



Evaluation of Some Chemical Quality Characteristics of Honey Produced in Iran

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HIGHLIGHTS

- Unacceptable hydroxymethylfurfural levels were found in 5% natural and 70% commercial honey samples.
- The average phenolic content was lower for commercial honeys than for natural honeys.
- There was a significant difference between chemical quality characteristics of honeys between two geographical regions.
- Chemical quality characteristics of Iranian natural honeys were more acceptable than commercial ones.

Article type

Original article

Keywords

Antioxidants
5-hydroxymethylfurfural
Honey

Article history

Received: 2 Sep 2017
Revised: 6 Nov 2017
Accepted: 2 Dec 2017

Acronyms and abbreviations

HMF=Hydroxymethylfurfural
DPPH=2,2-diphenyl-1-picrylhydrazyl
GAE=Gallic Acid Equivalents

ABSTRACT

Background: Iran could be considered as one of the most important producers of honey all over the world. This investigation was conducted to evaluate some chemical quality characteristics of honey produced in Iran.

Methods: Totally, natural (n=80) and commercial (n=20) honey samples were randomly collected from North-West and South-West regions of Iran. Hydroxymethylfurfural (HMF) levels, phenolic contents, antiradical activity, and antioxidative potency of the samples were analyzed. Data were statistically analyzed using SPSS version 16.

Results: The HMF level in 4 out of 80 (5%) natural honey samples and 14 out of 20 (70%) of commercial honey samples was higher than the recommended safety limit set by the Iran national standard. The average phenolic content was significantly ($p<0.05$) lower for commercial honeys (20.51 mg of Gallic Acid Equivalents (GAE)/100 g of honey) than for natural honeys (55.37 mg of GAE/100 g of honey). The mean IC_{50} of natural honey samples was 40.63 mg/ml with a range of 14.2-84.1 mg/ml; however, the mean IC_{50} of commercial honey samples was 341.96 mg/ml, with a range of 115-997.4 mg/ml. The difference between mean values of β -carotene bleaching inhibition of natural honey (54.54 mg/ml) and commercial ones (25.37 mg/ml) was found to be statistically significant ($p<0.05$). Also, there was a significant ($p<0.05$) difference between chemical quality characteristics of honeys between two geographical regions.

Conclusion: In conclusion, HMF levels, phenolic contents, antiradical activity, and antioxidative potency of Iranian natural honeys were remarkably more acceptable and suitable than commercial honeys produced in the country.

Introduction

Honey is composed of around 200 different compounds. Such compounds are usually a complex mixture of sugars and a little amount of other compounds like

minerals, proteins, vitamins, organic acids, flavonoids, phenolic acids, enzymes, and other chemical and herbal substances (Ferreira et al., 2009). Physical and chemical

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To cite: Ghorbani M., Saei-Dehkordi S.S., Mohebbi A., Pak A. (2017). Evaluation of some chemical quality characteristics of honey produced in Iran. *Journal of Food Quality and Hazards Control*. 4: 113-118.

compounds in honey depend first of all on its herbal origins and then on the environmental factors, climatic conditions, geographical region, methods of processing and storage, etc. (Erejuwa et al., 2012; Liu et al., 2013).

Honey is considered as one of the most important natural antioxidant sources among the foods. It is effective against human-threatening health substances such as oxidant agents. In addition, honey consumption can substantially decrease the risk of cardiovascular diseases, cancers, cataract, and inflammatory diseases (Belay et al., 2013; Da Silva et al., 2016). Honey includes antioxidant enzymes such as catalase, peroxidase, glucose oxidase; and non-enzyme antioxidants such as acid ascorbic, tocopherol, carotenoids, amino acids, proteins, and organic acids (Ferreira et al., 2009; Gambacorta et al., 2014).

Thermal processes are often applied in food industries in order to obtain healthy products with extended shelf life. Of course, in addition to having beneficial effects, some of negative impacts of thermal processes have to be taken into consideration (Capuano and Fogliano, 2011). The thermal processes applied on honey may destroy vitamins and biological nutrients and at the same time, reduce the activity of diastase enzyme and increase Hydroxymethylfurfural (HMF) content (Tosi et al., 2002). HMF is a colorless solid with a high degree of solubility in water. When honey is heated and kept for a time, its sucrose level decreases, while HMF content increases. Then, it can be metabolized within the body into other substances and make side reactions with DNA or proteins (Bogdanov et al., 2004). So, in the current study, we investigated some chemical quality characteristics of honey produced in Iran.

Materials and methods

Honey samples

From July 2015 to September 2016, the natural honey samples were randomly collected from the apiaries from the mountainous regions in North-West (East Azarbaijan province) and South-West (Chaharmahal and Bakhtiyari province) of Iran. Furthermore, the commercial and artificial honey samples were randomly gathered from the stores located in two mentioned regions. The numbers of natural and commercial samples per each region were 40 and 10, respectively. The samples were kept under the conditions of 4 °C and %25 humidity until conducting the process of analysis.

