Jundishapur J Nat Pharm Prod. 2019 November; 14(4):e64920. doi: 10.5812/jjnpp.64920.

Published online 2019 September 1. **Research Article**

# Physicochemical Properties of Royal Jelly and Comparison of Commercial with Raw Specimens

Vida Kazemi [1], Moein Eskafi [2], Mina Saeedi [1, 3], Azadeh Manayi [1, *] and Abbas Hadjiakhoondi [1, 2, **]

[1] Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[2] Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[3] Persian Medicine and Pharmacy Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

[*] *Corresponding author*: Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. Tel/Fax: +98-2164121229, Email: manayi@sina.tums.ac.ir
[**] *Corresponding author*: Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. Tel/Fax: +98-2164121229, Email: abbhadji@tums.ac.ir

**Received** 2017 December 09; **Revised** 2019 May 27; **Accepted** 2018 June 24.

## Abstract

**Background:** Royal jelly is an exclusive diet of the queen larvae of honey bee, *Apis mellifera*, which affects the body size, development time, lifespan and reproductive output of the queen relative to workers. The chemical composition of royal jelly is complex and it possesses diverse pharmacological activities. In addition to the chemical composition of royal jelly, other indirect parameters such as color, viscosity, sugar and protein content have also been proposed in evaluating the quality of royal jelly.
**Methods:** The present study described total phenolic compounds, proteins, and polysaccharide contents of two samples of royal jelly using spectrophotometric methods along with their total lipid, ash and moisture by gravimetric analysis.
**Results:** The results showed that similar amounts of phenol and polysaccharide were present in the commercial and raw samples of royal jelly (phenol: 22.98 $\pm$ 0.34 and 21.99 $\pm$ 0.41 $\mu$g/mg gallic acid equivalent; polysaccharide: 12.67 $\pm$ 0.00 and 12.63 $\pm$ 0.00%, respectively). Whereas, lipid (12.00 $\pm$ 0.00%) and protein (11.57 $\pm$ 0.00%) content of raw sample was calculated to be significantly higher compared to those in the commercial sample, the commercial sample has higher moisture than the raw specimen (61.03 $\pm$ 0.00 and 59.01 $\pm$ 0.00%, respectively. The similar amounts of ash were analyzed in both of the tested samples.
**Conclusions:** Although, the content of analyzed components was different in analyzed samples, both of them contained comparable amounts of desired compounds. Therefore, the Iranian raw sample of royal jelly could be a suitable source to produce commercial preparations compared to the formulated royal jelly.

*Keywords:* Royal Jelly, Physicochemical Properties, Spectrophotometry, Protein Content, Polysaccharide Content

## 1. Background

Royal jelly (RJ) is a secretion from the hypopharyngeal glands of worker bees, which serves as food for the queen bee and the growing larva. The product is a yellowish, creamy, acidic material with a slightly pungent odor and taste, which is primarily involved in prolonging the lifespan of the queen bee and feeding of young larvae. The composition of RJ varies depending on seasonal and regional conditions; it may contain different amount of proteins, lipids, sugars, vitamins and essential amino acids, particularly cystine, lysine and arginine (1). From the healthcare point of view, RJ is considered as an efficient supplement for treatment of diabetes and metabolic syndrome (2), acne (3), and premenstrual syndrome (4).

Because of royal jelly's relatively high price, and its popularity in public as a supplement or even food, it is an obvious candidate for fraud and adulteration (5). In Iran there is no regulation on royal jelly production and sale so it is necessary to accurately determine the composition of Iranian royal jelly which is essential for preparation of commercial supplements using national sources. In this study, two specimens of royal jelly have been compared with each other to see whether their compositions are varying or not. Therefore, freshly harvested royal jelly from beekeepers of Damavand reign, Tehran province (35.7013° N, 52.0586° E) was used as raw sample and a commercial royal jelly (1000 mg vials, Spain) as commercial specimen.

