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Physicochemical Characterization and Antioxidant Activity of Royal Jelly from Northwestern Bosnia and Herzegovina

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Abstract

With the discovery of the main bioactive compounds, royal jelly (RJ) takes a significant role in the food and pharmaceutical industry. One of the most important ingredients of RJ is 10-hydroxy-2-decenoic acid (10-HDA). In this paper, ten samples of RJ were analyzed, which were collected from the area of northwestern Bosnia and Herzegovina. In addition to 10-HDA, glucose, fructose, sucrose, and maltose content, physicochemical parameters were analyzed: pH value, total acidity, water content, protein content, and antioxidant activity of RJ. The obtained results show that samples of RJ meet international standards with regard to the content of 10-HDA. Considering the established quality and very high antioxidant activity of the analyzed samples (analyzed with DPPH and FRAP methods) they have significant potential in development of functional products with pronounced nutritional and biological capacity. This work is first attempt in establishment of RJ quality criteria in this area.

Keywords: Royal jelly; 10-HDA; Physicochemical characteristics; Antioxidant activity; Carbohydrates

Introduction

Royal jelly (RJ) is a complex biological mixture produced by the mandibular and hypopharyngeal glands of young worker bees of the *Apis mellifera* species. It is the main food for the queen bee throughout her life and is essential for the development of larvae in the colony. This white, light yellow or orange substance has a gelatinous-viscous texture, a peculiar sour taste and a recognizable phenol smell [1-4]. In addition to being rich in nutrients, royal jelly also possesses numerous biologically active components, which makes it the subject of much scientific research and important for both the use and commercial purposes.

The biological functions of royal jelly include growth promotion, development of immune system and reproductive functions, as well as queen longevity. Due to its exceptional nutritional value and biological activity, royal jelly is used in human nutrition as a food supplement, and in the pharmaceutical and cosmetic industries. Of particular importance are studies of its immunomodulatory, antitumor, antimicrobial, and antioxidant effects [5,6]. The increased interest in royal jelly in the scientific and industrial community is fueling the need for detailed study of its composition and quality standardization.

The chemical composition of royal jelly varies depending on numerous factors, including bee diet, bee species, climate, season, harvesting techniques, and larval age [7]. At the macro level, royal jelly is relatively stable, consisting of 60 %–70 % of water, 9 %–18 % of protein, 7 %–18 % of carbohydrates, 3

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%–8 % of lipids, 3 %–8 % of minerals and traces of vitamins, phenols, and amino acids [4,8].

The most important authentic honeybee proteins are royal jelly (RJ) proteins. They are the most abundant organic component in royal jelly, accounting for 11 % to 14 % of the total amount of royal jelly [9]. In RJ, the main proteins belong to the Major royal jelly proteins family (MRJPs, apalbumins). They represent up to 90 % of the total proteins in RJ, with MRJP1 (known as royalactin) being the most abundant (31-66 % of total RJ proteins), followed by MRJP3, MRJP2 and MRJP5. All MRJPs belong to a protein family consisting of nine members with Mr of 49–87 kDa, and have 1-8 predicted *N*-glycosylation sites. Water-soluble protein accounts for 46 % to 89 % of the total protein content [9,10]. Minor RJ proteins are mainly the homologues of apalbumins, antimicrobial peptides, and enzymes [11]. In RJ, a series of short peptides: Jelleines-1, Jelleines-II, Jelleines-III, and Jelleines-IV were identified [12]. The activities of enzymes invertase, amylase, ascorbinoxidase, catalase, acid phosphatase, and insulin-like peptid in RJ were determined [13]. Arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are essential amino acids commonly found in MRJPs; necessary for nourishing the queen bee and larvae [12]. MRJPs have important healthcare effects, exhibited mainly as antibacterial activity, strong immune activity, and promotion of special cell production possibly by acting as bio-similars or substitutes for growth factors [14]. MRJP1 and MRJP2 act as allergens in some people, posing a higher risk to individuals who already have other allergies [12].

