

Full Length Research Paper

Biochemical properties and microbial analysis of honey from North-Western regions of Iran: Seasonal effects on physicochemical properties of honey

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In the present study, physicochemical properties (pH, ash, commercial glucose, starch, reducing sugars and moisture) and microbial (yeast and enterobacterial) contaminations of 263 honey samples from North-western regions of Iran were evaluated in a 2 year period in different seasons of 2010 and 2011. Levels of reducing sugars and sucrose showed significant seasonal differences, with the highest levels observed in summer samples. No commercial glucose or starch was detected in any of the samples; but levels of reducing sugars and sucrose content of 1.52 and 6.84% samples were unacceptable, respectively. Moisture, ash content ($0.4 \pm 0.01\%$) and pH values (4.44 ± 0.02) of all samples were in the required standard range and did not significantly vary in different seasons. Of all the samples evaluated, only seven samples (2.66%) contained yeast, and two samples (0.76%) were contaminated with bacteria from family *Enterobacteriaceae* (2 samples with both contaminations). None of the isolates were found to be of major pathogenic importance.

Key words: Honey, physicochemical, microbial, north-western, Iran.

INTRODUCTION

Honey is an aromatic and sweet food having many nutritional benefits, and has been used for a long time. The major ingredients of honey include a mixture of carbohydrates, organic acids, amino acids, proteins, minerals, vitamins and lipids (White, 1975).

Defined by Codex, honey is the natural sweet substance produced by bees from the nectar of flowers or secretions of living parts of plants or waste, caused by sucking insects (aphids), and plants are part of the live material that honeybees collect, and transport materials and combine them with specific materials from their own bodies and store it in honey combs, so that it is

processed and matures (Codex Alimentarius Commission, 2001).

Honey is a nutrient with remarkable energy and is used as a major ingredient in producing many ready to eat foods, particularly the products based on grain because of having such characteristics as sweetness, colour, flavor, caramelization and viscosity (Rodriguez et al., 2004). Commercial samples of honey available in various parts of the world are of highly different quality, on the basis of factors like geographical conditions, production season, processing, and source of nectar, packaging and storage period. Given the importance of honey as a nutrient full of energy and prebiotic compounds and its usage in disease treatment, the necessity of identifying the physicochemical and qualitative properties of this valuable nutrient is obvious.

Quality of honey is mostly related to organoleptic,

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physicochemical and microbiological characteristics; qualitative physicochemical features of honey are indicated in European directive and codex food commission (Codex, 1996; EU, 2001). This feature includes content of moisture, ash, reducing and non-reducing sugars, acidity, starch, commercial sugar and content of hydroxymethylfurfural (HMF) (Gomes et al., 2010). Honey composition is mainly related to the type of flowers used by honeybees, climatic and storage conditions (Abu-Tarboush et al., 1993; Guler et al., 2007) and approximately consists of 80% carbohydrates (35% glucose, 40% fructose and 5% sucrose) and 20% water. It also contains more than 180 ingredients including amino acids, vitamins, minerals, enzymes, organic acids and phenolic compounds. Honey pH is about 4 (Ouchemoukh et al., 2007; Blasa et al., 2006). Physicochemical characteristics of honey from different regions of the world have been widely evaluated by many scientists (Mendes et al., 1998; Ouchemoukh et al., 2007; Przybylowski and Wilczynska, 2001; Singh and Bath, 1997; Unal and Kuplulu, 2006; Yilmaz and Yavuz, 1999). Honey consist vast amount of different compounds that can be of nutritional and health benefits. Its therapeutic potential has been credited to its antimicrobial, anti-inflammatory, anti-oxidant properties, as well as boosting of the immune system and treatment of wounds (Manyi-Loh et al., 2011).

Despite the large amounts of honey produced and extensively consumed in Iran, there is very little information about the physicochemical and ingredients prosperities of honey are in different regions of Iran.

This study was conducted with the aim of evaluating the physicochemical and microbiological quality of honey produced in Northwest of Iran, compared to international standards available in this field and the effect of season on these characteristics.

MATERIAL AND METHODS

Honey samples

A total number of 263 honey samples were collected from various areas of Northwest of Iran during different seasons in the years 2010 and 2011 (the honey samples used in this study were obtained from East Azerbaijan (n: 95), West Azerbaijan (n: 90) and Ardabil (n: 78) province, Northwest of Iran). 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.

Physicochemical analysis

Moisture

The moisture percentages were evaluated using refractometer unit at 20°C, and calculated from obtained refraction index using Wedmore table (Association of Analytical Communities (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) (Gomez et al., 2010).

Starch

Measurement of starch was done according to iodine method (TS2419, 2001).

Commercial glucose

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

pH

pH measurements were conducted using a digital pH meter (Metrohm Herisua, Switzerland); 10 g of homogenised 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. Ash was measured in triplicate and the mean values were expressed in g (%) (AOAC, 1990).

