Wageningen Academic Publishers

Assessment of the physicochemical quality of Iranian honey

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Received: 6 June 2014 / Accepted: 2 September 2014

RESEARCH ARTICLE

Abstract

Honey samples supplied by Iranian beekeepers in different regions of Iran were analysed for moisture, acidity, diastase activity, hydroxymethylfurfural (HMF), invert sugar, sucrose, ash and proline content. The mean values found were 18.35±5.6% for moisture, 24.45±12 meq/kg for acidity, 199.87±96.55 DN for diastase activity, 17.66±15.35 mg/kg for HMF, 37.31±17.13% for invert sugar, 2.62±1.9% for sucrose, 0.53±0.21% for ash, and 20.96±11.6 mg/kg for proline. The honey sample from Markazi province had the highest proline content and the highest diastase activity was observed in the honey sample from Isfahan. Honey samples from Mazandaran, Tabriz, and Ardebil had moisture contents that were greater than the EU standard (20%). The highest quality honey samples were from Kurdistan followed by Kermanshah, which showed higher quality in terms of moisture content, proline, HMF, and diastase activity.

Keywords: food quality, honey, hydroxymethylfurfural, physicochemical, Iran

1. Introduction

Honey is a naturally sweet substance produced by honey bees from the nectar of plants, the secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants. The bees collect these and process them by combining with substances from their bodies and then deposit it in a honeycomb to ripen and mature. Honey is used as an effective traditional healing agent and to prevent illness.

Honey is used to treat sore throats, wounds, and burns (Lay-flurrie, 2008). According to previous studies, honey is a mixture of fructose (about 38.4%), glucose (about 30.3%), sucrose (about 1.3%), other carbohydrates (about 12%), minerals (about 0.169%) and proteins (169 mg/100g), with a water content of about 17.2%. The pH of honey ranges from 3.4 to 6.1 with an average of 3.9. Water activity varies from 0.5 to 0.6 (Iurlina and Fritz, 2005; Kretavicius *et al.*, 2010).

The properties and composition of honey vary and depend primarily on the floral source, season, variety of bee, length of storage in the honeycomb, mode of harvesting, and postharvest storage (Moniruzzaman *et al.*, 2014). Physicochemical criteria for honey are specified in detail by the European Union. The major criteria are moisture content, electrical conductivity, ash content, reducing and non-reducing sugars, free acidity, diastase activity, and hydroxymethylfurfural (HMF) content (EC, 2001). Acidity and pH of honey are the most important factors for antibacterial activity of honey (Moussa *et al.*, 2012). Determination of the honey moisture content is necessary because of resistance to spoilage by yeast fermentation during storage (Ahmed, 2012). HMF content in fresh honey is very low or nonexistent, its concentration increases in the course of storing (in relation to pH, the length of storing) and also in the course of the honey heating (BartáKoVá *et al.*, 2011). The level of diastase is relatively easy to measure and have been used to estimate the extent of heating to which a honey has been exposed (Bogdanov *et al.*, 1999). Prolin content is a measure of the level of total amino

acids present (Meda *et al.*, 2005). It can also serve as an additional determinant of quality and in some case also as a criterion for estimating the maturity of honey as well as an indicator for detecting sugar adulteration (Bogdanov *et al.*, 2002; Meda *et al.*, 2005). Comparative physical and chemical characterisation of different types of honey have has been studied extensively (Azeredo *et al.*, 2003; Guler *et al.*, 2007; Ouchemoukh *et al.*, 2007; Rodriguez-Otero *et al.*, 1994; Sancho *et al.*, 1992). The present study investigated the variation in the physicochemical properties of honey produced in different geographical regions of Iran. Of the measures for the differentiation of this study in comparison with other local studies which have been done in Iran (Aarabi *et al.*, 2013; Mahmoudi *et al.*, 2012), the present study includes the north, west, east and centre of Iran.

2. Materials and methods

Honey samples

A total of 60 honey samples (each samples 500 g) were collected directly from beekeepers in 10 provinces of Iran (6 different beekeepers in each province), including Mazandaran, Ardabil, Zanjan, Tabriz, Hamedan, Kurdestan, Kermanshah, Markazi, Isfahan and Qom with different climates (dry, wet, cold, tropical, etc.). Ardabil, Tabriz, Zanjan, Hamedan, Mazandaran and Markazi are cold provinces and Mazandaran province is wetter than others. Qom province is tropical and the others are moderate. All of the honey samples were poly-floral and stored at 4 °C away from light until analysis. Then the samples were analysed to determine the following physicochemical characteristics: moisture, ash, sucrose, free acidity, HMF, proline, and diastase activity. The analysis was performed in triplicate.

