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Study on Predictive Models Relating Physico-chemical Properties of Iranian Royal Jelly and it's Sensory Evaluation

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ABSTRACT: Royal jelly is a substance of complex physico-chemical structure that have been used as a nutritional supplement and functional food for many years. In this paper, the physico-chemical analysis and organoleptic characteristics of royal jelly obtained from different climatic regions of Iran including Ardebil (cold and dry), Amol (wet and moderate), and Mashhad (hot and dry) have been evaluated. The average of Iranian royal jelly composition consisted of moisture content 64.03±3.67 %; proteins 14.13±2.36 %; carbohydrates 13.92±1.67 %; reducing sugars 8.78±1.55 %; fats 6.17±1.45 %; ash 2.08±0.85 %, pH 3.94±0.34 and acidity 31.34±4.90 mL/100g. The one-way analysis of variance (ANOVA) has illustrated that environmental factors had a significant influence on physico-chemical characteristics of Iranian royal jelly ($P<0.05$). Regarding the influence of temperature and relative humidity on the composition of royal jelly some valid prediction models have been provided. Test panel group evaluated the samples by using 5 points Hedonic and descriptive scales. In sensory evaluation, Ardebil royal jelly with a 4.64±0.230 score evaluated as a good quality royal jelly.

Keywords: Environmental Factors, Iranian Royal Jelly, Physico-chemical Characteristics, Predictive Modelling, Sensory Evaluation.

Introduction

Royal jelly as a principal food source of queen honeybee is produced by hypopharyngeal and mandibular glands on top of the heads of young nurse honeybees (Apis mellifera). Royal jelly is a substance of complex physico-chemical structure, also produced as larva food (Mutsaers *et al.*, 2005; Barnuțiu *et al.*, 2011).

Royal jelly is an emulsion of proteins, carbohydrates, and lipids in water. People have used royal jelly as a nutritional supplement and functional food for many years (Marghitaş *et al.*, 2010). The lipid fraction is mostly composed of polar acidic compounds (Bloodworth *et al.*, 1995). Water-soluble vitamins consist of B-complex (B_1, B_2, B_3, B_5, B_6, B_7, B_9), and traces of vitamin C. In addition, it contains significant amount of fat soluble vitamins, like vitamins A, D, and E. Royal jelly contains different minerals namely Potassium, Calcium, Magnesium, Phosphorus, Sulfur, Sodium, Zinc, Iron, Copper, Manganese. The trace elements with biological function are Aluminium, Barium, Strontium, Tin, Titanium, Chromium, Nickel, Cobalt, and

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Molybdenum. Royal jelly includes other components such as royalisin and apisimin (Stocker *et al*., 2005; Konings, 2006; Ostiguy *et al*., 2019). The health benefits of royal jelly are attributed to various bioactive compounds present in royal jelly namely B-complex vitamins, fatty acids, acetylcholine, adenosine, and trace minerals (Stocker *et al*., 2005; Bărnuțiu *et al*., 2011). The most unique and chemically interesting bioactive component in royal jelly is a fatty acid named trans-10-hydroxy-2-decanoic acid (10-HDA). This fatty acid forms half of royal jelly's fatty acid content. 10-HDA has an important role in the growth and balance of the hormone system and the immune system. Royal jelly is the only natural source of pure acetylcholine. Reducing acetylcholine levels is one of the most important causes of neurological diseases (Bloodworth et al., 1995; Zhou *et al*., 2007; Munoz *et al*., 2011).

In terms of the effects of environmental factors on products specially royal jelly, preliminary reports have shown that environmental factors such as climatic region and season can change the physico-chemical composition of royal jelly. There is a remarkable increase in free fatty acid in Thai royal jelly obtained during the summer season as compared to the other seasons (Haemmerli *et al*., 1980; Barnuțiu *et al*., 2011).

The impact of environmental factors on product quality of greenhouse vegetables for fresh consumption has been investigated. As well visual characteristics, properties such as texture, the content of minerals and vitamins, flavor and other organoleptic characteristics has been considered. Many research studies have documented methods for achieving a high-quality vegetable product. Indoor production for fresh vegetables offers advantages compared to outdoor production with regard to quality assurance principally, because the products are not exposed directly to the rapid changes of climate conditions. On the other hand, vegetable cultivation in a greenhouse under artificially created conditions also affects the internal quality of the product. This is reflected in a different taste and flavor compared with field vegetables. Changes in external as well internal quality attributes of greenhouse vegetables subjected to light intensity, temperature, vapor pressure deficit (VPD), and CO_2 enrichment of the atmosphere concentration are discussed (Gruda, 2005).

In 2019 the effect of environmental factors on honeybee activity and onion (Allium cepa L.) seed yield was considerd. Pollinators are required to produce onion seeds. This specie is one of the main vegetable crops. Two types of onion varieties are mainly grown worldwide: hybrids and open pollination (OP) cultivars. Although hybrids offer advantages to bulb growers, seed yields of hybrids are lower than OP cultivars and that is a significant problem. The influence of environmental factors (temperature, radiation, rainfall, relative humidity (RH) and wind speed) was determined, as well honeybee attraction and seed production in three locations of the main onion seed production area in Argentina. Nine male sterile lines (MSL) and one OP were used. The results obtained showed a marked variability in the attraction of honeybees and seed production between the OP and MSL and within MLS. In addition, environmental factors such as minimun temperature or RH were determinant to modify honeybee foraging behavior, where values lower than 9°C and 22%, respectively, caused that honeybees stop their activity (Caselles *et al*., 2019).

