

Journal of Chemical Health Risks

www.jchr.org



ORIGINAL ARTICLE

Evaluation of Authenticity in Honey Samples from Qazvin, Iran

Masoud Kazeminia¹, Razzagh Mahmoudi^{*2}, Ehsan Aali³, Peyman Ghajarbeygi⁴

¹PhD Student of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
²Professor, Medical Microbiology Research Center, Qazvin University of Medical Sciences, Qazvin, Iran
³Assistant Professor, Faculty of Medicine, Dept. of Pharmacology, Qazvin University of Medical Sciences, Qazvin, Iran
⁴Assosiate Professor, Health Products Safety Research Center, Qazvin University of Medical Sciences, Qazvin, Iran

(Received: 3 January 2020 Accepted: 7 July 2021) ABSTRACT: Adulteration of honey is a major problem in the world, due to its high nutritional value and the **KEYWORDS** expensive cost of honey. Thus, the quality of honey produced in different regions must be assessed to protect the Honev: rights of consumers. The study aims to investigate the physicochemical (hydroxymethylfurfural: HMF, moisture, ash, Authenticity; electrical conductivity, pH, total acidity, diastase activity, and reduction sugar), and microbiological (clostridium Fraud; perfringens, molds, and osmotolerant yeasts) parameters of 43 honey samples. All the honey samples were collected Physicochemical; from Qazvin province, Iran. Our results demonstrate that pH and acidity values in all of the honey samples were in the Microbiological; accepted limit and other physicochemical parameters include HMF (44.18%), reduction sugar (9.30%), moisture Qazvin (2.32%), sucrose (53.48%), diastase activity (58.13%), fructose/glucose ratio (25.58%), electrical conductivity (9.30%) and ash (4.65%) were below the acceptable quality level. All the honey samples were in the acceptable range in terms of microbial quality (yeast, fungi and, Clostridia). All the honey samples are within expected microbial levels but in non-standard physicochemical conditions. Our results indicate that you can use fast, inexpensive and safe tests for identifying the adulteration in a variety of honeys (commercial and non-commercial). These measurements should be widely practiced by governmental organizations determine a fair and reasonable price for each product.

INTRODUCTION

Honey

Honey is a sweet and viscous liquid produced by bees (*Apis mellifera*) from plants nectar [1] that is used as a natural food [2, 3]. Honey has many complex compounds that are related to botanical and geographical origin, climatic conditions at harvest, climate conditions of the region and beekeeping management, specifically during honey harvest and storage [4]. This product is a valuable source of compounds for human such as biologically active substances, macro, and micro elements [5] carbohydrates, water, organic acids (gluconic acid, acetic acid, etc.), enzymes (inverts, glucose oxidase, catalase, and phosphatases), minerals, vitamins (ascorbic acid, niacin,

pyridoxine, etc), proteins, pigments, antioxidant substances, aromatics and flavorings substances, sugar alcohols, colloids and phytochemicals [6-8]. Having antimicrobial activity, honey is capable of inhibiting the growth of many foodborne pathogens. Other important factors for the human health in honey include: antiinflammatory, anti-mutagenic, anti-tumor, anti-fungal, and anti-viral [8-13].

Food fraud

Honey production is a costly process, thus producers prefer to produce honey with cheaper substances in order to

^{*}Corresponding author: r.mahmodi@yahoo.com (R. Mahmoudi) DOI: 10.22034/jchr.2021.1889948.1082

reduce the cost of honey production [14]. The most common fraud method is the over feeding of bees with sucrose, corn syrup, High Fructose Corn Syrup (HFCS), and inverts syrups, which are inexpensive sweeteners. The use of Fructose/Glucose ratio (F.G r) [15], and aldehyde 5-HydroxyMethylFurfural (HMF) have been considered as important parameters in assessing the authenticity of honey [16].

Iran's geographical structure and climatic conditions is one of the best regions of the world for the production of honey. In recent years, the presence of unnatural honey in the global markets has become a major problem. Due to the increasing awareness of consumers about hygienic and safety foods, the importance of quality control of honey is necessary. Unfortunately, any particular research has been conducted in Qazvin province, Iran to determine the microbial quality and physicochemical parameters (P-CHPs). Thus, in the current study, was conducted to assess the physicochemical and microbiological parameters of different types of honey samples (commercial and noncommercial) to detect fraud and evaluate quality for the first time in Qazvin province.

MATERIALS AND METHODS

In the present study HMF, pH, total acidity (TA), reduction sugar (RS), moisture, sucrose, diastase activity (DA), F.G r, electrical conductivity (EC), and ash of honey samples were analyzed for fraud detection [17, 18].

Honey samples

All of the honey samples collected from Qazvin province, Iran and then transferred to the food quality control laboratory of Food and Drug Administration Qazvin, Iran for analysis.

Sampling

Commercial honey samples were collected from packaged and labeled honeys (packaged by industrial companies). Non-commercial honey samples were collected from bulk, unpackaged and unlabeled honeys (packaged by beekeepers). All of the honey samples from 2017 to 2018 have been collected from Qazvin province, Iran.

