

## Hygienic quality of the honey samples produced in the Iran in comparison with international standards

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### Abstract

Honey is a sweet, viscous liquid that bees produce from nectar collected from plant nectarines and store as food. In this study, some physicochemical properties (pH, ash, reducing sugars, sucrose, moisture, electrical conductivity, diastase activity, hydroxymethylfurfural (HMF) and commercial glucose) and microbial contaminations of 180 honey samples from North-western regions of Iran (Ardabil province) were evaluated in one year period in different seasons of 2012. The levels of reducing sugars, sucrose content and HMF of 6.11%, 8.33% and 3.33% samples were unacceptable, respectively. Diastase activity of 4 samples (2.22%) was negative and 5 samples (2.77%) had commercial glucose. But moisture, ash content, pH and electrical conductivity values of all samples were in the required standard range. The amounts of moisture and electrical conductivity value during various seasons show statistically significant differences ( $P \leq 0.05$ ). Microbial analyse results showed that of all the samples evaluated, only 13 samples (7.22%) contained mold, and 10 samples (5.55%) were contaminated with bacteria. The standard plate counts (SPC) were found in low numbers in most samples of honey with a mean count of 2.6 Log<sub>10</sub> CFU.g<sup>-1</sup>. The results of this study may help improve researchers' understanding of honey properties and their impact on consumer preference.

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### Keywords

Honey

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### Introduction

Honey is a natural supersaturated sugar solution, which is mainly composed of a complex mixture of carbohydrates. Besides this, it also contains certain minor constituent's proteins, enzymes (invertase, glucose oxidase, catalase, and phosphatases), amino and organic acids (gluconic acid, acetic acid, etc.), lipids, vitamins (ascorbic acid, niacin, pyridoxine etc.), volatile chemicals, phenolic acids, flavonoids, and carotenoid like substances and minerals (Blasa *et al.*, 2006). In almost all honey types, fructose predominates, glucose being the second main sugar. These two account for nearly 85–95% of the honey carbohydrates. More complex sugars made up of two or more molecules of glucose and fructose constitute the remaining carbohydrates, except for a trace of polysaccharide (Bogdanov *et al.*, 2004).

Moisture level of honey represents a major importance to its stability against fermentation and granulation. The low moisture content protects honey from microbiological activity and thus it can be preserved for longer periods (Akhtar *et al.*, 2014; El-Metwally, 2015). The quality of honey is mainly determined by its sensorial, chemical, physical and

microbiological characteristics (Erkan *et al.*, 2015). The composition of honey depends on the plant species visited by the honeybees and the environmental, processing and storage conditions (Bertoncelj *et al.*, 2007; Guler *et al.*, 2007). Blossom or nectar honey is derived from the nectaries of flowers and honeydew honey comes from the sugary excretion of some hemipterous insects on the host plant or from the exudates of the plants. It is rich in flavonoids and phenolic acids that exhibit a wide range of biological effects and act as natural antioxidants (Da Silva *et al.*, 2016). Since ancient times honey has been used as natural unprocessed food and medicine (Jeffrey *et al.*, 1996). Some honeys produced in different areas had different qualities and this can be because of climate, origin of plants, season, processing and other factors. This factor can affect the physicochemical and microbial properties of honey (Bansal *et al.*, 2005; Mahmoudi *et al.*, 2012). Microorganisms in honey may influence the stability of the products and its hygienic quality (Erkan *et al.*, 2015).

Honey has two sources of contamination with microorganisms: primary sources include pollen, the digestive tracts of honey bees, dust, air, soil and nectar; secondary sources are those

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arising from honey manipulation by people, they include air, food handlers, cross-contamination, equipment and buildings (Aureli *et al.*, 2005). Primary sources of honey contamination are very difficult to control. Conversely, secondary sources of honey contamination can be controlled by good manufacturing practices (Fenicila *et al.*, 2009). The microbes of concern in honey are fungi, yeasts and spore-forming bacteria. Fungi and yeasts are responsible for honey fermentation when the moisture content is high (i.e., above 21%). *Penicillium* and *Mucor* are microorganisms usually found in honey (Migdal *et al.*, 2000).

