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(RESEARCH ARTICLE)

Physicochemical characteristics of local royal jelly produced in Al-Baha region, Saudi Arabia

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### Abstract

Royal jelly (RJ) consumption is expanding because of its high nutritional and bioactive content. However, there is a lack of information to standardize the limit of properties of Saudi Royal Jelly for regulation. The aim of this study is to investigate the quality of Saudi royal jelly associated with the international standard. Twelve fresh royal jelly samples were analyzed for moisture, pH value, total acidity, protein, carbohydrate composition, and 10-hydroxydec-2-enoic acid (10-HAD) concentration. The result of physicochemical properties was varied from 61.70 % to 76.80 % for moisture; 3.14 to 3.83 for pH; 39.4 to 45.0 mL for free acidity; 0.1 N NaOH/100 g; 571.60 to  $745.80 \mu$ S/cm for electrical conductivity (EC); 6.73% to 13.27% for crude protein content. Moreover, the 10-HDA, fructose, glucose, sucrose, maltose, and lactose content were ranged from 1.68% to 6.36%, 2.51 to 5.71%, 0.00% to 2.64%, 0.00% to 2.01% and 0.00 to 0.69%, respectively. The obtained results indicated that all samples matched the international standard and Gulf Standardization Organization (GSO ISO 12824:2021) for royal jelly requirements in terms of 10-HDA concentration, one of the most critical quality parameters. The statistical analysis showed a significant positive correlation (r=0.487, P < 0.01) between the EC and the total protein content while negative correlation between EC and sucrose content (r=-0.825, P < 0.01) and 10HDA (r= -0.699, P < 0.01); fructose and glucose content were shown to be statistically significant (r=0.887). The obtained results of present study will assist to the establishment of a standards of the Saudi royal jelly.

Keywords: Royal Jelly; Quality; Physicochemical Characteristics; 10HDA; Saudi Arabia

### 1. Introduction

Honeybees (*Apis mellifera*) produce honey, pollen, propolis, venom, and royal jelly [1, 2]. Bee products composition have different compounds including carbohydrates, proteins, amino acids, lipids, vitamins, phenolics and minerals [3, 4]. Moreover, due to their health-promoting properties, bee products are increasingly used as dietary supplements in apitherapy, an alternative medicine. Royal jelly (RJ) is a prominent bee product; it is a yellowish-creamy acidic and has a sour and strong odor plus a sour and sweet flavor [5]. It is mainly secreted by cephalic glands; predominantly by two primary glands, hypopharyngeal and mandibular glands of nursing honeybee workers of *Apis mellifera* L [6], and serves as sustenance for the bee queen and larvae up to three days old. Royal jelly is secreted by 5-15-day old worker bees and is only fed to queen larvae during their development [7]. All larvae are given royal jelly for the first three days. Then only queen larvae are given royal jelly continuously, while drone and worker larvae are served pollen and honey [8, 9]. During the feeding of queen larvae, nurse bees give additional quantities of royal jelly which can be collected by beekeepers for commercial consumption [10]. Also, royal jelly is not a typical beekeeping product because it is fed immediately to the queen or larvae and not preserved [9].

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The morphology, life span, and behavior of queen and worker bees are assumed to be influenced by the most important difference in larval nutrition [8]. Regardless of bee breeds, various colonies, or temperature differences, the chemical makeup of royal jelly remains stable [11]. Different feeding (carbohydrates and/or proteins supplements), ambient environments, hive cell sanitary conditions, and manipulation are all affect chemical composition. Changing chemical compositions, on the other hand, has an influence on food type (carbohydrates and/or proteins), meteorological circumstances, hive sanitary conditions, and apiary management [12, 13]. The component of fresh royal jelly is 60–70% water, 18–28% protein, 7–18% carbohydrates and 3–8% fats and vitamins [14]. Consumption of royal jelly in various forms (direct or as a functional component of several food items) is steadily increasing. The presence of 10-HDA in royal jelly has been demonstrated to have antibacterial, anti-inflammatory, antioxidant, antitumoral, antiaging, and immune stimulating characteristics [15-18]. This unsaturated fatty acid (10HDA) is unique to royal jelly and regarded as an indicator of its authenticity and quality [14]. However, discovery of synthetic 10-HDA show that 10-HDA content cannot be utilized as the only authenticity measure. Aside from 10-HDA concentration, moisture, 13C/12C isotopic ratio, and furosine content are commonly measured to assess guality and validity [13, 14, 19]. Internationally, the first step on specifications of royal jelly was submitted by International Honey Commission's royal jelly working group on 2009 [14]. After that, the International Organization for Standardization (ISO) developed an international standard for royal jelly in 2016 [20]. Currently, few countries have established and authorized their national quality requirements for royal jelly [15].