Analytical methods to determine P-CHPs in honey samples

P-CHPs of honey samples were evaluated according to the International Honey Commission [19]. The parameters of P-CHPs examined included HMF (mg kg⁻¹), moisture (g 100g⁻¹), ash (g 100g⁻¹), EC (ms cm⁻¹), pH, TA (meq kg⁻¹), DA (Gothe scale) and RS (g 100g⁻¹) [20].

HMF

HMF content in honey samples were determined by High-Performance Liquid Chromatography (HPLC) coupled with UV spectrometry. A 5% (w/v) solution of the honey sample was prepared in distilled water and the solution was filtered through 0.45 mum filter paper and then injected into the HPLC system (WATERS _1515, USA). The injection volume was 20 mul, the column temperature 25°C and detection at 280 nm [20].

Moisture

Honey moisture content was determined by the refractometry method. All measurements were performed at 20°C [21].

Ash

Ash content of honey samples were measured by placing a crucible containing honey at 100°C oven for 1 h. After cooling the ash was weighed. Aliquots of 5 g of honey were placed into a crucible and then incinerated at 500°C Muffle furnace for 2 h and then reweighed [19]. Finally, ash percentage was calculated.

Electrical conductivity

Honey EC was examined in a 20% (w/v) honey dilution in distilled water using a cyber-scan waterproof (Inolab, Germany) series digital conductometer [19].

pH and TA

TA values were measured by the titrimetric method. The amount of 10 g of honey sample was dissolved in 75 ml distilled water, and this dilution was titrated with 0.05 M NaOH until the pH reached to 8.50. Then 10 ml of 0.05 M NaOH was added immediately and back-titrated with 0.05 M HCl solution until the pH reached to 8.30 (based on lactone acidity) and finally TA was determined. A cyberscan waterproof digital pH meter (Mettler, Swiss) model MP220 series was used to measure the pH of honey samples [22].

Diastase activity

Honey sample to amount of 5 g was placed into a 20 ml beaker and diluted in 10 ml distilled water and 2.50 ml of acetate buffer (1.59 M, pH 5.30). This solution was transferred to a 20 ml volumetric flask containing 1.50 ml of 0.50 M NaCl. Then 10 ml of honey dilution was incubated in a thermostatic bath at 40°C along with a second flask containing 100 ml of 1% (w/v) starch dilution. After 5 min, 5 ml of starch dilution was added to the honey dilution. After 5 min, 1 ml of this combination was mixed with 10 ml of 0.0007 M iodine dilution, and DA was measured at 660 nm in a spectrophotometer (Shimadzu model UV-1601, Japan) [23].

Sugar contents

Sugar contents (include fructose, glucose, maltose, and sucrose) of honey samples were analyzed by HPLC coupled with refractive index detector. Honey dilution to amount of 5% (w/v) was prepared in distilled water and dilution was filtered by a paper filter 0.45 mum and injected to HPLC system (WATERS _1515, USA) [20].

Microbiological parameters

All analyses of microbiological parameters take place in a duplicate method. An average number of colonies, multiplied by the dilution factor, was considered for the counting of microbe colonies (include *Clostridia*, yeast, and fungi). Results were expressed as colony forming units (CFU) of *Clostridia*, yeast, and fungi per gram of honey samples.

Yeast and fungi counting

Honey sample to amount of 10 g taken from the surface of the container were diluted in 90 ml of phosphate buffer, pH 5.30, containing 0.10 g of agar. A series of dilutions include 10^{-2} and 10^{-3} were obtained from these solutions. One ml of each dilution include 10^{-1} , 10^{-2} , and 10^{-3} was

mixed with 12 ml of culture medium (pH 3.50) that containing yeast extract, glucose, minerals, and chloramphenicol (10 mg ml⁻¹) and then was placed in petri dishes. Finally, these petri dishes incubated at 25°C for 5 days [24].

Clostridia counting

Vegetative cells isolation of *Clostridia* (anaerobic bacteria) take place in Sulfite Polymyxin Sulfadiazine Agar (SPSA) culture media based on sulfite-reducing (Liofilchem, Italy). Therefore 20 g of a honey sample was suspended in 150 ml of peptone water and then homogenized and centrifuged at 8500 rpm at 4°C for 60 min. The sediment was re-suspended in 7 ml of 1% peptone in water. Finally, a series of dilutions were cultured in Miller Pricket tubes (MPts) containing SPS culture medium, these tubes sealed by Vas Par and then incubated at 37°C for 48 h. Black colonies in SPSA culture media indicated the presence of *Clostridia* [24].

Isolation of spores based on sulfite-reducing Clostridia

The above dilutions were heated at 80°C for 20 min and then rapidly cooled in water to acquire *Clostridia* spores based on sulfite-reducing, and cultured in MPts containing SPS medium. These tubes were sealed by Vas Par and then incubated at 45°C for 48 h [24].

Statistical analysis

The mean value obtained from duplicate replications of each experiment were reported as mean±SD. The collected data were analyzed using SPSS statistical program version 19.

RESULTS AND DISCUSSION

Accepted limits of physical, chemical, and microbial properties of honey presented in Tables 1 and 2.

P-CHPs of honey samples (N=43) were compared with the Iranian national standardization organization showed in Figure 1.

The values of the P-CHPs for commercial (N=10) and non-commercial (N=33) honey samples examined in the current study presented in Tables 3 and 4.