The Effects of different environmental conditions on the cognitive function of honeybee (Apis mellifera L.) and mineral content of honey has been studied. The aim of the study was to determine the effect of local area inhabited by honeybee colonies on regional efficiency, foraging behavior and the content of certain metal elements in honey. Honeybee colonies from the same genetic source in different regions demonstrated significant variation ($P<0.001$) in behavior and performance. Initially, the number of forager worker honeybees exiting and entering the hive was approximately equal to each other. However, over time a significant difference ($P<0.001$) occurred between regions. Varying regional conditions caused considerable difference ($P<0.001$) in the average honey yields of colonies (between 28.60 ± 3.27 and 0.571 ± 2.76 kg/colony). Significant differences ($P<0.01$) in the amount of wax produced were also observed between regions. These regional differences were further reflected in concentrations of certain heavy metals in centrifugal honey samples. Environmental effects were determined to be the most important reason for the differences in all phenotypes, such as behavior, honey yield and heavy metal concentrations in honey. Those colonies inhabiting industrial or polluted areas destroyed before the winter. Therefore, colonies are only productive when provided with appropriate environments or conditions (Nisbet *et al.*, 2019).

The impact of environmental conditions on heavy metal concentration in honey samples was considered. The aim of the study is determination of heavy metals in honey samples with different botanic origin produced by Kosovo farmers and as well the comparison of gathered results with the specific environmental conditions, with particular emphasis on industrial and agricultural areas where the concentration of heavy metals is expected to be higher than at forester areas. Concentrations of thirty-four heavy metals were investigated in 30 different honey samples but are reported only the most important elements. For this reason, Inductively coupled plasma mass spectrometry (ICP-MS) techniques was used. All metals were detected in 78.49% of the honey samples. Values of pH for tested honey samples show that they are all acidic, with range of pH values from 3.41 to 4.97, with mean value of pH 4.07 ± 0.33. The most abundant element in investigated honey was Mg (44.831 mg kg^{-1}), followed by Al (12.013 mg kg^{-1}), Fe (8.859 mg kg^{-1}), Zn (4.814 mg kg^{-1}), Mn (3.378 mg kg^{-1}), Cr (2.472 mg kg^{-1}), Se (1.599 mg kg^{-1}) and Cu (1.105 mg kg^{-1}), the mean of others elements was less than 1 mg kg^{-1} (Co, Ni, As, U and Cd). In neither of the analysed samples has not been found the presence of any of the lead isotopes: 206, 207 or 208. In general, the heavy metal concentrations in investigated honey samples collected in regions of Kosovo generally fit international standards but the concentration of heavy metals in honey samples is strongly dependent on the environmental conditions (Aliua *et al*., 2020).

Iran has a suitable potential to produce royal jelly, due to the desirable climatic conditions and diversity of vegetation particularly because of it’s medicinal plants. Honey production in Iran is about 111,000 tons which has occurred by 11,000,000 colonies of honeybees and more than 300,000 beekeepers in 2022. Iran in terms of honey production has ranked in third place in the world after China and Turkey, in 2022 (Anon, 2023). Iran’s contribution to global honey production was 114,000 tons and as well 1,460 tons wax, 300 tons of propolis, 122 tons of pollen, and 3,400 tons of royal jelly in 2020 (Anon,, 2020).

The daily dose of royal jelly consumption is 1 mg per 1 kilogram of body weight (mg/kg) for a four weeks consumption period. Royal jelly in Iran is employed as an anti-cancer treatment, as well it promotes digestive health, boosts fertility, aids diabetes treatment, cures neurological diseases, boosts hair growth, and is beneficial for the skin (Mofidi *et al*., 2016).

Today, there are no standards at the European or international level for honeybee products other than honey, but several countries have established national standards or guidelines. The first country that set the criteria for royal jelly was Argentina in 1979, followed by Bulgaria in 1984, Poland in 1996, Turkey in 2000, Brazil in 2001, Serbia in 2003, Switzerland in 2005, Japan and China in 2008, India in 2012, and Korea in 2014 (Kanellis *et al*., 2015; Hu *et al*., 2017). A few years ago, a working group of the International Honey Commission (IHC) prepared a preliminary proposal for the standardization of royal jelly based on information they collected.

The objective of this paper is to investigate the physico-chemical characteristics of Iranian royal jelly obtained from different climatic regions of Iran. The effects of environmental factors on the physico-chemical characteristics of royal jelly are evaluated. It is statistically investigated whether the environmental factors have a significant impact on the composition of royal jelly. Some valid prediction models have been proposed in this study. As well organoleptic characteristics namely color, taste, odor, texture, and the absence of foreign objects like larvae remains were determined by filling out the questionnaire form by the test panel group. They evaluated the samples by using 5 points Hedonic (1= dislike extremely; 2= dislike slightly; 3= neither like nor dislike; 4= like slightly; 5= like extremely) and descriptive scales.

Materials and Methods

- *Sample collection*

Samples of royal jelly from three different climatic regions of Iran, Mashhad (hot and dry), Amol (wet and moderate), and Ardebil (cold and dry) were collected (15^{th} May) at the beginning of the beekeeping season. Royal jelly samples were obtained from these regions after 72 hours of honeybee larvae grafting, at 8:00 A.M. on the 15^{th} May, simultaneously. In order to carry out laboratory examinations and sensory evaluation, 60 grams of royal jelly from 10 hives for each region (180 grams of royal jelly from 30 hives) were collected. Finally, three samples of royal jelly were obtained from three beekeeping sites which represent three different climatic regions of Iran. The GPS coordinates for these studied beekeeping sites were about Mashhad FXPG+QH, 36.486969, 58.976481, 36°29'13.1"N 58°58'35.3"E, Ferizi, Razavi Khorasan Province, Iran, about Ardebil 63C3+98, 38.220954, 48.053269, 38°13'15.4"N 48°03'11.8"E, Atashgah, Ardabil Province, Iran and finally about Amol F857+MP, 36.459217, 52.314342, 36°27'33.2"N 52°18'51.6"E, Tajanjareolya, Mazandaran Province, Iran. Before the experimental work, royal jelly samples were stored at -20º C in the freezer.

The royal jelly during studied days concerned with the grafting of honeybee larvae until closing the queen cell (after 72 hours) was exposed to the environmental treatments. The digital thermohydrometer (RST model), which was placed on the beekeeping site recorded the temperature and relative humidity of each region. The obtained information was recorded over three days and on nine sections of time in the morning, noon and night. The major vegetation and honeybee breeds were detected by field surveys, observing, and consulting with an apiarist.