Saudi Arabia regulation of royal jelly quality follow the values that recommended by the Gulf Cooperation Council (GCC) under Gulf Standardization Organization (GSO ISO 12824:2021). However, there is limited data on the quality and authorization of bee products in Saudi Arabia [21]. Hence, there shall be a step ahead to start characterize and identify the products thereby to enhance the benefits obtained from the sub-sector. Consequently, the aims of current research are to determine the physicochemical characteristics of Saudi royal jelly in order to start establish a database of Saudi royal jelly and thereby contribute to create national royal jelly quality requirements. These physicochemical characteristics included moisture, pH value, protein content, total free acidity, carbohydrate composition, and 10-HDA concentration.

# 2. Material and methods

### 2.1 Royal jelly samples

The physiochemical analysis of local royal jelly was conducted on 12 fresh royal jelly samples collected randomly from two locations at Al-Baha area of Saudi Arabia's southwest. Al-Baha Province (longitudes 41° to 42°, and latitudes 19° to 21°). The fresh RJ samples were produced in Buljarshi and Tehama of Al-Baha region. Six samples (KSA1-KSA6) were collected from Tehama at latitude 20°11' 38.2"N and longitude 40°25'15.5"E while the other six samples (KSA7-KSA12) collected from Buljarshi (Hijaz) at latitude 19°51'05.0"N and longitude 41°35'10.5"E with associated of Beekeepers' Cooperative Association in Al-Baha Province during the season 2020 (Fig.1). After 72 hours of grafting 24hrs age larvae, samples were collected from queen cells. The RJ sample was promptly frozen after collecting in dark glass vials (10 g) and delivered to the laboratory in a frozen state until analysis.



Figure 1 Map of the samples two locations

## 2.2 Reagent

All standards (Glucose, Fructose, Sucrose, Maltose, and Lactose, the 10-hydroxy-2-decenoic acid (10-HDA) standard) were obtained from Sigma–Aldrich GmbH (Steinheim, Germany). The other reagents (Methanol, Acetonitrile, Dichloromethane, Chloroform and Acetone) were of analytical grade. All aqueous solutions were prepared with ultrapure deionized water.

### 2.3 Physiochemical Analysis

For quality assessment of RJ, the following major parameters of RJ were carried out including: free acidity level, 10HDA content, pH and electric conductivity, moisture, content, sugar content, were determined following the [20]. Every sample was tested in triplicate for every parameter and their average values were taken.

### 2.3.1 Water content

The water content of RJ samples was measured using a refractive index (Hamann® honey refractometer, Germany) in accordance with Sesta and Lusco [22]. The RI was calibrated using deionized water to get a zero reading before being tested on an aliquot of adequately homogenized materials without any additional treatment. Before taking the reading, the sample was kept in the refractometer for two minutes to thermally equilibrate. The refractometer was cleaned and dried before measuring the next sample.

#### 2.3.2 PH value measurement

The pH determined using pH meter device (Orion, thermo technology). Royal jelly diluted by adding 2 g in 10 mL of distilled water (pH 7.00), then mixed for 10 minutes according to Nabas, Haddadin [23].

### 2.3.3 The electrical conductivity (EC)

The electrical conductivity was determined using Benchtop pH Meter (Thermo Scientific<sup>M</sup> Orion Star<sup>M</sup> A211). Electrical conductivity meter was first calibrated with manufacture calibration solution then washed with deionized water after which conductance cell was dipped into RJ Solution (10.0%) and reading was recorded after stabilization of the instrument.

#### 2.3.4 Free acidity determination

Titration method using NaOH was used to report the acidity of the sample. 0.5g of the RJ samples was titrated using NaOH (0.1N) solution according to Popescu, Dezmirean [24].