M. Kazeminia et al / Journal of Chemical Health Risks 13(1) (2023) 73-83

Parameters	HMF	рН	ТА	\mathbf{RS}^*	Moisture	Sucrose	DA ^{**}	F.G r	EC****	Ash
(Unit)	(mg kg ⁻¹)	No unit	meq kg ⁻¹	(%)	(%)	(%)	(D N ^{***})	No unit	(mS cm ⁻¹)	(%)
Accepted limit	≤40	3.5>	<40	65>	<20	<5	3>	0.9>	<0.8	<0.6

ISIRI: Iranian national standardization organization [25] *Reducing Sugars **Diastase Activity ***Diastase Number ****Electrical Conductivity

Table 2. Accepted limits of clostridium perfringens, molds and osmotolerant yeasts of honey based on the ISIRI

Parameters	clostridium perfringens	molds	osmotolerant yeasts		
Accepted limit	Negative in per 1 to 2 g	100< in per g	10< in per g		

ISIRI: Iranian national standardization organization [25]



Figure 1. physicochemical parameters of honey samples (N=43) in comparison with the Iranian national standard [25].

Parameters	Min	Max	$\mathbf{Mean} \pm \mathbf{SD}^*$	A L**	N-A L***
HMF	0.30	334.30	127.55±109.32	3	7
				(30%)	(70%)
рН	3.93	4.70	4.38±0.27	10	0
I				(100%)	(0%)
ТА	10.50	14.89	13.59±1.52	10	0
171	10.50	14.09	15.57±1.52	(100%)	(0%)
RS	26.80	82.00	64.10±17.41	8	2
Ro	20.80	82.00	04.10±17.41	(80%)	(20%)
Moisture	13.80	61.65	20.98±14.34	9	1
Moisture	15.80	01.05	20.98±14.54	(90%)	(10%)
Sucrose	0.50	8.76	5 26 2 28	5	5
Sucrose	0.50	8.70	5.26±2.38	(50%)	(50%)
DA	5.00	8.00	6.25 . 0.02	0	10
DA	5.00	8.00	6.25±0.92	(0%)	(100%)
E.C.	0.65	1.70	1 22 . 0 22	9	1
F.G r	0.65	1.70	1.23±0.33	(90%)	(10%)
D C	0.10	10		10	0
EC	0.19	0.61	0.36±0.12	(100%)	(0%)
	0.00	0.51	0.20 . 0.10	10	0
Ash	0.08	0.51	0.30±0.18	(100%)	(0%)

Table 3. Physicochemical properties of commercial honey samples (packed by industrial companies) (N=10)

*SD (standard deviation) **acceptable limit, ***no acceptable limit

Parameters	Min	Max	Mean±SD	A L	N-A L
HMF	0.00	230.00	52 52 60 65	21	12
пмг	0.00	250.00	53.52±60.65	(63.36%)	(36.36%)
	3.56	4.60	4.03±0.26	33	0
рН	5.50	4.00	4.05±0.20	(100%)	(0%)
ТА	9.70	15.83	12 21 1 64	33	0
IA	9.70	15.85	12.21±1.64	(100%)	(0%)
RS	60.26	81.40	71.63±4.76	31	2
Kð	00.20	81.40	/1.03±4./0	(93.93%)	(6.06%)
Moisture	13.60	18.45	15.85±1.17	33	0
Woisture	15.00	16.45	15.65±1.17	(100%)	(0%)
Sucrose	0.09	12.90	5.89±3.38	15	18
Sucrose	0.09	12.90	J.09±3.30	(45.45%)	(54.54%)
Diastase activity	1.90	4.69	3.28±0.80	18	15
Diastase activity	1.90	4.09	5.28±0.80	(54.54%)	(45.45%)
F.G r	0.32	3.45	1.52±0.84	23	10
F.G I	0.32	5.45	1.52±0.64	(69.69%)	(30.30%)
EC	0.10	0.98	0.46±0.24	29	4
EC	0.10	0.98	0.40±0.24	(87.87%)	(12.12%)
Ash	0.00	0.84	0.21±0.22	31	2
ASII	0.00	0.04	0.21±0.22	(93.93%)	(6.06%)

Table 4. Physicochemical properties of non-commercial honey samples (packed by beekeepers) (N=33)

In the present study, pH and TA in all the honey samples were at the acceptable limit and other parameters include HMF, RS, moisture, sucrose, DA, F.G r, EC, and ash were below the acceptable quality level. The highest nonstandard values were observed for parameters include DA, sucrose, HMF, and F.G r.