- Sensory or questionnaire study

Organoleptic characteristics namely color, taste, odor, texture, and the absence of foreign objects like larvae remains were determined by filling out the questionnaire form by the test panel group. Twenty persons participated in the sensory study. The judges were healthy food scientists and did not use tobacco and stimulants such as spices.

They evaluated the samples by using 5 points Hedonic (1= dislike extremely; 2= dislike slightly; 3= neither like nor dislike; 4= like slightly; 5= like extremely) and descriptive scales.

According to the GSO[1] standard, in descriptive scale the royal jelly should be a white or cream-colored product and alcohol free. By increasing the time of storage, the color of royal jelly becomes yellower. Royal jelly should be without signs of fermentation and free of external materials and foreign flavor. It should be pure and without pollen grains, wax particles, and residues of larvae bodies. It should be with special flavor characteristics of royal jelly. It has a sour and rancid odor and also a sour and sweet taste. The texture of royal jelly is viscous and non-homogeneous. The formation of two phases and syneresis in royal jelly are unacceptable (Marine, 2013).

- Laboratory analysis

Determination of physico-chemical properties of royal jelly consisting of moisture content, proteins, carbohydrates, reducing sugars, lipids, pH, and acidity were carried out as follows. Moisture content was determined by a gravimetric method in a vacuum oven at 65º C (Marghitaş *et al.*, 2010). Proteins were determined by the Kjeldahl method based on total nitrogen according to AOAC 962.18. The Lane and Eynon volumetric method was applied to measure the reducing sugar of royal jelly according to AOAC 920.183. Total carbohydrates were obtained by the difference according to AOAC, 1980. Total lipids were determined by extraction of dry matter with petroleum ether according to AOAC 990.28. Minerals were determined by the ashing method in an electric furnace at 500-550˚ C followed by application to atomic absorption spectroscopy according to the standard method of AOAC 923.03. pH value was determined by potentiometric method with a C532 pH meter according to AOAC 994.16. The acidity of the royal jelly was determined by titration with sodium hydroxide solution using phenolphthalein as an indicator according to AOAC 962.16. All the solvents used were of analytical grade, obtained from Merck and Sigma-Aldrich companies. All the determinations were performed in triplicate.

- Data Analyzing Procedure

In data analysis, a randomized complete block design with three experimental groups was used (Ardebil, Amol, and Mashhad). The effects of environmental factors on physico-chemical properties of royal jelly were analyzed by using SPSS software version 22.

In statistical analysis of environmental factors, the case of quantitative variables (temperature and relative humidity), the one-way analysis of variance (ANOVA) was employed to investigate their effects on physico-chemical properties (moisture content, proteins, carbohydrates, reducing

[1] Persian Gulf Standardization Organization

sugars, fats, minerals, pH and acidity) of royal jelly. The average of relative humidity and temperature (quantitative variables) which were correlated (Pearson correlation is non-zero and statistical significance is less than 0.05), was considered to reduce the computational errors.

In the case of quantitative variables to confirm the proposed prediction models, the normal distribution of errors was determined by P-P plot[1] and Kolmogorov-Smirnov standardization test. Also, the independence of errors was determined by the Durbin-Watson statistic which must place between 1.5< Durbin-Watson statistic <2.5. And finally, the errors must have constant variance.

In the case of vegetation, climatic region, and type of honeybee colony (nominal variables) after confirming their correlation (Pearson correlation is non-zero and statistical significance is less than 0.05), one of them was considered. In the statistical analysis of nominal variables, Levene's test was employed. If statistical significance is more than 0.05, the assumption of variance equality was accepted and then the Tukey test was employed, and if statistical significance is less than 0.05, the assumption of variance equality was rejected and the Tamhane test was employed. Tamhane and Tukey tests were employed to investigate if the nominal variables of Ardebil, Amol, and Mashhad had significant effects on the physico-chemical properties of royal jelly.

Results and Discussion

Table 1 represents different environmental variables in three consecutive days for harvested royal jelly. As presented in Table 1 the vegetation and colony calendar in the mid-spring in Ardebil is Russian silver colony of honeybee or Silvarum breed which is hybrid with the local breed, and as well the major vegetation is Pennyroyal, Hound's-tongue, and Acacia. In rainy regions namely Amol type of honeybee colony is a yellowish Italian or Ligostica breed which is hybrid and the major vegetation is orange bloom and forest plants. In Mashhad the major breed of a honeybee colony is brown central European or Carnica and it is a hybrid. The major vegetation is Thyme, Jujube, Oregano, and Camel's Thorn.

The case of the organoleptic tests about Iranian royal jelly, on a descriptive scale, the samples have a thick, jelly and creamy form, with a milky color from white to white-yellow with an aromatic, pungent odor and sweet, sour, spicy, and slightly bitter taste, generally.

Table 1 presents and investigates the environmental variables in three consecutive days at different periods in Ardebil, Amol, and Mashhad.

Table 2 shows the sensory evaluation of the Iranian royal jelly. The sensory properties of royal jelly which tend to change with environmental factors are taste, odor, texture, and partly color Regarding Tables 1 and 2 concerned with the mountainous and desert regions of Iran namely Ardebil, and Mashhad due to the existence of aromatic medicinal herbs, the obtained royal jelly had a pleasant odor and taste. Amol royal jelly due to the acidity of forest soils and the presence of surface water, the acidity and sour taste of royal jelly was stronger than in other regions. Higher relative humidity of Amol caused less viscous royal jelly as compared to the other regions. The color intensity of Mashhad royal jelly has increased due to the higher levels of oils and carotenoids in the vegetation.

