Biological Forum – An International Journal **6**(2): 203-207(2014)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Physico-chemical Properties in Honey from Different Zonal of East Azerbaijan

***Abolfazl Asadi-Dizaji*[*], *Fatemeh Moeni-Alishah*[**], *Yadollah Yamini*[***], *Yahya Ebrahimnezhad*[*], *Ali Asghar Yari*[*] *and Shahnaz Rouhnavaz*[*]**

**Department of Animal Science, Shabestar Branch, Islamic Azad University, Shabestar, IRAN*
***Tabriz University of Medical Sciences, Shabestar Hospital, IRAN*
****Department of Chemistry, Faculty of Sciences, Tarbiat Modares University,*

(Corresponding author: Abolfazl Asadi-Dizaji)
(Received 08 August 2014, Accepted 24 October 2014)

ABSTRACT: The present study was undertaken to determine the physico-chemical parameters of honey samples obtained from different zonal of East Azerbaijan (Iran). The 60 samples were analyzed for parameters including moisture, ash, total acidity, diastase activity, invertase activity hydroxymethylfurfural (HMF) and sucrose. Average moisture was, the ash content was 0.47%, the total acidity was 17.59 meq/kg, the diastase activity was 18.59 DN, invertase activity was 11.40 IN, the sucrose was 4.06% and the HMF was 6.03mg/kg. The results of study indicated that 92.5% of honey samples were at good quality. It is important that the essential precautions should be taken to ensure standardization and rationalization of beekeeping techniques, manufacturing procedures and storing processes to improve honey quality.

Key words: Honey, Physico-chemical, composition, Iran

INTRODUCTION

East Azerbaijan is a suitable environment for apiculture; honey production has been well developed. There are about 300,000 hives in East Azerbaijan that the total production of honey is estimated at 2,500 tons. Honey is the natural sweet substance produced by *Apis mellifera* from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature (European Union, 2002). It plays an important part in our nutrition and it is well-known for its positive effects on health. Honey contains approximately 80% carbohydrates (35% glucose, 40% fructose, and 5% sucrose) and 20% water, serving as an excellent source of energy. Also, it contains more than 180 substances, including amino acids, vitamins, minerals, enzymes, organic acids phenol compounds. Its pH is approximately 4.0 (Ouchemoukh *et al.,* 2007). The composition of honey depends on the plant species visited by the honeybees and the environmental processing and storage conditions (Guler *et al.,* 2007).

The purpose of this study was undertaken to study physicochemical quality of honey purchased in different zonal of East Azerbaijan.

MATERIALS AND METHODS

This study was realized in the food-biochemistry laboratory of the University of Shabester in 2013. A number of 60 honey samples were obtained directly from beekeepers of East Azerbaijan and kept at 4–5°C until analysis.

A. Biochemical Analysis

Total acidity, 10g of the honey samples were dissolved in 75 ml CO_2 with free distilled water and titrated with 0.1 N NaOH (AOAC, 2000) Methods No. 962.19.

The moisture content was determined by drying a weighed amount of the product at 105°C until a constant weight was obtained (AOAC, 2000) Methods No. 969.38.

Ash: Five gram of each honey sample was separately weighed out into a porcelain crucible previously ignited and weighed. Organic matter was charred by igniting the sample on a hot plate in the fume cupboard. The crucible were then placed in the in the muffle furnace and maintained at 6000C for 6 h. They were then cooled in a desiccator and weighed immediately (AOAC, 1990). The percent Ash was calculated as:

$$\text{Ash (\%)} = \frac{\text{(Weight of crucible + ash) – (Weight of empty crucible) x 100}}{\text{Sample weight}}$$

Diastase activity: Determination of diastase activity was done in order to compare the activity of both enzymes. It was evaluated spectrophotometrically using the Shade method. The diastase activity is calculated as diastase number (DN). DN expresses units of diastase activity (Go the unit). One unit is defined as the amount of enzyme that will convert 0.01 g of starch to the prescribed end-point in 1h at 40°C under the conditions of test (Bogdanov *et al.*, 1997).

Invertse activity was determined according to the method of Siegenthaler (1977) which is based on the spectrophotometric measurement of decomposition of p-nitrophenyl-α-d-glucopyrinoside (p-NPG) in pnitrophenol and is determined spectrophotometrically at 400 nm. The honey invertase activity was calculated from the measured absorbency multiplying by the factor of 158.94 and calculated to a kilogram of honey. Then the value was expressed as invertase number (IN). The IN indicates the amount of sucrose per gram hydrolyzed in 1h by the enzymes contained in l00g of honey under test conditions. Each sample was analyzed two times. The results are expressed in units of the enzyme per kilogram (U/Kg).