The portion of carbohydrates accounts for 30 % of the dry matter of RJ. In qualitative terms, the carbohydrate components are highly constant, while a quantitative composition is very variable. The monosaccharides fructose and glucose are the main sugars, and they often account for over 90 % of the total sugars. The fructose is prevalent. Sucrose is always present but in highly variable concentrations. It is also possible to find oligosaccharides such as trehalose, maltose, gentiobiose, isomaltose, raffinose, erlose, melezitose; though present in very small concentrations, they are useful for identifying a characteristic pattern of RJ [15].

The proportion of lipids in RJ is relatively low (8-19 % of dry matter), but it is the most important fraction of RJ. It consists of organic acids (80-90 %), most of which are mono- and dihydroxy acids and dicarboxylic acids with 8 and 10 carbon atoms. Hydroxy acids with 10 carbon atoms (10-hydroxydecenoic and 10-hydroxy-2-decenoic acid) can be found in high concentrations. Not only as a marker component of RJ, but they have also been identified as responsible for important biological activities. The adulteration of RJ is the most important quality problem, and the most important quality criteria for RJ adulteration is 10-HDA. The 10-HDA content decreases with storage of RJ [15].

Previous research has shown that the quality and composition of royal jelly vary across geographical regions, implying the need to define local quality criteria. Microcomponents such as specific proteins, fatty acids, and bioactive compounds can vary significantly, affecting its biological properties and potential therapeutic value. In many countries that are major producers of royal jelly, such as China and Japan, quality standards have been established that include minimum content of protein, 10-HDA, and other key components [9,16]. In Bosnia and Herzegovina, research on royal jelly composition is still in its infancy, although this country has a great potential for producing high-quality royal jelly due to its favorable climate and biodiversity. Setting royal jelly quality standards in Bosnia and Herzegovina is not only important for consumer protection and health promotion, but also for the economic development of the beekeeping industry in the region. Therefore, the aim of this research was to determine the physicochemical properties of royal jelly produced in the northwestern part of Bosnia and Herzegovina (the Una-Sana Canton) in order to take the first steps in establishing royal jelly quality criteria at the national level. This research represents a foundation for further research that will contribute to the understanding of local variability in royal jelly composition and enable the establishment of adequate quality standards in accordance with national and international norms.

Materials and Methods

Royal jelly samples

In this study, ten samples of fresh royal jelly collected directly from beekeepers from different locations in the northwestern part of Bosnia and Herzegovina were analyzed: Velika Kladuša (RJ1, RJ2), Cazin (RJ3, RJ4), Bihać (RJ5, RJ6), Ključ (RJ7), Sanski Most (RJ8), Bosanska Krupa (RJ9, RJ10). The samples were stored under hygienic conditions in glass vials, protected from light. The samples were transported to the laboratory frozen to preserve their quality and prevent changes in composition. They were stored at a temperature of -20 °C until analysis.

Chemicals and Instruments

The used analytical grade chemicals in this work were: 5,5-Dithiobis(2-nitrobenzoic acid) DTNB, 2,4,6-Tris(2-pyridyl)-s-triazine TPTZ, bovine serum albumine (BSA), Glucose, Fructose, Sucrose, Maltose (Sigma-Aldrich, GmbH, Steinheim, Germany); BHA (Butylated hydroxyanisol), BHT (Butylated hydroxytoluene), Sodium carbonate, Sodium hidroxide (Acros Organics, USA); Gallic acid (Carl Roth, GmbH); Folin-Ciocalteu's reagent (Darmstadt, Germany); Ethanol 96 %, HCl, $FeCl_3$ x $6H_2O$, $FeSO_4$ x $7H_2O$, $K_4Fe(CN)_6$ x $3H_2O$, $Zn(CH_3COO)_2$ x $2H_2O$ (Kemika, Zagreb). The HPLC grade chemicals were: Water, Acetonitrile, Methanol, 10-HDA (Cayman Chemicals, USA). The spectrophotometric

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measurements were performed on a photoLab 6600 UV-VIS spectrophotometer. Data for the devices used for chromatographic analyzes are given in the description of methods for the determination of 10-HDA and individual carbohydrates.

pH value

The pH value was determined according to the method described in the international standard for royal jelly [17], while the total acidity was determined by the titrimetric method [18].