[1] probability–probability plot or percent–percent plot or P-value ***plot***

Table 1. Investigation of environmental variables in three consecutive days at different periods in Ardebil, Amol, and Mashhad

City name	**Environmental variables**	**Date and time period**								
		12th May			**13th May**			**14th May**		
		8:00 A.M.	**12:00 P.M.**	**8:00 P.M.**	**8:00 A.M**	**12:00 P.M.**	**8:00 P.M.**	**8:00 A.M.**	**12:00 P.M.**	**8:00 P.M.**
Ardebil	Temperature (ºC)	15	27	21	15	26	18	17	28	20
	Relative Humidity (%)	80	47	68	80	47	57	80	47	52
	Type of honeybee colony	Russian silver (Silvarum) & hybrids			Russian silver (Silvarum) & hybrids			Russian silver (Silvarum) & hybrids		
	Major vegetation	Pennyroyal,Hound's-tougue, Acacia			Pennyroyal, Hound's-tougue, Acacia			Pennyroyal, Hound's-tougue, Acacia		
Amol	Temperature (ºC)	25	31	26	24	30	25	25	31	26
	Relative Humidity (%)	92	70	83	91	62	92	85	59	69
	Type of honeybee colony	Italian yellow (Ligostica) & hybrids			Italian yellow (Ligostica) & hybrids			Italian yellow (Ligostica) & hybrids		
	Major vegetation	Orange bloom, Forest plants			Orange bloom, Forest plants			Orange bloom, Forest plants		
Mashhad	Temperature (ºC)	29	35	29	31	38	27	32	39	29
	Relative Humidity (%)	20	8	20	20	7	24	16	6	18
	Type of honeybee colony	Central European brown (Carnica) & hybrids			Central European brown (Carnica) & hybrids			Central European brown (Carnica) & hybrids		
	Major vegetation	Thyme, Jujube, Oregano, Camel's thorn			Thyme, Jujube, Oregano, Camel's thorn			Thyme, Jujube, Oregano, Camel's thorn		

Table 2 presents the results of the sensory evaluation of royal jelly samples from Ardebil, Amol, and Mashhad. According to the Hedonic and descriptive scales, Ardebil royal jelly in cases of color (milky-white), odor (pungent and aromatic), texture (thick and jelly), and absence of foreign objects (no pollon, no larvea) scored 5 (like extremely), and regarding the taste (sweet and a little sour) scored 4 (like slightly). Mashhad royal jelly was placed after Ardebil royal jelly where in the case of texture (homogenous, thick, and jelly) scored 5 (like extremely), regarding taste (sweet, sour, and spicy), odor (aromatic), and absence of foreign objects (a little) scored 4 (like slightly) and concerning the color (yellow with little brown spots) scored 2 (dislike). Sensory evaluation of Amol royal jelly in cases of color (white-yellow), texture (jelly and less viscous) and absence of foreign objects (a little) scored 4 (like slightly), and regarding taste (sour, rancid, bitter) scored 3 (neither like nor dislike) and in case of odor (pungent) scored 2 (dislike).

Table 2. Sensory evaluation of Iranian royal jelly

City name	Judge's name: 20 member								Date of evaluation: 15 May
	Run	**Characteristics evaluated**	**Qualitative ranking based on 0 to 5 point**						**Score coefficient**
Ardebil	1	Color (milky-white)	0	1	2	3	4	5	3
	2	Taste (sweet and a little sour)	0	1	2	3	4	5	4
	3	Odor (pungent and aromatic)	0	1	2	3	4	5	2
	4	Texture (thick and jelly)	0	1	2	3	4	5	1
	5	Absence of foreign objects (no pollon, no larvea)	0	1	2	3	4	5	2
		Royal jelly general acceptability= Sum of scores/ Sum of coefficient→4.64±0.23							Sum of coefficient=12
Amol	1	Color (white-yellow)	0	1	2	3	4	5	3
	2	Taste (sour,rancid, and bitter)	0	1	2	3	4	5	4
	3	Odor (pungent)	0	1	2	3	4	5	2
	4	Texture (jelly and less viscous)	0	1	2	3	4	5	1
	5	Absence of foreign objects (a little)	0	1	2	3	4	5	2
		Royal jelly general acceptability= Sum of scores/ Sum of coefficient→3.33±0.38							Sum of coefficient=12
Mashhad	1	Color (yellow with few brown spots)	0	1	2	3	4	5	3
	2	Taste (sweet, sour, and spicy)	0	1	2	3	4	5	4
	3	Odor (aromatic)	0	1	2	3	4	5	2
	4	Texture (homogenous, thick, and jelly)	0	1	2	3	4	5	1
	5	Absence of foreign objects (a little)	0	1	2	3	4	5	2
		Royal jelly general acceptability= Sum of scores/ Sum of coefficient→4.05±0.26							Sum of coefficient=12

Table 2 summarizes the sensory characteristics of Iranian royal jelly in terms of appearance, color, taste, odor, texture, and absence of foreign objects. The acceptability of Ardebil royal jelly was the highest (4.64±0.23; 4=like slightly, 5=like extremely). Ardebil royal jelly was milky-white, sweet and a little sour, pungent, aromatic and thick, and the jelly was without pollon and larvae. Mashhad royal jelly with an acceptability of 4.05±0.26 (4= like slightly) and finally, Amol royal jelly with a score of 3.33±0.38 (3=neither like nor dislike, 4= like slightly) followed Ardebil royal jelly.

Currently, there are a few reports concerned with the sensory preferences of royal jelly. In 2009 Persian gulf standardization organization (GSO 2097: Royal Jelly) prepared a standard for royal jelly. It should be noted that long storage of royal jelly turns color to yellow and tastes rancid. The viscosity of royal jelly increases with aging especially the royal jelly that is kept in an unsuitable condition. For maintaining the optimum quality of royal jelly, it should be lyophilized or stored in a frozen condition (Kim & Lee, 2010; Marine, 2013; Hu *et al*., 2017).

The appearance of Iranian royal jelly is illustrated in Fig.1. In terms of decreasing color intensity, Ardebil royal jelly followed by Amol, and Mashhad royal jellies are placed, respectively. It should be noted that the color of royal jelly also turns yellow by aging. Besides the temperature, relative humidity, major vegetation, and climatic region, the type of honeybee colony also affect the royal jelly's sensory characteristics (Bloodworth *et al*., 1995; Mofidi *et al*., 2016).