Hydroxymethylfurfural (HMF), was done using the Winkler method where the solution of the tested honey when reacting with p-toluidin and barbituric acid and in the presence of hydroxymethylfurfural (HMF) gives a wine-red compound (Bogdanov *et al.*, 1997). The absorption was measured at 550 nm on a one-ray Lambda II (fu Perkin Elmer, USA). Concentration of HMF was determined with the help of a calibrated line using the method of linear regression. Each sample was analyzed in three parallel determinations.

Sucrose was determined according to AOAC (2000).

RESULTS AND DISCUSSION

There were significant differences ($P<0.05$) in Moisture, ash, total acidity, invertase and diastase activity, Sucrose and HMF in the different zonal of East Azerbaijan (Table 1).

Table 1: Means of physico-chemical Different zonal of E. Azerbaijan.

Different zonal of E.Azerbaijan	Moisture (%)	Ash (%)	Total acidity (meq/ Kg)	Diastase activity (DN)	Invertase activity (IN)	Sucrose (%)	HMF (mg/kg)
Ajabshir	16.33^{d}	0.50^{abcd}	17.62^{c}	15.00	5.50^{ab}	4.87^{d}	5.62^{bcde}
Azarshah	17.30^{c}	0.450^{edf}	18.01^{ab}	14.25	2.05^{bc}	3.50^{c}	4.61^{a}
Hashtrod	15.98^{d}	0.413^{ef}	18.19^{a}	23.25	18.25^{a}	2.40^{ab}	5.12^{abcd}
Jolfa	18.17^{ab}	0.465^{ced}	16.36^{e}	12.75	2.00^{bc}	6.66^{ef}	5.24^{abcde}
kalaybar	18.62^{a}	0.448^{edf}	16.20^{e}	14.50	2.50^{bc}	6.98^{f}	8.34^{g}
Maragheh	16.00^{d}	0.470^{cde}	18.15^{ab}	15.30	8.25^{ab}	2.64^{ab}	4.90^{abc}
Marand	16.55^{d}	0.543^{ab}	18.03^{ab}	22.67	16.30^{a}	3.42^{c}	5.89^{bcde}
Meyaneh	18.49^{a}	0.329^{g}	16.55^{e}	22.25	15.40^{a}	6.11^{e}	8.42^{g}
Oskou	16.43^{d}	0.492^{bcd}	18.10^{ab}	23.25	18.01^{a}	2.92^{bc}	6.61^{ef}
Sarab	18.13^{ab}	0.394^{f}	17.06^{d}	16.72	11.74^{ab}	5.37^{d}	6.17^{cdef}
Shabesta	16.38^{d}	0.562^{a}	18.08^{ab}	16.77	14.12^{a}	3.07^{bc}	7.44^{fg}
Sofyan	17.33^{c}	0.437^{def}	18.17^{a}	20.30	15.30^{a}	2.45^{ab}	6.44^{def}
Tabriz	17.62^{bc}	0.548^{ab}	17.69^{abc}	24.12	21.02^{a}	3.61^{c}	5.58^{b}
Tasouj	18.45^{a}	0.517^{abc}	18.23^{a}	23.00	17.00^{a}	1.98^{a}	5.79^{bcde}
Zonouz	17.58^{bc}	0.529^{abc}	17.49^{cd}	14.75	3.60^{abc}	4.95^{d}	4.23^{a}
SEM	0.2310	0.0203	0.1675	3.0567	0.4661	0.2453	0.4191
P-Value	<.0001	<.0001	<.0001	0.0602	<.0001	<.0001	<.0001
Mean of East Azerbaijan	17.29	0.473	17.59	18.59	11.40	4.06	6.0313

Means with different superscripts in the same column represent significant difference at ($P < 0.05$)

The water content of honey varied from 15.98 % in Hashtrod, to 18.62% in kalaybar. The average was 17.29%. This moisture variation can be explained by the composition and floral origin of honey samples. The strong interaction of sugar with water molecules may decrease the water available for microorganisms. Honey is hygroscopic and will remove moisture from the air. The low moisture content of honey also forms an important part of the system which protects honey from attack by microorganisms. The hyper osmotic nature of honey would prevent the growth of bacteria and yeasts as it draws water out of the organism, killing them by desiccation.

