Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 7977 - 7997
Received 16 February 2021; Accepted 08 March 2021.

# Quality Parameters of Royal Jelly in national and international standards: Specifications, differences and suggestions

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**ABSTRACT**

Royal Jelly is one of the most important beehive products, which has been known for centuries, known as "Honeybee's milk" or "youth elixir" due to its high nutritional value and numerous health promoting properties, RJ is secreted by the hypopharyngeal and mandibular glands of young worker bees (*Apis mellifera*). The chemical composition of Royal jelly varies according to different factors including but not limited to honeybee races, geographical origins, harvesting seasons, foraging sources and feed supplementation offered by beekeepers. Lack of National quality standards for royal jelly in many countries and the variation between the requirements of different national standards for royal jelly in others has a negative impact on the beekeeping industry because it may impede the trade of royal jelly between countries with different standards for RJ and discourages producers and beekeepers of expanding their businesses. This research article presents a detailed comparison between the quality parameters and requirements imposed by the national standards for royal jelly in Egypt compared with the international standards for RJ which was recently issued in 2016 by the international organization for standardization (ISO 12824:2016). We also investigated the chemical composition and quality parameters of Egyptian royal jelly samples (n=9) which was produced and analyzed during the honey flow seasons in Egypt. This article aims to highlight the differences between national and international standards for Royal jelly, and shows the necessity to update the national dated standards, states and suggests the adoption of some modern methods for determining authenticity, origin and freshness, which are applicable, cheap, fast and reliable.

**Keywords**
Royal Jelly, quality, National Standards, ISO, 10-HDA; Furosine ; melissopalynology

## 1. Introduction

Royal jelly is a highly nutritious substances that is produced by the hypopharyngeal and mandibular glands of young worker honeybees (*Apis mellifera*), all honeybee larvae are being fed in their first three days on royal jelly, only the honeybee queen is being nurtured on RJ solely throughout her entire life span **(Ramadan MF et al., 2012; Johansson TSK, 1955)[44, 28]**, despite that the workers and queen honeybees are genetically identical, such miraculous food provides the queen honeybee with all required nutrients to increase her body size, extends her age and life span and becomes sexually mature to lay several thousand eggs everyday for many years (**Maghsoudhou A. et al., 2019; Kamakura M., 2011; Spannhoff A. et al., 2011; Bogdanov S., 2012)[38, 29, 48, 11]**.

Royal jelly has many health promoting effects, such as antiaging, antioxidant, anti inflammatory, anti diabetic, immunomodulatory, neurotrophic, hypotensive, and general tonic, these biological effects are attributed to the presence of many active ingredients in RJ such as 10-hydroxy-2-decenoic acid (10-HDA), active peptides such as major Royal Jelly proteins (MRJPs), Royalactin, Steriods, acetyl choline and other compounds which might be of high importance in modern medicine for the development of new drugs **(Maghsoudhou A. et al., 2019; Khazaei M., et al.,2017; Bogdanov S., 2017; El-Guendouz S. et al.,2020)[38, 33, 12, 19].**

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The purified Royal Jelly proteins (MRJP2) and its isoform (MRJP2 XI) have shown antiviral effect against enveloped RNA Viruses such as Hepatitis C Virus (HCV) and Human Immuodeficiency Virus (HIV), studies suggest that MRJPs may be effective against the newly emerging Severe Acute Respiratory Syndrome-Coronavirus (SARS-CoV-2) as they are supposed to be efficient in preventing virus cell attachment due to interaction with binding sites of the virus, also these proteins may prevent viral health complications in the lungs **(Habashy NH et al., 2020)[22]**., further investigations and clinical trails are required to confirm such mechanisms, many authors reported that apicultural products including royal jelly, honey, propolis and other products are potential promising sources of pharmaceuticals and nutraceuticals for the treatment or prophylaxis of the coronavirus (COVID-19) pandemic **(Lima WG et al, 2020)[36]**.

The demand for royal jelly is growing worldwide, RJ is an internationally traded commodity which is being used as a dietary supplement and an ingredient in the food, cosmetics and pharmaceuticals industries, this emphasizes the need to establish and adopt consistent quality parameters and standards within producing countries **(Mureşan CL et al., 2016**)**[42]**.

The main forms of pure royal jelly in the market are Fresh frozen RJ and lyophilized RJ, also RJ could be used in combination with other honeybee products, mainly honey and bee pollen, and also added to different consumer ready products including yoghurt, jellies and soft caramels, juice preparations **(Bogdanov S., 2017; Clarke M. et al., 2017)[12, 15]**.

In Some Asian countries like Japan, Royal jelly is being added to beverages including soft and medicinal energy drinks **(Moriyama H. et al., 2017)[41]** which are popular among athletes, labor intensive workers and younger generations to revitalize strength and enhance stamina and vitality (**Moriyama H. et al., 2017)[41]**. Royal jelly is being used as an ingredient in many cosmetics and skin creams for the treatment of wounds and burns with other honeybee products such as beeswax **(Bogdanov S., 2017)[12]**.

Market Data provided from a range of unofficial sources shows that the global production of royal jelly have increased six fold in about three decades (between 1984 and 2012), from about 700 tons o about 4000 tons per annum **(Clarke M. et al., 2017)[15]**, (unfortunately, we couldn't find an official market data. as RJ was not included in the Database of **(FAO stat)[4]** which is provided by the Food and Agriculture Organization (FAO) about the agricultural and food production statistics in each country, but statistics about honey and beeswax are included for each country). China is the undisputed world main producer and exporter of royal jelly producing several thousand tons per year; other major producers are Vietnam, Taiwan, Japan and Korea **(Clarke M.et al., 2017)[15]**.

The Chinese beekeeping industry was the most efficient during the last 3 decades, and it made a notable contribution to the global RJ production, consequently the prices of RJ became more reasonable. the prices of RJ have dwindled since the early days of RJ production in late 50th and early 60th of the twentieth century, in that time, prices were very volatile and the quantity produced was considered very little compared with the existing demand **(Inoue T. et al, 1964) [25],** unsurprisingly, until early 60th of the last century, RJ prices were considered extremely expensive and unaffordable for the majority of middle class consumers.

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**1.1 The commercial production of Royal jelly**

The royal jelly could be produced in commercial quantities by stimulating queenless colonies with movable frames to produce RJ for feeding queen Honeybees larva, Simply, the process is carried out by grafting the 24 hours old honeybee larvae into the artificial queen cells, introducing the frame containing grafted larvae into the colony, harvesting the deposited RJ from queen cups after 48 to 72 hours of grafting **(Bogdanov S., 2012**)**[11]**, RJ is harvested from queen cells which are the only cells containing relatively large quantities of deposited RJ (500 – 600 mg) per queen cup **(Isidorov VA et al., 2012)[26]**.

China has implemented a well developed system for maximizing the commercial production ofRJ which is characterized by many unique features throughout the RJ production cycle, which includes maintaining high yield strain of honeybee through selection over generations, introduction of New equipments (queen cup plastic strips for collecting high amount of RJ from grafted larvae), prolonging the RJ production period by keeping the honeybee colonies in a flourishing phase as long as possible, transferring the larvae from worker cells to queen cups during grafting at the right age which is no more than 24 hours, as well implementing supplementary feeding programs to the colonies, providing a huge number of worker larvae in the RJ producing colonies through using assistant honeybee colonies **(Chen S. et al., 2002; Jianke L. et al., 2005; Bogdanov S., 2012)[14, 27, 11]**.

In 1984, the Production of RJ for one colony over 3 days (72 Hour period) was about 20 g, where after implementing these high yielding production methods, average RJ production per colony over 3 days reached 150 g (maximum was more than 200 g). **(Chen S. et al., 2002)[14]**.

The Chinese beekeepers provide artificial feeding programs to honeybees which usually consistof sugar syrups, isolated soy protein, brewers yeast, palm oil and linseed oil.

**1.2 Royal Jelly "Authentic versus Commercial" or "Quantity Quality tradeoff" Dilemma**

Despite of all such efforts which were exerted to achieve the highest possible quality practices concerning different steps of production, harvesting, conservation and trade, still there is a furious debate between researchers and experts regarding the commercial production practices of RJ which is widely used nowadays, such controversy arises due to the artificial feeding or supplementation of sugar syrups and protein supplements (pollen substitutes) to feed honeybee colonies and increase RJ production, we will present the different view regarding the commercial honeybee feeding in details.

In order to produce commercial quantities of RJ, honeybee colonies require a diet rich in sugars, vitamins, fatty acids, minerals and all amino acids, since the amount of stored pollen is very limited and inadequate, so protein supplementation is very crucial in commercial production of Royal jelly **(Sereia MJ et al., 2013; Sahinler N. et al., 1997)[47, 46]**.