#### 2.3.5 Sugar content determination

Sugar profiles of different royal jelly samples were identified using High Performance Liquid chromatography (Agilent Technologies® HPLC with RID detector and carbohydrate column) device according to Mureşan, Mãrghitaş [25] with modification. The samples were prepared by adding 1g of RJ to 5 mL of ultrapure water/ methanol (v/v 3:1) then 0.1 mL Carrez I and Carrez II reagent were added of each. The samples were then centrifuged (4000rpm) for 30 minutes and the supernatant was collected in a new tube and washed with dichloromethane for 2-3 minutes before being filtered through Millipore (0.45  $\mu$ m). The filtrate was then put into the autosampler of HPLC system which was connected with Zorbax Carbohydrate column (4.6 diameter, 250mm length 5 Micron particle size (P.N. 840300-908)) using acetonitrile/water (75:25, v/v) as mobile phase. At a flow rate of 1 ml/min, column oven temp 30°C, and 5  $\mu$ L injection volume in HPLC-RID. The results were presented as percentages.

#### 2.3.6 Total nitrogen and protein content

The total crude protein content in RJ sample was determined using the total nitrogen method according to Sidor, Miłek [18]. TOC (Total Organic Carbon) analyzer (Multi N/C 3100, Jena Co., and Germany) instrument was used to measure the concentration of nitrogen in unknown samples. The total nitrogen calibration curve was conducted using potassium nitrate. Total nitrogen converted to protein using factor of 6.25 for conversion to protein content according to Thompson, Owen [26].

#### 2.3.7 The 10-HDA content of RJ:

The 10-hydroxy-2-decenoic acid (10-HDA) content of RJ was determined by high performance liquid chromatography with diode array detector DAD (Water 2545 Quaternary Gradient Module with RP C-18 Supelcosil column). According to Antinelli, Zeggane [27] ultrapure water of pH 2.5 (acidified by Phosphoric acid) and methanol (60:40, v/v) were used

as mobile phases. The injection volume was  $20\mu$ L, with a flow rate of 1 mL/min. The detection was carried out at 210nm. The standard concentration ranged from 0.2 to 200g/mL for the calibration curve. The R<sup>2</sup> coefficient was 0.9997, indicating that the readings were well correlated. The results were presented as percentages.

### 2.4 Statistical analysis

Statistical analyses were performed using the SPSS® Version 25. The significance was calculated for P < 0.05 and the results were presented in "mean± Standard Deviation (SD)". Analysis of variance (ANOVA) was used to compare the quantified variables of the samples.

# 3. Results

### 3.1 Moisture content

Results indicating the moisture content is implied in Table 1 bellow. Variations among the samples were significantly (P<0.05) different. Moisture content in royal jelly samples ranged from 61.70 % to 76.80 % with an overall average of 66.53±4.19%. Table 3 illustrated negative significant correlation between moisture content and 10HDA (r=-0.621, P < 0.01) and sucrose (r=-0.494, P < 0.01), while positive significant correlation with fructose (r=0.472, P < 0.01) and glucose (r=0.478, P < 0.01).

## 3.2 The pH Value

The average pH value of fresh local RJ samples was 3.54±0.2 (range: 3.14-3.83) (Table 1). These values fall within standard pH range (3-4) and Although the royal jelly samples harvested from Hijaz had higher pH values than those harvested from Tehama still, all samples were in accordance with the international standards [14].

## 3.3 Free acidity

In this study, the results indicated a statistically significant difference among the royal jelly relating to free acidity (P < 0.05). Free acidity of RJ samples varied between 3.94 and 4.50, with an average value of (42.4±0.16 mL 0.1 N NaOH/100g) (Table 1).

### 3.4 The electrical conductivity (EC)

The electrical conductivity (EC) of royal jelly samples ranged between (571.60 and 745.80  $\mu$ S/cm) with an average 646.96±62.91 $\mu$ S/cm, (Table 1). The results showed significant differences (571.33±0.85-745.80±0.25  $\mu$ S/cm) between the samples.

### 3.5 Crude protein content

Protein content ranged from 6.73% to 13.27% with an average of 9.88 $\pm$ 2.01. The statistical analysis showed positive correlation (r=0.487, P < 0.01) between the EC and the total protein content (Table 3).

