cells in rats. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, *15*(1), 63-69.

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

Sugiyama, T., Takahashi, K., Kuzumaki, A., Tokoro, S., Neri, P., Mori, H. (2013). Inhibitory mechanism of 10 hydroxy trans 2 decenoic acid (royal jelly acid) against lipopolysaccharide and interferon beta induced nitric oxide production. *Inflammation*, *36*(2), 372-378. DOI: 10.1007/s10753-012-9556-0

Suzuki, K. M., Isohama, Y., Maruyama, H., Yamada, Y., Narita, Y., Ohta, S., ... Mishima, S. (2008). Estrogenic activities of fatty acids and a sterol isolated from royal jelly. *Evidence-Based Complementary and Alternative Medicine, 5*(3), 295-302. DOI: 10.1093/ecam/nem036

Takaki-Doi, S., Hashimoto, K., Yamamura, M., & Kamei, C. (2009). Antihypertensive activities of royal jelly protein hydrolysate and its fractions in spontaneously hypertensive rats. *Acta medica Okayama*, *63*(1), 57-64. DOI: 10.18926/AMO/31859

Tamura, S., Kono, T., Harada, C., Yamaguchi, K., & Moriyama, T. (2009). Estimation and characterisation of major royal jelly proteins obtained from the honeybee *Apis merifera*. *Food Chemistry*, *114*, 1491-1497. DOI: org/10.1016/j.foodchem.2008.11.058

Teixeira, R. R., Souza, A. V., Peixoto, L. G., Machado, H. L., Caixeta, D. C., Vilela, D. D., ... Espindola, F. S. (2017). Royal jelly decreases corticosterone levels and improves the brain antioxidant system in restraint and cold stressed rats. *Neuroscience Letters.* 17 pages. DOI: 10.1016/j.neulet.2017.07.010

Tatsuhiko, T., Naoko, K., & Yuko, H. (2011). Application of the material of honeybee origin. Application of the consmetic material of the honeybee origin (Japanese). *Frag Journal*, *30*, 17-24.

Terada, Y., Narukawa, M., & Watanabe, T. (2011). Specific hydroxy fatty acids in royal jelly activate TRPA1. *Journal of Agricultural and Food Chemistry, 59*, 2627-2635. DOI: 10.1021/jf1041646

Trombetta, D., Castelli, F., Sarpietro, M. G., Venuti, V., Cristani, M., Daniele, C., ... Bisignano, G. (2005). Mechanisms of antibacterial action of three monoterpenes. *Antimicrobial Agents and Chemotherapy,* 2474-2478.

Viuda-Martos, M., Ruiz-Navajas Y., Fernández-López, J., & Pérez-Alvarez, J. A. (2008). Functional properties of honey, propolis, and royal jelly. *Journal of Food Science*, *73*(9), 117-24. DOI: 10.1111/j.1750-3841.2008.00966.x

Wei, W., Wei, M., Kang, X. J., Deng, H. H., Lu, Z. H. (2009). A novel method developed for acetyl choline detection in royal jelly by using capillary electrophoresis coupled with electrogenerated chemiluminescence based on a simple reaction. *Electrophoresis*, *30*(11), 1949-1952. DOI: 10.1002/elps.200800721

Wongcha, 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.

Wu, L., Chen, L., Selvaraj, N. J., Wei, Y., Wang, Y., Li, Y., Zhao, J., Xue, X. (2015). Identification of the distribution of adenosine phosphates, nucleosides and nucleobases in royal jelly. *Food Chemistry, 173*, 1111-1118. DOI: 10.1016/j.foodchem.2014.10.137

Xue, X. F., Zhou, J. H., Wu, L. M., Fu, L. H., Zhao, J. (2009). HPLC determination of adenosine in royal jelly. *Food Chemistry*, 115, 715-719. DOI:org/10.1016/j.foodchem.2008.12.003

Yamaura, K., Tomono, A., Suwa, E., & Ueno, K. (2013). Topical royal jelly alleviates symptoms of pruritus in a murine model of allergic contact dermatitis. *Pharmacognosy Magazine*, *9*(33), 9-13. DOI: 10.4103/0973-1296.108127

Yang, X .Y., Yang, D. S., Wei, Z., Wang, J. M., Li, C.Y., Ye, H., ... Wang, J. G. (2010). 10-Hydroxy-2-decenoic acid from royal jelly: a potential medicine for RA. *Journal*

of Ethnopharmacology, *128*(2), 314-321. DOI: 10.1016/j.jep.2010.01.055

Zamami, Y., Takatori, S., Goda, M., Koyama, T., Iwatani, Y., Jin, X. (2008). Royal jelly ameliorates insulin resistance in fructose-drinking rats. *Biological and Pharmaceutical Bulletin*, *31*(11), 2103-2107. PMID: 18981581