The Chinese beekeeping industry which is the largest producer and exporter of RJ in the world adopts and supports the use of artificial honeybee feeding and supplementation in commercial RJ production, such practice is one of the main success factors of commercial RJ production in china

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and indispensable part of the commercial RJ production process in china. **(Chen S. et al., 2002; Jianke L. et al., 2005)[14, 27]**.

On the contrary, there are many critics of such commercial beekeeping practices, for instance, Marine Wytrychowski and her Research team believe that authentic RJ originates from natural feeding of bees on nectar and pollen, and the only allowed supplements are honey and pollen **(Wytrychowski M. et al, 2013)[49]**. They clearly stated that "Natural Royal jelly is produced by the transformation of nectars and pollen collected in the environment of the hives during the foraging activity of the honeybees", according to them artificial feeding on cheap sugar syrups andproteins is some sort of temptation or adulteration, They even carried out a huge research for the French ministry of Agriculture to construct a RJ Database and detect the commercial RJ that originates from artificial feeding of honeybees **(Wytrychowski M. et al, 2013)[49]**, the French production of Royal jelly was estimatedto be only 1 to 2 tons annually.

Such raging controversy between the previous contradicting views has evoked some Countries to control the artificial honeybee feeding practices in their national standards, for example the national standards of RJ in Brazil have considered both views and adopted a balanced view that maintained commercial production while ensuring safety and quality at the same time, as they didn't prohibit artificial supplementation to honeybees colonies completely during RJ production, provided that such supplementation Doesn't alternate the physicochemical composition and the final characteristics of RJ, consequently protecting the health of the consumers and honeybees **(Sereia MJ et al., 2013)[47]**.

### 1.3 Lack of Common and Consistent Royal Jelly standards around the World

The chemical composition of Royal jelly varies according to different factors, such as Honeybee races, floral origin, age of bee larva, beekeeping practices (including the supplementary feeding practices and age at which RJ was harvested from grafted larva, geographical and environmental conditions **(Balkanska R. et al., 2013; Brouwers EVM et al., 1987; Zheng HQ et al., 2011)[10, 13, 50]**.

The growing demand for RJ in the last few decades had urged some countries to establish their own national standards that contain specifications and quality criteria for this miraculous product, the first country that established national standards for royal jelly was Argentina in 1979, Some countries have established their national standards such as Bulgaria, Turkey, Brazil, Japan, China, Switzerland. **(IHC publications; Kanelis D. et al, 2016)[6, 31]** while other countries didn’t impose any national standards for RJ until now, however, these national standards are inconsistent, as there are many differences which exist between the ranges imposed by these standards, which impede the trade of royal jelly, also dated standards fail to ensure the authenticity of RJ and cope with new methods of adulteration.

In an attempt to alleviate the barriers that impede the trade of royal jelly between countries, the International Honey Commission **(IHC)[5]** have published a research paper proposing the specifications of fresh and freeze dried RJ (lyophilized) in 2009 through their experts **(IHC publications; Sabatini AG et al., 2009)[6, 45]**,while recently in September 2016, the international organization for standardization (ISO) have issued its first version for the Royal jelly specifications **(ISO 12824:2016)[7]** which specified the production and sanitary requirements for

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fresh royal jelly and established a series of organoleptic and chemical test methods to control royal jelly quality criteria, moreover, it specified the requirements for proper transport, storage, packaging and marking of royal jelly that shall be discussed later.

### 1.4 The chemical Composition and quality parameters of Egyptian Royal Jelly

Beekeeping was an indispensable and intrinsic part of the ancient kingdom of Egypt, which had the longest documented history of beekeeping and use of honeybee products **(Al Naggar Y. et al, 2018; Gupta et al., 2014)[8, 21],** that can be traced back to king Menes, who was very curious about his bees so he was called "the beekeeper", Ancient Egyptians reared their pure honeybee race (*Apis meliferra lamarckii*) in mud hives, this indigenous honeybee race still exist in isolated Siwa oasis and being reared in traditional mud hives until now, moreover, In Egypt nowadays, there are 2 common honeybee races, the carniolan race, (*Apis mellifera carnica*) , and the Italian race, (*A. m.ligustica*) and their hybrids **(Al Naggar Y. et al, 2018; Elaidy WKM et al, 2017)[8, 18]**.

According to the published Egyptian official statistics **(CAPMAS, 2019)[1]** in 2017, there were about 821 thousand beehives in Egypt producing about 4147 tons of honey and 132 tons of beeswax, however, no official statistics about the quantity of produced royal jelly.

The aim of this study is to reveal and assess the chemical composition and quality parameters of the Royal Jelly which was produced in the three main honey flow seasons of Egypt in the light of national and international standards, firstly, we should present and compare between the ISO standards **(ISO 12824:2016)[7]** with the current Egyptian national standards for RJ **(ES: 2914/2005)[2]**, and highlight the main differences between them, It worth's to mention that Egypt has introduced its first version of national standards for RJ in 1995, which was abolished and replaced by the current version in 2005 **(ES: 2914/2005)[2]**, However this standard is still voluntary (not obligatory for beekeepers or producers of RJ to comply with), contrarily, the national standards for honey in Egypt **(ES: 355-1/2005)[3]** is mandatory, and all honey producers and processors in Egypt must comply with it.

We may recognize many differences between the chemical quality parameters of the Egyptian and ISO standards, the minimum limit of 10-HDA is higher in the ES versus ISO (2 vs 1.4 %), while the maximum water content is lower in ES than ISO (66 vs 68.5 %), also the minimum requirement for protein is higher in ES than ISO (12 vs 11 %).

On the other hand we can notice that ES have only determined the total sugar content without setting any value regarding the individual sugars included, while fructose and glucose are considered the major sugars, other minor sugars such as sucrose, isomaltose, ,erolose, maltose, maltotriose and others could be very useful in verifying the genuineness of RJ, especially to detect the of supplementation of colonies with $C_3$ syrups as cereal starch hydrolysates and others **(Wytrychowski M. et al., 2013; Sabatini A.G. et al., 2009)[49, 45]**.

Also the Egyptian standards didn't mention the use of Carbon isotopes ($C^{13}$ / $C^{12}$ isotope Ratio) for detecting the use of sugarcane or Corn syrup or in feeding the honeybees.

A very promising and reliable indicator was also mentioned in ISO standards for detecting the freshness of royal jelly, which is furosine, unfortunately no limits had been set yet, however the International Honey commission (IHC) had proposed a furosine content of less than 50 mg per 100 gm protein in fresh RJ samples.

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**Table 1.** comparison between the Specifications of Royal jelly in ES and ISO

| | Egyptian standards of RJ | ISO standards of Royal Jelly |
|---|---|---|
| **Definition and Product Description** | One of the honeybee products produced by honeybee colonies which is known synonymously as bee's milk, secreted from the hypopharyngeal and mandibular glands of worker bees.<br>Fresh Royal jelly is a gelatinous thick substance creamy or white in color, Consistent due its colloidal nature, forms a suspension in water. Free from any additives, preservatives and coloring agents. The constituents of RJ as follows: water, protein, lipids, reducing sugars, vitamins, minerals and its salts, other compounds acetyl choline, Adenosine triphosphate, Adenosine diphosphate and hormones. | Mixture of secretions from hypopharyngeal and mandibular glands of worker bees.<br>Milky white or pale yellow, with luster Pasty or jelly like at room temperature with fluidity, minor crystallization may occur naturally in royal jelly during storage, RJ shall be free from bubbles and foreign substances. |
| **Royal Jelly types** | the Egyptian standards recognized the use of supplementary feeding for bee colonies, but it didn't set any differences in the required physicochemical properties and quality parameters of RJ produced from honeybee colonies, whether supplemented or not. | The ISO standard distinguishes RJ into 2 types, type1 RJ which is produced by bee's fed with only bee's natural food (pollen, nectar and honey), while type 2 RJ is produced by bee's natural food and other nutrients (proteins, carbohydrates, etc…). |
| **10–Hydroxy-2-Decenoic acid** | The main quality parameter and Characteristic Material of Royal jelly. | |
| **Color** | Creamy or white in color. | Milky white, pale yellow in color. |
| **Odor and taste** | Pungent sour taste. | Pungent, unfermented, and shall not be rancescent, spicy, brings acerb taste to palate and mouth. |
| **Packaging of Royal jelly** | Packaging in contact with royal jelly shall be of a food grade.<br>ES stipulated that containers shall be non light transmitting, brown or blue in color, It is recommended to be completely filled with royal jelly entirely to avoid the effect of air on Royal jelly constituents. | |
| **Storage Requirements** | Short term storage, storage at -4 °C for a Maximum duration of 2 months only.<br>Long term storage, storage at -18 °C for a maximum duration of 12 months.<br>No instructions are included for storage during transportation. | The temperature for storage shall be between +2 and +5 °C Or preferably, less than -18 °C for long term storage.<br>RJ shall be transported at low temperature and shall not be stored and transported with toxic, corrosive material or material with peculiar smell or that might cause contamination. |