### 3.6 The 10-HDA content of royal jelly

Royal jelly quality and authenticity is depending on the content of 10-hydroxy-2-decenoic acid (10-HDA). In this study, 10-HDA ranged from 1.68% to 6.36% with mean of ( $3.83\pm1.56$ ) (Table 2). The royal jelly was significant different in 10HDA (P < 0.05) content with a high level in the samples that collected from Tehama (KSA1-KSA6) except sample KSA6 has the close content to the samples collected from Buljarshi (Fig. 2). Statistical analysis (Table 3) revealed significant positive correlation between 10HDA (r=0.791, P < 0.01) and sucrose content, but significant negative correlation to EC (r=-0.699, P < 0.01), moisture (r=-0.621, P < 0.01), glucose % (r=-0.494, P < 0.01), maltose % (r=-0.468, P < 0.01), fructose % (r=-0.467, P < 0.01) and pH (r=-0.446, P < 0.01).

### 3.7 Sugar content

Table 2 showed the concentration of sugar content in RJ samples. The fructose concentrations ranged from 2.51 to 5.71% with a mean value of  $3.67\pm0.81\%$ , while the glucose concentrations were between 1.98 to 5.59% with a mean of  $3.25\pm1.01\%$ . High significant positive correlation was determined between fructose and glucose content (r=0.887, P < 0.01) (Table 3). The sucrose contents of the investigated samples were in the range of 0.00% to 2.64% (Table 2), with mean of  $0.91\pm0.82\%$ . Maltose values ranged between 0.00% to 2.01% with a mean of  $0.45\pm0.53\%$ , while lactose values ranged between 0.00 to 0.69% with a mean of  $0.05\pm0.12\%$  (Table 2). Table 3 shows negative significant correlation between sucrose and maltose (r=-0.486, P < 0.01) content.

#### 3.8 Correlations of physicochemical parameters



Figure 2 The mean and standard deviations of 10-hydroxydecenoic acid (10-HDA) content royal jelly samples

Table 1 PH, electrical conductivity (EC), total protein acidity, moisture of the royal jelly san	nples. (n=36)
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RJ sample	рН	EC μS/cm	Acidity*	Moisture	Protein	
KSA1	3.14±0.01f	572.27±0.681	41.7±0.1fe	70.45±2.50b	9.15±0.56f	
KSA2	3.40±0.01d	577.73±0.28k	43.8±0.06abc	65.37±0.15de	11.87±0.23c	
KSA3	3.30±0.01e	596.43±0.20i	43.4±0.06abcd	61.80±0.10h	8.22±0.23g	
KSA4	3.55±0.01c	588.48±0.37j	41.2±0.10fe	63.53±0.06g	8.59±0.65g	
KSA5	3.39±0.01d	615.19±0.73h	40.3±0.10f	61.97±0.15h	9.35±0.06f	
KSA6	3.67±0.02b	621.06±0.30g	42.9±0.06bcd	63.80±0.20fg	7.48±0.04h	
KSA7	3.55±0.01c	641.16±0.22e	42.6±0.06cde	67.63±0.15c	6.82±0.07i	
KSA8	3.82±0.01a	632.62±0.39f	40.3±0.10f	65.30±0.10de	10.35±0.00e	
KSA9	3.67±0.02b	715.33±0.85d	42.0±0.10de	64.90±0.10ef	13.27±0.00a	
KSA10	3.55±0.01c	722.17±0.35c	44.7±0.06a	76.70±0.10a	11.18±0.00d	
KSA11	3.82±0.01a	745.53±0.25a	44.3±0.06ab	66.47±0.06cd	9.49±0.00f	
KSA12	3.65±0.01b	735.60±0.46b	42.0±0.10de	70.50±0.10b	12.82±0.00b	
Mean	3.54	646.96	42.4	66.53	9.88	
SD	0.20	62.91	0.16	4.19	2.01	
Min	3.14	571.60	39.4	61.70	6.73	
Max	3.83	745.80	45.0	76.80	13.27	
ISO, 2016	NA	NA	30-53	60-68.5%	11-18%	

Min-minimum, Max-maximum, SD-standard deviation; Values represent the average of triplicates ± standard deviation. NA= Not available \*[(1mol/1N NaOH) ml/100g}] EC: electrical conductivity

Statistical analysis (Table 3) revealed significant positive correlation between 10HDA (r=0.791, P < 0.01) and sucrose content. On the contrary it showed significant negative correlation to EC (r=-0.699, P < 0.01), moisture (r=-0.621, P < 0.01), glucose % (r=-0.494, P < 0.01), maltose % (r=-0.468, P < 0.01), fructose % (r=-0.467, P < 0.01) and pH (r=-0.446, P < 0.01). High significant positive correlation was determined between fructose and glucose content (r=0.887, P < 0.01) (Table 3). The statistical analysis showed positive correlation (r=0.487, P < 0.01) between the EC and the total protein content. However, statistical analysis revealed there was a significant negative correlation between EC and sucrose content (r=-0.825, P < 0.01) and 10HDA (r= -0.699, P < 0.01).