Water content

The water content of royal jelly was determined by measuring the refractive index using the Abbe refractometer (OPTECH, model RMT), according to Sesta and Lusco (2008) at a constant temperature of 20 °C [19]. Before analysis, the sample was stored in a refrigerator for up to 30 minutes, after which it was allowed to equilibrate to room temperature. The refractive index was measured on an aliquot of the carefully homogenized sample, without any prior treatment. After the sample was allowed to equilibrate in the refractometer for two minutes, a reading was taken. The refractometer was then cleaned and dried before measuring the next sample. All measurements were performed in triplicate.

Total protein content analysis

Total protein content was determined according to the method by Lowry et al. (1951) [20]. A 0.05 g sample of RJ was dissolved in 10 mL of redistilled water and centrifuged for 25 min at 2300 g (4500 rpm, radius 100 mm). To 1 ml of sample, 5 ml of alkaline solution was added – which was obtained by mixing 50 ml of solution I (20 g/L Na_2CO_3 in 0.1 mol/l NaOH) and 1 ml of solution II ($CuSO_4$ x 5 H_2O 5 g/l in 10 g/L Na,K-tartrate). After mixing, the solution was left at room temperature for 10 min. Then 0.5 ml of Folin-Ciocalteu reagent was added with simultaneous mixing. After 30 min at room temperature, the absorbance was read at 750 nm with a blank assay. To create the calibration curve, the standard solution of albumin in the concentration range of 0.1 to 1 % w/w (0, 0.1, 0.3, 0.5, 0.75, 1 %) was used, which was analyzed in the same way as previously described. Results are expressed as mass fraction w/w.

Analysis of total phenolic content

The total phenolic content of royal jelly was determined by the Folin-Ciocalteu method [21], and the results were expressed in mg of gallic acid per 100 g royal jelly. 5 g of royal jelly were weighed and dissolved in approximately 20 mL of water using an ultrasonic bath. The dissolved samples were then quantitatively transferred to 50 mL volumetric flasks and filled up to the mark with distilled water. For analysis, 100 µL of sample was measured, to which 1 mL of Folin-Ciocalteu reagent (diluted with distilled water in a ratio of 1:10) was added, after which the contents of the tube were mixed intensively for 2 minutes. After 20 minutes of standing at room temperature, the absorbance was measured at a wavelength of 750 nm. Measurements were performed three times for each sample. The concentration of total phenols was read from the calibration curve of gallic acid, which was analyzed in the concentration range from 8 to 120 mg/L. The results are expressed as mg of GAE/g of royal jelly.

Determination of glucose, fructose, sucrose and maltose content

The content of glucose, fructose, maltose and sucrose was determined using the HPLC technique; the method described in Sesta (2005) [22]. In brief, about 9 mL of water/methanol solution (3:1) was added to a 2 g RJ sample in a 10 mL volumetric flask. After the sample was homogenized by mixing, 0.2 mL of Carrez I reagent (distilled water solution of potassium hexacyanoferrate (II), 15 g/100 mL) and 0.2 mL volume of Carrez II reagent (distilled water solution of zinc acetate, 30 g/100 mL) were added to the flask and mixed. The flask was filled up to 10 mL volume with the water/methanol solution and mixed. The solution was then centrifuged at 4000 rpm for 15 minutes to remove the proteins. An aliquot of 5 mL of the supernatant was transferred to a 10 mL glass vial. Five mL of dichloromethane was added, and the vial was vortexed for five minutes. After separation of the layers, the upper layer with the sugar fraction was collected. The extraction from the upper layer was repeated twice more. The extract was filtered through a 0.45 µm disposable syringe filter. The sample was stored at 4 °C until further analysis. Sugar determination was performed on a Shimadzu liquid chromatograph consisting of a solvent delivery module (LC-2040C), oven (CTO-20AC), autosampler (SIL-20AC), pump (LC-20AD) and differential refractometric detector RID-20A. The instrument was supported by LabSolution software. Carbohydrate separation was performed on a 5 µm diameter column (Shim-pack GIST NH2) measuring 250 x 4.6 mm. The mobile phase flow rate (acetonitrile/water, 85:15 ratio) was 0.9 mL/min. The injection volume was 10 µL, and the column temperature was set at 33 °C. Qualitative and quantitative determination was based on the injection of an external standard prepared as follows: 100 mL water/methanol solution (3:1) containing 1.1 g of fructose, 1.1 g of glucose, 0.3 g of sucrose and 0.3 g of maltose. The results are expressed as a w/w percentage (g/100 g) of each sugar on the crude RJ.