Chemicals and reagents

The chemicals and reagents used in the current study were analytical grades and obtained from Merck,

Darmstadt, Germany, including sodium bi-sulfite, potassium ferrocyanide with three water molecules, zinc acetate with two water molecules, ethylalcohol, HMF, 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol, β -carotene, chloroform, tween 80, and gallic acid. Linoleic acid and folin-ciocalteu phenol reagent were prepared from Sigma-Aldrich (USA).

HMF determination

The quantitative determination of HMF was conducted according to the method proposed by White (1979). Briefly, 5 g honey was transferred into a 50 ml volumetric flask. Then, 25 ml distilled water was added to the flask and honey was completely dissolved in it. Next, 0.5 ml Carrez I and II solutions were added and stirred well. The volume was increased to 50 ml. The contents of the flask were mixed completely and then filtered through Whatman paper. The first 10 ml was rejected and the rest were collected. Five ml of the filtrate was added to two test tubes. In the first tube (i.e., the sample tube), 5 ml distilled water and in the second (i.e., the reference tube), 5 ml sodium bi-sulphite were added. After stirring the content of both tubes, the absorbance of the sample solution was determined against the reference solution using UV/Visible spectrophotometer at 284 nm. In order to subtract the amount of absorbance for the background substance, the sample absorbance was conducted on the reference materials after resetting the device. The same procedure was repeated at 336 nm and its absorbance was recorded.

$$\text{HMF concentration (mg/kg)} = (A_{284} - A_{336}) \times 149.7 \times 5 / W$$

A_{284} and A_{336} are the absorbance at 284 and 336 nm, respectively; W is the weight of analyzed honey sample in g and the factor 149.7 is a theoretical value derived from molar absorptivity of HMF (i.e. 16800) at 284 nm. Accuracy of the method applied to determine HMF was confirmed by spiking of increasing amounts of standard solutions of HMF to different types of honey solutions. As seen in Table 1, the recoveries were acceptable.

Determination of total phenolic content

Total phenolic content was evaluated based on methods by Tenore et al. (2012) with a little modification and the use of gallic acid as the reference. Briefly, 20 μ l of each honey sample in addition to the gallic acid calibration solutions (having concentrations equal to 20, 40, 60, 80, and 100 mg/L) were added to a container (with the capacity of 25 ml) containing 1.16 ml ultrapure water (ddH₂O). Also, a reference reagent solution was provided using ultrapure water. One hundred μ l Folin-Ciocalteu's phenol reagent was added to the mixture and stirred well. After 8 min, 300 μ l Na₂CO₃ (%20) solution was added to the mixture and stirred. The mixture was

reached its final volume by the addition of double-distilled water and then was stirred well. The resulting solution was kept for 30 min under the temperature of 40 °C in a stove and then, its absorption was read using spectrophotometer at the wavelength of 675 nm. The total phenolic content was evaluated in the form of mg Gallic Acid Equivalents (GAE)/100 g honey.

DPPH free-radicals scavenging assay

The DPPH radical scavenging activity of honey samples was evaluated according to the method applied by Khalil et al. (2011). The capacity of honey samples to donate electron or lose hydrogen atoms was investigated through the bleaching of the purple DPPH solution. Firstly, the DPPH solution was obtained by dissolving the DPPH powder in methanol with the concentration of 0.024 mg/ml. Then, several concentrations of pure water, including 0.125, 0.25, 0.5, and 0.75 g/ml were provided for each honey sample. One ml of each honey sample was mixed with 2.7 ml methanol containing DPPH radicals and stirred well. The mixture was kept for 15 min in darkness and at room temperature. The reduction of DPPH radicals was determined through the measurement of absorbance at 517 nm.

Antiradical activity(%)=100×(absorbance of control–absorbance of sample/absorbance of control)

The results were reported according to IC₅₀, which refers to the minimum concentration of honey (mg/ml) that has %50 antiradical effects.

β-carotene bleaching assay

The antioxidant activity of honey was evaluated using β-carotene–linoleic-acid system (Ferreira et al., 2009). Firstly, a concentration of honey equal to 0.5 mg/ml distilled water was provided from each sample and 0.2 ml of them was transferred into each test tube. Then, 2 mg β-carotene was dissolved in 10 ml chloroform in order to obtain the β-carotene solution. Two ml of this solution was transferred into a 100 ml flat-bottom container. Chloroform was evaporated at 40 °C in vacuum. This part of the study was conducted through the application of incubator device and vacuum pump. Then, 40 mg linoleic acid, 400 mg tween 80 (as emulsifier), and 100 ml distilled water were added to the mixture and stirred well. Aliquots of this emulsion (each equal to 4.8 ml) were added to each tube containing 0.2 ml honey in various densities. The tubes were stirred and kept at 50 °C in a hot water bath. It should be mentioned that as soon as the emulsion was added to each tube, the zero-time absorbance at 470 nm was determined using spectrophotometer. Determining the subsequent absorbance intensity was conducted every 20 min until the discoloration of the control sample. In order to eliminate the background

effect, a control sample that was free of β-carotene was applied. Inhibition of lipid peroxidation in the samples was evaluated using the following formula:

Lipid peroxide inhibition = (β-carotene content after 2 h of assay/initial β-carotene content)×100

Statistical analysis

Statistical analysis of the data of this survey were conducted by the application of SPSS software version 16.

Results

The results showed that the mean HMF content in honeys produced in South-West of Iran (16.9 mg/kg) was significantly ($p<0.05$) higher than those in North-West of Iran (2.85 mg/kg). As shown in Figure 1, mean HMF concentration in commercially produced honeys was too different with ($p<0.05$) this rate in naturally produced honeys. The HMF level in 4 out of 80 (5%) natural honey samples and 14 out of 20 (70%) of commercial honey samples was higher than the recommended safety limit set by the Iran national standard.

The honeys from the North-West of Iran had higher mean phenolic content than the honeys from the South-West of Iran with a significant difference ($p<0.05$). Total phenolic content of natural honey samples was ranged from 19.0 to 55.7 mg of GAE/100 g of honey, whereas these rates for commercial honey samples was ranged from 8.45 to 32.63 mg of GAE/100 g of honey. Also, the average phenolic content was significantly ($p<0.05$) lower for commercial honeys (20.51 mg of GAE/100 g of honey) than for natural honeys (55.37 mg of GAE/100 g of honey).

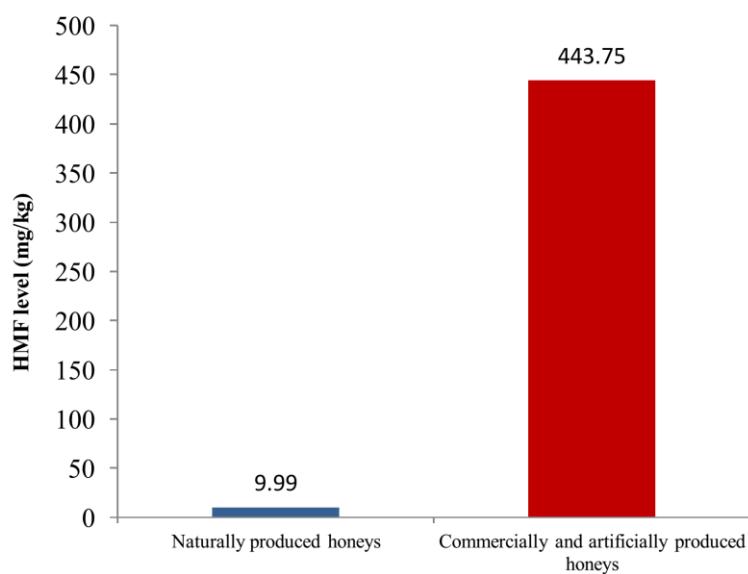
There was significant ($p<0.05$) difference between DPPH radical scavenging activity of honey samples of North-West (IC₅₀ = 50.96 mg/ml) and South-West regions (IC₅₀ = 30.31 mg/ml). Also, the mean IC₅₀ of natural honey samples was 40.63 mg/ml with a range of 14.2-84.1 mg/ml; however, the mean IC₅₀ of commercial honey samples was 341.96 mg/ml, with a range of 115-997.4 mg/ml.

β-carotene bleaching inhibition rate in honeys obtained from North-West of Iran was significantly ($p<0.05$) higher than the values recorded for honey samples of South-West of Iran.

For natural and commercial honey samples, the β-carotene bleaching inhibition ranges were 32.15 -78.96 mg/ml as well as 10.15-37.69 mg/ml, respectively. The difference between mean values of β-carotene bleaching inhibition of natural honey (54.54 mg/ml) as well as commercial ones (25.37 mg/ml) was found to be statistically significant ($p<0.05$).