## 2. Methods

### 2.1. Sample Preparation

Royal jelly is a water soluble component and it could be solved in deionized water for further analysis. One hundred mg of raw and commercial samples were put on a

small amount of water to dissolve using an ultrasonic homogenizer, subsequently the volume reached 100 mL with deionized water in a volumetric flask to obtain the concentration of 1 mg/mL. All determinations were carried out in triplicate and the mean value ± standard deviation (SD) we presented.

### 2.2. Total Phenol

As a standard, the absorption changes of gallic acid at 765 nm wavelength is used to assay total phenols in the royal jelly samples and the amount of phenol represented as gallic acid equivalent (GAE) (6). The samples (1 mL) were transferred to glass tubes, to which 5 mL Folin-Ciocalteu reagent (diluted 1:10) was subsequently added and incubated at room temperature for 10 minutes. Four milliliters of sodium bicarbonate (75 mg/ mL) was added to the mixture and it was made up to 10 mL with distilled water. Each solution was incubated for 30 minutes at room temperature, and then its absorbance was measured using UV-Vis spectrometer (Optizen, Korea).

### 2.3. Total Polysaccharide

In order to assay total polysaccharide, the changes in absorption of solution on 490 nm, in the presence of acidic phenol were observed (7). The basis of this method is that all the glycoside linkage broke in the presence of sulfuric acid and an atomic complex is made between polysaccharide and phenol, afterwards the light absorbance of that complex is measured. Each sample (100 $\mu$L) with 500 $\mu$L of phenol 4% were added to 2.5 mL of sulfuric acid 96% and after 5 minutes the absorption of mixture was measured. The calibration curve using different concentrations of glucose were depicted to calculate polysaccharide contents of the samples as glucose equivalent (GE).

### 2.4. Total Proteins

Total protein contents of the samples were analyzed using the Bradford reagent. The basis of the Bradford method is actually on the complex of reagents with proteins which cause the light absorption of Bradford reagent (Coomassie Brilliant Blue G-250, Sigma-Aldrich, USA) change from 465 nm to 595 nm (8). Light absorptions of a solution of 100 $\mu$L of each sample together with 900 $\mu$L of deionized water and 5 mL of Bradford reagent was evaluated at 595 nm UV-Vis spectrometer (Optizen, Korea). The absorptions of each sample were compared to the bovine serum albumin standard curve to determine the amount of proteins.

### 2.5. Total Lipids

Each sample of royal jelly (1 g) was weighted and homogenized with 100 mL of distilled water using an ultrasonic homogenizer for 30 minutes until the compound was completely dispersed. In the next step, equal amounts of chloroform and ethanol were added to the mixture and filtered through a Whatman filter paper No. 1 (Sigma-Aldrich, USA). Buchner tunnel and vacuum were applied for more rapid performance of filtration. After filtration, the chloroform phase was separated and transferred to a beaker on a warm water bath to evaporate the chloroform. Finally, the beaker was cooled and weighed to calculate the amount of lipid present in 1 g of royal jelly (9).

### 2.6. Moisture and Ash Contents

The amounts of ash in each royal jelly sample (each 1 g) were measured after being in the oven at 550°C for 8 hours. After 1 hour of losing heat, the crucible was weighted and the difference between initial and final weights indicated the amount of ash present in 1 g of royal jelly specimens.

To calculate the moisture percentage of royal jelly samples, 1 g of each specimen was weighed in a crucible to put in an oven of 105°C for 3 hours. The crucible was carefully weighed after cooling. The samples remained in the oven for 1 more hour to examine if the weights decrease. The process have to be repeated again until the evaporation of water is complete with constant weight (10).

### 2.7. Statistical Analysis

After calculating the amount of each group of components in three specimens of raw and commercial samples of royal jelly, the amounts were reported as mean ± standard deviation (SD). The statistical method of *t*-test has been applied using Excel 2013 to compare the means. P values $< 0.05$ was considered as statistically significant.