**Table 2.**The required information that shall be marked on each package or a label in each of the standards

| Marking Requirements | ES | ISO |
|---|---|---|
| **The following information shall be marked on each package or on a label (minimum)** | | |
| The Name of the product and trade name or brand name. | √ | √ |
| The Name and address of the producer and/or packer. | √ | √ |
| The Net Weight. | √ | √ |
| The harvesting country/countries (origin). | √ | √ |
| The storage Mode and instructions. | √ | √ |
| The Date of Minimum Durability. | √ | √ |
| The type of RJ (according to ISO standards whether type 1 or type 2) | | √ |
| The Batch Number. | | √ |
| The production date and expiry date. | √ | |
| The harvesting year. | | √ |
| The freezing month (if any). | | √ |
| The trade and handling license. | √ | |

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**Table 3.** The quality parameters of Royal Jelly in Egyptian standards and ISO standards

| Parameters | | ES | | ISO | |
|---|---|---|---|---|---|
| | | Fresh | Lyophilized | Type 1 | Type 2 |
| Moisture Content (%) | Min.<br>Max. | ---<br>66 | ---<br>5 | 62.0<br>68.5 | |
| 10 HDA (%) | Min. | 2 | 6 | 1.4 | |
| Protein (%) | Min.<br>Max. | 12<br>-- | 32<br>--- | 11<br>18 | |
| Total Sugar (%) | Min.<br>Max. | 12<br>--- | --- | 7<br>18 | |
| Fructose (%) | Min.<br>Max. | --- | --- | 2<br>9 | |
| Glucose (%) | Min<br>Max | --- | --- | 2<br>9 | |
| Sucrose (%) | | --- | --- | <3.0 | --- |
| Erlose (%) | | --- | --- | <0.5 | --- |
| Maltose (%) | | --- | --- | <1.5 | --- |
| Maltotriose (%) | | --- | --- | <0.5 | --- |
| pH | Min.<br>Max. | 3<br>4 | 3<br>4 | --- | |
| Total acidity (0.1N NaOH/ g) | Min.<br>Max. | --- | --- | 30.0<br>53.0 | |
| Total lipids (%) | | 5.5 | --- | 2<br>8 | |
| $C^{13}$ / $C^{12}$ Isotope Ratio | | --- | --- | -29 to -20 | -29 to -14 |
| Furosine (*) (Freshness indicator) | | --- | | Recommended (while no values have been introduced yet) | |
| Concentration of heavy metals | | The Lead Concentration Shall not Exceed 0.5 Part Per Million (PPM). The Copper Concentration Shall not Exceed 1 Part per Million (PPM). The Arsenic Concentration Shall not Exceed 1 Part per Million (PPM). | | --- | |
| Ash Content | | Not more than (0.8%) in fresh RJ | | --- | |
| Pollen Screening (authenticity and determining geographical origin) (*) | | --- | | Recommended | |

(*) furosine and pollen screening (melissopalynology) are still regarded as optional quality parameters in ISO standards.

Despite melissopalynology or analysis of the pollen sediments is a well known technique for more than 4 decades, it remained the most reliable and rapid method for detecting the geographical origin of Honeybee products (**Sabatini AG et al., 2009; Louveaux J. et al., 1978**) **[45, 37]**, analysis of pollen spectrum is not mentioned at all in the Egyptian Standards of Honey **(ES: 355/2005)[3]** or ES of royal jelly **(ES: 2914/2005)[2]**,, while it is recognized and recommended by ISO as an additional optional quality parameter (alongside with furosine), but not an obligatory quality parameter.

Until now, although many studies have examined the pollen spectrum in authentic honey androyal jelly samples in some countries **(Dimou M. et al., 2007; Dimou M. et al, 2013)[16, 17]** it is not

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commonly used by commercial honey processing facilities and traders to detect the geographic and botanical origin of RJ or honey.

The conditions of handling and transportation should be included in the Egyptian Standard because RJ is a rapidly perishable matrix; many active compounds lose their biological health promoting benefits when RJ is subjected to high temperature during transportation or poor storage conditions. **(Ramadan MF et al., 2012; Maghsoudlou A. et al, 2019**)**[44, 38]** .

**Table 4.** The hygienic requirements of royal jelly according to the Egyptian standards and the ISO standards

| | ES of Royal Jelly | ISO standards of Royal Jelly |
|---|---|---|
| **Basic requirements** | Unfermented and free from any signs of Deterioration. | Unfermented and shall not be rancescent |
| **Max. Colony Count** | 10.000 | 500 |
| **Enterobacteriaceae (CFU /g)** | Absent | Absent in 10 g. |
| **Salmonella (CFU/g)** | Absent | Absent in 25 g. |
| **Anaerobic bacteria spores** | Absent (absolutely free from anaerobic bacteria spores) | --- |
| **Mould and yeast, their spores and toxins** | No signs of yeast and mould growth, RJ shall be completely free from any spores or toxins | --- |

## 2. Materials and Methods

Fresh royal jelly samples (n=9) were collected from local beekeepers whose apiaries are located at the northern of Egypt (northern delta, mainly kafrelsheik and Behera), samples were harvested in 2019 at the main honey flow seasons, in April June and September which are the honey flow seasons for citrus, Egyptian clover and cotton respectively. All fresh royal jelly samples were stored in food grade dark plastic containers and transported immediately to the laboratory in an icebox; samples of each season have been analyzed as soon as they were transported to the lab.

**Moisture content** was calculated by determining weight loss upon the drying of fresh RJ samples in a vacuum oven at 65 °C, each Fresh RJ sample (1 g) was weighted precisely, speeded uniformly on a Petri dish and weighted again, then placed in the vacuum drying oven which was set at 65°C under vacuum (0.0005 pa) for about 2 hours, then the weighting dish was taken out to cool down, weighted and inserted again to the vacuum drying oven for 2 hours, until a constant mass is retained, difference in weight before and after drying is calculated **(ISO 12824:2016; Hu F. et al., 2019)[7, 24],** by using the following formula

Water content % = [(weight before drying – weight after drying) / (weight before drying)] × 100

**Lipid content** was determined by soxhelt extraction method, the lipid content was extracted from the dry matter of each sample using petroleum ether as an organic solvent **(Mărghitaş LA. et al.,2010)[39]**.

**pH** was measured in a diluted water solution of Royal Jelly (containing RJ and distilled water 1:5, g/ml) by using pH meter **(Kolayli S. et al, 2015)[35]**.

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**Protein content** was calculated by using kjeldahl method, 0.5 g RJ was weighted, digested by using concentrated sulphuric acid, followed by distillation and titration of the sample solution, the result was multiplied by 6.25 to get an estimate for the crude protein content **(kanelis D. et al., 2015)[31].**

**10-Hydroxy-2-decenoic acid (10-HDA)** was determined using high performance liquid chromatography (HPLC) system, a reversed phase column supelco $C_{18}$ was used (150 × 4.6 mm, 5 µm i.d.)equipped with a variable wavelength ultraviolet absorbance detector, the column temperature was adjusted at 25 °C, the mobile phase is a mixture of ultra pure water, methanol and phosphoric acid (55:45:2.5 V:V:V, and the flow rate was 0.75 ml/min, the UV detector was adjusted at 215 nm,, the total run time was 15 minutes and the injection volume was 20 µl for each sample.
0.1 g of methyl hydroxybenzoate (MHB) had been dissolved in a solvent that consist of methanol and water (50:50 V/V) to produce a final volume of 1000 ml with a concentration of 100 µg/ml, 0.016 g of 10-HDA was completely dissolved in a solvent (methanol and water, 50:50 V/V) to produce a 100 ml of stock solution with a concentration of 160 µg/ml, by diluting this stock solution a series of 10-HDA solutions were prepared. Solutions for the construction of a standard curve were prepared by mixing an equal amount MHB and 10-HDA solutions. **(Kolayli S. et al, 2015; Kim J. et al., 2010)[35, 34]**

**Ash content** was determined by removing the moisture and organic matter through placing the sample in a crucible and heating the furnace at 550° C to a constant weight (2 consecutive weights are equal). **(Garcia-Amoedo LH et al., 2007)[20]**

**Carbohydrates** were calculated as a difference using the following formula:
Carbohydrates = 100 – (moisture % + protein % + lipids % + ash %) **(Garcia-Amoedo L.H et al., 2007)[20]**

## 3. Results and discussion

In order to compare the composition Royal jelly from different geographical origins with our findings, so we gathered data reported in recent studies (from different origins to be more reliable), which is presented in the following table