RJ sample	10HDA %	Fructose%	Glucose%	Sucrose%	Maltose%	Lactose%
KSA1	2.84±0.21ef	5.15±0.83a	4.86±1.14a	1.24±0.26cd	0.37±0.30d	0.23±0.40b
KSA2	6.25±0.11a	3.07±0.18e	2.59±0.15e	2.18±0.13b	0.14±0.01e	ND
KSA3	6.17±0.06ab	2.72±0.16e	2.11±0.12e	1.39±0.08c	ND	0.02±0.00ab
KSA4	5.53±0.74bc	2.66±0.15e	2.13±0.12e	2.5±0.14a	0.36±0.02d	ND
KSA5	4.93±0.16cd	4.08±0.01cd	3.24±0.01d	0.9±0.00f	0.09±0.00e	ND
KSA6	4.70±0.17d	4.19±0.01bc	3.59±0.01d	1.16±0.00de	.6±0.00de 0.13±0.00e	
KSA7	2.66±0.29ef	2.99±0.01e	2.34±0.01e	0.12±0.00gh	0.12±0.00gh 2.00±0.01a	
KSA8	2.81±0.98ef	2.86±0.01e	2.02±0.01e	1.01±0.01ef 0.04±0.00e		ND
KSA9	3.07±0.07e	3.64±0.02d	4.47±0.02ab	0.16±0.00gh 0.55±0.00d		0.04±0.00ab
KSA10	2.61±0.12ef	4.21±0.02bc	4.24±0.02bc	ND 0.75±0.00		0.05±0.00ab
KSA11	2.15±0.12f	3.84±0.02cd	3.73±0.02cd	0.22±0.00g 0.62±0.00c		0.01±0.00ab
KSA12	2.20±0.48f	4.58±0.09b	3.74±0.07cd	0.06±0.00gh 0.39±0.01d		0.03±0.00ab
Mean	3.83	3.67	3.25	0.91 0.45		0.05
SD	1.56	0.81	1.01	0.82 0.53		0.12
Min	1.68	2.51	1.98	0.00	0.00	0.00
Max	6.36	5.71	5.59	2.64	2.64 2.01	
ISO, 2016	>1.4	2-9	2-9	<3.0	<1.2	NA

**Table 2** The relative concentrations (%) of 10-HAD and different sugar (fructose, glucose, sucrose, maltose, andLactose). (n=36)

Min-minimum, Max-maximum, SD-standard deviation; Values represent the average of triplicates ± standard error. NA= Not available. ND = Not detected (below 0.01%) 10HDA

	рН	EC	Acidity	Moisture	Fructose	Glucose	Sucrose	Maltose	Lactose	Protein%	10HDA %
pН	1.000										
EC	0.652**	1.000									
Acidity	0.072	0.344*	1.000								
Moisture	0.007	0.464**	0.306*	1.000							
Fructose	-0.197	0.275	0.017	0.472**	1.000						
Glucose	-0.113	0.421**	0.162	0.478**	0.887**	1.000					
Sucrose	-0.390**	-0.825**	-0.213	-0.494**	-0.414**	-0.484**	1.000				
Maltose	0.128	0.287*	0.185	0.394**	-0.095	-0.011	-0.486**	1.000			
Lactose	-0.290*	-0.103	-0.160	0.372*	0.035	0.000	-0.082	0.458**	1.000		
Protein%	0.196	0.487**	0.056	0.310*	0.216	0.383*	-0.246	-0.263	-0.189	1.000	
10HDA %	-0.446**	-0.699**	0.018	-0.621**	-0.467**	-0.494**	0.791**	-0.468**	-0.225	-0.226	1.000

**Table 3** Pearson's correlation coefficients between analyzed physicochemical parameters

\*\* Correlation is significant at the 0.01 level (2-tailed). \* Correlation is significant at the 0.05 level (2-tailed).