Determination of 10-HDA content

The 10-HDA content was determined on a Shimadzu liquid chromatograph consisting of an LC-20AD solvent delivery module, a CTO-20AC column oven, a SIL-10AF autosampler, and an SPD-M20A photodiode array detector

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(for 10-HDA determination). The instrument was supported by LabSolution Lite software (version 5.52). The analysis was performed according to the method described in the international standard ISO 12824 (ISO, 2016) and optimized as described in our previous work [23].

Determination of antioxidant activity

The antiradical activity of royal jelly samples was determined according to the Brand-Williams et al. (1995) method with certain modifications [24]; 800 µL of acetate buffer (100 mM, pH 5.5) and 1 900 µL of DPPH reagent were added to 300 µL of royal jelly solution (10 %). Absolute alcohol was used to adjust the zero of the apparatus, and the absorbance of the DPPH reagent was measured. For blank tests, acetate buffer replaced the DPPH for each sample. The prepared samples were left in the dark for 60 minutes, after which the absorbance was read at 517 nm. The radical scavenging ability of the tested samples was calculated using the following formula: % inhibition = [(A0 – Asample) / A0] x 100; A0 – absorbance of the DPPH ethanol solution measured at the beginning at 517 nm; Asample – absorbance of the sample measured after 60 minutes. Obtained results were expressed as the percentage of inhibition of DPPH radicals.

The total reduction potential of royal jelly samples was determined by the FRAP method [25]. The method is based on electron transfer, using the iron complex with 2,4,6-tris(2-pyridyl)-s-triazine (Fe(III)-TPTZ complex) as the oxidant. Upon reduction of the yellow-colored complex in the presence of antioxidants and at low pH, the reaction mixture changes color to blue, with an absorbance maximum at a wavelength of 593 nm. By comparing the change in absorbance of the reaction mixture at λ = 5 93 nm, with that of solutions of ferrous ions of known concentration ($FeSO_4$ x $7H_2O$; 50, 100, 150, 250, 500, 750 µM), the calibration curve shows the total reducing capacity of antioxidants in the reaction solution. The detailed procedure is described in Oraščanin et al. (2023) [26]. The results were expressed as Fe(II) µM/g of RJ.

Statistical data analysis

The results of the analyzed samples are presented as mean value ± standard deviation. One-way analysis of variance (ANOVA) and multiple comparisons (Duncan's Post Hoc Test) were used to assess the significant difference in data at the $p<0.05$ significance level. Statistics were implemented using Microsoft Office 2014 and the demo version of the statistical package MS Office XLSTAT-Pro 2014 [27]. A principal component analysis (PCA) was also performed. Measurements for all methods used in this research were done in three repetitions for each sample.

Results and Discussion

This study analyses the physicochemical characteristics and quality parameters of ten royal jelly samples from the northwestern part of Bosnia and Herzegovina. The results indicated significant variability among the samples, which reflects the influence of local conditions, such as bee diet, seasonal changes, and collection techniques. The results for pH values, total acidity, water content, and sugar content (fructose, glucose, and sucrose) are presented in Table 1. The results for 10-HDA, DPPH, FRAP, proteins and total phenols are presented in Table 2, while the results of statistical analysis are presented in Table 3 and Figure 1.

The pH values of the analyzed samples ranged from 5.13 to 5.70, which is higher than the standard range for royal jelly, which is between 3.5–4.5 [23,28,29]. These elevated values may indicate reduced acidity, which may affect product stability. Given that acidity is crucial for preserving freshness and biological activity, samples with lower pH values, such as RJ4, showed potentially better-quality conditions. Total acidity varied between 15.09 and 36.36 mL NaOH/100 g, while it is usually between 30 and 40 mL in other studies. [23] Lower acidity values in sample RJ10 (15.09 mL) may indicate longer storage or differences in collection conditions.