**Table 5.** Comparison between the Chemical compositions of Fresh Royal Jelly samples from different countries published in recent Research articles

| | **Mean** | **Range** |
|---|---|---|
| **French RJ** | **N=500 authentic samples (French)** | **(Wytrychowski M. et al., 2013)** |
| Moisture content, % | 65.03 | (60.7 – 69.9) |
| Protein, % | 13.9 | (11.4 – 16.5) |
| 10-HDA, % | 2.3 | (1.3 – 3.3) |
| Total sugars | 13.1 | (8.0 – 18.3) |
| **Asian Commercial RJ** | **N= 142 Commercial samples** | **(Wytrychowski M. et al., 2013)** |
| Moisture content, % | 64.3 | (61.8 – 68.2) |
| Protein, % | 14.7 | (11.6 – 16.8) |
| 10-HDA, % | 1.5 | (0.9 – 2.9) |
| Total sugars | 15.35 | (8.58 – 19.18) |
| **Italian RJ** | **N= 14 authentic samples (Italian)** | **(Wytrychowski M. et al., 2013)** |

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| | | |
|---|---|---|
| Moisture content, % | 64.7 | (61.1 – 67.5) |
| Protein, % | 15.0 | (13.5 – 16.5) |
| 10-HDA, % | 2.4 | (1.1 – 3.2) |
| Total sugars | 12.09 | (9.89 – 14.43) |
| **French RJ** | **N= 32 samples (French RJ produced by beefeeding)** | **(Wytrychowski M. et al., 2013)** |
| Moisture content, % | 66.7 | (63.6 – 71.6) |
| Protein, % | 13.3 | (11.3 – 15.6) |
| 10-HDA, % | 2.4 | (1.5 – 3.1) |
| Total sugars, % | 13.11 | (9.68 – 15.74) |
| **Anatolian (Turkish) RJ** | **N= 18 samples** | **(Kolayli S. et al., 2015)** |
| Moisture content, % | 66.8 | (61.6 – 73.6) |
| Protein, % | 14.1 | (11.4 – 15.8) |
| 10-HDA, % | 2.7 | (1.0 – 3.9) |
| Fructose + glucose + sucrose, % | 13.9 | (8.2 – 19.0) |
| pH | 4.2 | (3.6 – 5.1) |
| **Greek RJ** | **N= 176 samples (Supplementary feeding)** | **(Kanelis D. et al., 2015)** |
| Moisture content, % | 66.1 | (46.8 – 73.2) |
| Protein, % | 13.6 | (10.5 – 19.6) |
| 10-HDA, % | 2.32 | (0.8 – 6.5) |
| Total sugars, % | 11.03 | (7.2 – 16.7) |
| **Bulgarian RJ** | **N= 5 samples (Sofia region)** | **(Balkanska R. et al., 2012)** |
| Moisture content, % | 62.72 | (61.70 – 65.20) |
| Protein, % | 16.05 | (11.3 – 15.6) |
| Total sugars, % | 9.31 | (7.2 – 16.7) |
| pH | 4.01 | (3.94 – 4.06) |
| **Bulgarian RJ** | **N= 5 samples (Lovech region)** | **(Balkanska R. et al., 2012)** |
| Moisture content, % | 60.78 | (59.10 – 62.7) |
| Protein, % | 18.49 | (16.84 – 19.63) |
| Total sugars, % | 11.22 | (8.12 – 13.48) |
| pH | 3.90 | (3.86 – 3.97) |
| **Bulgarian RJ** | **N= 3 samples (Varna region)** | **(Balkanska R. et al., 2012)** |
| Moisture content, % | 64.80 | (63.30 – 65.80) |
| Protein, % | 12.64 | (12.23 – 13.08) |
| Total sugars, % | 11.05 | (9.15 – 12.87) |
| pH | 3.67 | (3.60 – 3.70) |
| **Romanian RJ (*)** | **N= 34 samples (Romania RJ)** | **(Balkanska R. et al., 2013)** |
| Moisture content, % | 62.5 | (54.47 – 69.74) |
| Protein, % | 13.04 | (9.54 – 17.13) |
| (fructose, glucose, Sucrose), % | 11.99 | (8.12 – 16.61) |
| pH | 3.99 | (3.74 – 4.12) |
| **Bulgarian RJ (*)** | **N= 35 samples (Bulgaria)** | **(Balkanska R. et al., 2013)** |
| Moisture content, % | 62.13 | (58.80 – 65.80) |
| Protein, % | 15.83 | (9.62 – 19.63) |
| (fructose, glucose, Sucrose), % | 11.27 | (8.05 – 15.47) |
| pH | 3.85 | (3.20 – 4.06) |
| **Brazilian RJ** | **N= 15 homogenized samples (feeding experiments)** | **(Sereia MJ et al., 2013)** |
| Moisture content, % | 67.04 | (66.23 – 67.81) |
| Protein, % | 14.24 | (13.17 – 14.76) |

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| | | |
|---|---|---|
| Lipids, % | 3.48 | (2.14 – 5.8) |
| Reducing sugars (fructose & glucose), % | 9.77 | (8.32 – 10.68) |
| Sucrose | 4.52 | (3.96 – 5.14) |
| pH | 3.97 | (3.92 – 4.02) |
| **Brazilian RJ** | **N=7 pure Samples** | **(Garcia-Amoedo LH et al., 2007)** |
| Moisture content, % | 63.17 | (61.45 – 67.58) |
| Protein, % | 13.12 | (11.99 – 14.01) |
| HDA, % | 2.53 | (1.58 – 3.39) |
| Lipids, % | 3.28 | (2.17 – 4.22) |
| Total sugars | 19.36 | (15.30 – 21.20) |
| **Iranian RJ** | **N=2 Samples (Ardebil)** | **(Kamyab S. et al., 2019)** |
| 10-HDA, % | 1.803 | |
| Protein, % | 14.5 | |
| **Iranian RJ** | **N=2 Samples, (Amol)** | **(Kamyab S. et al, 2019)** |
| 10-HDA, % | 0.676 | |
| Protein, % | 11.5 | |
| **Iranian RJ** | **N=2 samples, (Mashad)** | **(Kamyab S. et al., 2019)** |
| 10-HDA, % | 2.443 | |
| Protein, % | 13.92 | |
| **American RJ** | **N=12 Samples (Pennsylvania state apiary)** | **(Howe SR et al., 1985)** |
| Moisture content, % | 67.1 | (66.3 – 67.7) |
| Protein, % | 11.9 | (11.6 – 12.2) |
| Lipids, % | 4.1 | (3.2 – 5.0) |
| 10-HDA, % | 2 | (1.54 – 2.47) (**) |
| **Turkish RJ** | **N= 2 samples** | **(Karaali A. et al., 1988)** |
| Moisture content, % | 65.32 | |
| Protein, % | 13.36 | |
| Lipids, % | 8.20 | |
| Total carbohydrates, % | 10.45 | |
| **African Royal Jelly (***)** | **N= 14 Samples From Kenya (Central, Coast and western areas)** | **(Mokaya HO et al, 2020)** |
| Moisture content, % | 65 | (58.1 – 71.5) |
| Protein, % | 5.9 | (3.79 – 8.0) |
| 10-HDA, % | 2.9 | (2.1 – 3.29) |
| Total Sugars, % | 8.0 | (6.3 – 10.7) |
| pH | 3.9 | (3.7 – 4.14) |

(*) according to the authors, both countries Romania and Bulgaria have similar weather conditions, flowering species and honeybee species (*Apis mellifera macedonica and A. melliferra carnica*)

(**) the 10-HDA was expressed as percent of fatty acids (crude lipid content), which ranged from 38.8 to 62.3 % with an average 50.3 %.

(***) Royal Jelly was harvested from different areas in Kenya where climate is different, Western (high rainfall), Coast (hot and wet), and Central (cool and wet).

Results in table (7) shows that the moisture content in fresh Egyptian royal jelly samples was the greatest component, which ranged between 63.5 and 68.4%, this results clearly demonstrated that all Egyptian RJ samples complied with the international standards, whether ISO or IHC, which proposed an upper limit of 68.5 and 70 % respectively, however, according to the Egyptian Standards, two samples out of nine exceeded the permissible moisture content limits for fresh RJ that set the maximum moisture content at 66%.

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The average moisture content of RJ samples harvested in April was the highest, followed by June and September respectively, which could be attributed to the prevailed temperature and climatic conditions during the honey flow season and their effect on the abundance of pollen and nectar that Honeybees are fed on, hence, in early spring (April) the nectar is very abundant and the weather conditions are mild, on the other hand, the summer season in Egypt (July through September) is very hot with less availability of nectar due to drought and excessive evaporation rates, which could reduce the moisture content in honey and RJ, this hypothesis agrees with previous studies reported that the climatic conditions, season and harvesting methods are among the factors that affect the chemical composition of Royal jelly **(Mureşan CL et al., 2016)**[42].