Microbial assessments

From a 10% solution of each honey sample in sterile distilled water, 50 µl was dispersed on culture media. For yeast isolation, potato dextrose agar (PDA) was used and for isolation of bacteria from family Enterobacteriaceae, Mac conkey agar and Salmonella shigella agar were used. Each isolated colony was differentially identified to species level using conventional biochemical tests and differential culture media.

Microbiological analysis

10 g of each honey sample were homogenized into 90 ml of sterile peptone water and appropriately diluted suspensions of samples (100 ul) were cultured in duplicate by the spread plate method. The standard plate count method was used for culturing and isolating the different micro-organisms. MacConkey agar was used as the medium for enterobacterial isolation and culture, while potato dextrose agar and Sabouraud dextrose agar were used for growing yeasts. Bacterial colonies resulting from the first culture after incubation at 37°C for 48 h were transferred to fresh media, streaked and incubated again. After successive transfers, the resulting pure isolates were Gram stained and identified, based on the color, size and shape. For yeasts, incubation was at room temperature (22°C) and 37°C for four days. The resulting colonies were examined, streaked and grown successively until pure cultures were obtained. Identification was based on the color and shape (Thapa et al., 2004). All microbial tests were performed in triplicate.

Table 1. Physicochemical analysis of spring (2011) honey samples (78 samples).

Parameter	Mean	SD	Maximum	Minimum	Satisfactory limit by EU	Unacceptable sample (%)
Moisture (%)	16.09	0.53	17.8	15.2	Almost 20%	ND
Reducing sugar (%)	70.51	3.92	78.3	58.3	Almost 60%	ND
Sucrose (%)	3.4	2.98	11.9	0.2	Almost 5%	ND
Ash (%)	0.39	0.05	0.41	0.32	Almost 0.6	ND
pH	4.42	0.45	5.14	3.55		-
Glucose	ND	-	ND	ND	Not found	ND
Starch	ND	-	ND	ND	Not found	ND

ND, Not detected; SD, standard deviation; EU, European Commission Regulation (2002).

Table 2. Physicochemical analysis of summer (2011) honey samples (49 samples).

Parameter	Mean	SD	Maximum	Minimum	Satisfactory limit by EU	Unacceptable sample (%)
Moisture (%)	16.2	0.57	16.8	14.5	Almost 20%	ND
Reducing sugar (%)	73.27	2.95	78.7	67.01	Almost 60%	2.04
Sucrose (%)	5.51	3	14.8	1.8	Almost 5%	10.2
Ash (%)	0.42	0.11	0.45	0.35	Almost 0.6	ND
pH	4.46	0.46	5.12	3.45		-
Glucose	ND	-	ND	ND	Not found	ND
Starch	ND	-	ND	ND	Not found	ND

ND, Not detected; SD, standard deviation; EU, European Commission Regulation (2002).

Table 3. Physicochemical analysis of autumn (2010) honey samples (136 samples).

Parameter	Mean	SD	Max	Min	Satisfactory limit by EU	Unacceptable samples (%)
Moisture (%)	16.15	0.61	18.7	14	Almost 20%	ND
Reducing sugar (%)	70.15	4.23	87.01	58.3	Almost 60%	2.2
Sucrose (%)	3.78	2.58	14.1	0.21	Almost 5%	10.29
Ash (%)	0.4	0.24	0.51	0.3	Almost 0.6	ND
pH	4.08	0.04	5.2	3.07		-
Glucose	ND	-	ND	ND	Not found	ND
Starch	ND	-	ND	ND	Not found	ND

ND, Not detected; SD, standard deviation; EU, European Commission Regulation (2002).

RESULTS AND DISCUSSION

Physicochemical and microbiological quality of honey is produced in different regions Northwest of Iran and the effect of season on these characteristics was evaluated. The physicochemical properties of honey samples are shown in Tables 1, 2, 3 and 4. According to available international standards (Codex, 1996 and EU, 2000), 22 samples (8.36%), from all analyzed honey samples lacked the acceptable qualitative specifications (including ten samples of the East Azerbaijan, eight samples of the

West Azerbaijan and four samples was related to the Ardabil province) (Table 4). There were no signs of fermentation or sugar crystal formation in honey samples before conducting microbial and physicochemical analysis. Inhibition of fermentation and sugar crystal formation and durability is mainly affected by moisture content of honey. Moisture content of honey is affected by environmental conditions and manipulations by the apiarists during honey harvest, and is highly variable from year to year (Acquarone et al., 2007). Mean values of moisture content of honey samples in this study (16.14%)

Table 4. Evaluation results of honey samples lacks standard in some physicochemical parameters.