Table 1: Recoveries of hydroxymethylfurfural spiked to honey (mean±SD; n=3)

Spiked concentration (mg/L)	Recovery concentration (mg/kg)	Recovery (%)
0.5	0.46	92
	0.43	86
	0.47	94
		90±4.16
1.5	1.60	106
	1.55	103
	1.58	105
		105±1.53
3	2.67	89
	3.00	100
	2.77	92
		94±5.69
6	5.97	95
	6.20	103
	5.71	99
		99±4.00
12	11.25	94
	11.50	96
	11.58	96
		95±1.33
24	23.00	95
	21.80	91
	22.10	92
		92±2.10

**Figure 1:** Comparison of mean hydroxymethylfurfural levels (mg/kg) in two different honey samples of Iran

Discussion

There are several types of natural honeys in Iran depending on the honey floral sources. However, unauthorized production or improper processing could decrease honey's quality and nutritional values. The safety and

quality of the commercial or the so-called artificially-produced honey are always questionable. In the case of commercially-produced honeys in Iran, direct incorporation of industrial carbohydrate syrups in honey and/or

heat treatment of honey could result in quality deterioration. In addition, heating the sugar solutions by adding unsafe or forbidden food colorants and essences to produce artificial honey could be regarded as an adulteration. In this survey, the variability of quantitative data regarding chemical characteristics of honey samples obtained from two different geographical regions of Iran were highlighted the influence of different factors such as botanical source, climatic conditions, etc.

Several investigations on honey HMF contents have been accomplished in recent years all over the world. According to Belay et al. (2013), the mean concentration of HMF in honey samples obtained from Bale, Ethiopia was 0.84 ± 0.46 mg/kg. Oddo et al. (2008) analyzed the HMF levels in honey samples obtained from various locations around Brisbane, Queensland, Australia. They reported the mean concentration and the range of HMF as 1.2 mg/kg and 0.4-1.2 mg/kg, respectively. Estevinho et al. (2012) reported that the HMF levels of the Portuguese organic honeys were ranged between 0.8 and 1.5 mg/kg with the mean level of 1.1 mg/kg. Comparing to the similar previous studies, the mean levels of HMF in honeys found in the current study were far above the mean concentrations reported in the some other countries. Since HMF as a cyclic aldehyde is formed from sugar, it is an indicator of overheating or long and inadequate storage condition. Due to its possible cytotoxicity, carcinogenicity, genotoxicity, mutagenicity, HMF is a major health concern worldwide (Pita-Calvo et al., 2017). The Institute of Standard and Industrial Research of Iran (ISIRI, 2009) has established that the HMF content in honey samples should be lower than 40 mg/kg. In the present survey, HMF concentrations in commercial honey samples were considerably high that was similar to the finding of a research conducted by Jalili (2016) who detected HMF in 43% of honeys from different supermarkets of Tehran, Iran. Therefore, it seems that adulteration actions in commercial honey marketed in Iran is common.

Total phenolic contents in honey samples of this study showed diverse ranges which was in accordance with some previous published investigations (Escuredo et al., 2012; Ferreira et al., 2009; Gambacorta et al., 2014; Khalil et al., 2011). The phenolic content in honey is remarkably influenced by the climatic conditions, floral sources, and geographical origin (Escuredo et al., 2012). There are roughly ten thousands of botanical phenolic compounds that could be divided into flavonoids and non-flavonoids. Many of these compounds could be transferred by honeybees to the final honey product. Phenolic acid content as an important fraction of phenolic compounds could result in antioxidative characteristics and lipid oxidation inhibitory activities. The major phenolic compounds in honey consist of gallic acid, cyringic acid, benzoic acid,

coumaric acid, cinnamic acid, caffeic acid, ferulic acid, and so forth. In addition, more than 50% of all natural phenolic compounds are comprised of flavonoids that could contribute to the antioxidative activity of honey (Alvarez-Suarez et al., 2012; Da Silva et al., 2016; Erejuwa et al., 2012).

In Iran, illegal heat processing of honey could result in a product temperature very close to the boiling point of water. In addition, adulteration of honey by adding different industrial sugar syrups or other additives causes significant alterations in the final product. The effect of heat treatment and storage time on the degradation of heat-labile phenolic compounds in some foodstuffs has been reported in recent years (Mrad et al., 2012; Spanos and Wrolstad, 1990). The DPPH radicals are not affected by chemical side reactions like chelating of metal ions as well as enzyme inhibition. On the other hand, the DPPH method has become one of the most frequently used procedures to assess the antioxidant properties (Khalil et al., 2011; Silici et al., 2010). According to scientific literature, antiradical activities varied markedly among the honey samples all over the world (Belay et al., 2013; Khalil et al., 2011). Ferreira et al. (2009) reported an average inhibition of β -carotene equal to 75.51, 39.25, and 37.03 mg/ml for light, amber, and dark honey samples, respectively in Portugal which their results are somewhat similar to the findings of the current study.

Conclusion

In conclusion, HMF levels, phenolic contents, antiradical activity, and antioxidative potency of Iranian natural honeys were remarkably more acceptable and suitable than commercial honeys produced in the country.

Conflicts of interest

There are no conflicts of interest.

Acknowledgments

The results of this article are related to MSc. thesis (No.170/2271) in the Faculty of Veterinary Medicine, Shahrekord University, Iran. This study was financially supported by Shahrekord University. This research was ethically approved by the local institutional review board.

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