

Chemical properties of the royal jellies in Turkish markets

Türkiye’de satışa sunulan arı sütlerinin kimyasal özellikleri

İbrahim YAVUZ¹, Fehmi GÜREL²

¹Akdeniz University, Vocational School of Technical Sciences, Program of Organic Agriculture, Antalya, Turkey

²Akdeniz University, Faculty of Agriculture, Department of Animal Science, Antalya, Turkey

Corresponding author (Sorumlu yazar): F. Gürel, e-mail (e-posta): fgurel@akdeniz.edu.tr

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ABSTRACT

Although the consumption of royal jelly has been rapidly increased in recent years, there is a lack of knowledge about the quality of commercial royal jelly purchased in Turkey. In order to evaluate quality properties, a total of thirteen different royal jelly samples, consisting of 12 commercial samples in Turkish markets, and 1 sample of known origin obtained freshly harvested from honeybee colony in Akdeniz University were analyzed for water, crude protein, acidity, pH, ash, total sugar, fructose, glucose, sucrose and 10-HDA content. Water, crude protein, pH values, ash, total sugars and 10-HDA content of the 13 royal jelly samples varied from 63.10 to 73.55%, 9.76 to 12.57%, 3.66 to 4.02, 0.92 to 1.17%, 7.68 to 11.66% and 0.57 to 3.11% respectively. Comparison of the 10-HDA values measured in this study with the Turkish (TS 6666) and currently available royal jelly international standard (ISO 12842) showed that 50% of the royal jelly samples had lower values than the allowed ISO and Turkish standards of 1.4%.

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ÖZ

Son yıllarda arı sütü tüketimi hızlı bir şekilde artmasına karşın, Türkiye’de satılan arı sütlerinin kaliteleri ile ilgili yeterli bilgi bulunmamaktadır. Kalite özelliklerini belirlemek için 12 adedi ticari firmalardan bir adedi ise Akdeniz Üniversitesi Ziraat Fakültesi Zootekni Bölümü bal arısı kolonilerinden üretilen (kaynağı bilinen, saf) olmak üzere toplam 13 farklı arı sütü örneği nem, ham protein, asitlik, pH, kül, toplam şeker, fruktoz, glukoz, sakkaroz ve 10-HDA içeriği bakımından analiz edilmiştir. Arı sütü örneklerinin nem içeriği % 63.10 ile % 73.55, ham protein içeriği % 9.76 ile % 12.57, pH değerleri 3.66 ile 4.02, kül içeriği % 0.92 ile 1.17, toplam şeker içeriği % 7.68 ile % 11.66 ve 10-HDA içeriği % 0.57 ile % 3.11 aralıklarında değişim göstermişlerdir. Bu çalışmada ölçülen 10-HDA değerleri yürürlükte olan ulusal (TS 6666) ve uluslararası arı sütü standardı (ISO 1842) ile karşılaştırıldığında arı sütü örneklerinin % 50 sinin % 1.4 olarak belirtilen ulusal ve uluslararası arı sütü 10-HDA standardı sınırının altında olduğu saptanmıştır.

1. Introduction

Royal jelly is a milky-white colored secretion produced by hypo-pharyngeal and mandibular glands in the head of young worker honeybees and is used to feed the larvae up to three days, and the queen throughout larval and adult stages (Munstedt and Von Georgi 2003). This exclusive food plays an important role in caste (queen-worker) differentiation, development, and reproduction of the queen. Royal jelly (RJ) is one of the most important bee products due to the unique chemical composition. The chemical composition of RJ has been studied by many authors since the 1950s (Jianke and Shenglu 2003). However, it is difficult to bring together the data collected by different authors into an organic whole, as the data themselves are not always comparable due to the lack of homogeneity among the materials used, the different sampling procedures and analytical methods (Sabatini et al. 2009). The

composition of RJ varies with seasonal, regional and production conditions (Jianke et al. 2005; Kösoğlu et al. 2013). Storage conditions and durations also affect the quality and composition of RJ (Chen and Chen 1995). Fresh RJ consists of water (60-70%), protein (9-18%), sugars (7-18%), lipids (3-8%), minerals (0.8-3%), small amounts of vitamins (B-complex vitamins, vitamin C, and vitamin E), free amino acids and other components (Sabatini et al. 2009). Biological activities of RJ are mainly attributed to the bioactive fatty acids, proteins and phenolic compounds (Ramadan and Al-Ghamdi 2012).