Physicochemical properties

Moisture content

Moisture was measured using the refractometric method. The refractive index generally increases as the solid content increases. The refractive indices of honey samples were measured at ambient temperature using an Abbe refractometer (Germany). All measurements were performed on 100 g honey at 20 °C by adding a correction factor of 0.00023 °C to obtain the corresponding percentage of moisture from the refractive index by consulting a standard table (AOAC, 1990).

Ash content

AOAC method 942.05 (AOAC, 2012) was followed: 5 g of honey was placed in a combustion pot and the sample was preheated using a gas flame to avoid foaming. Afterward, the samples were incinerated at a high temperature (550 °C) in a muffle for 5 h. The ash was cooled to room temperature and weighed.

Free acidity

Free acidity was determined by potentiometric titration (AOAC method 962.19; AOAC, 1990). Before analysis, the honey sample was homogenised in a water bath and filtered through gauze. Then, 10 g of honey were dissolved in 75 ml of distilled water and a solution of phenolphthalein was added. The solution was titrated with 0.1 N NaOH. The volume of alkali used was calculated as follows:

$$\text{percentage titratable acidity} = (1 \times \text{EW acid} \times \text{normality of NaOH} \times \text{titre} \times 100)/\text{weight of fresh sample} \quad (1)$$

Where EW is the equivalent weight.

Diastase activity

Diastase activity (AOAC method 958.09; AOAC, 1990) was determined using a buffered solution of soluble starch and honey incubated in a thermostatic bath at 40 °C. Thereafter, 1 ml aliquot of the mixture was removed at 5 min intervals and the absorption of the sample was examined at 660 nm in a PerkinElmer luminescence spectrophotometer (PerkinElmer, Norwalk, CT, USA). The diastase value was calculated using the time taken for absorbance to reach 0.235. The results were expressed in degrees as the amount (ml) of 1% starch hydrolysed by an enzyme in 1 g of honey for 1 h.

Hydroxymethylfurfural

HMF was determined using the standard AOAC method (AOAC method 980.23; AOAC, 1990) where 5 g of honey were dissolved in 25 ml of distilled water and treated with a clarifying agent (0.5 ml of Carrez I and 0.5 ml of Carrez II solution), The amount of solution was then increased to 50 ml. The solution was filtered and the first 10 ml was discarded. The absorbance of the filtered solution was measured at 284 and 336 nm against an aliquot of filtered solution treated with $NaHSO_3$. The HMF was determined as:

$$\text{HMF in 100 g of honey} = (\text{Abs}_{284} - \text{Abs}_{336}) \times 14.97 \times (5 \text{ g of sample}) \quad (2)$$

Where Abs_{284} and Abs_{336} are the absorptions at the wavelength of 284 and 336 nm, respectively.

Apparent sucrose

The apparent sucrose were determined by potentiometric titration using the Fehling test (Lane and Eyon modified method; ISIRI, 1998).

Proline content

The proline content was determined using the standard AOAC method (AOAC method 979.20; AOAC, 2005): 2.5 g honey was weighed into a baker and dissolved in about 25 ml distilled water. The solution was then transferred quantitatively to a 100 ml volumetric flask, diluted using distilled water and shaken. Afterward, 1 ml of the sample solution was poured into each of 2 tubes with 0.5 ml of formic acid (98-100%) and 2 ml ninhydrin solution (3% in ethylene glycol monomethylether). The tubes were capped carefully and shaken vigorously.

Then, 1 ml of distilled water was added to 1 tube instead of sample solution and the procedure was repeated. The tubes were placed in a boiling water bath for 15 min and then transferred to a water bath at 22 °C for 10 min. Afterward, 10 ml of 2-propanol-water solution (1:1) was added to each tube at regular intervals. At 22 °C, the tubes were removed for 35 min and the absorbance was determined at 520 nm. Strict control of the timing of each step was critical. The honey colour was corrected by determining the absorbance of the solution containing 1 ml of sample solution, 2.5 ml distilled water and 10 ml 2-propanol. This value was substarted from the absorbance of the sample before estimation. Blank reactions were also made and considered.

Statistical analysis

Statistical analysis was done using SAS software (version 9; SAS Institute Inc., Cary, NC, USA). For the results, $P<0.05$ was considered to be significant.

3. Results and discussion

Table 1 shows the means, standard deviations, and ranges for the different parameters. The differences between physicochemical parameters in the honey by region are also listed in Table 1.