## 3. Results

The chemical content of an Iranian raw and a commercial sample of royal jelly including total phenol, protein, polysaccharide, lipid, moisture, and ash were successfully calculated using relevant spectrophotometric and gravimetric methods. The results showed that similar amounts of phenol and polysaccharide were present in the commercial and raw samples of royal jelly (phenol: 22.98 ± 0.34 and 21.99 ± 0.41 $\mu$g/mg GAE; polysaccharide: 12.67 ± 0.00 and 12.63 ± 0.00%). Contents of lipid (12.00 ± 0.00%) and protein (11.57 ± 0.00%), two other noteworthy classes of compounds in Iranian raw sample were calculated to be significantly higher than those in commercial sample (lipid: 11.00 ± 0.00 and protein: 8.21 ± 0.00%). The moisture of commercial sample (61.03 ± 0.00%) was significantly higher than moisture in raw royal jelly (59.01 ± 0.00%). The similar amounts of ash were analyzed in the both tested samples (0.1 ± 0.00%).

**Table 1.** Chemical Contents of Commercial and Raw Samples of Royal Jelly

| Samples | Phenol[a] | Polysaccahride[b] | Protein[b] | Lipid[b] | Moisture[b] | Ash[b] |
|---|---|---|---|---|---|---|
| **Commercial** | 22.98 ± 0.34 | 12.67 ± 0.00 | 8.21 ± 0.00[c] | 11.00 ± 0.00[c] | 61.03 ± 0.00[c] | 0.1 ± 0.00 |
| **Raw** | 21.99 ± 0.41 | 12.63 ± 0.00 | 11.57 ± 0.00[c] | 12.00 ± 0.00[c] | 59.01 ± 0.00[c] | 0.1 ± 0.00 |

[a] $\mu$g/mg gallic acid equivalent.
[b] Values are expressed as percentage.
[c] Statistically different.

## 4. Discussion

Royal jelly is a natural and the most important product of *A. mellifera*, honey bee. Several studies varying from specific component analysis or total analysis of royal jelly have been performed on the product since 1955. The analysis and comparative studies become more accurate over time. According to the previous study, the freshly harvested royal jelly contained 61% water, 12% proteins, 7.6% lipids and up to 18% of polysaccharide (11). According to Table 1, except for comparable moisture, other parameters including protein and polysaccharide in the both tested samples of royal jelly in the present study were lower than those reported previously. While, higher lipid contents in the commercial and raw samples were analyzed in the current study. Another study compared contents of raw samples of royal jelly from different ecosystems in South America to Europe suggesting wider standard ranges of components present in royal jelly (60% to 70% water, 8 to 18% proteins, 9% to 18% total polysaccharide, 3% to 11% lipids and 0.3% to 0.5% ash) (12). Considering these wide-ranging chemical parameters of royal jelly, both of the tested samples are comparable with those examined samples of different origins. The amounts of ash in both samples of our study were lower (0.1%) than those reported previously (12, 13). The quantities of phenolic compounds in our samples were similar to that found previously by Nagai and Inoue (21.2 $\mu$g/mg) and Nabas et al. (23.3 $\mu$g/mg) (13, 14). The origin and the amount of phenolic compounds in royal jelly could be affected by the plant species that used by the bees, health of the plant, season, and environmental factors (15). Collection time of RJ after larval transfer could impact the phenolic contents of the product (16). These compounds have a reputation for their antioxidant, immunomodulation and anti-inflammatory activities (14). Overall, both of the analyzed RJ samples contained comparable amounts of desired compounds with previous reported specimens. Therefore, Iranian raw sample of royal jelly could be a suitable source to produce commercial preparations compared to the formulated royal jelly.

## Acknowledgments

This study was supported by Tehran University of Medical Sciences.

## Footnotes

**Authors' Contribution:** Study concept and design: Abbas Hadjiakhondi and Azadeh Manayi; analysis and interpretation of data: Azadeh Manayi and Mina Saeedi; drafting of the manuscript: Azadeh Manayi and Vida Kazemi; critical revision of the manuscript for important intellectual content: Azadeh Manayi, Mina Saeedi, Vida Kazemi, Abbas Hadjiakhondi, Moein Eskafi; statistical analysis: Moein Eskafi.