In recent years, the physiological functionality of foods has received much attention, due to increasing interest in human health. Among them RJ is one of the most attractive products. It has been widely used in commercial medical products, healthy

foods and cosmetics in many countries. This has resulted in large-scale importation in countries where production is insufficient to meet domestic demand (Ramadan and Al-Ghamdi 2012). However, much less is known regarding chemical compositions and quality of royal jelly products despite their increasing consumption. RJ adulteration is the most important quality problem (Sabatini et al. 2009). RJ is the only product that contains 10-Hydroxy-2-Decenoic Acid (10-HDA) naturally. Therefore, 10-HDA is the most important quality criteria for RJ adulteration and is mostly used for routine testing of RJ authenticity (Sabatini et al. 2009). On the other hand, this acid produced synthetically and is widely available in international trade in recent years. Another important quality control parameter for RJ is the freshness (Marconi et al. 2002). 10-HDA content also tends to be accepted as a freshness indicator. However, no significant correlation was found between 10-HDA content and storage duration whatever the storage temperature (Antinelli et al. 2003). Therefore, recent studies have focused on identifying different markers or indicators of RJ freshness such as, furosine (Marconi et al. 2002; Messia et al. 2005; Wytrychowski et al. 2014), major royal jelly proteins (Buttstedt et al. 2014; Shen et al. 2015), adenosine triphosphate (Wu et al. 2015), amino acid composition (Wu et al. 2009) and color changes (Zheng et al. 2012).

Turkey has great beekeeping potential having very rich flora, suitable ecology and just about 7 million beehives, but the production of RJ is quite low (about 500 kg per year). Because of high domestic demand, Turkey imports large quantities of RJ, mainly from China. RJ is predominately produced in China and other far eastern countries and is marketed world-wide at highly competitive prices. As there is increasing interest in RJ with respect to human health, it is necessary to assess the quality parameters of commercial royal jelly products before selling. Therefore, the aim of this work is to evaluate the chemical properties of a total of thirteen different royal jelly samples, consisting of 12 commercial samples, and 1 sample of known origin obtained freshly harvested from honeybee colony in Akdeniz University. We analyzed water, crude protein, acidity, pH, ash, total sugars, fructose, glucose, sucrose and 10-HDA contents which are the most common criteria used to determine RJ properties and compared the chemical compositions of commercial samples with the Turkish (TS 6666) and the currently available royal jelly international standard (ISO 2016).

2. Materials and Methods

2.1. Samples

The study was carried out totally on thirteen pure different royal jelly samples. One sample (identified as S1) was obtained freshly harvested from apiary of Animal Science Department in Akdeniz University. Three samples (S2, S3 and S4) were provided by Turkish royal jelly producers. Nine commercial samples (S5, S6, S7, S8, S9, S10, S11, S12 and S13) originating from China were purchased from different import companies or distributors. All samples were kept at -18 °C until analyses.

2.2. Chemical analysis

Moisture content of the samples was measured by weight loss upon drying at 70 °C ± 2 °C in a vacuum drying oven (Mettler, Schwabach, Germany). Ash content was determined gravimetrically using an oven at 550 °C until constant mass

(Turkish Standard 2000). pH was determined using a digital pH meter (WTW 537 model, Weilheim, Germany). Acidity was determined by automatic titration with 0.1 N NaOH. The total nitrogen content was determined by the Kjeldahl method. The quantity of crude protein was calculated using the factor of 6.25 for conversion to protein content.

2.3. Glucose, fructose and sucrose analysis

Glucose, fructose and sucrose content of the samples were determined chromatographically. The analyses were carried out using an HPLC system (Shimadzu, Kyoto, Japan). The elution was performed on a size exclusion column (CARBOsep Coregel 87P, Transgenomic, Omaha, NE, USA) connected to a guard column at 85 °C of column oven temperature. HPLC grade water as mobile phase was allowed to flow at the rate of 0.6 ml min⁻¹ with a 20 µL sample injection volume. All the samples diluted with HPLC grade water and passed through 0.45 µm syringe filter (CHROMAFIL, PET-45/25; Macherey-Nagel, Düren, Germany) before injection. External standard method was used for the calculation of the sugar concentration of the samples. All the sugar standards were purchased from Sigma-Aldrich Chemie (St Louis, MO, USA).