The water content of the samples ranged from 64.94 % to 76.00 %, with an average of 68.61 % (Table 1). The variations in water content among the samples were statistically significant ($p<0.05$). The water content was higher compared to the results obtained in other studies [30]. High water content may indicate insufficiently dried samples, altitude, collection period or contamination [18]. The international standard (ISO, 12824: 2016) defines the moisture content of fresh royal jelly as 62.00 % and 68.50 % [17]. Ideally, royal jelly should have a water content of less than 60 %, and high values may negatively affect the quality and stability of the product. Table 3 shows a positive significant correlation of moisture content with 10-HDA ($r=0.733$, $p<0.05$) and sucrose ($r=-0.626$, $p<0.05$), while a negative significant correlation with glucose ($r=-0.4130$, $p<0.05$).

The proportion of fructose and glucose (Table 1) can be compared with studies showing typical values for these sugars in royal jelly. Sabatini et al. (2009) reported that the total content of fructose, glucose and sucrose in RJ is 7 to 18 %. Based on the available literature, the range of content of fructose (3-13 %), glucose (4-8 %) and sucrose (0.5-2 %) in RJ is given [15,16]. The average glucose content of 5.16 % is consistent with the literature, while the fructose content can vary. The comparison can reveal how similar these samples from the northwestern part of Bosnia and Herzegovina are to the samples from other regions in terms of carbohydrate content, which is an important indicator of authenticity. The fructose content varied from 2.85 % to 5.23 %, with an average of 3.82 %. The glucose content varied from 4.11 % to 6.25 %, with an average of 5.16 %. Fructose and glucose are the key sugars in royal jelly. The results presented indicate

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Table 1: Physicochemical parameters of fresh RJ samples.

RJ	pH	Total acidity mL	Water %	Fructose %	Glucose %	Sucrose %
RJ1	5.37±0.08$^{b.c.d}$	33.87±0.10^{c}	64.94±0.12^{a}	2.85±0.01^{g}	5.04±0.12^{d}	3.60±0.05^{a}
RJ2	5.24±0.10$^{f.g}$	29.25±0.10^{e}	64.93±0.07^{a}	5.23±0.10^{a}	5.74±0.03^{b}	1.73±0.04^{d}
RJ3	5.28±0.05$^{e.f}$	29.78±0.01^{d}	67.00±0.01^{b}	3.72±0.01^{d}	5.05±0.02^{d}	3.00±0.01^{b}
RJ4	5.16±0.05^{g}	34.03±0.01^{b}	70.00±0.03^{f}	2.96±0.02^{f}	5.43±0.01^{c}	2.96±0.01^{b}
RJ5	5.21±0.01$^{f.g}$	36.36±0.02^{a}	69.00±0.01^{e}	2.90±0.03$^{f.g}$	4.11±0.01^{f}	2.45±0.02^{c}
RJ6	5.29±0.02$^{d.e.f}$	26.67±0.01^{f}	67.90±0.01^{c}	4.07±0.02^{c}	5.18±0.10^{d}	1.27±0.02^{g}
RJ7	5.42±0.01 $^{b.c}$	19.98±0.01^{h}	68.10±0.01^{c}	4.12±0.10^{c}	4.71±0.20^{e}	1.38±0.10^{f}
RJ8	5.45±0.01^{b}	17.80±0.01^{i}	68.47±0.35^{d}	3.60±0.10^{e}	4.60±0.20^{e}	1.49±0.10^{e}
RJ9	5.35±0.01$^{c.d.e}$	21.95±0.10^{g}	69.80±0.02^{f}	3.74±0.01^{d}	5.52±0.02^{c}	1.05±0.03^{h}
RJ10	5.70±0.01^{a}	15.09±0.02^{j}	76.00±0.02^{g}	5.00±0.02^{b}	6.25±0.01^{a}	0.17±0.01^{i}
Average	5.71	26.48	68.61	3.82	5.16	1.91
SD	0.15	7.16	3.027	0.79	0.59	1.02
Min	5.13	15.07	64.85	2.84	4.1	0.16
Max	5.71	36.38	76.02	5.33	6.27	3.65

Min-minimum. Max-maximum. SD-standard deviation; Values represent the average of triplicates ± standard deviation; a.b.c.d.e.f.g.h.i.j - Mean values in the same row marked with different letters are statistically significantly different according to Duncan's test ($p<0.05$).