Zheng, H. Q., Wei, W. T., Wu, L. M., Hu, F. L., Dietemann, V. (2012). Fast determination of royal jelly freshness by a chromogenic reaction. *Journal of Food Science*, *77*(6), 247-252. DOI: 10.1111/j.1750-3841.2012.02726.x

Supplementary Table 1. Fatty Acids found in RJ

Fatty acids	References
Eptanedioic acid, Hexanedioic acid, Nonanedioic acid, 6-Hydroxydecanoic acid, 9-Hydroxynonanoic acid, 3-hydroxyundecanoic acid, 5,10-dihydroxydecanoic acid	Garcia-Amoedo et al., 2002.
10,11-Dihydroxydodecanoic acid	Moselhy et al., 2013; Isidorov et al., 2011, 2012.
5,10-Dihydroxydecanoic acid, 8,9-dihydroxydecanoic acid, 3-hydroxydecanedioic acid, 1,12-dodecanedioic acid, 7-hydroxy-2-octenoic acid, 3,10-dihydroxydodecanoic acid, 8-hydroxy-2-octenoic acid, 2-octene-1,8-dioic acid, 8-hydroxy-2-decenoic acid, (Z)-9-hydroxy-2-decenoic acid, (Z)-9-HDA, 3,9-dihydroxydecanoic acid, 8,9 dihydroxydecanoic acid, 10-hydroxy-2-dodecenoic acid, 11-hydroxy-2-dodecenoic acid, 3-hydroxydecanedioic acid, 1,12-dodecanedioic acid, 10,12-dihydroxydodecanoic acid, (E)-9-Hydroxy-2-decenoic acid, (E)-9-HDA, 9-hydroxydecanoic acid	Isidorov et al., 2011, 2012; Garsia-Amoedo et al., 2002.
Suberic acid (octanedioic acid), 7-Hydroxyoctanoic acid, 2-Decene-1,10-dioic acid	Isidorov et al., 2011.
12-Hydroxydodecanoic acid, 12-hydroxy-2-dodecenoic acid, 3-hydroxyoctanoic acid, 13-hydroxytetradecanoic acid, 2-dodecene-1,12-dioic (traumatic) acid, 10-hydroxydodecanoic acid, 12-hydroxydodecanoic acid, 12-hydroxy-2-dodecenoic acid, 11-hydroxyundecanoic acid, (E)-9-Hydroxy-2-decenoic acid, (E)-9-HDA, 9-hydroxydecanoic acid	Isidorov et al., 2011, 2012.
10-Hydroxydecanoic acid, 10-HDAA, (E)-10-hydroxy-2-decenoic acid, 10-HAD, 8-Hydroxyoctanoic acid, 3,12-dihydroxydodecanoic acid, 3,13-dihydroxytetradecenoic acid	Moselhy et al., 2013; Isidorov et al., 2011; Ferioli et al., 2007.
10-Acetoxy-2-decenoic acid, 10-acetoxydecanoic acid, 3-hydroxydecanoic acid methyl ester, 11-oxododecanoic acid	Moselhy et al., 2013; Pavel et al., 2014.
Butyric acid, octanoic acid, oleic acid, succinic acid, octadecanoic acid, eicosanoic acid, tetracosanoic acid, hexadecanoic acid, 7-oxooctanoic acid, 3-methyl-3-hydroxyglutaric acid, 9,10-dihydroxy-2-decenoic acid, 9,10-dihydroxydodecanoic acid, 9-oxo-2-decenoic acid (9-ODA), 11,12-dihydroxy-2-dodecenoic acid, 13-hydroxy-2-tetradecenoic acid, 2 hydroxyoctanoic acid, 3-methyl-3-hydroxyglutaric acid, 14-hydroxytetradecanoic acid	Isidorov et al., 2011; Amoedo et al., 2002.
Sebacic acid (decanedioic acid), 3,10-dihydroxydecanoic acid; 3,11-Dihydroxydodecanoic acid, 3-hydroxydecanoic acid, 3-HDA, 11,12-dihydroxydodecanoic acid, 3-hydroxydodecanedioic acid, 11-hydroxydodecanoic acid	Moselhy et al., 2013; Dzopalic et al., 2011; Isidorov et al., 2012; Pasupuleti et al., 2017.
(E)-9,10-dihydroxy-2-decenoic acid	Tani et al., 2009.