2.4. Determination of 10-HAD

The 10 HDA contents of the samples were determined using an Ultra High Pressure Liquid Chromatography–Tandem Mass Spectrophotometry (UHPLC- MS/MS) (Thermo Scientific, CA, USA). About 12.6 mg 10-HDA was weighed into a 10 ml volumetric flask and was adjusted with ultrapure water to prepare the stock solution of 10-HDA at a concentration of 1234.8 mg kg⁻¹. Then, working stock solution at a 10 mg kg⁻¹ concentration was prepared diluting the first stock solution. The last stock solution was diluted ten times again and the infusion sample was obtained at a concentration of 1 mg kg⁻¹ of concentration to introduce to the MS/MS at a 10 µl min⁻¹ flow rate. The ionization of analyze was observed in the negative mode. It is observed that the molecule was negative while the molecular mass of 10-HDA 186.3 m z⁻¹ was decreased to 185.2 with a hydrogen loss. Fragment ions were obtained from main molecule (185.2 m z⁻¹) belonging with 111.4 m z⁻¹ and 134.2 m z⁻¹ masses in MS/MS. An analysis method was established including these ions. Then, the 10-HDA standard at a concentration of 1 mg kg⁻¹ was eluted on a C18 column by UHPLC and was detected in MS/MS using established method. The mobile phase was acetonitrile at a 400 µl min⁻¹ flow rate in 5 minutes' analysis time. The retention time of 10-HDA was 0.68 min in these conditions. The external standard method was used for the quantification of the 10-HDA content of the samples. The regression coefficient of the calibration curve of the standards obtained from the injections was 0.9997. The 10-hydroxy-2-decenoic acid (10-HDA) standard was obtained from Cayman Chemicals (item no: 10976, purity >98%, Michigan, USA). The stock solution (10 mg kg⁻¹) was used for the preparation of 7 standard solutions containing 50 µg kg⁻¹ to 2 mg kg⁻¹ of 10 HDA.

2.5. The extraction procedures of the samples

Approximately, 200 mg RJ samples was weighed into 50 ml volumetric flask and added 25 ml water. The flasks were shaken gently to dissolve the RJ samples in water. 0.5 ml of 2 M NaOH solution was added and then it was adjusted with water after ten minutes waiting (250 fold dilutions). 4 ml of this solution was transferred to a tube and 27 ml saturated NaCl was added. pH

value of the samples was adjusted to 2-2.5 using with 1 ml 0.1 M HCl. The mixtures were rapidly shaken after 8 ml diethyl ether addition (10 fold dilution). The tubes were centrifuged at 3000 rpm. The 4 ml of organic phase was transferred to a tube and was dried at under nitrogen atmosphere. The dried sample was dissolved in 1.6 ml acetonitrile (1.6/4 fold concentration). The last solution was diluted 150 fold with acetonitrile and was injected to UHPLC-MS/MS. The total dilution factor was 150000 fold (250 x 10 x 1.6/4 x 150) in this extraction procedure (Antinelli et al. 2003; Ferioli et al. 2007).

2.6. Statistical analysis

Data were expressed as mean \pm standard deviation. Descriptive statistics of traits were calculated and one-way analyses of variance (ANOVA) were performed to test for significant differences between the samples of each parameter by using Minitab Statistical Software (Version 16.2.4).

3. Results

In order to evaluate chemical composition, a total of thirteen different royal jelly samples, (consisting of 12 commercial samples, and 1 sample of known origin obtained from honeybee colony) were analyzed for water, crude protein, acidity, pH, ash, total sugars, fructose, glucose, sucrose and 10-HDA contents and the results are listed in Table 1 and Table 2. It was found that water, crude protein and ash contents in royal jelly samples ranged from 63.10 to 73.55% (ANOVA df=12, F=519.97, P<0.01), 9.76 - 12.57% (ANOVA df=12, F=21.89, P<0.01), and 0.92-1.17% (ANOVA df=12, F=9.98, P<0.01) respectively. Similarly, the mean values of 10-HDA content in royal jelly samples ranged from 0.57-3.11 % (ANOVA df=12, F=3492.86, P<0.01). For the three main sugars and the total amount of the sugars, the minimum and maximum values are as follows; fructose 3.58-4.87% (ANOVA df=12, F=3.91, P<0.01), glucose 2.95-6.78% (ANOVA df=12, F=37.19, P<0.01), sucrose not detected to 1.93 % (ANOVA df=12, F=636.62 P<0.01) and total sugars content 7.68-11.66 % (ANOVA df=12, F=8.27, P<0.01).

