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 µS/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 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 quality 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](image-url)
2.2 Reagent
All standards (Glucose, Fructose, Sucrose, Maltose, and Lactose, the 10-hydroxy-2-decenolic 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™ Orion Star™ 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, Dezmişean [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, Mârghitaş [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 µm). 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 µL 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-decenolic 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 20µL 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 R² 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 below. 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 µS/cm) with an average 646.96±62.91µS/cm, (Table 1). The results showed significant differences (571.33±0.85 - 745.80±0.25 µS/cm) between the samples.

3.5 Crude protein content

Protein content ranged from 6.73% to 13.27% with an average of 9.88±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±1.56) (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±0.81%, while the glucose concentrations were between 1.98 to 5.59% with a mean of 3.25±1.01%. 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±0.82%. Maltose values ranged between 0.00% to 2.01% with a mean of 0.45±0.53%, while lactose values ranged between 0.00 to 0.69 % with a mean of 0.05±0.12% (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-hydroxydecanoic acid (10-HDA) content royal jelly samples

Table 1 PH, electrical conductivity (EC), total protein acidity, moisture of the royal jelly samples. (n=36)

<table>
<thead>
<tr>
<th>RJ sample</th>
<th>pH</th>
<th>EC µS/cm</th>
<th>Acidity*</th>
<th>Moisture</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>KSA1</td>
<td>3.14±0.01f</td>
<td>572.27±0.68l</td>
<td>41.7±0.1fe</td>
<td>70.45±2.50b</td>
<td>9.15±0.56f</td>
</tr>
<tr>
<td>KSA2</td>
<td>3.40±0.01d</td>
<td>577.73±0.28k</td>
<td>43.8±0.06abc</td>
<td>65.37±0.15de</td>
<td>11.87±0.23c</td>
</tr>
<tr>
<td>KSA3</td>
<td>3.30±0.01e</td>
<td>596.43±0.20i</td>
<td>43.4±0.06abcd</td>
<td>61.80±0.10h</td>
<td>8.22±0.23g</td>
</tr>
<tr>
<td>KSA4</td>
<td>3.55±0.01c</td>
<td>588.48±0.37j</td>
<td>41.2±0.10fe</td>
<td>63.53±0.06g</td>
<td>8.59±0.65g</td>
</tr>
<tr>
<td>KSA5</td>
<td>3.39±0.01d</td>
<td>615.19±0.73h</td>
<td>40.3±0.10f</td>
<td>61.97±0.15h</td>
<td>9.35±0.06f</td>
</tr>
<tr>
<td>KSA6</td>
<td>3.67±0.02b</td>
<td>621.06±0.30g</td>
<td>42.9±0.06bcd</td>
<td>63.80±0.20g</td>
<td>7.48±0.04h</td>
</tr>
<tr>
<td>KSA7</td>
<td>3.55±0.01c</td>
<td>641.16±0.22e</td>
<td>42.6±0.06cde</td>
<td>67.63±0.15c</td>
<td>6.82±0.07i</td>
</tr>
<tr>
<td>KSA8</td>
<td>3.82±0.01a</td>
<td>632.62±0.39f</td>
<td>40.3±0.10f</td>
<td>65.30±0.10de</td>
<td>10.35±0.00e</td>
</tr>
<tr>
<td>KSA9</td>
<td>3.67±0.02b</td>
<td>715.33±0.85d</td>
<td>42.0±0.10de</td>
<td>64.90±0.10f</td>
<td>13.27±0.00a</td>
</tr>
<tr>
<td>KSA10</td>
<td>3.55±0.01c</td>
<td>722.17±0.35c</td>
<td>44.7±0.06a</td>
<td>76.70±0.10a</td>
<td>11.18±0.00d</td>
</tr>
<tr>
<td>KSA11</td>
<td>3.82±0.01a</td>
<td>745.53±0.25a</td>
<td>44.3±0.06ab</td>
<td>66.47±0.06cd</td>
<td>9.49±0.00f</td>
</tr>
<tr>
<td>KSA12</td>
<td>3.65±0.01b</td>
<td>735.60±0.46b</td>
<td>42.0±0.10de</td>
<td>70.50±0.10b</td>
<td>12.82±0.00b</td>
</tr>
<tr>
<td>Mean</td>
<td>3.54</td>
<td>646.96</td>
<td>42.4</td>
<td>66.53</td>
<td>9.88</td>
</tr>
<tr>
<td>SD</td>
<td>0.20</td>
<td>62.91</td>
<td>0.16</td>
<td>4.19</td>
<td>2.01</td>
</tr>
<tr>
<td>Min</td>
<td>3.14</td>
<td>571.60</td>
<td>39.4</td>
<td>61.70</td>
<td>6.73</td>
</tr>
<tr>
<td>Max</td>
<td>3.83</td>
<td>745.80</td>
<td>45.0</td>
<td>76.80</td>
<td>13.27</td>
</tr>
<tr>
<td>ISO, 2016</td>
<td>NA</td>
<td>NA</td>
<td>30-53</td>
<td>60-68.5%</td>
<td>11-18%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>RJ sample</th>
<th>10HDA %</th>
<th>Fructose%</th>
<th>Glucose%</th>
<th>Sucrose%</th>
<th>Maltose%</th>
<th>Lactose%</th>
</tr>
</thead>
<tbody>
<tr>
<td>KSA1</td>
<td>2.84±0.21ef</td>
<td>5.15±0.83a</td>
<td>4.86±1.14a</td>
<td>1.24±0.26cd</td>
<td>0.37±0.30d</td>
<td>0.23±0.40b</td>
</tr>
<tr>
<td>KSA2</td>
<td>6.25±0.11a</td>
<td>3.07±0.18e</td>
<td>2.59±0.15e</td>
<td>2.18±0.13b</td>
<td>0.14±0.01e</td>
<td>ND</td>
</tr>
<tr>
<td>KSA3</td>
<td>6.17±0.06ab</td>
<td>2.72±0.16e</td>
<td>2.11±0.12e</td>
<td>1.39±0.08c</td>
<td>ND</td>
<td>0.02±0.00ab</td>
</tr>
<tr>
<td>KSA4</td>
<td>5.53±0.74bc</td>
<td>2.66±0.15e</td>
<td>2.13±0.12e</td>
<td>2.5±0.14a</td>
<td>0.36±0.02d</td>
<td>ND</td>
</tr>
<tr>
<td>KSA5</td>
<td>4.93±0.16cd</td>
<td>4.08±0.01cd</td>
<td>3.24±0.01d</td>
<td>0.9±0.00f</td>
<td>0.09±0.00e</td>
<td>ND</td>
</tr>
<tr>
<td>KSA6</td>
<td>4.70±0.17d</td>
<td>4.19±0.01bc</td>
<td>3.59±0.01d</td>
<td>1.16±0.00de</td>
<td>0.13±0.00e</td>
<td>ND</td>
</tr>
<tr>
<td>KSA7</td>
<td>2.66±0.29ef</td>
<td>2.99±0.01e</td>
<td>2.34±0.01e</td>
<td>0.12±0.00gh</td>
<td>2.00±0.01a</td>
<td>0.18±0.00ab</td>
</tr>
<tr>
<td>KSA8</td>
<td>2.81±0.98ef</td>
<td>2.86±0.01e</td>
<td>2.02±0.01e</td>
<td>1.01±0.01ef</td>
<td>0.04±0.00e</td>
<td>ND</td>
</tr>
<tr>
<td>KSA9</td>
<td>3.07±0.07e</td>
<td>3.64±0.02d</td>
<td>4.47±0.02a</td>
<td>0.16±0.00gh</td>
<td>0.55±0.00c</td>
<td>0.04±0.00ab</td>
</tr>
<tr>
<td>KSA10</td>
<td>2.61±0.12ef</td>
<td>4.21±0.02bc</td>
<td>4.24±0.02bc</td>
<td>ND</td>
<td>0.75±0.00b</td>
<td>0.05±0.00ab</td>
</tr>
<tr>
<td>KSA11</td>
<td>2.15±0.12f</td>
<td>3.84±0.02cd</td>
<td>3.73±0.02cd</td>
<td>0.22±0.00g</td>
<td>0.62±0.00c</td>
<td>0.01±0.00ab</td>
</tr>
<tr>
<td>KSA12</td>
<td>2.20±0.48f</td>
<td>4.58±0.09b</td>
<td>3.74±0.07cd</td>
<td>0.06±0.00gh</td>
<td>0.39±0.01d</td>
<td>0.03±0.00ab</td>
</tr>
</tbody>
</table>