HMF

HMF value is a quality indicator for checking the freshness and high temperature processing of honey [26]. HMF is a by-product of fructose decomposition and it is generated during storage or overheating. Thus, its presence is considered as the spoilage of honey. The excessive HMF value indicates long-term storage, overheating or invert sugar adulteration [27]. In the current study, almost half of the honey samples (44.18%) were below the acceptable quality level. The average amount of HMF in commercial honey samples was measured 127.55±109.32 mg kg⁻¹ while in non-commercial samples it was 53.52±60.65 mg kg⁻¹. 24 of 43 honey samples considered at the acceptable quality level ($\leq 40 \text{ mg kg}^{-1}$), although in a study which was undertaken in Azerbaijan [28], 4 of 53 samples exceeded the acceptable limit and the average amount of HMF in 34 samples was lower than 10 mg kg⁻¹ indicating that they were fresh and unheated. According to the other studies conducted in Argentina [29] and Uttarakhand (north

Indian) [30], HMF value in the examined honeys were at the acceptable limit. Another experiment in Iran [31] reported HMF value of honey samples in the range of 2.20 to 39.40 mg kg⁻¹ and the other study in Northwestern Argentina [11] reported the HMF value of 13 honey samples in the range of 4 to 28.20 mg kg⁻¹. The HMF value in all samples were below the limits established by the Codex Alimentarius (lower than 40 mg kg⁻¹). In a study conducted on honey samples from Shariatpur, Bangladesh [32] the HMF content was 32.48 mg kg⁻¹ which was much lower than our study samples. Low HMF value (0 to 0.20 mg kg⁻¹) is the indicator of freshly harvested honey, while high HMF value indicates the prolonged exposure of honey to heat or poor storage condition.

In general, HMF content is known as an indicator of the freshness of honey with low HMF value. HMF content increases during food processing and aging. High value of HMF due to caramelization of carbohydrates, Maillard reaction, and the decomposition of fructose in the acidic environment can lead to changes in the composition honey [31].

pH and TA

Honey is naturally acidic thus its pH is extremely low, between 3 to 4.50, which inhibits the growth of bacteria and other spoil-ready microorganisms [39]. The pH of examined honey samples in this study were in range of 3.56 to 4.70 that demonstrate all samples were in the acceptable pH range recommended by Codex Alimentarius (pH: 3.40 to 6.10) [39]. In Telangana, India reported pH values of all samples in the range of 3.70 and 3.90 [33] which is consistent with our study. The maximum TA was observed in commercial honey samples. A high strong negative correlation was found between pH and TA. In Shariatpur, Bangladesh reported pH and acidity values 4 and 42.50 meq kg⁻¹, respectively [32] that pH values were consistent with our study, while acidity values were much higher than our study. The values of pH examined in the current study were agreement with the other results reported in the continent of America include Brazil [34], Mexico [35] and Argentina and also the studies that conducted in other areas such as Algerian [37], Indian [38] and Azerbaijan [28]. It can be concluded that geographical conditions cannot have a significant effect on pH values. Maximum TA was observed in the commercial honey samples. TA values in all the honey samples were fall under the described limit of 40 meq kg-1. These results were in agreement with the study conducted in Azerbaijan [28]. The high acidity values of honey samples in the present study was related to the fermentation of honey sugars to organic acid, thus it can provide resistance against spoilage microorganisms.

Total RS

Total RS value in examined commercial honey samples ranged from 26.80 to 82.00%, and in non-commercial honey samples ranged from 60.26 to 81.40% which is in agreement with the standards proposed by the Iranian Standard Organization [25]. A study conducted in India reported total RS between 71 to 80 that consistent with our study [33]. Total RS value of commercial honey samples more than 80% and of non-commercial more than 93% were at the acceptable limit. Similar results were obtained in the studies conducted in Kashmir valley (north India) [39] and Algerian [40].

Moisture percentage

The moisture percentage or water content of honey reported as an important quality parameter for honey since lower moisture content of honey demonstrate longer shelf life [41]. In the current study the most of honey samples had the level of moisture of 18.20 to 19.11 which was lower than the limit described by Iranian Standard Organization [25]. Similar results were observed in India [33] and Nigerian [42]. The results of our study indicate that honey in Qazvin province has long-term storage conditions. Because during storage, high humidity can lead to fermentation function by osmotolerant yeast. As a result, the pH decreases [43]. Our results were in agreement with the previous studies conducted in eastern Anatolia (Turkey) [44], China [45], Kars city (Turkey) [46], and Kashmir valley (north India) [39]. In a study, moisture content was obtained 18.35%, which corresponded to the international limit set by Codex Alimentarius, i.e. 20% [32] and in another study conducted in Shahr kourd, Iran, moisture content was reported ranged from 14.30 to 16% [31]. These results were in agreement with our current study. Honey moisture content was affected by various factors such as climatic conditions, nectar features, and the treatment of honey during extraction, storage, maturity period and harvesting time [8, 38] thus honey quality and water content can vary widely from hive to hive and even from cell to cell [47]. If the moisture content of honey is higher, honey can be fermented and granulated during storage. Thus, a low moisture content (<20%) is necessary to increase the shelf life of honey during storage [48].