Table 1. 10-HDA and sugar contents in royal jelly samples.

Samples	10-HDA (%)	Total Sugars (%)	Fructose (%)	Glucose (%)	Sucrose (%)
S1	2.48 \pm 0.04	9.11 \pm 1.73	4.39 \pm 0.92	4.69 \pm 0.78	0.04 \pm 0.01
S2	2.29 \pm 0.04	9.41 \pm 1.42	4.53 \pm 0.56	4.60 \pm 0.73	0.29 \pm 0.10
S3	2.42 \pm 0.04	9.49 \pm 0.30	4.57 \pm 0.21	4.92 \pm 0.07	n.d.
S4	3.11 \pm 0.03	10.24 \pm 0.10	4.70 \pm 0.03	5.54 \pm 0.12	n.d.
S5	0.75 \pm 0.02	7.68 \pm 0.93	4.52 \pm 0.45	2.95 \pm 0.47	0.21 \pm 0.01
S6	0.69 \pm 0.02	11.37 \pm 0.39	4.42 \pm 0.34	4.97 \pm 0.05	1.98 \pm 0.08
S7	2.38 \pm 0.04	11.66 \pm 0.30	4.87 \pm 0.15	6.78 \pm 0.16	0.00 \pm 0.00
S8	2.36 \pm 0.04	8.69 \pm 0.21	3.53 \pm 0.14	3.29 \pm 0.06	1.93 \pm 0.03
S9	0.88 \pm 0.02	9.66 \pm 0.32	4.56 \pm 0.11	3.51 \pm 0.24	1.58 \pm 0.01
S10	0.75 \pm 0.03	9.89 \pm 0.48	4.57 \pm 0.19	3.70 \pm 0.29	1.61 \pm 0.02
S11	0.76 \pm 0.02	8.64 \pm 0.30	4.01 \pm 0.11	3.31 \pm 0.14	1.33 \pm 0.06
S12	0.57 \pm 0.02	10.04 \pm 0.10	3.93 \pm 0.03	4.32 \pm 0.04	1.79 \pm 0.04
S13	2.14 \pm 0.03	9.54 \pm 0.73	4.45 \pm 0.32	3.50 \pm 0.27	1.59 \pm 0.14

Results are expressed as means and standard deviations (n=4). n.d= Not detected- below 0.01%.

Table 2. Values of water, protein, ash, pH, and acidity in royal jelly samples.

Samples	Water (%)	Protein (%)	Ash (%)	pH	Acidity ml 1N NaOH 100g ⁻¹
S1	64.50 \pm 0.18	11.26 \pm 0.15	1.05 \pm 0.09	3.80 \pm 0.05	40.36 \pm 0.47
S2	66.80 \pm 0.22	9.76 \pm 0.10	0.92 \pm 0.02	3.70 \pm 0.01	43.81 \pm 0.12
S3	66.03 \pm 0.28	10.65 \pm 0.12	1.00 \pm 0.06	3.82 \pm 0.02	41.98 \pm 1.53
S4	63.10 \pm 0.29	12.10 \pm 1.06	1.06 \pm 0.04	3.83 \pm 0.01	41.05 \pm 1.22
S5	71.03 \pm 0.43	11.19 \pm 0.07	0.95 \pm 0.01	3.93 \pm 0.05	36.02 \pm 1.29
S6	72.13 \pm 0.33	12.55 \pm 0.03	1.17 \pm 0.10	3.98 \pm 0.01	28.36 \pm 0.24
S7	65.25 \pm 0.26	11.97 \pm 0.28	1.09 \pm 0.01	4.01 \pm 0.01	35.88 \pm 2.56
S8	65.37 \pm 0.25	12.57 \pm 0.35	1.12 \pm 0.02	3.66 \pm 0.01	40.53 \pm 0.02
S9	70.68 \pm 0.26	12.48 \pm 0.01	1.14 \pm 0.02	3.81 \pm 0.01	37.49 \pm 0.08
S10	71.85 \pm 0.25	12.35 \pm 0.31	1.14 \pm 0.01	3.82 \pm 0.02	37.46 \pm 2.02
S11	70.80 \pm 0.29	12.04 \pm 0.22	1.14 \pm 0.06	3.85 \pm 0.01	35.74 \pm 0.26
S12	73.55 \pm 0.26	12.15 \pm 0.35	1.15 \pm 0.04	4.02 \pm 0.01	27.94 \pm 0.97
S13	67.36 \pm 0.31	12.21 \pm 0.20	1.16 \pm 0.02	3.89 \pm 0.01	37.66 \pm 1.69

Results are expressed as means and standard deviations (n=4).