These results are comparable with the average moisture content reported through the analysis of 500 authentic French RJ samples (Table 5) which have shown a wider range from 60.7 to 69.9 % with an average moisture content of 65.03%.

The protein content varied between 9.8 and 13.8 % in all examined samples, with an average of 12.1, 11.5 and 11.8 % throughout April, June and September, all of examined samples, are in accordance with the IHC proposal which proposed a range of 9 to 18 % . While 2 samples out of nine didn't comply with the ISO standards which stipulated a minimum protein content of 11 %.

**Table 6.** the chemical composition of Royal Jelly samples from Egypt throughout the main Honey flow seasons.

| **Analytical Criteria** | **April** | | | **June** | | | **September** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Mean** | **Min** | **Max** | **Mean** | **Min** | **Max** | **Mean** | **Min** | **Max** |
| **Water content, %** | 66.5 ± 1.27 | 64.4 | 68.4 | 64.4 ± 0.47 | 63.9 | 65.1 | 64.2 ± 0.62 | 63.5 | 65.1 |
| **Proteins, %** | 12.1 ± 0.75 | 10.8 | 13.8 | 11.5 ± 0.78 | 9.8 | 13.1 | 11.8 ± 0.22 | 11.3 | 12.2 |
| **Lipids, %** | 5.34 ± 0.21 | 4.83 | 5.62 | 5.52 ± 0.25 | 4.95 | 5.89 | 5.25 ± 0.08 | 5.16 | 5.35 |
| **10-HDA, %** | 3.2 ± 0.12 | 2.96 | 3.37 | 2.92 ± 0.17 | 2.58 | 3.21 | 3.1 ± 0.10 | 2.92 | 3.32 |
| **Carbohydrates, %** | 15.39 ± 1.00 | 15.27 | 15.55 | 17.86 ± 0.83 | 16.51 | 19.51 | 18.12 ± 0.59 | 17.83 | 18.3 |
| **Ash, %** | 0.67 ± 0.02 | 0.62 | 0.71 | 0.72 ± 0.04 | 0.64 | 0.77 | 0.63 ± 0.03 | 0.61 | 0.65 |
| **pH** | 3.63 ± 0.11 | 3.6 | 3.7 | 4.07 ± 0.15 | 4.0 | 4.1 | 3.73 ± 0.05 | 3.7 | 3.8 |

**Values are expressed as Mean ± Standard Deviation, n=9**

**Table 7.** The average chemical composition of investigated Royal Jelly samples from Egypt

| **Contents** | **Overall Mean ± SD** | **Min** | **Max** |
|---|---|---|---|
| **Water content, %** | 65.03 ± 1.49 | 63.5 | 68.4 |
| **Proteins, %** | 11.8 ± 1.11 | 9.8 | 13.8 |
| **Lipids, %** | 5.37 ± 0.34 | 4.83 | 5.89 |
| **10-HDA, %** | 3.07 ± 0.23 | 2.58 | 3.37 |
| **Carbohydrates, %** | 17.12 ± 1.43 | 15.27 | 18.3 |
| **Ash, %** | 0.67 ± 0.05 | 0.61 | 0.77 |
| **pH** | 3.81 ± 0.19 | 3.6 | 4.1 |

The average protein content was 11.8 %, Only 3 RJ samples out of 9 were in accordance with the national ES that set the lower threshold at 12 %, a rational explanation could be the insufficiency of pollen reserves collected by honeybees or harvesting of a large proportion of bee pollen gathered by the honeybees through the use of pollen traps by Egyptian beekeepers and the lack of complementary protein supplementation for honeybees to replenish harvested pollen, so

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honeybee workers didn’t receive enough protein and sequentially produced RJ with relatively low protein content.

Many previous Studies on Mediterranean and European RJ have shown larger protein proportions, as illustrated in table (5), the average protein content in Greek RJ was 13.6 % **(Kanelis D. et al., 2015)[31],** authentic French and Italian RJ samples have shown higher averages achieving 13.9 and 15% respectively **(Wytrychowski M. et al., 2013)[49]** , the highest reported average protein content in RJ samples was from Bulgaria (lovech Region) which was 18.49% **(Balkanska R. et al., 2012)[9]**, on the other hand, the African RJ from Kenya have shown the least protein content, its mean content was only 5.9%, and values ranged between 3.79 and 8 % **(Mokaya HO et al, 2020)[40]**.

The lipid portion of royal jelly is very important quality criteria for royal jelly as it compromises many unique hydroxy and dicarboxylic short chain fatty acids which are uncommon in nature that contain 8 to 10 carbons atoms, including the biological fingerprint of RJ **(Ramadan MF et al., 2012)[44]** which is 10-hydroxy-2-decenoic acid (10-HDA) and several organic acids that could be used for authenticity determination of Royal Jelly.

The lipid content in all investigated RJ samples ranged between 4.83 and 5.89 %, all values coincides with the ranges proposed by the international standards, as IHC have proposed a lower range of 3 to 8%, ISO proposed a range between 2 and 8%. However, the minimum content imposed by the national ES was 5.5% which is quite high, only 3 out of the 9 samples complied with such limits.

As previously mentioned, 10-HDA is considered the biomarker and main quality parameter for fresh and freeze dried (lyophilized) RJ, the content of 10-HDA in all investigated fresh RJ samples ranged between 2.58 and 3.37% the average content throughout the honey flow seasons was 3.2, 2.92, 3.1 % respectively, Needless to say that these values are comfortably high enough to meet all the international specifications of ISO and IHC proposal which both reported a lower limit of 1.4%, also values comply with the requirements of the Egyptian standards which clearly stated that 10-HDA shall not be less than 2%. So all the samples met the national and international requirements regarding 10-HDA content.

Referring to previous studies in table (5), 10-HDA content in authentic French RJ samples was ranging between 1.4 and 3.7 with a mean of 2.3 % **(Wytrychowski M. et al., 2013)[49]**, Anatolian RJ average content of 10-HDA was 2.7, where samples ranged between 1.0 and 3.9%, the highest mean value of 10-HDA in fresh RJ samples was from Kenya as values fluctuated between 2.1 and 3.29 **(Mokaya HO et al, 2020)[40]**.

On the other hand, in table (5) we can see the widest range attained from investigated RJ samples was reported from Greece, where the content of 10-HDA ranged between 0.8% and 6.5% with an average content of 2.32% **(Kanelis D. et al., 2015)[31]**, the authors have reported that very high content may be an indication of adulteration through the addition of synthetic 10-HDA, and they suggested an upper limit of 6.0%.

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The ash content in all investigated RJ samples ranged between 0.61 and 0.77, which was in accordance to the Egyptian standards that had set an upper limit of 0.8%, on the other hand, IHC had proposed a different range which is (0.8 to 3.0 %).

pH values in Egyptian RJ samples varied from 3.6 to 4.1 with a mean value of 3.63, 4.07 and 3.73 during the seasons of April, June and September, all samples harvested during June had the highest pH values and were higher that the pH levels imposed by the ES that set a pH range between (3 and 4), still, all samples were in accordance with the international standards proposed by IHC which set a pH range between 3.4 and 4.5.

The acidic pH of royal jelly is derived from the organic acids, fatty acids, phenolic acids that exist naturally in Royal jelly **(Kolayli S. et al, 2015)**[35], also our study agrees with an explanation indicated by a previous study of fresh RJ samples in Kenya that indicated that there is a negative correlation between acidity and increased active ingredient (10-HDA), which means the lower the pH, the higher the amount of 10-HDA **(Mokaya HO et al, 2020)**[40].