Sucrose content and F.G r

Honey contains a concentrated water solution of two main sugars, include fructose and glucose, with small amounts of different complex sugars [11]. In the current study, the sucrose content and F.G r in commercial honey samples ranged from 0.05 to 8.76 and 0.65 to 1.70, respectively, and the sucrose content and F.G r in non-commercial honey samples ranged from 0.09 to 12.90 and 0.32 to 3.45, respectively. In a study, glucose, fructose, and F.G r value in honey samples were reported ranged from 19.20 to 31.80%, 25.40 to 39.20%, and 1.10 to 1.50, respectively [31]. In a study conducted in the north-west of Spain reported glucose, fructose, and F.G r values of 34 honey samples ranged from 24.40 to 35.20, 33.10 to 42.10, and 0.90 to 1.70%, respectively [49]. The results of the previous two studies were inconsistent with our study. In the current study, honey samples with F.G r outside the

range was observed in the non-commercial honey samples more than commercial honey samples, revealing that fraud in non-commercial honey is more than commercial honey. High sucrose content in honey indicates the fraud above 50%. Thus, it can be concluded that the measuring sucrose in honey will help to confirm authenticity of natural honey. The F.G r parameter gives information about the crystallization state of honey. Crystallization occurs when F.G r is 1.10 or less but does not occur at values greater than 1.50 due to the presence of higher fructose value, there is no tendency to crystallize. As well as, F.G r affects the honey taste due to fructose is much sweeter than glucose. This ratio can be mainly dependent on the nectar source [31].

Diastase activity

Diastase is one of the prime enzymes found in honey. Diastase (a mixture of α -amylase and β -amylase) is a natural enzyme catalyzing the degradation of starch and viscosity loss in honey. DA in honey depends on the amount of nectar the bee processes in each period, geographic and floral origins of the product. DA and HMF content can be used as criteria to recognize the honey quality [50]. Diastase activity value, shows honey exposure to high temperature. Our study demonstrate that the DA value in the commercial and non-commercial honey samples ranged from 5 to 8 DN (Diastase Number) and 1.90 to 4.69 DN, respectively. DA values in the commercial honey samples were almost twice more than the non-commercial honey samples. The most of the honey samples demonstrate DA values within the Iranian Standard for DA i.e. lower than 8 DN [25]. High DA values in the examined honey samples may be due to mountain climatic conditions of Qazvin province. DA in honey depends on the intensity of the nectar flow and nectar amount processing by the honey bees. Thus, honey from very rich nectar sources often shows low natural enzyme activities. When honey is adulterated by the addition of inverted sucrose (also called invert syrup and invert sugar, is an edible mixture of two simple sugars, glucose and fructose, that is made by heating sucrose with water) or hydrolyzed starch namely HFCS, then such dilution of honey leads to the reduction of DN [51].

Generally, very low and/or very high DA, both are undesirable in honey. Large amounts of DA may be due to the formation of fermented acid, and then the acid helps break down the enzyme starch [50].

Electrical conductivity

EC is proportional to dissolved solids values in the mixture. According to the Codex Alimentarius, EC is an advantageous parameter for distinguish the quality of honey sample, specifically in ensuring its floral origin for the correct labeling purposes [52]. In previous studies, the EC parameter has been used as an evaluation of eligible honey [53]. All of the examined commercial honey samples were at the acceptable limit for EC whereas, 12.12% (4 to 29) of non-commercial honey samples were in unacceptable limit. EC value for the nectar honey must be less than 0.80 mS cm⁻¹ according to the EU standards for honey [47]. In a study conducted in the Kashmir valley (north India) [39] was in agreement with our result, while another study was conducted in the Shariatpur, Bangladesh [32] EC values higher than our results. In a study, EC was reported 1.20 mS cm⁻¹ which was higher than our result [52]. However, mismatch of EC values among different types of honey is possible due to different ecological and botanical conditions such as honey source (floral or honeydew), season, acidity, moisture, viscosity and salt content [54].

Ash

The percentage of ash is an indicator of mineral content, plant origin, and quality index in the honey samples [31]. The average ash value in the commercial and noncommercial honey samples were 0.30 ± 0.18 and 0.21 ± 0.22 , respectively. The ash content of the most honey samples were at the acceptable range (95.34%). Similar values for honey ash content were observed in Argentina honey (0.11%) [55], Algeria honey (0.14%) [56], Ankara city (Turkey) honey (0.15%) [57], and Garmsar city (Iran) honey (0.28%) [58]. Differences in ash content are probably related to several factors, include differences in soil and climate conditions, the type and physiology of each plant, and the botanical and geographic origins of honey samples [31].

Microbial

All of the honey samples (commercial and noncommercial) were in the acceptable range for microbial quality, include *clostridium perfringens*, molds, and osmotolerant yeasts. Similar results for honey microbial quality were observed in Nigerian [42] and in southwest of Antioquia, Colombia [59], but in a study conducted in Argentina, it did not match our study [29]. In general, honey is not a suitable medium for microbial growth due to its high sugar content and low water activity.

Pearson correlation coefficients between P-CHPs

It was interesting that a positive significant correlation found between pH and HMF, and between DA and TA, and between EC and DA measurements of commercial honey samples (p<0.05) (Table 5). A positive significant correlation was found between TA and pH, and among RS with sucrose and HMF, and between F.G r and DA measurements of non-commercial honey samples (p<0.05) (Table 6).