4. Discussion

National royal jelly standards have been established some countries such as Bulgaria, Poland, Switzerland, Turkey, Japan, China, Korea (Kanelis et al. 2015), and a group of the International Honey Commission (IHC) prepared a preliminary proposal for the standardization of royal jelly (Sabatini et al.

2009). In 2016, the International Organization for Standardization issued royal jelly international standard (ISO 2016). Comparison of the values obtained in this study with the international royal jelly standard (ISO 2016) showed that all pH, ash, total sugars values found in the samples were within the international standards. But, there was a large variation in

glucose (2.95-6.78%) and sucrose (not detected to 1.93%) contents amongst the samples. These changes are mainly caused by the hydrolyses of sucrose to fructose and glucose (Chen and Chen 1995). All the protein values were in accordance with the limits recommended by ISO 12842 (ranging from 9 to 18%). Considering Turkish legislation (TS 6666) only two royal jelly samples (S2- 9.76% and S3- 10.65%) were not within the allowed limits, ranging from 11 to 14.5% (Turkish Standard 2000).

As royal jelly is the unique natural product that has 10-HDA, this acid has been used as a main marker of freshness, quality and authenticity for pure royal jelly (Sabatini et al. 2009; Wytrychowski et al. 2013). The minimum limits of 10-HDA content range from 1.4% to 2% depending on the national legislations (Kanelis et al. 2015). According to the standards of the ISO and Turkish, 10-HDA content should be at least 1.4% for fresh royal jelly to attend quality control parameters. The 10-HDA contents of royal jelly samples measured here also showed great variability, ranging from 0.57 to 3.11%. 10-HDA contents of six royal jelly samples (S5, S6, S9, S10, S11, and S12) had lower values than the allowed ISO and Turkish limit of 1.4%. Similarly, the water contents of samples followed the same pattern. According to the standards, the water content of the fresh royal jelly should be in the range 60-70%. Water contents of same six samples (S5, S6, S9, S10, S11, and S12) were higher than the upper limit of standard.

Royal jelly adulteration is the most important quality problem (Ramadan and Al-Ghamdi 2012). The quantity of 10-HDA decreases in proportion to the degree of adulteration (Vujic and Pollak 2015). Garcia- Amoedo and Almeida-Muradian (2007) adulterated experimentally royal jelly with yogurt, egg white, water and corn starch slurry and found that adulteration with more than 25% of yogurt, egg white, water and corn starch slurry can be detected by the enhancement of moisture, diminishing in lipid, protein, 10-HDA contents and insolubility in alkaline medium. Adulteration with honey results in a general decrease of proteins and 10-HDA and a relative increase of sugars (Serra-Bonvehí 1991). It seems that the easiest way to adulterate RJ is the adding of synthetically produce 10-HDA which is widely available in international trade in recent years. Our results show that all royal jelly samples did not meet the national and international limits were imported from China. Although official estimates are not available, more than 80% of the royal jelly sold in Turkey is imported mainly from China mostly in bulk. Therefore, we don't know precisely where the royal jelly was adulterated. Previous studies have also demonstrated that lipid, 10-HDA and total polyphenols contents were significantly higher in local royal jelly samples than in commercial samples (Ferioli et al. 2007; Ferioli et al. 2014; Pavel et al. 2014).

5. Conclusion

Our data clearly demonstrated that there were significant differences between the royal jelly samples with regard to the chemical properties determined in this work and in terms of 10-HDA and water contents, 50% of all royal jelly samples did not meet the limits permitted by international and Turkish royal jelly standards. We obtained royal jelly samples from all import companies and main local distributors. Therefore, imported royal jelly products must comply with the regulations and quality standards and its quality must be regularly and deeply monitored before selling. Further research is needed to develop fast and low cost methods to detect non-compliance with

regulations and quality standards. Moreover, intensive standardization studies should be also made for improving royal jelly international standards

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