Ardabil province's honeys are recognized throughout the country because of their quality. This province has the third position of honey production in the country (Moghadamnia *et al.*, 2012). However, there is little scientific research published on Ardabil's honey on its physicochemical and microbiological quality. The quality of honey is mainly determined by its sensorial, chemical, physical and microbiological characteristics. Internationally, honey quality criteria are specified in Regulatory Standards, compiled in a Codex Alimentarius standard which at present is under revision (Bogdanov *et al.*, 2004). The Codex Alimentarius Standard for honey quality includes several chemical and physical parameters, comprising moisture content, mineral content, acidity, HMF content, diastase activity, apparent sugar content, and water insoluble solids content (Codex, 2011). These analyses help the food analyst to determine the "chemical" quality of the honeys analyzed (Cantarelli *et al.*, 2008).

The aim of the present work was to analyze the physicochemical and microbiological quality of some honey samples produced in Ardabil's province apiaries in warm and cold seasons. The results were compared to international standards available in this field and the effect of seasons on these parameters were studied.

## Materials and Methods

### Honey samples

A total number of 180 honey samples were collected from various areas of Ardabil province in Iran during different seasons in the year 2012 (Select the number of honey samples based on similar studies has been done in the field). 90 samples belonged to the first half of 2012 and 90 samples to the second half. Samples were transferred to laboratory under appropriate conditions for conducting physicochemical and microbial analysis, and were stored at 4°C until analysis time. All physicochemical

and microbial tests were conducted in triplicate.

### Moisture

The moisture percentages were evaluated using refractometer refractometer (ATAGO, ATC-1E, Japan) unit at 20°C, and calculated from obtained refraction index using Wedmore table (AOAC, 1990).

### Reducing sugars and sucrose

Reducing sugars and apparent sucrose were determined by potentiometric titration, using the Fehling's test (Lane and Eyon modified method) (Gomes *et al.*, 2010).

### pH

pH measurements were conducted using a digital pH meter (Metrohm Herisua, Switzerland); 10 g of homogenized honey and 90 ml of distilled water was added, and the pH was read directly from the pH meter. The instrument was calibrated with standard buffer solutions of pH 7 and 4 prior to measuring the pH of samples (Saxena *et al.*, 2010).

### Ash

In order to determine ash content of honey samples, 3 g of each sample was weighted in a Chinese crucible and put in an electric furnace at 640°C for 6 h. Then, the amount of Ash was measured (AOAC, 1990).

### Electrical conductivity

The measurement of electrical conductivity is based on the determination of the electrical resistance. The electrical conductivity was measured using a conductivity bridge (type CLOI/02A) for a 20% (w/v) solution of honey suspended in milli Q water (Bogdanove *et al.*, 1997). The electrical conductivity of the milli Q water was < 10 IS/cm. Each sample was analyzed in triplicate and the mean was expressed in mS/cm.

### Diastase activity

Diastase activity was measured using Phadebas method based on the procedure of Siegenthaler (Singh *et al.*, 1998), modified by Bogdanov (Bogdanove *et al.*, 1987) and harmonized by the European Honey Commission (Bogdanove *et al.*, 1997). Adsorption was determined using a spectrophotometer UV/VIS at  $\lambda = 620$  nm.

### HMF

HMF was determined by using the standard method AOAC (1990) Official Method 980.23. Five grams of honey were dissolved in 25 mL of

distilled water, treated with a clarifying agent (0.5 mL of Carrez I and 0.5 mL of Carrez II solutions) and volume made up to 50 mL. The solution was filtered, and the first 10 mL discarded. The absorbance of the filtered solution was measured at 284 and 336 nm against an aliquot of the filtered solution treated with NaHSO<sub>3</sub>. HMF was determined as:

$$\text{HMF/ 100g of honey} = (\text{Abs}_{284} - \text{Abs}_{336}) \times 14.97 \times (5 \text{ g of honey}).$$

#### Commercial glucose

The amount of commercial glucose was determined on the proposed method of AOAC, NO. 959,12, (AOAC, 2000).