The moisture content of honey is widely related to the harvest season in East Azerbaijan and the level of maturity released in the hive. This parameter is highly important for the shelf life of the honey during storage. According to Codex Alimentarius (2001) and EU (2000) standard of honey samples, the maximum value of moisture content in honey is 21%, this is in contrast to our findings in the present work. The moisture content of the present study similar results was detected by (Al-Khalifa and Al-Arify, 1999; Duman Aydin, et al., 2008; Nanda, *et al.*, 2003).

The floral origin has been reported to be responsible for the differences in ash content (Fredes and Montenegro, 2006) and it is also a quality criterion for honey botanical origin (European Commission, 2000). Results of the ash content obtained in this study were varied from 0.329 % in Meyaneh, to 0.562% in Shabesta. This result similar results was detected by (Karabournioti and Zerualaki, 2001; Mouteria, *et al.*, 2003). These differences in mineral content are dependent on the type of soil in which the original nectar bearing plant was located (Anklam, 1998).

The total acidity of honey varied from 16.20 (meq/ Kg) in Kalaybar, to 18.23 (meq/ Kg) in Tasouj. The average was 17.59 (meq/ Kg). Similar results were detected by Rameres et al., (2000); Ozcan, *et al.*, (2006). The acidity of honey is due to the presence of organic acids, particularly the gluconic acid, in equilibrium with their lactones or esters and inorganic ions such as phosphate and chloride (Al-Khalifa and Al-Arify, 1999). The acidity of honey developed due to the presence of organic acids. A high total acidity may mean that the honey had fermented at some time, and that the resulting alcohol was converted into organic acid (Rodgers, 1979)

The results from invertase determinations in different zonal of East Azerbaijan honeys are presented in Table 1. It was estimated that invertase activity in the different zonal honeys was 11.40 IN on average. The minimum value of the invertase activity in the Jolfa honeys was 2 IN and the maximum value Tabriz honeys was 21.02 IN. The use of diastase activity as an indicator of freshness, as is common practice for *Apis mellifera* honeys. However, invertase is a parameter that is not normally considered for these ends, in spite of being more sensitive to heat, and it rather therefore a parameter to be used for measuring the quality of honey. White *et al.*, (1964) and White (1994) demonstrated that invertase was destroyed more quickly than amylase when honey was heated, so invertase activity could be a better indicator of honey quality than diastase activity. Dustmann (1993) states that invertase, in combination with other analytical criteria, is able to detect damage caused to the quality of the honey due to overheating or to long periods of storage.

The variability in enzyme activity found in the different honey types is probably due to a series of factors, such as: nectar collection period (and consequently the physiological stage of the colony); abundance of nectar flow and its sugar content (a high flow of concentrated nectar lead to lower enzyme content); age of the bees (when the honey bee becomes a forager its glands produce more digestive enzymes); pollen consumption, etc (Simpson *et al.*, 1968; Fluri *et al.*, 1982; Brouwers, 1982, 1983; Huang *et al.*, 1989a, 1989b).

The Sucrose of honey varied from 1.98 % in Tasouj, to 6.98 % in kalaybar. The average was 4.06%. Our findings showed approximately similarity with the results of Gul and Sahinler (2002), Mouteria *et al.*, (2003) and Tchoumboue *et al.*, (2007). The level of sucrose differs according to the maturity degree and origin of the nectar compound of the honey.

The minimum value of the HMF in the Zonouz honeys was 4.23 mg/kg and the maximum value Meyaneh honeys was 8.42(mg/kg). The average was 6.03 mg/kg. Our findings were appropriate to TSE, CODEX and EU standards and lowest Italy honeys (7.80 mg/kg, Esti *et al.*, 1997) and Turkey 25.9 mg/kg, Akyuz, *et al.*, (1995). The diastase activity and the HMF content are widely recognized as parameters indicating the freshness of honey (Mendes et al., 1998; Terrab *et al.*, 2002). The variation in the HMF may be related to source of honey as well as climate of region (Singh and Bath, 1997).

CONCLUSION

The analytical work was carried out on 60 samples in this study completely agree with the European Commission and the Codex Alimentarius indicating adequate processing, good maturity and freshness. Honey samples that are available commercially different quality on account of various factors like seasons, packaging and processing conditions, floral source, geographical origin, and storage period. The results obtain of this study very important for the commercialization of the Iran honey.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support of Shabestar Branch Islamic Azad University. This work is a part of the research project (Research project code 51952890526005).

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