Table 5. Correlation between physicochemical properties of co	ommercial honey samples (N=10)
---	--------------------------------

parameters		HMF	pН	TA	RS	Moisture	Sucrose	DA	F.G r	EC
	PC^*	.711**								
pН	P-value	.021***								
ТА	PC	.599	.597							
IA	P-value	.067***	.069***							
RS	PC	.047	.223	073						
K3	P-value	.897***	.535***	.841						
Maiatana	PC	.502	.297	.271	.096					
Moisture	P-value	.139***	.405***	.450***	.792***					
Sucrose	PC	.353	.385	.135	439	062				
Sucrose	P-value	.317***	.272***	.709***	.204***	.865***				
DA	PC	.457	.595	.700**	171	.307	.334			
DA	P-value	.184***	.070***	.024***	.637***	.389***	.345***			
F.C	PC	157	112	.146	448	288	093	.472		
F.G r	P-value	.665***	.759***	.688***	.195***	.419***	.798 ***	.168 ***		
EC	PC	.190	.435	.240	189	.430	013	.679**	.442	
EU	P-value	.599***	.209***	.504***	.602***	.215***	.972***	.031***	.201***	
A alı	PC	.506	.523	.604	382	154	.261	.323	.220	.20
Ash	P-value	.136***	.121***	.064***	.275***	.670***	.466***	.363***	.541***	.579

*Pearson Correlation **Correlation is significant at the 0.05 level ***No significant difference (p>0.05)

Table 6. Correlation between physicochemical properties of non-commercial honey samples (N=33)

param	eters	HMF	pH	TA	RS	Moisture	Sucrose	DA	F.G r	EC
pН	PC	.458								
рп	P-value	.007								
ТА	PC	.503	.924*							
IA	P-value	.003**	$.000^{**}$							
RS	PC	349*	371	389						
	P-value	.046**	.034**	.025**						
Moisture	PC	.162	.114	.051	075					
Moisture	P-value	.369**	.526**	.779**	.677**					
Sucrose	PC	.429*	.298	.344	740*	.182				
Sucrose	P-value	.013**	.092**	$.050^{**}$	$.000^{**}$.311**				
DA	PC	255	087	124	.117	.198	174			
DA	P-value	.152**	.631**	.491**	.515**	.270**	.333**			
F.G r	PC	141	.126	.074	.235	181	358	$.488^{*}$		
r.G ľ	P-value	.435**	.484**	.681**	.189**	.314**	.041**	.004**		
EC	PC	008	.260	.326	308	118	.431	191	103	
EC	P-value	.966**	.145**	.064**	.082**	.514**	.012**	.288**	.568**	
Ash	PC	263	.189	.081	.107	085	024	.044	.105	.152
ASN	P-value	.140**	.292**	.656**	.555**	.637**	.896**	.806**	.562**	.399

*Correlation is significant at the 0.05 level **No significant difference (p>0.05)

CONCLUSIONS

The present study attempted to evaluate and compare the physicochemical and microbial properties of commercial and non-commercial honey samples from Qazvin province of Iran. The results demonstrate that most of the honey samples failed to meet the acceptable quality level. Detection of honey adulteration by means of fast, inexpensive, and safe tests should be widely practiced by responsible organizations. The parameters used to verify natural honey include: hydroxymethylfurfural, moisture, ash, electrical conductivity, pH, total acidity, diastase activity, and reduction sugar. At the end of the discussion, the government subsidies for beekeeping and honey supply chain can be the main incentive against honey adulteration.

ACKNOWLEDGMENTS

The authors thank Qazvin Food and Drug Administration and Department of Food Safety of Health Faculty, Qazvin University of Medical Sciences for giving technical and financial support.

Conflict of interests

The authors declare that there is no conflict of interests.

REFERENCES

1. Rodríguez-Flores M.S., Escuredo O., Míguez M., Seijo M.C., 2019. Differentiation of oak honeydew and chestnut honeys from the same geographical origin using chemometric methods. Food chemistry. 297124979.

2. Bryant Jr V.M., Jones G.D., 2001. The r-values of honey: Pollen coefficients. Palynology. 25(1), 11-28.

3. Akbulut M., Özcan M.M., Çoklar H., 2009. Evaluation of antioxidant activity, phenolic, mineral contents and some physicochemical properties of several pine honeys collected from Western Anatolia. International Journal of Food Sciences and Nutrition. 60(7), 577-589.

4. Escuredo O., Seijo M.C., in, Multidisciplinary Digital Publishing Institute, 2019.

5. Smanalieva J., Senge B., 2009. Analytical and rheological investigations into selected unifloral German honey. European Food Research and Technology. 229(1), 107-113.

Bogdanov S., Haldimann M., Luginbühl W., Gallmann P., 2007. Minerals in honey: environmental, geographical and botanical aspects. Journal of Apicultural Research. 46(4), 269-275.

7. Alvarez-Suarez J.M., González-Paramás A.M., Santos-Buelga C., Battino M., 2010. Antioxidant characterization of native monofloral Cuban honeys. Journal of Agricultural and Food Chemistry. 58(17), 9817-9824.

 Al M.L., Daniel D., Moise A., Bobis O., Laslo L., Bogdanov S., 2009. Physico-chemical and bioactive properties of different floral origin honeys from Romania. Food Chemistry. 112(4), 863-867.

 Lachman J., Orsák M. Hejtmánková A., Kovářová E., 2010. Evaluation of antioxidant activity and total phenolics of selected Czech honeys. LWT-Food Science and Technology. 43(1), 52-58.

10. Liu J.R., Ye Y.L., Lin T.Y., Wang Y.W., Peng C.C., 2013. Effect of floral sources on the antioxidant, antimicrobial, and anti-inflammatory activities of honeys in Taiwan. Food chemistry. 139(1), 938-943.