#### Microbiological analysis

Ten grams of each honey sample were homogenized into 90 mL of peptone water solvent (Gomes *et al.*, 2010) in room temperature (25°C). Preparing decimal dilutions, the appropriate medium was inoculated by standard analysis methods. Aerobic mesophilic bacteria were counted onto PCA and incubated at 30°C for 48 h (NP 3788:2002). Moulds and yeasts counts followed the protocol of ISO 21527-2:2008. Microbial counts were expressed as Log of colony-forming units per gram of honey (Log<sub>10</sub> CFU.g<sup>-1</sup>). Fecal coliforms were enumerated by the Most Probable Number technique defined in the protocol ISO 4831:2006. All microbial tests were performed in triplicate.

## Results

#### Moisture content

The moisture content (%) in the investigated samples ranged from 3.8 to 19.15 (Table 1 and 2). All of the samples moisture was less than 20%. The amounts of moisture during various seasons show statistically significant differences ( $P \leq 0.05$ ).

#### Reducing sugars and sucrose

Based on the results of the analysis of sugar compounds of honey samples, total reducing sugar levels were in the range of 58.91 to 85.05% (Table 1 and 2). The sucrose contents were between 1.2% to 14.8%. According to EU (2001), 11 samples of total samples had unacceptable value for reducing sugar and 15 samples for sucrose.

#### pH

All analyzed samples of honey in this study had an acidic pH in the range of 3.65 to 4.81 (Table 1 and 2).

#### Ash

The values of ash content varied in the range of 0.2% to 0.62% (Table 1 and 2).

#### Electrical conductivity

Values were between 0.03 and 0.19 mScm<sup>-1</sup> (Table 1 and 2).

#### Diastase activity

In this study, four samples exceeded the limits of European Community Regulation with values less than 8° Gothe (Table 1 and 2).

#### HMF

The European Union (EU Directive 110/2001) fixed a HMF limit in honey of 40 mg/kg. Values were between 1.54 and 45.1 mg/kg (Table 1 and 2). Only 3 samples had more than 40 mg/kg HMF which could be because of temperature abuse during processing and/or bad storage practices.

#### Commercial glucose

In this study, five samples had commercial glucose.

#### Microbial analyze

Microbial analyze results are shown in Table 3 and 4. The SPC were found in low numbers in most samples of honey with a mean count of 2.6 Log<sub>10</sub> CFU.g<sup>-1</sup>. Few samples of honey contained detectable levels of yeasts, below 2.3 Log<sub>10</sub> CFU.g<sup>-1</sup>.

## Discussion

#### Moisture content

Moisture content is one of the most important compositions to be considered as a quality parameter of honey since it affects storage life and processing characteristics. Our results are in agreement with the findings of Cantarelli *et al.* (2008) who reported that the moisture content in honey was recorded in the range of 14 to 18%; however it depends upon the season and geographic condition. Moisture values were within the values found in Algerian honeys (between 14.64% and 19.04%) (Ouchemoukh *et al.*, 2007). Chakir *et al.* (2011) in Morocco obtained similar result. It is less than those found in Northwest Moroccan honeys (between 14% and 24.1%) (Terrab *et al.*, 2004). The low moisture content of honey also forms an important part of the system which protects honey from attack by microorganisms (Omafuvbe *et al.*, 2009). Similar results as our study (Nanda *et al.*, 2003; Gomes *et al.*, 2005) and higher (Przybylowsky *et al.*, 2001; Rodriguez *et al.*, 2004; Guler *et al.*,

Table 1. Physicochemical analysis of first half of 2012 honey samples (90 samples).