Table 2: Bioactive compounds and antioxidant activity of RJ samples.

RJ	10-HDA %	Proteins %	TPC mg GAE/100 g	DPPH % for 10 % solution	FRAP Fe2+µM/g
RJ1	3.65±0.02^{a}	20.03±1.17^{a}	0.31±0.01^{b}	41.94±0.78^{a}	134.78±0.10^{a}
RJ2	3.43±0.09^{b}	14.07±1.71$^{c.d}$	0.21±0.01^{e}	38.40±1.18$^{b.c.d}$	129.03±0.10^{b}
RJ3	2.68±0.03$^{e.f}$	13.67±0.78$^{c.d}$	0.37±0.01^{a}	38.91±1.00$^{b.c}$	125.76±0.20^{c}
RJ4	3.15±0.08^{c}	10.14±0.39^{e}	0.19±0.02^{f}	37.97±0.05$^{c.d.e}$	104.41±0.17^{g}
RJ5	3.14±0.03^{c}	8.36±2.47^{e}	0.27±0.01^{c}	36.61±0.05^{e}	106.19±0.18^{f}
RJ6	2.62±0.02$^{e.f}$	14.70±0.61$^{c.d}$	0.18±0.00^{f}	39.76±1.26^{b}	119.30±0.74^{d}
RJ7	2.82±0.05^{d}	17.89±0.18^{b}	0.23±0.00^{d}	39.94±1.10^{b}	125.71±0.26^{c}
RJ8	2.61±0.04^{f}	13.80±0.44$^{c.d}$	0.23±0.00^{d}	38.52±0.82$^{b.c.d}$	134.07±0.10^{a}
RJ9	2.71±0.04^{e}	12.64±1.28^{d}	0.27±0.00^{c}	36.98±0.05$^{d.e}$	109.81±1.10^{e}
RJ10	2.19±0.04^{g}	15.79±0.11^{c}	0.21±0.01^{e}	38.73±1.39$^{b.c}$	134.84±2.60^{a}
Average	2.9	14.11	0.25	38.78	122.39
SD	0.42	3.41	0.06	1.66	11.46
Min	2.14	5.51	0.17	36.58	104.23
Max	3.68	21.38	0.38	42.39	137.44

10-HDA: 10-Hydroxy-2-decenoic acid; TPC: Total phenolic content. Min-minimum. Max-maximum. SD-standard deviation; Values represent the average of triplicates ± standard deviation; $^{a.b.c.d.e.f.g}$ - Mean values in the same row marked with different letters are statistically significantly different according to Duncan's test ($p<0.05$).

variability in content, with sample RJ2 having the highest fructose content. The high content of fructose in regard to glucose may be an indicator of the specific diet of the bees and the quality of the nectar. The low sucrose concentration (average 1.91 %) is in line with other studies, where sucrose is present in smaller amounts compared to fructose and glucose [31]. The proportion of sucrose ranges from 0.17 % to 3.60 %, with an average of 1.91 %. A low concentration of sucrose is desirable, as high values may indicate added sugar or adulteration. Sample RJ10 shows an extremely low concentration of sucrose, which may suggest good quality. Samples RJ1, RJ5 and RJ6 contain the maltose in the values of 0.19 %, 0.08 % and 0.12 %, respectively, while no maltose was detected in other samples. Maltose can be present in royal jelly samples [32]. The statistical analysis indicated a high significant positive correlation between fructose and glucose content ($r=0.6441$, $p<0.05$) (Table 3). The protein content in RJ, analyzed from ten different locations in northwestern BiH, was 8.36 ± 2.47 % for the sample from the area of the city of Bihać, while the highest value in protein content was

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measured in samples from the area of Velika Kladuša, 20.03 ± 1.17 % (Table 2). Given that the protein content in RJ ranges from 9 to 18 % [4,8,9,10], it can be concluded that the majority of samples, i.e. the average protein content in the analyzed samples, is in accordance with the aforementioned literature data, and that the analyzed samples represent a significant source of RJ protein. In eight RJ samples, a high protein content (>11 %) was determined, which is in accordance with the international standard (11-15 %) (ISO, 12824: 2016) [17]. The high content of RJ proteins indicates their potential as food supplements with regard to their nutritional value and biological activity [1]. The statistical analysis showed a positive correlation of protein content with DPPH ($r=0.7078$, $p<0.05$) and FRAP (0.7449, $p<0.05$) (Table 3).