11. Isla M.I., Craig A., Ordoñez R., Zampini C., Sayago J., Bedascarrasbure E., Alvarez A., Salomón V., Maldonado L., 2011. Physico chemical and bioactive properties of honeys from Northwestern Argentina. LWT-Food Science and Technology. 44(9), 1922-1930.

12. Cushnie T.T., Lamb A.J., 2005. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents. 26(5), 343-356.

13. Vorlova L., Karpiskova R., Chabiniokova I., Kalabova K., Brazdova Z., 2005. The antimicrobial activity of honeys produced in the Czech Republic. Czech Journal of Animal Science. 50(8), 369.

14. Sivakesava S., Irudayaraj J., 2002. Classification of simple and complex sugar adulterants in honey by mid-infrared spectroscopy. International Journal of Food Science & Technology. 37(4), 351-360.

15. Puscas A., Hosu A., Cimpoiu C., 2013. Application of a newly developed and validated high-performance thinlayer chromatographic method to control honey adulteration. Journal of Chromatography A. 1272132-135.

16. Spiteri M., Jamin E., Thomas F., Rebours A., Lees M., Rogers K.M., Rutledge D.N., 2015. Fast and global authenticity screening of honey using 1H-NMR profiling. Food Chemistry. 18960-66.

17. Wang J., Li Q.X., 2011. Chemical composition, characterization, and differentiation of honey botanical and geographical origins. Advances in food and nutrition research. 6289-137.

18. Lazarević K.B., Andrić F., Trifković J., Tešić Ž., Milojković-Opsenica D., 2012. Characterisation of Serbian unifloral honeys according to their physicochemical parameters. Food Chemistry. 132(4), 2060-2064.

Bogdanov S., Martin P., Lullmann C., 2002.
Harmonised methods of the international honey commission. Swiss Bee Research Centre, FAM, Liebefeld.
Mendes E., Proença E.B., Ferreira I., Ferreira M., 1998. Quality evaluation of Portuguese honey. Carbohydrate polymers. 37(3), 219-223.

21. Commission C.A., 2001. Revised Codex Standard for Honey, Codex Standard 12-1981, Rev. 1 (1987), Rev. 2 (2001), Joint FAO/WHO Food Standards Programme. 24th Session, FAO Headquarters, Rome, Italy.

22. Horwitz W., Albert R., 2006. The Horwitz ratio (HorRat): a useful index of method performance with respect to precision. Journal of AOAC International. 89(4), 1095-1109.

23. Vorlova L., Pridal A., 2002. Invertase and diastase activity in honeys of Czech provenience. Acta Universitatis Agriculturae et Silviculturae Sbornik Mendelovy Zemedelske a Lesnicke Mendelianae Brunensis. 557-66.

24. Fernández R., Ciccarelli A., de Centorbi O., Centorbi H., Rosetti F., de Jong L., Demo N., 1999. Infant Botulism in Argentina, 1982—1997. Anaerobe. 5(3-4), 177-179.

25. ISIRI, 2013. Honey- Specification and test methods. Institute of Standards and Industrial Research of Iran, 92, 7th revision.

26. Tosi E., Ciappini M., Re E., Lucero H., 2002. Honey thermal treatment effects on hydroxymethylfurfural content. Food Chemistry. 77(1), 71-74.

27. Olawode E.O., Tandlich R., Cambray G., 2018. 1H-NMR profiling and chemometric analysis of selected honeys from South Africa, Zambia, and Slovakia. Molecules. 23(3), 578.

28. Karimov E., Xalilzad Z., Hobbi P., Alekperov J., 2014. Quality evaluation of honey from the different region of Azerbaijan. Journal of Food Chemistry and Nutrition. 2(2), 71-79.

29. Finola M.S., Lasagno M.C., Marioli J.M., 2007. Microbiological and chemical characterization of honeys from central Argentina. Food Chemistry. 100(4), 1649-1653.

30. Gairola A., Tiwari P., Tiwari J., 2013. Physicochemical properties of Apis cerana-indica F. honey from Uttarkashi district of Uttarakhand, India. Journal of Global Biosciences ISSN. 2(1), 20-25.

31. Khalafi R., Goli S.A.H., Behjatian M., 2016. Characterization and classification of several monofloral Iranian honeys based on physicochemical properties and antioxidant activity. International Journal of Food Properties. 19(5), 1065-1079.

32. Tasnim T., Inam A., Hossain M., Akhter P., 2018. Evaluation of Physiochemical Properties of Nigella Honey from Shariatpur, Bangladesh. 5(1&2), 1-7.

33. Shobham K.K.C., Nayar J., 2017. Physicochemical analysis of some commercial honey samples from Telangana. Indian Journal of Nutrition. 4(1), 1-4.

 34. da C Azeredo L., Azeredo M., De Souza S., Dutra V.,
2003. Protein contents and physicochemical properties in honey samples of Apis mellifera of different floral origins.
Food Chemistry. 80(2), 249-254.

35. Cervantes M.R., Novelo S.G., Duch E.S., 2000. Effect of the temporary thermic treatment of honey on variation of the quality of the same during storage. Apiacta. 35(4), 162-70.