Parameter	Mean	SD	Min	Max	Satisfactory limit by EU	Unacceptable samples
Moisture (%)*	16.02	0.61	14.5	17.8	Almost 20%	-
Reducing sugars (%)	71.24	4.06	59.7	78.3	Almost 60%	6
Sucrose (%)	5.34	2.75	1.2	14.8	Almost 5%	7
pH	4.93	0.26	3.65	4.67	At least 3.5	-
Ash (%)	0.37	0.09	0.21	0.62	Almost 0.6	-
Electrical	0.06	0.01	0.03	0.09	Almost 0.8	-
Conductivity(mS/cm)*						
Diastase activity(*Gothe)	-	-	-	-	At least 8	1
HMF(mg/kg)	9.7	2.02	1.87	45.1	Almost 80	2
Commercial glucose	-	-	-	-	Should not be found	1

Table 2. Physicochemical analysis of second half of 2012 honey samples (90 samples).

Parameter	Mean	SD	Min	Max	Satisfactory limit by EU	Unaccepta ble samples
Moisture (%)*	15.64	1.16	3.8	19.15	Almost 20%	-
Reducing sugars (%)	71.8	5.37	58.91	85.05	Almost 60%	5
Sucrose (%)	4.96	2.91	1.34	11.88	Almost 5%	8
pH	4.03	0.25	3.65	4.81	At least 3.5	-
Ash (%)	0.33	0.07	0.2	0.5	Almost 0.6	-
Electrical	0.07	0.03	0.03	0.19	Almost 0.8	-
conductivity(mS/cm)*						
Diastase activity(*Gothe)	-	-	-	-	At least 8	3
HMF(mg/kg)	8.04	2.12	1.54	42.21	Almost 80	1
Commercial glucose	-	-	-	-	Should not be found	4

\* the amount of moisture and electrical conductivity in honey samples in various seasons showed statistically significant differences.

2005) results were detected in previous studies.

#### Reducing sugars and sucrose

These results are more than those Olugbemi *et al.* (2013), Rodriguez *et al.* (2004) and Cantarelli *et al.* (2008) obtained, but in agreement of Mahmoudi *et al.* (2012). Also the results of this study agreement with EL Sohaimi *et al.* (2016) research, Based on their findings the value of reducing sugars of Saudi, Egyptian and Yemeni honey samples were  $72.36 \pm 0.32$  g/100 g,  $69.84 \pm 0.31$  and  $64.21 \pm 0.18$  g/100 g respectively, and this rate in our study was 71. 24%. Reducing sugars value of 169 (95.89%) samples was accepted by Codex Alimentations (2001).

#### pH

The results are completely similar to the findings of Ouchemoukh *et al.* (2007), Azeredo *et al.* (2003),

Kayacier and Karama (2003) and Mahmoudi *et al.* (2012). The pH of honey is low enough to slow down or prevent the growth of many species of bacteria, but this acidity may be neutralized in the body by the buffering liquid fluids. The results of this study are also in agreement with those of Adenekan *et al.* (2010) and White (1975), who reported the pH of 3.0 to 5.0 in pure honey,. These pH ranges are mainly due to the variation of different acid and minerals present in the honey.

#### Ash

These results are in agreement with those of White (1975) who worked on different varieties of honey and obtained ash content in the range of 0.020 to 1.028%. Adenekan *et al.* (2010) (0.18% - 0.5%), Olugbemi (0.33% - 0.37%) (2013), Adebisi *et al.* (2004), Jeffery and Echazarreta (1996) and Malika

Table 3. Microbial results (bacterial, fungal and yeast)

	Number of contaminated samples	Mean (Log <sub>10</sub> cfu.g <sup>-1</sup> )
Bacterial contamination	10	2.6
Fungal contamination	8	2.3
Yeast contamination	5	2.3
Total coliforms	Not found	-

*et al.* (2005) also obtained such results. The variation may be due to many factors such as soil conditions, atmospheric conditions and physiology of each plant.