(a) (b) (c)

Fig. 1. Appearance of Iranian royal jelly from Ardebil (a), Amol (b), and Mashhad (c) cities.

In statistical analysis of environmental factors, in the case of nominal variables (type of vegetation, climatic region, and colony type of honeybee) because of the relation between them, one variable should be included in the model (if one type of honeybee or vegetation existed in three different locations, it was possible to examine each factor separately). Since P<0.05, at least one average of each physico-chemical property related to nominal factors of the three cities is different.

As Table 3 showes the Levene test on physico-chemical characteristics of royal jelly. In order to investigate the effect of nominal variables on physico-chemical properties of Iranian royal jelly after the Leven’s test, regarding proteins (P=0.07, P<0.05) and reducing sugars (P=0.02, P<0.05), since P<0.05 it followed Tamhane test. Other physico-chemical properties of Iranian royal jelly such as moisture content (P=0.136, P>0.05), carbohydrates (P=0.352, P>0.05), fats (P=0.049, P>0.05), minerals (P=0.195, P>0.05), pH (P=0.688, P>0.05) and acidity (P=0.115, P>0.05), Tukey test was employed since P>0.05.

The statistical analysis of physico-chemical characteristics of royal jelly namely protein, reducing sugar, and fat declared as instances (Tables 4, 5, 6). Tamhane and Tukey tests were employed to investigate if the nominal variables of Ardebil, Amol, and Mashhad had significant effects on the physico-chemical properties of royal jelly.

Table 3. Levene test on physico-chemical characteristics of royal jelly namely moisture content, protein, carbohydrate, reducing sugar, fat, ash, pH, and acidity

Analytical criteria	F	Df_1	Df_2	Sig.
Moisture content	2.837	2	6	0.136
Protein	12.582	2	6	0.007
Carbohydrate	1.247	2	6	0.352
Reducing sugar	8.097	2	6	0.020
Fat	2.663	2	6	0.149
Ash	2.175	2	6	0.195
pH	0.398	2	6	0.688
Acidity	3.163	2	6	0.115

The correlation coefficients of temperature and relative humidity at three different times for three consecutive days on the physico-chemical properties of royal jelly have been investigated (Table 7). The correlation coefficients of temperature and relative humidity are non-zero and correlated (statistical significance is less than 0.05) (Table 7). The values of N=9 refer to three different times (morning, noon, and night) for three consecutive days (12, 13, and 14 of May). Since the quantitative variables were correlated, their averages were considered. The temperature and relative humidity of each city has a significant effect on the concentration of the physico-chemical compounds (moisture content, protein, carbohydrate, reducing sugar, fat, minerals, pH, and acidity) of royal jelly.

Table 4. Tamhan test comparison of Ardebil, Amol, and Mashhad nominal variables on the protein of royal jelly

	Nominal variables (I)	Nominal variables (J)	Mean Difference (I-J)	Std. Error	Sig.
Tukey HSD	Ardebil	Amol	5.4600	0.25036	0.000
		Mashhad	1.0867	0.25036	0.012
	Amol	Ardebil	-5.4600	0.25036	0.000
		Mashhad	-4.3733	0.25036	0.000
	Mashhad	Ardebil	-1.0867	0.25036	0.012
		Amol	4.3733	0.25036	0.000
Tamhane	Ardebil	Amol	5.4600	0.30625	0.008
		Mashhad	1.0867	0.03844	0.001
	Amol	Ardebil	-5.4600	0.30625	0.008
		Mashhad	-4.3733	0.30459	0.014
	Mashhad	Ardebil	-1.0867	0.03844	0.001
		Amol	4.3733	0.30459	0.014

Table 5. Tamhan test for comparison of Ardebil, Amol, and Mashhad nominal variables on the reducing sugar of royal jelly

	Nominal variable (I)	Nominal variable (J)	Mean Difference (I-J)	Std. Error	Sig.
Tukey HSD	Ardebil	Amol	-3.6900	0.18866	0.000
		Mashhad	-1.0567	0.18866	0.003
	Amol	Ardebil	3.6900	0.18866	0.000
		Mashhad	2.6333	0.18866	0.000
	Mashhad	Ardebil	1.0567	0.18866	0.003
		Amol	-2.6333	0.18866	0.000
Tamhane	Ardebil	Amol	-3.6900	0.22903	0.011
		Mashhad	-1.0567	0.03528	0.000
	Amol	Ardebil	3.6900	0.22903	0.011
		Mashhad	2.6333	0.23039	0.020
	Mashhad	Ardebil	1.0567	0.03528	0.000
		Amol	-2.6333	0.23039	0.020

Table 6. Tukey test for comparison of Ardebil, Amol, and Mashhad nominal variables on the fat of royal jelly

	Nominal variable (I)	Nominal variable (J)	Mean Difference (I-J)	Std. Error	Sig.
Tukey HSD	Ardebil	Amol	2.7067	0.34757	0.001
		Mashhad	-0.6533	0.34757	0.224
	Amol	Ardebil	-2.7067	0.34757	0.001
		Mashhad	-3.3600	0.34757	0.000
	Mashhad	Ardebil	0.6533	0.34757	0.224
		Amol	3.3600	0.34757	0.000
Tamhane	Ardebil	Amol	2.7067	0.38753	0.055
		Mashhad	-0.6533	0.18049	0.168
	Amol	Ardebil	-2.7067	0.38753	0.055
		Mashhad	-3.3600	0.42387	0.016
	Mashhad	Ardebil	0.6533	0.18049	0.168
		Amol	3.3600	0.42387	0.016

Table 7. The correlation coefficient of temperature and relative humidity at three different times for three consecutive days concerned with the physico-chemical properties of three samples of royal jelly with three replicate measurements