36. Mouteira M., Malacalza N., Lupano C., Baldi B., 2003. Analysis of honey produced in the province of Buenos Aires, Argentine, from 1997 to 2000. Proc. 37th Int. Apic. Congr., 28 Oct – 1 Nov 2001, Durban, South Africa.

37. Ouchemoukh S., Louaileche H., Schweitzer P., 2007. Physicochemical characteristics and pollen spectrum of some Algerian honeys. Food Control. 18(1), 52-58.

38. Saxena S., Gautam S., Sharma A., 2010. Physical, biochemical and antioxidant properties of some Indian honeys. Food Chemistry. 118(2), 391-397.

39. Nayik G.A., Nanda V., 2015. Physico-chemical, enzymatic, mineral and colour characterization of three different varieties of honeys from Kashmir valley of India with a multivariate approach. Polish Journal of Food and Nutrition Sciences. 65(2), 101-108.

40. Khalil M.I., Moniruzzaman M., Boukraâ L., Benhanifia M., Islam M.A., Islam M.N., Sulaiman S.A., Gan S.H., 2012. Physicochemical and antioxidant properties of Algerian honey. Molecules. 17(9), 11199-11215.

41. Fredes C., Montenegro G., 2006. Heavy metal and other trace elements contents in honey bee in Chile. Cien Inv Agr.(in English) 33(1), 50-58. Ciencia e Investigación Agraria. 33(1), 50-58.

42. Omafuvbe B., Akanbi O., 2009. Microbiological and physico-chemical properties of some commercial Nigerian honey. African Journal of Microbiology Research. 3(12), 891-896.

43. Gomes S., Dias L.G., Moreira L.L., Rodrigues P., Estevinho L., 2010. Physicochemical, microbiological and antimicrobial properties of commercial honeys from Portugal. Food and Chemical Toxicology. 48(2), 544-548.

44 Yilmaz H., Kufrevioglu I., 2000. Composition of honeys collected from eastern Anatolia and effect of storage on hydroxymethylfurfural content and diastase activity. Türk J Agric Forst. 33347-349.

45 Junzheng P., Changying J., 1998. General rheological model for natural honeys in China. Journal of Food engineering. 36(2), 165-168.

46. Duman A., Sezer C., Oral N., 2008. Kars' ta Satis a Sunulan Suzme Ballarin Kalite Niteliklerinin Aras tirilmasi. Kafkas Universitesi Veteriner Fakultesi Dergisi. 1489-94.

47. Alimentarius C., 2001. Revised codex standard for honey. Codex Stan. 121982.

48. Baroni M.V., Arrua C., Nores M.L., Fayé P., del Pilar Díaz M., Chiabrando G.A., Wunderlin D.A., 2009. Composition of honey from Córdoba (Argentina): Assessment of North/South provenance by chemometrics. Food Chemistry. 114(2), 727-733.

49. Escuredo O., Seijo M.C., Fernández-González M., 2011. Descriptive analysis of Rubus honey from the north-west of Spain. International Journal of Food Science & Technology. 46(11), 2329-2336.

50. Pasias I.N., Kiriakou I.K., Proestos C., 2017. HMF and diastase activity in honeys: A fully validated approach and a chemometric analysis for identification of honey freshness and adulteration. Food Chemistry. 229425-431.

51. Voldřich M., Rajchl A., Čížková H., Cuhra P., 2009. Detection of Foreign enzyme addition into the adulterated honey. Czech J Food Sci. 27280-282.

52. Machado A.M., Miguel M.G., Vilas-Boas M., Figueiredo A.C., 2020. Honey Volatiles as a Fingerprint for Botanical Origin—A Review on their Occurrence on Monofloral Honeys. Molecules. 25(2), 374.

53 Mateo R., Bosch-Reig F., 1998. Classification of Spanish unifloral honeys by discriminant analysis of electrical conductivity, color, water content, sugars, and pH. Journal of Agricultural and Food Chemistry. 46(2), 393-400.

54. Španik N.J.O.V.I., 2014. Identification of volatile organic compounds in honeydew honeys using comprehensive gas chromatography. Journal of Food and Nutrition Research. 53(4), 353-362.

55. Cantarelli M., Pellerano R., Marchevsky E., Camiña J., 2008. Quality of honey from Argentina: Study of chemical composittion and trace elements. The Journal of Argentine Chemical Society. 96(1-2), 33-41.

56. Zerrouk S.H., Fallico B.G., Arena E.N., Ballistreri G.F., Boughediri L.A., 2011. Quality evaluation of some honey from the central region of Algeria. Jordan Journal of Biological Sciences. 4(4), 243-248.

57. Ünal C., Küplülü Ö., 2006. Chemical quality of strained honey consumed in Ankara. Ankara Universitesi Veteriner Fakultesi Dergisi. 531-4.

58. Jahed K.G.R., Kamkar A., 2005. A survey of physicochemical properties of produced honey in garmsar city in 2003. Iranian Journal of Food Science and Technology. 2(1), 35-41.

59. Velásquez Giraldo A., Vélez Acosta L., Zuluaga Gallego R., 2013. Physicochemical and microbiological characterization of Apis mellifera sp. honey from southwest of Antioquia in Colombia. Ingeniería y Ciencia. 9(18), 61-74.