## 4. Discussion

Some countries have been established their national royal jelly standards, such as Bulgaria, Poland, Switzerland, Turkey, Japan, China, Korea and Egypt [15]. Saudi Arabia and other Arabic gulf countries were followed the International Organization for Standardization issued of royal jelly (ISO 2016). Recently, the Gulf Cooperation Council (GCC) issued a new standard of royal jelly under Gulf Standardization Organization (GSO ISO 12824:2021) which is adoption the international standard without modification. However, the physicochemical values obtained in this study was within the international royal jelly standard (ISO 2016), but, there was a large variation within the samples due to the different locations of the produced samples. Moisture content in RJ samples probably due to disparities in time of sampling and climatic variations due to altitudinal change. The results were consistent with the previous published ones in literature [15, 19, 28, 29]. The international standard (ISO, 12824: 2016) ranged the moisture content in fresh royal 62.0% and 68.5%. The result of the minimum moisture content was like the standard while the maximum is 12% more than the value prescribed in the standard. The moisture content of royal jelly is substantially due to different factors: e.g., the time of collection after the grafting of young larvae [29-31]. On the other hand, mixing fresh royal jelly with queen bee larvae triturate or/and fresh drone bee larvae could increase the amount of moisture in royal jelly [25]. pH values in royal jelly samples was closed to the range reported in literatures [14, 29, 32, 33]. The same result of total free acidity was obtained (36.5 to 43.2 mL 0.1 N NaOH/100g) by Al-Kahtani and Taha [29], who tested RJ samples collected from Al-Ahsa. Saudi Arabia during the spring of 2018. According to the international standard (ISO, 12824; 2016) and data in the literature, the acidity of the same solution (same concentration) of royal jelly was between 30% and 60% [14, 32, 34]. In general, the pure royal jelly samples have higher total acidity compared to the RJ adulterated samples. Thus, this parameter could be used for identification purposes [34]. Electrical conductivity in royal jelly depending on the chemical composition of the product, mainly mineral elements, organic acids, amino acids, and protein contents. Moreover, Balkanska, Karadjova [32] recommended using this parameter for identification purposes. Unexpectedly, the EC petameter is not include in the international quality and standardization of the royal jelly (ISO, 12824: 2016). However, statistical analysis showed there was a significant negative correlation between EC and sucrose content (r=-0.825, P < 0.01) and 10HDA (r= -0.699, P < 0.01) (Table 3). Because, EC is simple, easy and available in routine analysis laboratory, we suggest this parameter can be used to estimate the quality of the royal jelly sample as a rapid method. To gain more confidential and validation of EC parameter, fresh royal jelly samples from different regions of Saudi Arabia should be analyzed. Low protein content (<11%) was detected in eight samples of local royal jelly which was lower than the level in international standard (11-15%) (ISO, 12824: 2016). Mokaya, Njeru [28] reported low protein content in African royal jelly (3.79-8.00%) while, high protein content reported by Al-Kahtani and Taha [29] for Saudi royal jelly and other kind of royal jelly reported by literatures [15, 19, 35].

10HDA result agreed with the international standard (ISO, 12824: 2016) and the data reported in the literature for royal jellies from different geographical origins [15, 19, 28, 31, 35]. The International standard [20] set the minimum content of the 10-HDA in fresh royal jelly as (1.4%), while others established higher minimal value for 10- HDA content., [13, 15, 34]. Kanelis, Tananaki [13] recommended that the upper limit of 10-HDA should not exceed 6.00% to avoid adulteration from adding synthetic 10-HDA, which is generally available in global trade. Therefore, more samples from various locations of Saudi Arabia and from different production seasons should be gathered and examined to establish the upper and lower limits of 10-HDA concentration in Saudi royal.

The content of glucose and fructose in all royal jelly samples was within the international standard range (2 to 9%) (ISO, 2016). According to the literature, royal jelly contains high amounts of fructose and glucose compared to other carbohydrates [36]. Relatively similar results were obtained by Al-Kahtani and Taha [29], Mokaya, Njeru [28], Flanjak, Primorac [19], Yavuz and Gürel [34], Balkanska, Karadjova [32] and Daniele and Casabianca [36]. Sucrose result was within the limits of international standard < 3% (ISO, 2016). In contrast, feeding honeybees colonies with beet or cane sugar syrups might increase sucrose content in royal jelly, while feeding the colonies with cereal and corn starch syrups increased the maltose content in royal jelly [9].