		Temp. 1st day	Temp. 2nd day	temp.3rd day	Humidity. 1st day	Humidity .2nd day	Humidity.3rd day
Temperature .1st day	Pearson Correlation	1	0.971**	0.986**	-0.604	-0.550	-0.650
	Sig. (2-tailed)		0.000	0.000	0.085	0.125	0.058
	N	9	9	9	9	9	9
Temperature .2nd day	Pearson Correlation	0.971**	1	0.997**	-0.776*	-0.733*	-0.812**
	Sig. (2-tailed)	0.000		0.000	0.014	0.025	0.008
	N	9	9	9	9	9	9
Temperature . 3rd day	Pearson Correlation	0.986**	0.997**	1	-0.729*	-0.682*	-0.769*
	Sig. (2-tailed)	0.000	0.000		0.026	0.043	0.015
	N	9	9	9	9	9	9
Humidity. 1st day	Pearson Correlation	-0.604	-0.776*	-0.729*	1	0.998**	0.998**
	Sig. (2-tailed)	0.085	0.014	0.026		0.000	0.000
	N	9	9	9	9	9	9
Humidity. 2nd day	Pearson Correlation	-0.550	-0.733*	-0.682*	0.998**	1	0.992**
	Sig. (2-tailed)	0.125	0.025	0.043	0.000		0.000
	N	9	9	9	9	9	9
Humidity. 3rd day	Pearson Correlation	-0.650	-0.812**	-0.769*	0.998**	0.992**	1
	Sig. (2-tailed)	0.058	0.008	0.015	0.000	0.000	
	N	9	9	9	9	9	9

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

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

The Ox axis presents the average temperature of each site at the time of royal jelly sampling and the Oy axis represents the analytical criteria (Figure 2). It has illustrated the temperature effect of Ardebil (20.77° C), Amol (27° C), and Mashhad (33.22° C) on physico-chemical properties of Iranian royal jelly. By reducing the temperature, some features of royal jelly namely proteins, minerals and pH were increased (Figure 2).

The Ox axis presents the average relative humidity of each site on the time of royal jelly sampling and the Oy axis represents the analytical criteria (Figure 3). It illustrates the relative humidity of Ardebil (15.44 %), Amol (62 %), and Mashhad (78.11 %) that significantly affects the physico-chemical composition of Iranian royal jelly. The results indicated that by increasing the relative humidity, some physico-chemical components of royal jelly namely moisture content, reducing sugars and acidity have increased (Figure 3). By increasing the temperature and decreasing the relative humidity some physico-chemical compounds of royal jelly namely lipids and carbohydrates have increased. By applying the statistical models and quantitative variables (temperature and relative humidity), the physico-chemical properties of Iranian royal jelly (moisture content, protein, carbohydrate, reducing sugar, fat, mineral, pH, and acidity) are predictable.

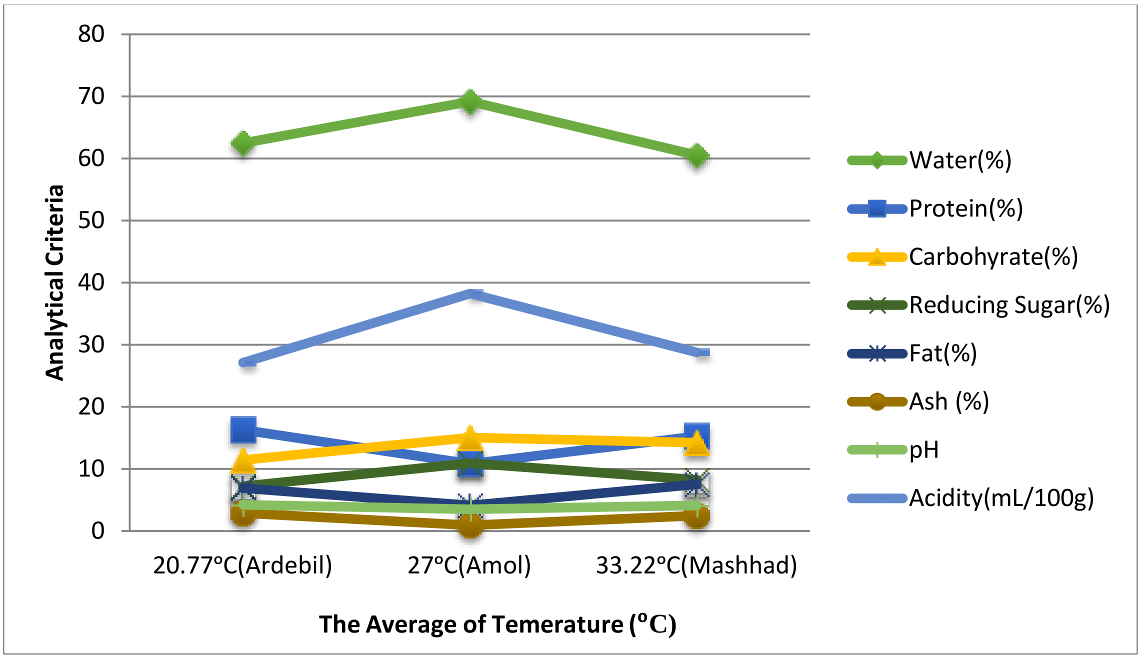

Fig. 2. Influence of temperature on physico-chemical properties of Iranian royal jelly.