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   2.01   0.69
ISO, 2016 >1.4  2-9   2-9   <3.0  <1.2   NA

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

Table 3 Pearson’s correlation coefficients between analyzed physicochemical parameters

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>EC</th>
<th>Acidity</th>
<th>Moisture</th>
<th>Fructose</th>
<th>Glucose</th>
<th>Sucrose</th>
<th>Maltose</th>
<th>Lactose</th>
<th>Protein%</th>
<th>10HDA %</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>1.00</td>
<td></td>
<td></td>
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<tr>
<td>EC</td>
<td>0.652**</td>
<td>1.000</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>0.007</td>
<td>0.464**</td>
<td>0.306*</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>-0.197</td>
<td>0.275</td>
<td>0.017</td>
<td>0.472**</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>-0.113</td>
<td>0.421**</td>
<td>0.162</td>
<td>0.478**</td>
<td>0.887**</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>-0.390**</td>
<td>-0.825**</td>
<td>-0.213</td>
<td>-0.494**</td>
<td>-0.414**</td>
<td>-0.484**</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maltose</td>
<td>0.128</td>
<td>0.287*</td>
<td>0.185</td>
<td>0.394**</td>
<td>-0.095</td>
<td>-0.011</td>
<td>-0.486**</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactose</td>
<td>-0.290*</td>
<td>-0.103</td>
<td>-0.160</td>
<td>0.372*</td>
<td>0.035</td>
<td>0.000</td>
<td>-0.082</td>
<td>0.458**</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein%</td>
<td>0.196</td>
<td>0.487**</td>
<td>0.056</td>
<td>0.310*</td>
<td>0.216</td>
<td>0.383*</td>
<td>-0.246</td>
<td>-0.263</td>
<td>-0.189</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>10HDA %</td>
<td>-0.446**</td>
<td>-0.699**</td>
<td>0.018</td>
<td>-0.621**</td>
<td>-0.467**</td>
<td>-0.494**</td>
<td>0.791**</td>
<td>-0.468**</td>
<td>-0.225</td>
<td>-0.226</td>
<td>1.000</td>
</tr>
</tbody>
</table>

** 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 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].

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