# 5. Conclusion

The health promoting of royal jelly requires setting the quality parameters limits at national and international level. The results of this study will contribute to creation of Saudi royal jelly database but further research must include more samples from different regions of Saudi Arabia to gain the limits for each physicochemical characteristic and finally suggest the quality requirements for royal jelly produced in Saudi Arabia.

#### **Compliance with ethical standards**

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#### Disclosure of conflict of interest

No conflict of interest.

#### References

- [1] Sforcin JM, Bankova V, Kuropatnicki AK. Medical benefits of honeybee products. Evidence-Based Complementary and Alternative Medicine. 2017 Jan 1;2017.
- [2] Yeung YT, Argüelles S. Bee products: royal jelly and propolis. In Nonvitamin and Nonmineral Nutritional Supplements 2019 Jan 1 (pp. 475-484). Academic Press.
- [3] Cornara L, Biagi M, Xiao J, Burlando B. Therapeutic properties of bioactive compounds from different honeybee products. Frontiers in pharmacology. 2017:412.
- [4] Matuszewska E, Klupczynska A, Maciołek K, Kokot ZJ, Matysiak J. Multielemental Analysis of Bee Pollen, Propolis, and Royal Jelly Collected in West-Central Poland. Molecules. 2021;26(9):2415.
- [5] Bogdanov S. The Royal Jelly Book. http://www.bee- hexagon.net/royal-jelly/. Accessed February 6, 2018.; 2016.
- [6] Knecht D, Kaatz H. Patterns of larval food production by hypopharyngeal glands in adult worker honey bees. Apidologie. 1990;21(5):457-68.
- [7] Haydak M. Larval food and development of castes in the honeybee. Journal of economic entomology. 1943;36(5):778-92.
- [8] Ferioli F, Marcazzan GL, Caboni MF. Determination of (E)-10-hydroxy-2-decenoic acid content in pure royal jelly: A comparison between a new CZE method and HPLC. Journal of separation Science. 2007;30(7):1061-9.
- [9] 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. Journal of Food Composition Analysis. 2013;29(2):126-33.
- [10] Clarke M, McDonald P. Australian Royal Jelly-Market Opportunity Assessment based on production that uses new labour saving technology. Rural Industries Research Development Corporation. 2017;4.
- [11] Barnuțiu LI, AL marghitas L, Dezmirean D, Bobis O, Bonta V, PAVEL C. Preliminary Study on Chemical Composition of Fresh Royal Jelly from Transylvania. J Bulletin of the University of Agricultural Sciences, Veterinary Medicine Cluj-Napoca Animal Science, Biotechnologies. 2012;69.
- [12] Isidorov V, Bakier S, Grzech I. Gas chromatographic-mass spectrometric investigation of volatile and extractable compounds of crude royal jelly. J Journal of Chromatography B. 2012; 885:109-16.
- [13] Kanelis D, Tananaki C, Liolios V, Dimou M, Goras G, Rodopoulou MA, et al. A suggestion for royal jelly specifications. Arhiv za higijenu rada i toksikologiju. 2015;66(4):275-84.
- [14] Sabatini AG, Marcazzan GL, Caboni MF, Bogdanov S, Almeida-Muradian L, Science A. Quality and standardisation of royal jelly. Journal of ApiProduct. 2009;1(1):1-6.
- [15] Arfa A, Riad YM, El Nikeety M. Quality Parameters of Royal Jelly in national and international standards: Specifications, differences and suggestions. J Annals of the Romanian Society for Cell Biology. 2021:7977-97.
- [16] Bouamama S, Merzouk H, Latrech H, Charif N, Bouamama A. Royal jelly alleviates the detrimental effects of aging on immune functions by enhancing the in vitro cellular proliferation, cytokines, and nitric oxide release in aged human PBMCS. Journal of Food Biochemistry. 2021;45(2): e13619.
- [17] Eslami-kaliji F, Sarafbidabad M, Kiani-Esfahani A, Mirahmadi-Zare SZ, Dormiani K. 10-hydroxy-2-decenoic acid a bio-immunomodulator in tissue engineering; generates tolerogenic dendritic cells by blocking the toll-like receptor 4. Journal of Biomedical Materials Research Part A. 2021.