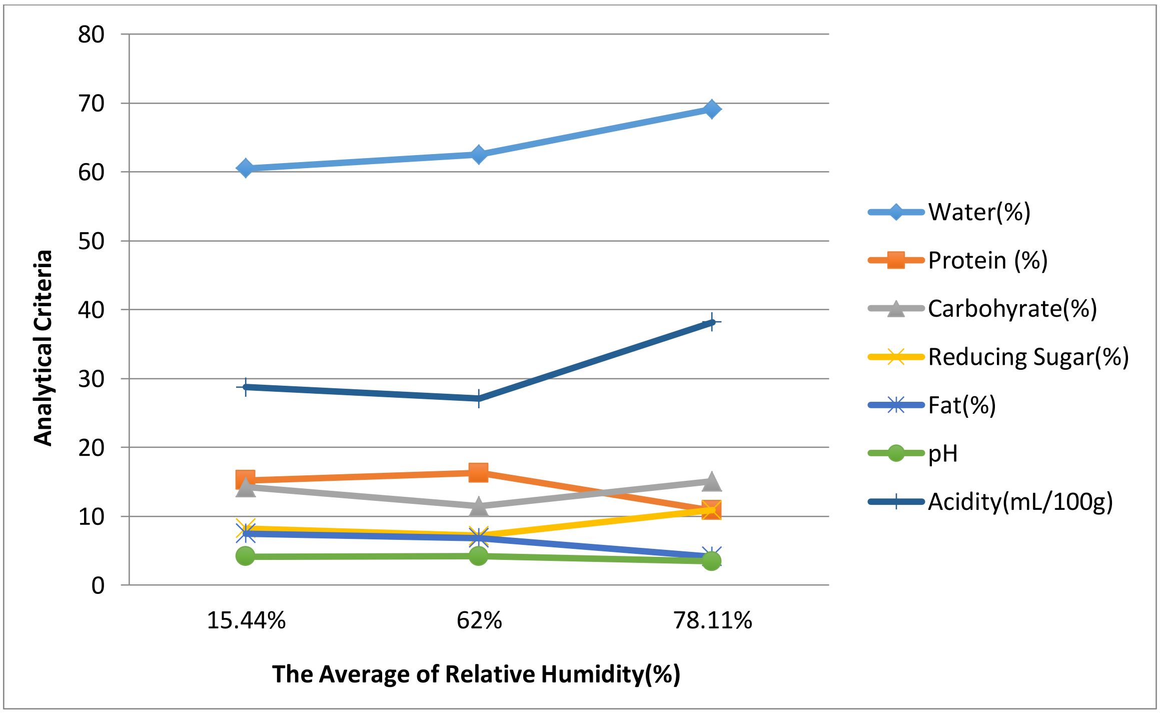

Fig. 3. Influence of relative humidity on physico-chemical properties of Iranian royal jelly.

The physico-chemical composition of the studied royal jelly samples is presented in Figure 2 and 3. The studied physico-chemical composition of royal jelly consists of moisture content, protein, carbohydrate, reducing sugar, lipid, minerals, pH and acidity. Moisture content, reducing sugars, and acidity are the highest in Amol royal jelly. The concentration of proteins, minerals, and pH is the highest in Ardebil royal jelly, while the concentration of carbohydrates and fats is the highest in Mashhad royal jelly. Carbohydrate levels decreased in cold conditions (Ardebil) and increased in hot and moderate conditions (Mashhad and Amol). The lipid concentration increased during the transition from the rainy region (Amol) to the hot region (Mashhad). As presented in Table 1 Ardabil, and Mashhad royal jellies, due to the lower relative humidity had lower moisture content as compared to the Amol royal jelly. This caused an increase in the proportion of solid content present in royal jelly. In the mountainous and desert regions of Iran, the majority of vegetation consists of medicinal plants. Therefore, due to the valuable micronutrients of these plants, the concentration of fats, proteins, reducing sugars, and minerals of Ardabil, and Mashhad royal jellies were increased. In the rainy region, Amol royal jelly had a lower pH and higher acidity in other regions. These changes were due to the acidity of surface water and forest soil, whereas, in the other regions, due to the presence of calcareous soils, the alkalinity of water was higher. In addition, the variation of the honeybee colony also affected the physico-chemical properties of royal jelly. It should be noted that the honeybee colonies were hybrids with local breeds, and were not pure in Iran.

By applying the statistical models and quantitative variables (temperature and relative humidity), the physico-chemical properties of royal jelly are predictable. The accuracy of the prediction models for spring Iranian royal jelly was investigated and confirmed. The temperature and relative humidity in average and punctual (3×3) forms were applied in prediction models. According to Table 1, the range of temperature and relative humidity were 15-39° C and 6-92 %. The observations showed that the laboratory surveys and predicted model results were almost similar.

For example, in the case of Ardebil royal jelly, the average temperature and relative humidity at three different times for three consecutive days were 20.3° C and 62 %. The calculated of moisture content in Ardebil royal jelly by using the prediction model was 62.22 % and the real moisture content according to laboratory tests was 62.50 %. In another case, the protein contents in royal jelly obtained Amol with climatic conditions T[1]= 24 °C and RH[2]= 91 % for laboratory research and by using the model were 10.85 % and 10.89 % respectively. The fat contents of Ardebil royal jelly with T= 15° C and RH= 80 % were 6.85 % and 6.82 %. The contents of carbohydrates in the case of Mashhad royal jelly with climatic conditions T= 32° C and RH= 16 % for laboratory research and by using the model were 15.25 % and 15.80 % respectively.

The bioactive compounds of Iranian royal jelly are 10-HDA, acetylcholine, soluble proteins namely albumin, beta-globulin, gamma globulin, and insoluble proteins, nucleotides, and ATP, free amino acids namely proline, lysine, aspartic acid, serine, threonine, alanine, cystine, valine, B-complex vitamins namely thiamine, riboflavin, pyridoxine, niacin, folic acid, and potassium, sodium, calcium, magnesium as important minerals (ISIRI, INSO:18385, 2014). The physico-chemical composition of global royal jelly on average is moisture content (60-70 %), lipids (3-8 %), 10-hydroxy-2-decanoic acid (at least 1.4 %), proteins (9-18 %), carbohydrates (7-18 %), reducing sugars (6-17 %), ash (0.8-3.0 %), pH of 3.4-4.5 and the acidity of 28.3-35.7 mL/100g (Hu *et al.*, 2017). Some differences between the physico-chemical components of

[1] Temperature
[2] Relative Humidity

Iranian royal jelly and other countries were observed which might be due to various conditions. These conditions are collection procedures and other factors that depend on soil fertility such as vegetation, climatic region, temperature, and relative humidity, season, floral sources, and type of honeybee colony. These factors could influence the composition of the incoming pollen and nectar and the food of nurse bees that secrete royal jelly (ISIRI, INSO: 18385, 2014; Kanellis *et al*., 2015; Hu *et al*., 2017). Considering the potential of royal jelly production in Iran, there is an expectation that by using good beekeeping practices and proper management of the apiary, the production of royal jelly is increased and the product is employed in the pharmaceutical and food industries.