Total phenols range from 0.18 mg GAE/g (to 0.37 mg GAE/g of RJ3. Phenols are important for their biological activity and antioxidant properties. The results obtained for total phenols in all samples were relatively low. The content of total phenols was lower compared to the results of previously published studies: 11.66 – 36.73 mg GAE/g [33], 1.82 to 8.61 mg GAE/g [34], 2.35 to 4.07 mg GAE/g [35], and similar to Turkish RJ result of 0.59 mg GAE/g [36] Lower results may be due to high water content in the samples. The content of 10-HDA in the samples ranged from 2.14 % to 3.68 %, with an average value of 2.90 % and a standard deviation of 0.42 %. The 10-HDA (10-hydroxydecanoic acid) is a key component used as an indicator of royal jelly quality. A higher content of this acid may indicate better quality and biological activity. Sample RJ1 with the highest content of 10-HDA (3.65 %) may be particularly useful in therapeutic and nutritional applications [37]. Although 10-HDA is recognized as a key parameter in detecting royal jelly adulteration, only a small number of countries have regulated requirements for the content of 10-HDA at the national level. At the international level, recommendations for certain quality parameters have been issued by the International Organization for Standardization. All samples met the recommendations of the international standard for the specification of fresh royal jelly (ISO, 2016), in which the prescribed minimum value for the 10-HDA content is 1.4 % [17]. The proportion of 10-HDA in the analyzed samples is slightly higher than that reported in Flanjak et al (2019) for Croatian royal jelly [23], and Kanelis et al (2015) for Greek royal jelly [18]. Slightly higher values (average 3.81 ±1.65 %) were obtained in Alattal et al (2025) for royal jelly samples from Saudi Arabia [38]. As stated by Alkidni et al (2024), differences in the proportion of 10-HDA, as well as other components, are influenced by environmental conditions, climate change, nectar source, extraction techniques and subsequent handling of royal jelly (especially storage conditions), as well as the bee species [39]. The statistical analysis (Table 3) revealed a significant positive correlation between 10-HDA with the content of sucrose ($r=0.7313$, $p<0.05$) and water ($r=0.7333$, $p<0.05$), as well as a significant negative correlation with fructose ($r=-0.3870$, $p<0.05$) and pH ($r=-0.5928$, $p<0.05$).

The DPPH test results showed that the samples have a significant free radical scavenging capacity (36.61 %–41.94 %), with the highest value in sample RJ1. FRAP analysis further confirmed the strong antioxidant capacity, with a range of 104.23 µM/g to 137.44 µM/g. These results indicate the potential of these royal jelly samples as functional products with antioxidant properties. The obtained results are higher compared to the samples of RJ from Bulgaria with the following ranges obtained: DPPH 10.17 – 39.39 % inhibition

Table 3: Pearson correlation coefficients of analyzed physicochemical parameters of fresh RJ samples.

	pH	TA[a]	Proteins	10-HDA	DPPH	FRAP	TPC[b]	Water	Fructose	Glucose	Sucrose
pH	1										
TA[a]	**-0.8022***	1									
Proteins	**0.4544***	-0.3348	1								
10-HDA	**-0.5928***	**0.7643***	0.0651	1							
DPPH	0.2823	-0.0085	**0.7078***	0.212	1						
FRAP	**0.6409***	**-0.4827***	**0.7449***	-0.117	**0.5926***	1					
TPC[b]	-0.071	0.2771	0.1094	0.1556	0.1535	0.1391	1				
Water	**-0.5950***	**0.5413***	0.2016	**0.7333***	0.3354	0.0918	0.3311	1			
Fructose	**0.4508***	**-0.5843***	0.2373	**-0.3870***	0.0025	**0.4618***	-0.355	-0.2274	1		
Glucose	**0.3972***	-0.3451	0.1978	-0.2482	0.0794	0.2286	-0.283	**-0.4130***	**0.6441***	1	
Sucrose	**-0.6201***	**0.8406***	-0.0534	**0.7313***	0.2571	-0.1738	**0.5353**	**0.6259***	**-0.7097***	**-0.4116***	1

* Correlation is significant at the 0.05 level. [a] TA – Total Acidity. [b] TPC – Total Phenolic Content.