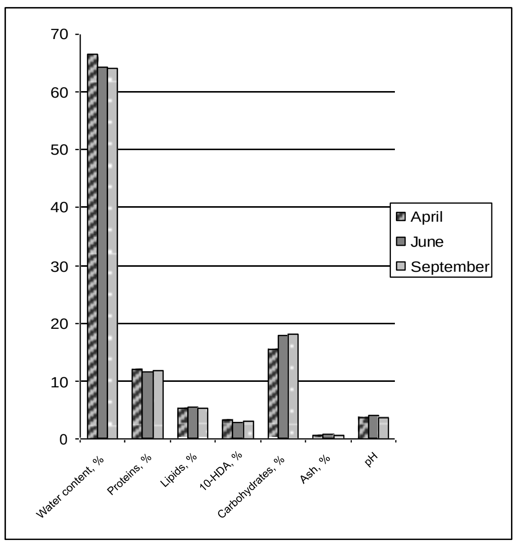


**Figure 1.** the following figure (Histogram) presents average chemical composition during the different main Honey flow seasons in Egypt

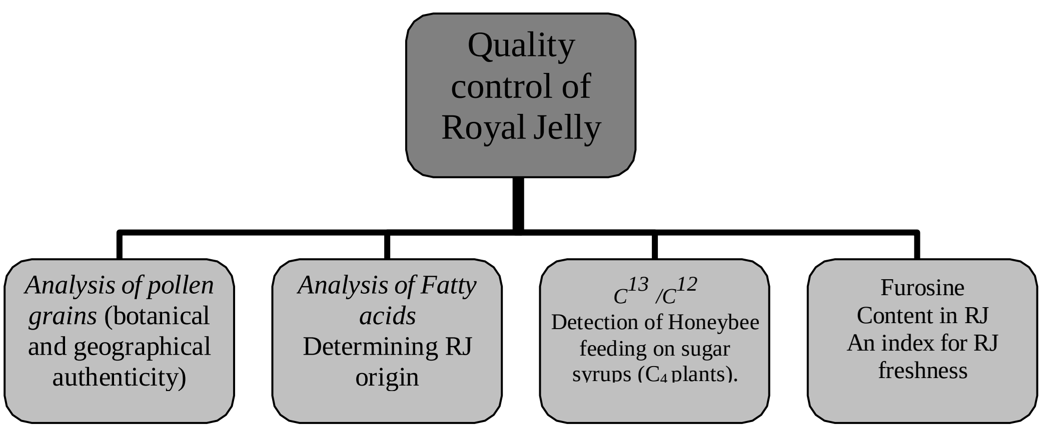


**Figure 2.** Additional parameters Recommended to Control RJ Quality (Summary)

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The fractionation and analysis of the lipid composition could be a marker for geographical origin of RJ (authenticity of origin), this was demonstrated by a study that was conducted in Egypt to differentiate between RJ from Egypt, Germany and China through the investigation of their organic acids content **(Nafea EA et al. 2011)[43]**, results show that there were 3 common organic acids within all investigated RJ samples which are shikmic, propionic and lactic acid, the difference between them was statistically significant. another 3 organic acids were distinctively present in Egyptian RJ which are malonic, maleic and succinic acid, moreover, Chinese and German RJ samples contained citric acid which was absent in Egyptian RJ, lastly, butyric acid was present only in Chinese RJ samples **(Nafea EA et al. 2011)[43].**

## 4. Conclusion

New techniques should be amended into the Egyptian Standards of Royal jelly for ensuring authenticity and freshness, such as the analysis of pollen grains which could be indispensable, simple and rapid method for ensuring the botanical and geographical origin of royal jelly or honey samples, the detection of furosine should be introduced to examine the freshness of royal Jelly, examining Carbon isotope Ratio ($C^{13}$ / $C^{12}$) is considered effective for detecting the use of $C_4$ Sugar syrups in honeybee feeding, while the analysis of organic acids in the lipids portion could be used as a useful and promising tool for verifying geographical origin of royal jelly.

Pollen analysis of honey and royal jelly samples constitutes a useful tool for the identification of the major floral sources upon which the bees forage in different regions, Research Centers should start to establish pollen reference slides and pollen books for each geographical regions containing the different shapes of pollen for different plants and their botanical origin and taxonomy, to facilitate the examination of honey and royal jelly samples for ensuring authenticity of such precious products and preventing falsification through misidentification of botanical origin of honey and royal jelly.

Until now, the traders are free to comply with the requirements of the Egyptian standards for RJ **(ES: 2914/2005),** this situation should change and the Egyptian RJ standards should be enforced as compulsory standards for producers, importers and processors, all products containing RJ in any form whether fresh or lyophilized should comply with the Egyptian Standards.

Modern Beekeeping practices should aim to increase RJ quantity without compromising quality, authenticity and safety, standards should allow honeybee supplementation with natural honey and pollen or allowed protein substitutes only, while restricting the addition of cheap sweeteners or foreign substances that could adversely affect RJ composition, stability and bioactivity, or deteriorate the health of customers or honeybees.

ISO standards should introduce the Standards for lyophilized Royal Jelly, and should introduce limits for furosine or adopt the limits set by IHC.

Data about the annual production of Honeybee products should be updated annually instead of relying on estimations, and be consistent in different sources.

## Conflict of interest

The authors declare that there is no conflict of interest.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 7977 - 7997
Received 16 February 2021; Accepted 08 March 2021.

## References

[1] CAPMAS (Central Agency of Public Mobilization and Statistics of Egypt), annual publications, Statistical Yearbook in Agriculture (2019). https://www.capmas.gov.eg/Pages/Publications.aspx?page_id=5104&Year=23496

[2] ES 2914/2005, (2005) Standards of Royal Jelly, Issued by the Egyptian Organization for Standards & Quality, (first edition was issued in 1995, which was abolished and replaced by the current version in 2005). http://www. eos.org.eg/ar/standard/4941

[3] ES 355-1/2005, (2005) Standards of Honey and methods of analysis, part one, Issued by the Egyptian Organization for Standards & Quality. . https://www.eos.org.eg/en/standard/4686

[4] FAO STAT Food and Agriculture Organization of the United Nations, provides access to food and agriculture data and statistics of different countries, http://www.fao.org/faostat/en/#data/QL

[5] IHC (International Honey Commission) - World Network of *Honey* and Bee Product Science. http://www.ihc-platform.net/

[6] IHC International Honey Commission Publications and proposals for standards of different Honeybee products. http://www.ihc-platform.net/publications.html

[7] ISO 12824:2016, (2016), Royal Jelly specifications, Issued by the International Organization for Standardization, https://www.iso.org/obp/ui/#iso:std:65648:en

[8] Al Naggar Y, Codling G, Giesy JP, Safer A. (2018). Beekeeping and the Need for Pollination from an Agricultural Perspective in Egypt. Bee World. 95. DOI: 10.1080/0005772X.2018.1484202.

[9] Balkanska R, Zhelyazkova I, Ignatova MN. (2012). Physico-chemical quality characteristics of royal jelly from three regions of Bulgaria. AGRICULTURAL SCIENCE AND TECHNOLOGY. 4(3), 302-305.

https://www.researchgate.net/publication/242012965_Physico-chemical_quality_characteristics_of_royal_jelly_from_three_regions_of_Bulgaria

[10] Balkanska R, Mărghitaş L, Pavel C, IGNATOVA M, Tomoş L (2013). Comparison of Physicochemical Parameters in Royal Jelly from Romania and Bulgaria. Bulletin UASVM Animal Science and Biotechnologies. 70(1), 117-121. DOI: 10.13140/2.1.1809.1846

[11] Bogdanov S. (2012). Royal Jelly, Bee Brood: Composition, Health, Medicine: A Review. https://www.semanticscholar.org/paper/Royal-Jelly%2C-Bee-Brood%3A-Composition%2C-Health%2C-A-Bogdanov/c674f36093a44dffd9923387e8df9d6b883d1b21

[12] Bogdanov S. (2017). Royal Jelly and Bee Brood: Harvest, Composition, Quality. Bee Product, Science. Access at: http://www.bee-hexagon.net/royal-jelly/

[13] Brouwers EVM, Ebert R, Beetsma J (1987) Behavioural and Physiological Aspects of Nurse Bees in Relation to the Composition of Larval Food During Caste Differentiation in the Honeybee, Journal of Apicultural Research, 26(1), 11-23. DOI:10.1080/00218839.1987.11100729

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 7977 - 7997
Received 16 February 2021; Accepted 08 March 2021.