Supplementary Table 2. Content of bio-active compounds of RJ and their effects on health

Bioactive compounds	Content	effects on health	References
Acetylcholine	1 mg/g dry weight,	Having a number of hormone-like effects in the central and vegetative nervous System, It is a nerve transmitter	Wei et al., 2009.
Proteins and peptides	9–18%	Anti-oxidative, immuno-modulating, mono-cyte-proliferation stimulating by the 350 kDa proteins with N-terminal amino acid sequence as apalbumin-α, antibacterial (royalisin, apisimin, jelleines I, II, III,IV, apalbuminaα), Anti-inflammato-ry, vitalisation and anti-fatigue, anti-hypertensive, anti-allergic, antidiabetes, collagen proliferating and skin fibroblast differentiating, Maintaining of the viability of rat primary cultured cells by apalbumin-β	Barnutiu et al., 2011; Takaki-Doi, 2009; Henshaw et al., 2014; Kim et al., 2010
Hormones, testosterone, progesterone, prolactine, estradiol	11.63 (ng/g) 36.16(ng/g lyophilized RJ)	Increasing of fertility and male power and endurance; Preventive effect against osteoporo-sis and amelioration of menopausal disorder.	Carvalho et al., 2011; Salem, 2013; Shirzad et al., 2013; Abdelatif et al., 2008; Takaki-Doi, 2009.
HDA (organic acids)	1.4- 3.5 %	Immuno-modulating by affecting dendritic cells, anti-cancer, collagen synthesis-inducing and MMP[1]-inhibitory activities and skin protecting, facilitates differentiation of brain cells, promotes endothelial health, antihypertensive, antihyperlipoidemia, an-ti-rheumatic, antibacterial and immuno activating, anti-diabetes, estrogenic effects, antidepressant in mice experiments, Increasing of neurogenesis and decreasing of glial generation, Alkylation of Cancerostatic 5-Fluorouridine	Mannoor et al., 2009; Takaki-Doi, 2009; Dzopalic, 2011; Sugiyama et al., 2013; Ito et al., 2011; Izuta et al., 2009; Matsubara et al., 2008; Moutsatsou et al., 2010; Yang et al., 2010; Park et al., 2010; Yang et al., 2010; Ramadan & Al-Ghamdi, 2012; Terada et al., 2011; Barnutiu et al., 2011; Ito et al., 2011; Ottenhaus & Rosemeyer, 2015; Pavel et al., 2014.
(Adenosine monophos-phate) AMP-N1 Oxide	Unknown	(A compound found only in RJ) It promots generation of all types of cells composing the central nervous system and stimulates neuronal differentiation.	Hattori et al., 2010; Xue et al., 2009; Park et al., 2010; Ito et al., 2011.
Polyphenols	23.3 (µg galic acid/ mg)	Antioxidant effect	Nabas et al., 2014b; Lopez-Gutierrez et al., 2014; Pasupuleti et al., 2017
Flavonoids	1.28 (µg rutin/mg)	Antioxidant effect	Nabas et al., 2014 b; Pasupuleti et al., 2017
Adenosine	5.9 to 2057.4 mg/kg	Hyperpolarising effect on the membrane potential of excitable cells, producing inhibition in vascular.	Xue et al., 2009; Xue et al., 2009; Wu et al., 2015; Kim & Lee ,2011

[1] Matrix Metalloproteinases (MMPs): The MMPs make up a family of structurally related matrix-degrading enzymes involved in tissue-destructive processes such as aging of the skin, arthritis, and tumor invasion. MMPs also degrade collagen and other extracellular matrix proteins (Park et al., 2010).

Supplementary Table 3. Criteria for RJ freshness

Criteria	Impact on freshness of RJ	References
Glucose oxidase enzyme	Decrease by increasing of storage temperature and time	Boselli et al., 2003; Abdelnur et al., 2011.
10-HAD	Decrease by increasing of storage time and temperature (It is difficult to use 10-HDA decrease as a freshness marker, because of its variable amount in fresh RJ)	Pavel et al., 2011; Henrique et al., 2003; Siavash et al., 2011.
Furosine	A product of Maillard's reaction, increased very low (from 0 to 10 mg/100g of protein) in freshly produced RJ samples, but increases quickly over time and in relation to temperature. (A limit of 50 mg furosine /100g protein could be used for fresh RJ)	Pavel et al., 2011; Wytrychowski et al., 2014.
proteins	Decrease by increasing of storage temperature and time. Modifications of the water-soluble proteins play an important role in the quality deterioration of RJ, and the enzymatic activity. The total amount of free amino acids of RJ stored at room temperature was also found to be decreased with time.	Kamakura et al., 2001; Zheng et al., 2012; Boselli et al., 2003; Pavel et al., 2011.
Titratable acidity	It increases with time of storage and temperature (Millard reaction or lipid oxidation)	Pavel et al., 2011.
Adenosine triphosphate	The concentrations of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (HxR), hypoxanthine (Hx), adenosine (Ao), and adenine (Ai) in RJ stored were measured at different temperatures and for different storage periods. Ratio between the sum of Ao, Ai, HxR, and Hx and the sum of ATP, ADP, AMP, IMP, HxR, Hx, Ao, and Ai defined as an F-value evaluated as an indicator of RJ freshness. TheF-value increases during storage and has good linear correlations with storage temperature and time.	Wu et al., 2015a; Wu et al., 2015b.