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for 10% RJ solution, and FRAP 0.44 – 8.49 mM Fe2+/g [33]. The RJ has antioxidant activity, and it was shown that the antioxidant activity derived from MRJPs and peptides. The peptides obtained from RJ hydrolyzed with proteases had a strong antioxidant effect against lipid peroxidation [40]. This claim is supported by the high protein content in the analyzed samples, as well as the results of the performed statistical analysis. In addition to proteins, peptides and enzymes such as catalase, glucose oxidase, and peroxidase, the antioxidant activity of RJ is obtained from phenolic acids, flavonoids, tocopherols, carotenoids and vitamins (thiamine, ascorbic acid and riboflavin) [41]. It has been stated that the antioxidant properties increase due to the increase in the total amount of phenolic substances [42]. The statistical analysis (Table 3) shows a significant positive correlation between DPPH ($r=0.7078$, $p<0.05$) and protein content, as well as a significant positive correlation between FRAP and protein ($r=-0.7449$, $p<0.05$). The positive association between protein and antioxidant activities (DPPH and FRAP) confirms the significance of protein as a factor contributing to the functional properties of royal jelly.

Correlation analysis revealed significant relationships between parameters. The negative correlation between pH and total acidity, as well as between pH and 10-HDA, suggests that lower pH and higher acidity positively affect quality. Furthermore, the positive correlation between protein and antioxidant activities (DPPH and FRAP) confirms the importance of protein as a factor contributing to the functional, and especially antioxidant, properties of royal jelly.

Principal component analysis (PCA, Figure 1) was conducted with the aim of studying the interrelationship of different variables, which in this case are the physicochemical parameters of the tested royal jelly samples. The first principal component (PC1) included 43.42 % of the total variability of the data, while the second principal component (PC2) amounted to 25.11 %. The results of PCA, more precisely the mutual projections for the first two components, are offered in Figure 1. According to the obtained results of fresh royal jelly samples according to PC1, the physicochemical parameters that correlate best are the amounts of TPC, 10 HDA, Sucrose (RJ1, RJ2, RJ3), and total acidity (RJ4, RJ5). According to PC2, the physicochemical parameters that are positively correlated are the amounts of DPPH, FRAP, fructose, glucose, and pH (RJ6, RJ7, RJ8, RJ9).

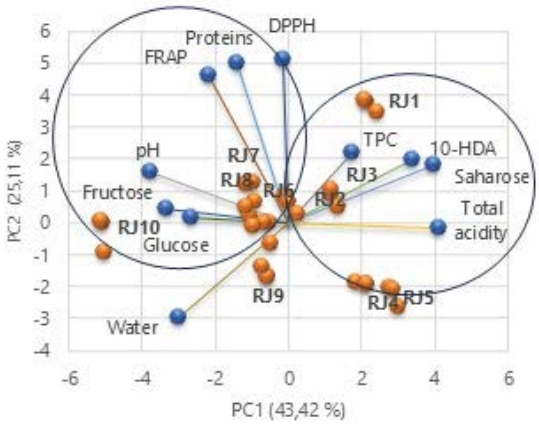

Figure 1: Principal component analysis of the physicochemical parameters of fresh RJ samples.

Conclusions

The study results highlight the need for further research to establish quality standards for royal jelly in Bosnia and Herzegovina. The high variability among samples indicates the significant influence of environmental and technical factors, as well as the importance of standardizing production and storage conditions. Sample RJ1 showed the best characteristics, including high protein content, 10-HDA and antioxidant activity, making it an ideal model for future quality model criteria. The components of royal jelly, as well as their variability under different conditions, make royal jelly a unique product. Additional research and quality standardization will facilitate its use in promoting human health and well-being.

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Conflict of interest

Authors declare: No conflict of interest.

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