[14] Chen S, Su S, Lin X. (2015). An introduction to high-yielding royal jelly production methods in China. Bee World. 83. 69-77. DOI: 10.1080/0005772X.2002.11099543

[15] Clarke Michael, McDonald Peter, (2017). Australian Royal Jelly: Market Opportunity Assessment based on production that uses new labour saving technology, Australian Government Rural industries Research and Development Corporation (RIRDC), https://www.agrifutures.com.au/product/australian-royal-jelly-market-opportunity-assessment-based-on-production-that-uses-new-labour-saving-technology/

[16] Dimou M, Goras G, Thrasyvoulou A (2007), Pollen analysis as a means to determine the geographical origin of royal jelly, Grana, 46(2), 118-122, DOI: 10.1080/ 00173130701393874

[17] Dimou M, Tananakia C, Goras G, Karazafirisa E, Thrasyvouloua A (2013), Melissopalynological analysis of royal jelly from Greece, Grana, 52(2), 106-112, DOI: 10.1080/00173134.2012.719538

[18] Elaidy WKM, Tolba A, (2017), Comparative Evaluation of Four Honeybee Races According To Pollen Storage and Worker Brood Rearing Activities Under Natural Conditions. IOSR Journal of Pharmacy and Biological Sciences. 12. 40-49. DOI: 10.9790/3008-1201044049

[19] El-Guendouz S, Lyoussi B, Miguel MG (2020): Insight into the chemical composition and biological properties of Mediterranean royal jelly, Journal of Apicultural Research, 59 (5), 890-909. DOI: 10.1080/00218839.2020.1744241

[20] Garcia-Amoedo LH, Almeida-Muradian LH. (2007). Physicochemical composition of pure and adulterated royal jelly. Quim. Nova. 30. 257-259. DOI: 10.1590/S0100-40422007000200002

[21] Gupta RK, Khan MS, Srivastava RM, Goswami V. (2014), History of Beekeeping in Developing World, Beekeeping for Poverty Alleviation and Livelihood Security, Vol. 1: Technological Aspects of Beekeeping, Springer, Chapter 1. p 3 – 62,

[22] Habashy NH, Abu-Serie MM (2020), The potential antiviral effect of major royal jelly protein2 and its isoform X1 against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): Insight on their sialidase activity and molecular docking, Journal of Functional Foods, 75(2) , 104282. https://doi.org/10.1016/j.jff.2020.104282

[23] Howe SR, Dimick PS, Benton AW (1985) Composition of Freshly Harvested and Commercial Royal Jelly, Journal of Apicultural Research, 24(1), 52-61, DOI: 10.1080/00218839.1985.11100649

[24] Hu F, Bilikova K, Casabianca H, Daniele G, Espindola FS, et al. (2017). Standard methods for Apis mellifera royal jelly research. Journal of Apicultural Research. 58(2) 1-68. DOI: 10.1080/00218839.2017.1286003.

[25] Inoue T, Inoue A, (1964). The World Royal Jelly Industry: Present Status and Future Prospects, Bee World, 45:2, 59-64. DOI: 10.1080/0005772X.1964.11097045

[26] Isidorov VA, Bakier S, Grzech I. (2012). Gas chromatographic-mass spectrometric investigation of volatile and extractable compounds of crude royal jelly. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences. 885-886. 109-16. DOI:10.1016/j.jchromb.2011.12.025

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 7977 - 7997
Received 16 February 2021; Accepted 08 March 2021.

[27] Jianke L, Aiping, W. (2005). Comprehensive technology for maximizing royal jelly production. 145. 661-664. https://www.researchgate.net/publication/292821721_Comprehensive_technology_for_maximizing_royal_jelly_production

[28] Johansson, T. S. K. (1955) Royal Jelly, Bee World, 36(2), 21-32. DOI:10.1080/0005772X.1955.11094863

[29] Kamakura M. (2011). Royalactin induces queen differentiation in honeybees. Nature 473, 478-483. DOI:10.1038/nature10093

[30] Kamyab S, Maryam Gharachorloo M, Honarvar M, Ghavami M (2019): Quantitative analysis of bioactive compounds present in Iranian royal jelly, Journal of Apicultural Research, 59(1). DOI: 10.1080/00218839.2019.1673964

[31] Kanelis D, Tananaki C, Liolios V, Dimou M, Goras G, Rodopoulou MA, Karazafiris E, Thrasyvoulou A. (2016). A suggestion for royal jelly specifications / Prijedlog globalnih standarda za matičnu mliječ. Arhiv za higijenu rada i toksikologiju. 66. 275-284. DOI: 10.1515/aiht-2015-66-2651.

[32] Karaali Artemis, Meydanoğlu Fügen, Eke Dilek (1988) Studies on Composition, Freeze-Drying and Storage of Turkish Royal Jelly, Journal of Apicultural Research, 27(3), 182-185, DOI: 10.1080/00218839.1988.11100799

[33] Khazaei M, Ansarian A, Ghanbari E. (2017). New Findings on Biological Actions and Clinical Applications of Royal Jelly: A Review. Journal of Dietary Supplements. 15, 1-19. DOI: 10.1080/19390211.2017.1363843

[34] Kim J, Lee J. (2010). Quantitative analysis of trans-10-hydroxy-2-decenoic acid in royal jelly products purchased in USA by high performance liquid chromatography. Journal of Apicultural Science. 54(1), 77-85. https://pdfs.semanticscholar.org/e85f/dfc1823d778a40cca2eefa4be8ba4b4d986c.pdf

[35] Kolayli S, Sahin H, Can Z, Yildiz O, Malkoç M, Asadov A. (2015). A Member of Complementary Medicinal Food: Anatolian Royal Jellies, Their Chemical Compositions, and Antioxidant Properties. Journal of Evidence-Based Complementary & Alternative Medicine. 21(4), 43-48. DOI: 10.1177/2156587215618832

[36] Lima WG, Brito JCM, da Cruz Nizer WS (2020). Bee products as a source of promising therapeutic and chemoprophylaxis strategies against COVID-19 (SARS-CoV-2). Phytotherapy Research. 35(2), 743-750. DOI: 10.1002/ptr.6872

[37] Louveaux J. Maurizio A. Vorwohl G. (1978) Methods of Melissopalynology, Bee World 59, 139–157. DOI: 10.1080/0005772X.1978.11097714

[38] Maghsoudlou A, Mahoonak AS, Mohebodini H, Fidel T. (2019). Royal Jelly: Chemistry, Storage and Bioactivities. Journal of Apicultural Science. Vol. 63(1). DOI: 10.2478/jas-2019-0007

[39] Mărghitaş LA, Morar O, Bobis O, Bonta V, Dezmirean DS. (2010). Comparative evaluation of chemical composition for three categories of royal jelly.

https://www.researchgate.net/publication/266287688_Comparative_evaluation_of_chemical_composition_for_three_categories_of_royal_jelly

[40] Mokaya HO, Njeru LK & Lattorff HMG., African honeybee royal jelly (2020),

Phytochemical contents, free radical scavenging activity, and physicochemical properties, Food Bioscience, 37, 100733. DOI: 10.1016/j.fbio.2020.100733.

[41] 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: 10.1016/B978-0-12-805413-0.00018-1

[42] Mureşan CL, MÃRGHITAŞ LA, DEZMIREAN DS, BOBIS O, BONTA V, ZACHARIAS, Mărgăoan R, PAŞCA C. (2016). Quality Parameters for commercialized Royal Jelly. Bulletin UASVM Animal Science and Biotechnologies. 73(1). DOI: 10.15835/buasvmcn-asb:11630

[43] Nafeaa EA, ElMmohandes S, (2011). Determination and identification of organic acids in three types of royal jelly, J. plant prot. and Path, Mansoura University, 2(10): 873 – 881. http://srv4.eulc.edu.eg/eulc_v5/Libraries/UploadFiles/DownLoadFile.aspx?RelatedBibID=NWFhMWNmODItMzc0Ny00MDU3LWIyNmItYTAxMDY1MjJlN2I3X2l0ZW1zXzEyMTMxNjM2XzM1Mjg4OV9f&filename=873-881.pdf

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

[45] Sabatini AG, Marcazzan GL, Caboni MF, Bogdanov SB, Almeida-Muradian L. (2009). Quality and standardisation of Royal Jelly. Journal of ApiProduct and ApiMedical Science. 1. 1-6. DOI: 10.3896/IBRA.4.01.1.04.

[46] Sahinler N, Kaftanoglu O (1997). , Effects of feeding, age of the larvae, and queenlessness on the production of Royal Jelly. Bee Products Properties, Applications, and Apitherapy, Springer (Conference Book), Chapter 21, P. 173-178. https://www.springer.com/gp/book/9780306455025

[47] Sereia MJ, Toledo VA (2013). Quality of royal jelly produced by Africanized honeybees fed a supplemented diet. Food Sci. Technol, Campinas, 33(2), 304-309, DOI: 10.1590/S0101-20612013005000039

[48] Spannhoff A, Kim YK, Raynal NJ, Gharibyan V, Su M, Zhou Y, Li J, Castellano S, Sbardella G, Issa J, Bedford M. (2011). Histone deacetylase inhibitor activity in royal jelly might facilitate caste switching in bees. EMBO reports. 12(3), 238-243 DOI:10.1038/embor.2011.9

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

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

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 7977 - 7997
Received 16 February 2021; Accepted 08 March 2021.