**Conflict of Interests:** There is nothing to declare.

**Funding/Support:** The manuscript was supported by Tehran University of Medical Sciences.

## References

1. Wytrychowski M, Chenavas S, Daniele G, Casabianca H, Batteau M, Guibert S, et al. Physicochemical characterisation of French royal jelly: Comparison with commercial royal jellies and royal jellies produced through artificial bee-feeding. *J Food Compos Anal*. 2013;**29**(2):126–33. doi: 10.1016/j.jfca.2012.12.002.
2. Nagai T, Sakai M, Inoue R, Inoue H, Suzuki N. Antioxidative activities of some commercially honeys, royal jelly, and propolis. *Food Chemistry*. 2001;**75**(2):237–40. doi: 10.1016/s0308-8146(01)00193-5.
3. Burlando B, Cornara L. Honey in dermatology and skin care: A review. *J Cosmet Dermatol*. 2013;**12**(4):306–13. doi: 10.1111/jocd.12058. [PubMed: 24305429].
4. Taavoni S, Barkhordari F, Goushegir A, Haghani H. Effect of Royal Jelly on premenstrual syndrome among Iranian medical sciences students: A randomized, triple-blind, placebo-controlled study. *Complement Ther Med*. 2014;**22**(4):601–6. doi: 10.1016/j.ctim.2014.05.004. [PubMed: 25146061].
5. Serra Bonvehi J. Study of adulteration of Royal Jelly with other honeybees products and water. *Investig Agrar Prod Sanid Anim*. 1991;**6**:99–111.
6. Pyrzynska K, Biesaga M. Analysis of phenolic acids and flavonoids in honey. *TrAC Trends Anal Chem*. 2009;**28**(7):893–902. doi: 10.1016/j.trac.2009.03.015.
7. Vazirian M, Dianat S, Manayi A, Ziari R, Mousazadeh A, Habibi E, et al. Anti-inflammatory effect, total polysaccharide, total phenolics content and antioxidant activity of the aqueous extract of three basidiomycetes. *Res J Phcog*. 2014;**1**(1):15–21.
8. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976;**72**:248–54. doi: 10.1006/abio.1976.9999. [PubMed: 942051].
9. Muller E, Berger R, Blass E, Sluyts D. Liquid-liquid extraction. *Ullmann's encyclopedia of industrial chemistry*. Wiley-VCH Verlag GmbH & Co. KGaA; 2000. doi: 10.1002/14356007.b03_06.
10. Horwitz W, Latimer G. *Official methods of analysis of AOAC international*. Gaithersburg MA, USA: Association of Official Analytical Chemist; 2000.

11. Johansson TSK. Royal Jelly. *Bee World*. 1995;**36**(2):21–32. doi: 10.1080/0005772x.1955.11094863.
12. Sabatini AG, Marcazzan GL, Caboni MF, Bogdanov S, Almeida-Muradian L. Quality and standardisation of Royal Jelly. *J ApiProd ApiMed Sci*. 2009;**1**(1):16–21. doi: 10.3896/ibra.4.01.1.04.
13. Nabas Z, Haddadin M, Haddadin J, Nazer I. Chemical composition of Royal Jelly and effects of synbiotic with two different locally isolated probiotic strains on antioxidant activities. *Pol J Food Nutr Sci*. 2014;**64**(3):171–80. doi: 10.2478/pjfns-2013-0015.
14. Nagai T, Inoue R. Preparation and the functional properties of water extract and alkaline extract of Royal Jelly. *Food Chem*. 2004;**84**(2):181–6. doi: 10.1016/s0308-8146(03)00198-5.
15. Kucuk M, Kolayli S, Karaoglu S, Ulusoy E, Baltaci C, Candan F. Biological activities and chemical composition of three honeys of different types from Anatolia. *Food Chemistry*. 2007;**100**(2):526–34. doi: 10.1016/j.foodchem.2005.10.010.
16. Liu JR, Yang YC, Shi LS, Peng CC. Antioxidant properties of royal jelly associated with larval age and time of harvest. *J Agric Food Chem*. 2008;**56**(23):11447–52. doi: 10.1021/jf802494e. [PubMed: 19007163].