#### Electrical conductivity

Electrical conductivity, closely related to the concentration of mineral and organic acids, shows great variability according to the floral origin. This results in this study was within the values found in Algerian (mean ranged between 0.02 and 0.16 mScm<sup>-1</sup>) (Ouchemoukh *et al.*, 2007) and Northwest Moroccan honeys (between 0.02 and 0.17 mScm<sup>-1</sup>) (Terrab *et al.*, 2004). The results of this study are less than results of Pavelková *et al.* (2013) in Liptov region (between 0.17 and 0.39 mScm<sup>-1</sup>), Adenekan *et al.* (2010) in Ibadan (between 0.25–0.64 mScm<sup>-1</sup>). Electrical conductivity very often used in routine honey control instead of the ash content. The amounts of Electrical conductivity during various seasons show statistically significant differences ( $P \leq 0.05$ ).

#### Diastase activity

Diastase is a natural enzyme of honey. Its level depends upon geographic and floral origins of the product, as well as on its freshness. As with HMF, diastase activity can be used as indicative of aging and temperature abuse, but with precaution, since its variability has been higher, confirmed in several honeys. These results are in agreement with those of Chakir *et al.* (2011) that work on seventy- three Moroccan honey samples. But all of the honey samples diastase activity was more than 8° Goethe (Gomes *et al.*, 2010).

#### HMF

HMF is formed during acid-catalysed dehydration of hexoses and, it is connected to the chemical properties of honey, like pH, total acidity, mineral content (Bertoncelj *et al.*, 2007; Saxena *et al.*, 2010; Chakir *et al.*, 2011; Olugbemi *et al.*, 2013). The

Table 4. *Bacillus* spp. detected from contaminated samples

<i>Bacillus</i> spp.	Contaminated samples (N)
<i>B. mycoides</i>	3
<i>B. cereus</i>	6
<i>B. circulans</i>	1

HMF content is widely recognized as a parameter of honey samples freshness, because it is absent in fresh honeys and tends to increase during processing and/or aging of the product. Several factors influence the levels of HMF, such as temperature and time of heating, storage conditions, pH and floral source, thus it provides an indication of overheating and storage in poor conditions (Fallico *et al.*, 2006).

#### Commercial glucose

Commercial glucose is an important factor in assessing the quality of honey. According to European Commission Regulation (EU) (2002), the presence of commercial glucose is not acceptable. Our result is in agreement of Mahmoudi *et al.* (2012) studies. But in disagreement of (Kayacier *et al.*, 2003).

#### Microbial analyze

Based on this study, the numbers of the average total bacteria count was found to be 2.6 Log<sub>10</sub> cfu.g<sup>-1</sup>. These results are in agreement with Malika *et al.* (2005) but less than results obtained by Omafuvbe (2009) and Erkan *et al.* (2015). The result of *Bacillus* spp that detected is presented in Table 4. The

Contamination rate of vegetative form of *Bacillus* spp. was found to be 5.55%, the study was carried out on honey samples in Turkey the rate of spor form bacteria was 4%, and the observed difference is minimal (Erkan *et al.*, 2015). The Total coliforms were not detected in any of the honey sample, similar as in study Adenekan *et al.* (2010) and Malika *et al.* (2005). The mold and yeast contamination levels in the honey samples analyzed were found in 13 honey samples (7.22%), While most fungal infections have been reported in many studies (Gomes *et al.*, 2010; Erkan *et al.*, 2015). The low number of moulds in this study would be most probably related to the environmental conditions during honey processing. Such results are shown in Malika *et al.* (2005) researches.

#### Conclusion

The results showed that the physicochemical and microbial properties of the samples of honey samples

produced in the Ardabil province during the year 2012 were acceptable. The low microbial and fungal contamination of honeis, affect their quality. High pH and low moisture are one of the reasons for this.

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