**Another form for table (5) in this article…**

**Comparison between the Chemical compositions of Fresh Royal Jelly samples from different countries published in recent Research articles**

| Analytical Criteria | RJ Origin | N | Values obtained | Range | Reference: |
|---|---|---|---|---|---|
| Moisture content, % | French (authentic) | 500 | 65.03 | 60.7 - 69.9 | (Wytrychowski M. et al., 2013) [49] |
| | Italian (Authentic) | 14 | 64.7 | 61.1 - 67.5 | (Wytrychowski M. et al., 2013) [49] |
| | Asian (commercial) | 142 | 64.3 | 61.8 - 68.2 | (Wytrychowski M. et al., 2013) [49] |
| | French (supplemetary bee feeding) | 32 | 66.7 | 63.6 - 71.6 | (Wytrychowski M. et al., 2013) [49] |
| | Anatolian (Turkish) | 18 | 66.8 | 61.6 - 73.6 | (Kolayli S. et al., 2015) [35] |
| | Greek | 176 | 66.1 | 46.8 - 73.2 | (Kanelis D. et al., 2015) [31] |
| | Bulgarian (Sofia) | 5 | 62.72 | 61.70 - 65.20 | (Balkanska R. et al., 2012) [9] |
| | Bulgarian (Lovech) | 5 | 60.78 | 59.10 - 62.70 | (Balkanska R. et al., 2012) [9] |
| | Bulgarian (Varna) | 5 | 64.8 | 63.30 - 65.80 | (Balkanska R. et al., 2012) [9] |
| | Romanian | 34 | 62.5 | 54.47 - 69.74 | (Balkanska R. et al., 2013) [10] |
| | Bulgarian | 35 | 62.13 | 58.80 - 65.80 | (Balkanska R. et al., 2013) [10] |
| | Brazilian | 15 | 67.04 | 66.23 - 67.81 | (Sereia M.J et al., 2013) [47] |
| | Brazilian | 7 | 63.17 | 61.45 - 67.58 | (Garcia-Amoedo LH et al., 2007) [20] |
| | American | 12 | 67.1 | 66.3 - 67.7 | (Howe SR et al., 1985) [23] |
| | Turkish | 2 | 65.32 | | (Karaali A. et al., 1988) [32] |
| | Kenyan | 14 | 65 | 58.1 - 71.5 | (Mokaya HO et al, 2020) [40] |
| Protein content, % | French (authentic) | 500 | 13.9 | 11.4 - 16.9 | (Wytrychowski M. et al., 2013) [49] |
| | Italian (Authentic) | 14 | 15 | 13.5 - 16.5 | (Wytrychowski M. et al., 2013) [49] |
| | Asian (commercial) | 142 | 14.7 | 11.6 - 16.8 | (Wytrychowski M. et al., 2013) [49] |
| | French (supplemetary bee feeding) | 32 | 13.3 | 11.3 - 15.6 | (Wytrychowski M. et al., 2013) [49] |
| | Anatolian (Turkish) | 18 | 14.1 | 11.4 - 15.8 | (Kolayli S. et al., 2015) [35] |
| | Greek | 176 | 13.6 | 10.5 - 19.6 | (Kanelis D. et al., 2015) [31] |
| | Bulgarian (Sofia) | 5 | 16.05 | 15.07 - 16.85 | (Balkanska R. et al., 2012) [9] |
| | Bulgarian (Lovech) | 5 | 18.49 | 16.84 - 19.63 | (Balkanska R. et al., 2012) [9] |
| | Bulgarian (Varna) | 5 | 12.64 | 12.23 - 13.08 | (Balkanska R. et al., 2012) [9] |
| | Romanian | 34 | 13.05 | 9.54 - 17.13 | (Balkanska R. et al., 2013) [10] |
| | Bulgarian | 35 | 15.83 | 9.62 - 19.63 | (Balkanska R. et al., 2013) [10] |
| | Brazilian | 15 | 14.24 | 13.17 - 14.76 | (Sereia M.J et al., 2013) |
| | Brazilian | 7 | 13.12 | 11.99 - 14.01 | (Garcia-Amoedo LH et al., 2007) [20] |
| | Iranian | 2 | 14.5 | | (Kamyab S. et al., 2019) [30] |
| | Iranian | 2 | 11.5 | | (Kamyab S. et al., 2019) [30] |
| | American | 12 | 11.9 | 11.6 - 12.2 | (Howe SR et al., 1985) [23] |
| | Turkish | 2 | 13.36 | | (Karaali A. et al., 1988) [32] |
| | Kenyan | 12 | 5.9 | 3.79 - 8.0 | (Mokaya HO et al, 2020) [40] |
| | French (authentic) | 500 | 2.3 | 1.4 - 3.7 | (Wytrychowski M. et al., 2013) [49] |
| | Italian (Authentic) | 14 | 2.4 | 1.1 - 3.2 | (Wytrychowski M. et al., 2013) [49] |
| | Asian (commercial) | 142 | 1.5 | 0.9 - 2.9 | (Wytrychowski M. et al., 2013) [49] |

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 7977 - 7997
Received 16 February 2021; Accepted 08 March 2021.

| | | | | | |
|---|---|---|---|---|---|
| **10-HDA, %** | French (supplemetary bee feeding) | 32 | 2.4 | 1.5 - 3.1 | **(Wytrychowski M. et al., 2013) [49]** |
| | Anatolian (Turkish) | 18 | 2.7 | 1.0 - 3.9 | **(Kolayli S. et al., 2015) [35]** |
| | Greek | 97 | 2.32 | 0.8 - 6.5 | **(Kanelis D. et al., 2015) [31]** |
| | Brazilian | 15 | 3.48 | 2.14 - 5.8 | **(Sereia MJ et al., 2013)** |
| | Brazilian | 7 | 2.53 | 1.58 - 3.39 | **(Garcia-Amoedo LH et al.,2007) [20]** |
| | Iranian | 2 | 1.803 | | **(Kamyab S. et al., 2019) [30]** |
| | Iranian | 2 | 0.676 | | **(Kamyab S. et al., 2019) [30]** |
| | American | 12 | 2 | 1.54 - 2.47 | **(Howe SR et al., 1985) [23]** |
| | Kenyan | 14 | 2.9 | 2.1 - 3.29 | **(Mokaya HO et al, 2020) [40]** |
| **Total Sugars, %** | French (authentic) | 500 | 13.1 | 7.9 - 17.9 | **(Wytrychowski M. et al., 2013) [49]** |
| | Italian (Authentic) | 14 | 12.09 | 9.89 - 14.43 | **(Wytrychowski M. et al., 2013) [49]** |
| | Asian (commercial) | 14 | 15.35 | 8.58 - 19.18 | **(Wytrychowski M. et al., 2013) [49]** |
| | French (supplemetary bee feeding) | 32 | 13.11 | 9.68 - 15.74 | **(Wytrychowski M. et al., 2013) [49]** |
| | Anatolian (turkish) | 18 | 13.9 | 8.2 - 19.0 | **(Kolayli S. et al., 2015) [35]** |
| | Greek | 176 | 11.03 | 7.2 - 16.7 | **(Kanelis D. et al., 2015) [31]** |
| | Bulgarian (Sofia) | 5 | 9.31 | 8.05 - 10.37 | **(Balkanska R. et al., 2012) [9]** |
| | Bulgarian (Lovech) | 5 | 11.22 | 8.12 - 13.48 | **(Balkanska R. et al., 2012) [9]** |
| | Bulgarian (Varna) | 5 | 11.05 | 9.15 - 12.87 | **(Balkanska R. et al., 2012) [9]** |
| | Romanian | 35 | 11.99 | 8.12 - 16.61 | **(Balkanska R. et al., 2013) [10]** |
| | Bulgarian | 34 | 11.27 | 8.05 - 15.47 | **(Balkanska R. et al., 2013) [10]** |
| | Brazilian | 7 | 19.36 | 15.3 - 21.20 | **(Garcia-Amoedo LH et al., 2007) [20]** |
| | Turkish | 2 | 10.45 | | **(Karaali A. et al., 1988)** |
| | Kenyan | 14 | 10 | 6.3 - 10.7 | **(Mokaya HO et al, 2020) [40]** |
| **pH** | Anatolian (Turkish) | 18 | 4.2 | 3.6 - 5.1 | **(Kolayli S. et al., 2015) [35]** |
| | Bulgarian (Sofia) | 5 | 4.01 | 3.94 - 4.06 | **(Balkanska R. et al., 2012) [9]** |
| | Bulgarian (Lovech) | 5 | 3.9 | 3.86 - 3.97 | **(Balkanska R. et al., 2012) [9]** |
| | Bulgarian (Varna) | 5 | 3.67 | 3.60 - 3.70 | **(Balkanska R. et al., 2012) [9]** |
| | Romainan | 35 | 3.99 | 3.20 - 4.06 | **(Balkanska R. et al., 2013) [10]** |
| | Bulgarian | 34 | 3.85 | 3.74 - 4.12 | **(Balkanska R. et al., 2013) [10]** |
| | Brazilian | 15 | 3.97 | 3.92 - 4.02 | **(Sereia MJ et al., 2013) [47]** |
| | Kenyan | 14 | 3.9 | 3.7 - 4.14 | **(Mokaya HO et al, 2020) [40]** |