- [18] Sidor E, Miłek M, Zaguła G, Bocian A, Dżugan M. Searching for Differences in Chemical Composition and Biological Activity of Crude Drone Brood and Royal Jelly Useful for Their Authentication. Journal of Foods. 2021;10(9):2233.
- [19] Flanjak I, Primorac L, Vukadin i, Kovacic M, Puskadija Z, rajs BB. Physicochemical characteristics of Croatian royal jelly. Croat J Food Sci Technol. 2019;11(2).
- [20] ISO IOfS. Royal jelly-specifications, ISO 12824:2016. 2016.
- [21] Bazeyad AY, Al-Sarar AS, Rushdi AI, Hassanin AS, Abobakr Y. Levels of heavy metals in a multifloral Saudi honey. Environmental Science and Pollution Research. 2019;26(4):3946-53.
- [22] Sesta G, Lusco L. Refractometric determination of water content in royal jelly. Apidologie. 2008;39(2):225-32.
- [23] Nabas Z, Haddadin MS, Haddadin J, Nazer IK. Chemical composition of royal jelly and effects of synbiotic with two different locally isolated probiotic strains on antioxidant activities. Polish Journal of Food Nutrition Sciences. 2014;64(3).
- [24] Popescu O, Dezmirean D, Laslo L. A characterization about physical-chemical composition of royal jelly. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca Animal Science and Biotechnologies. 2008;65(1-2).
- [25] Mureşan CI, Mãrghitaş LA, Dezmirean DS, Bobiş O, Bonta V, Zacharias I, et al. Quality Parameters for commercialized Royal Jelly. Bulletin of the University of Agricultural Sciences, Veterinary Medicine Cluj-Napoca Animal Science, Biotechnologies. 2016;73(1).
- [26] Thompson M, Owen L, Wilkinson K, Wood R, Damant A. A comparison of the Kjeldahl and Dumas methods for the determination of protein in foods, using data from a proficiency testing scheme. The Analyst. 2002;127(12):1666-8.
- [27] Antinelli J-F, Zeggane S, Davico R, Rognone C, Faucon J-P, Lizzani L. Evaluation of (E)-10-hydroxydec-2-enoic acid as a freshness parameter for royal jelly. Food chemistry. 2003;80(1):85-9.
- [28] Balkanska R, Karadjova I, Ignatova M. Comparative analyses of chemical composition of royal jelly and drone brood. Bulg Chem Commun. 2014;46(2):412-6.
- [29] Mokaya HO, Njeru LK, Lattorff HMG. African honeybee royal jelly: Phytochemical contents, free radical scavenging activity, and physicochemical properties. Food Bioscience. 2020; 37:100733.
- [30] Al-Kahtani S, Taha E-KA. Effect of Harvest Time on Royal Jelly Yield and Chemical Composition. Journal of the Kansas Entomological Society. 2021;93(2):132-9.
- [31] Zheng H-Q, Hu F-L, Dietemann V. Changes in composition of royal jelly harvested at different times: consequences for quality standards. Apidologie. 2010.
- [32] Kanelis D, Tananaki C, Liolios V, Rodopoulou M-A, Goras G, Argena N, et al. Investigating the Effect of Supplementary Feeding on Carbohydrate Composition and Quantity of Royal Jelly. Open Journal of Applied Sciences. 2018;8(4):141-9.
- [33] Kolayli S, Sahin H, Can Z, Yildiz O, Malkoc M, Asadov A. A member of complementary medicinal food: anatolian royal jellies, their chemical compositions, and antioxidant properties. Journal of evidence-based complementary alternative medicine. 2016;21(4):48.
- [34] Yavuz I, Gürel F. Chemical properties of the royal jellies in Turkish markets. Mediterranean Agricultural Sciences. 2017;30(3):281-5.
- [35] Garcia-Amoedo LH, Almeida-Muradian LB. Physicochemical composition of pure and adulterated royal jelly. Química Nova. 2007; 30:257-9.
- [36] Sereia MJ, Toledo VdAAd, Technology. Quality of royal jelly produced by Africanized honeybees fed a supplemented diet. Food Science. 2013;33(2):304-9.
- [37] Kamyab S, Gharachorloo M, Honarvar M, Ghavami M. Quantitative analysis of bioactive compounds present in Iranian royal jelly. Journal of Apicultural Research. 2020;59(1):42-52.
- [38] Daniele G, Casabianca H. Sugar composition of French royal jelly for comparison with commercial and artificial sugar samples. Journal of Food chemistry. 2012;134(2):1025-9.