In order to validate the proposed models, the normal distribution of errors by P-P plot, Kolmogorov-Smirnov normality test, and also the independence of errors (based on Durbin-Watson statistic should be between 1.5-2.5), and constant error of variance exist. Therefore, the confirmation of all these three definitions is required.

The physico-chemical composition of Iranian royal jelly consists of moisture content, protein, carbohydrate, reducing sugar, lipid, minerals, pH, and acidity (Fig. 2 and 3). The average Iranian royal jelly contains moisture content (64.03±3.67 %), proteins (14.13±2.36 %), carbohydrates (13.92±1.67 %), reducing sugars (8.78±1.55 %), fats (6.17±1.45 %), minerals (2.08±0.85 %), pH of 3.94±0.34 and acidity of 31.34±4.90 mL/100g (Fig. 2 and 3).

Table 8 declared the Durbin-Watson test for the influence of temperature and relative humidity on the physico-chemical characteristics of royal jelly. According to Table 8 the “R-Square Statistics change” of physico-chemical properties of royal jelly are R^2_{ash}=99.8 %, $R^2_{moisture\ content}$=99.1 %, R^2_{pH}=99.0 %, $R^2_{protein}$=98.9 %, $R^2_{reducing\ sugar}$=98.5 %, $R^2_{acidity}$=98.2 %, R^2_{fat}=94.6 %, $R^2_{carbohydrate}$=90.2 %. The percentage of “R-Square Statistics change” indicates the amounts of physico-chemical properties of royal jelly which are explained by the temperature and relative humidity of the region. If the content of “R-Square Statistics changes” close to 100 %; it has indicated that the proposed model is more accurate and reliable. Therefore, due to the results of “R-Square Statistics change” the prediction models were more valid and reliable for ash, moisture content, pH, protein, reducing sugar, acidity, fat, and carbohydrate respectively.

As shown in Table 8 Durbin-Watson statistics about physico-chemical characteristics of royal jelly were between 1.5-2.5.

Table 8. Durbin-Watson test for the influence of temperature and relative humidity on physico-chemical characteristics of royal jelly

Model	R Square Change	F Change	df_1	df_2	Sig. F Change	Durbin-Watson
Reducing sugar	0.985[a]	202.918	2	6	0.000	2.499
Moisture content	0.991[a]	330.696	2	6	0.000	2.331
Protein	0.989[a]	266.525	2	6	0.000	2.298
Carbohydrate	0.902[a]	27.642	2	6	0.001	2.000
Fat	0.946[a]	52.542	2	6	0.000	2.168
Ash	0.998[a]	1660.977	2	6	0.000	1.685
pH	0.990[a]	291.148	2	6	0.000	2.190
Acidity	0.982[a]	167.344	2	6	0.000	2.011

The percentage of “R-Square Statistics change” obtained from *Durbin-Watson* tests which investigated the existence of auto *correlation* in the errors of regression models. The prediction models achieved from unstandardized regression coefficients results.

Approved models with the following three definitions are as follows:

$RJ_{Moisture\ content} = 38.878 + 0.561\ a + 0.193\ b$

$RJ_{Protein} = 35.568 - 0.554\ a - 0.125\ b$

$RJ_{Carbohydrate} = - 1.023 + 0.434\ a + 0.056\ b$

$RJ_{Reducing\ Sugar} = - 5.774 + 0.385\ a + 0.080\ b$

$RJ_{Fat} = 16.525 - 0.236\ a - 0.077\ b$

$RJ_{Ash} = 9.749 - 0.198\ a - 0.045\ b$

$RJ_{Acidity} = - 12.260 + 1.113\ a + 0.262\ b$

$RJ_{pH} = 6.958 - 0.076\ a - 0.019\ b$

Where “RJ” is the royal jelly, “a” is the temperature and “b” is the relative humidity.

Since the absolute value of the temperature coefficient is larger than the relative humidity coefficient in the proposed models, therefore temperature has been more effective than the relative humidity on the physico-chemical properties of Iranian royal jelly.

The prediction models by using the punctual values (3×3) and also the averages of temperature and relative humidity had suitable answers nearby the laboratory result. But placing the averages of temperature and relative humidity are suggested due to the higher accuracy.

Temperature and relative humidity are the important environmental factors affecting the physico-chemical properties of Iranian royal jelly, this might be due to the valid prediction models obtained between temperature and relative humidity and the composition of royal jelly. These quantitative variables (temperature and relative humidity) have relative effects on other environmental parameters. Since in data statistical analysis, the absolute value of the temperature is larger than relative humidity in the proposed models, therefore the temperature had more influence on the royal jelly characteristics among the studied environmental factors.

Conclusion

In this study, physico-chemical analysis, test panel group method, and hedonic and descriptive scales have been employed. In sensory evaluation, Ardebil royal jelly with a 4.64±0.230 score scored as a good quality royal jelly. The results indicated that the concentration of proteins, minerals, and pH of Ardebil royal jelly, moisture content, reducing sugars, and acidity of Amol royal jelly, and the concentration of carbohydrates and fats of Mashhad royal jelly are the highest. The lipid concentration increased during the transition from the rainy region (Amol) to the hot region (Mashhad). In the mountainous and desert regions of Iran, the majority of vegetation is medicinal plants. Therefore, due to the valuable micronutrients of these plants, the concentration of fats, proteins, reducing sugars, and ash of Ardabil (cold and dry), and Mashhad royal jellies are increased.

One-way analysis of variance (ANOVA) has specified that environmental factors namely vegetation, climatic region, and type of honeybee colony (nominal variables), and temperature and relative humidity (quantitative variables) have significantly affected the physico-chemical properties of royal jelly ($P<0.05$). In association with the influence of temperature and relative humidity on the composition of royal jelly namely moisture content, proteins, carbohydrates, reducing sugars, fats, minerals, pH, and acidity some valid prediction models have been provided and are confirmed.

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