Provenience of the sampling	East Azerbaijan (90 samples)			West Azerbaijan (95 samples)			Ardabil (78 samples)		
	Autumn	Spring	Sumer	Autumn	Spring	Summer	Autumn	Spring	Summer
Seasons (2010 to 2011)									
Sample	50	25	15	45	35	15	36	18	19
Unacceptable samples (Sucrose parameter)	8	ND	ND	7	ND	ND	3	ND	ND
Unacceptable samples (Reducing sugar parameter)	ND	ND	2	1	ND	ND	ND	ND	1

ND, Not detected.

were in the limit of acceptable international standards of honey moisture content ($\leq 20\%$). This shows appropriate durability and quality of these products, because moisture content levels higher than international standards limit leads to crystallization, increase of the water activity appropriate for yeast growth, fermentation during storage period and decrease in durability of honey. The range of moisture level in honey samples in this study was 14 to 18.70%, which are similar to the findings of other researchers (Rodriguez et al., 2004; Duman et al., 2008; Nanda et al., 2003; Kahraman et al., 2010). According to results, the mean values of moisture in honey samples from different seasons showed no statistically significant differences.

Based on the results of the analysis of sugar compounds of honey samples, total reducing sugar levels were in the range of 58.30 to 87.01%. According to international standards in this field ($\leq 60\%$), four samples (1.52%) among all analyzed samples were not acceptable. The results obtained for reducing sugar levels are completely consistent with the study of Ouchemoukh et al. (2004), Yilmaz and Yavuz (1999), Erdogan et al. (2004) and Przybyłowski and Wilczynska (2001). The amounts of reducing sugars during various seasons show statistically significant differences ($P \leq 0.05$). The highest levels belonged to summer (87.01%) and the lowest ones were associated with samples of spring (58.30%). The

level of sucrose varied according to maturity degree and source of nectar compounds of honey.

In other studies, the mean values of sucrose were reported as 4.05% (Rodriguez et al., 2004) and 2.21 to 5.52% (Cantarelli et al., 2008). The findings of the present study show that the mean level of sucrose (4.64 ± 1.48 , Mean \pm SD) and range (0.2 to 14.80%) is consistent with the results of the above-mentioned studies. The highest levels and significantly different levels of sucrose were observed in summer (5.51 ± 3.00 , mean \pm SD), while sucrose levels of spring and autumn (3.78 ± 2.58 and 3.40 ± 2.89 , mean \pm SD, respectively) showed no statistically significant differences ($P \leq 0.05$). A higher level of sucrose in honey samples could be related to honey production in early stages, because in this condition, sucrose has not been converted into fructose and glucose (Azeredo et al., 2003).

All analyzed samples of honey in this study had an acidic pH in the range of 3.07 to 5.20, and this is completely similar to the findings of Ouchemoukh et al. (2007), Azeredo et al. (2003) and Kayacier and Karama (2008). In general, regardless of the geographical origin of honey, it has naturally acidic pH (Saxena et al., 2010). This feature is extremely important during the honey harvest and storage, its effect on preventing growth of microorganisms, improving the stability and durability of honey. Mean pH value of honey

samples in this study (4.32 ± 0.23) was similar to the results by other researchers in different areas of the world (Andrade et al., 1999; Terrab et al., 2002; Gomes et al., 2010). According to the findings of this study, synthetic glucose and starch did not exist in any of the analyzed samples. As another physicochemical parameter, ash content of honey is affected by geographical and climatic conditions of the production site. The ash content of honey is generally low and mainly dependent upon the nectar ingredients of the plants used for honey production (Al-Khalifa and Al-Arif, 1999). In the present study, ash content of samples was within acceptable range ($0.4 \pm 0.01\%$, mean \pm SD). These results are completely consistent with the ash contents of honey samples measured in the studies by Nanda et al. (2003) and Mendes et al. (1998). In addition, the amount of ash in honey samples in various seasons showed no statistically significant differences ($P \leq 0.05$). Commercial glucose and starch are important factors in assessing the quality of honey.

According to European Commission Regulation (EU) (2002), the presence of starch is not acceptable. In this study, all samples were acceptable. Similar findings were obtained in the studies of Kahraman et al. (2010) and Aydogan et al. (1990).

In microbial assessments, yeasts were only isolated from seven samples (2.66%) and bacteria from family Enterobacteriaceae were present in

two samples (0.76%); neither of them were among significant pathogens (with biological and biochemical properties consistent with properties of genera *Providencia* and *Buchnera*). Among these contaminated samples, two samples had both yeast and bacterial contamination. These contaminated samples were all among the ones rejected in terms of physicochemical qualities. Physicochemical standards, including moisture (Singh and Bath, 1997) and sucrose (Rodrigoz et al., 2004) are directly related to the survival of micro-organisms.

Results of the present study also confirm this relationship. Although there are very few papers discussing the defined standard levels for honey microbial contents (Finola et al., 2007), the microbial evaluations showed the proper and suitable level of microbial safety of the evaluated samples. Lack of microbial contamination, antibacterial properties and desirable effect of honey produced in Northwest areas of Iran on healing injuries were confirmed in several studies (Jalali and Tajik, 2009).

Conclusion

The results of this study show that a high proportion of honey produced in Northwest of Iran had appropriate physicochemical and hygienic qualities. Continuous monitoring of quality of the honey and upgrading standards of production, processing, packaging and distribution conditions are completely necessary.

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