The moisture content of honey depends on the harvesting season, maturity of the hive, and environmental conditions and can vary from year to year (Finola *et al.*, 2007; Gomes *et al.*, 2010). The mean moisture content of the samples varied from 12 to 28% (Table 1). The samples from Mazandaran, Tabriz and Ardebil had means of 26.08, 22.47 and 28%, respectively, but all other samples had mean moisture contents of <20%, which is the maximum prescribed limit for Codex and EC standards (Codex, 2001; EC, 2001). The high values for the first three samples are probably a result of the unusually wet conditions in north-western Iran and early extraction of honey from the hives.

High moisture content generally causes the honey to spoil and lose flavour, decreasing quality (Costa *et al.*, 1999). It accelerates crystallisation in certain types of honey and increases water activity to allow the growth of yeasts and fermentation during storage (Gomes *et al.*, 2010). Previous studies have reported moisture contents well below the recommended limit of 20% (EC, 2001), which is in agreement with the present study for most cities (Esti *et al.*, 1997; Gomes *et al.*, 2010; Islam *et al.*, 2012; Kirs *et al.*, 2011; Mateo and Bosch-Reig, 1998).

The ash content is an indicator of mineral content. It is considered to be a quality scale that points to the botanical and geographical origins of the honey (Saxena *et al.*, 2010). The mean ash content of the honey samples varied from

Table 1. Physicochemical parameters of honey samples (mean ± standard deviation (SD), n=6).

	Moisture (%)	Ash (%)	Acidity (meq/kg)	Diastase (DN)	HMF (mg/kg)	Invert sugar (%)	Sucrose (%)	Proline (mg/kg)
Kurdistan	13.85±3.75^{a}	0.51±0.33^{a}	23.05±2.29^{d}	29.7±0.92^{d}	0.92±0.38^{a}	21.8±3.33^{a}	0.13±0.04^{a}	231.83±35.39^{cdf}
Zanjan	17±5.41^{ab}	0.35±0.21^{a}	34±2.85^{e}	3.72±0.55^{a}	22.5±2.68^{c}	34±2.92^{b}	1.45±0.26^{ab}	167.67±64.95^{ad}
Mazandaran	26.08±6.04^{d}	0.26±0.22^{a}	39.92±3.09^{f}	15.63±1.48^{b}	16.13±2.08^{b}	54.27±1.6^{d}	5.53±1.07^{f}	354.17±64.51^{g}
Isfahan	19.2±3.02^{ac}	0.73±0.48^{a}	8.55±1.57^{a}	42.93±1.97^{e}	41.52±5.61^{e}	16.57±0.63^{a}	3.58±1.24^{ce}	192.33±70.88^{bcde}
Tabriz	22.47±3.07^{bcd}	0.17±0.17^{a}	21.73±2.19^{cd}	30.88±2.58^{d}	37.98±4.65^{e}	55.63±3.85^{d}	0.29±0.18^{a}	85.53±17.15^{a}
Kermanshah	12.07±0.74^{a}	0.8±0.71^{a}	32.02±2.24^{e}	22.55±1.98^{c}	11.47±1.41^{b}	65.33±5.54^{e}	0.61±0.26^{a}	270.67±59.73^{efg}
Markazi	15.27±2.08^{a}	0.68±0.26^{a}	18.12±2.26^{c}	17.9±1.02^{b}	1.75±0.6^{a}	19.35±2.34^{a}	3.38±1.58^{cd}	333.17±34.35^{g}
Ardabil	28±4.62^{d}	0.5±0.25^{a}	42.25±1.81^{f}	6.7±1.04^{a}	0.45±0.24^{a}	23.28±2.95^{a}	4.63±0.76^{def}	124±22.17^{ab}
Hamedan	17.52±1.88^{a}	0.62±0.58^{a}	11.89±0.86^{ab}	21.62±0.99^{c}	32.15±3.62^{d}	41.02±5.95^{bc}	3.98±0.72^{cf}	77.83±18^{a}
Qom	12±0.87^{a}	0.68±0.52^{a}	13±0.85^{b}	18.02±1.12^{b}	11.73±0.97^{b}	41.87±6.02^{c}	2.63±0.84^{bc}	161.5±51.76^{ac}
Mean ± SD	18.35±5.6	0.53±0.21a	24.45±12	20.96±11.6	17.66±15.35	37.31±17.13	2.62±1.9	199.87±96.55
Satisfactory limit by EU	≤20	≤0.6	≤40	≥8	≤40	≥60	≤5	≥180

0.17 to 0.8% (Table 1). The ash content of all samples, except for those from Isfahan, Kermanshah, Markazi, Hamedan, and Qom were in accordance with EC standards of 0.6% (EC, 2001). The mean ash content can be affected by harvesting methods, beekeeping techniques, and material that collect on the bees during their exploration of flora (Finola *et al.*, 2007). Also there has been found a straight linear relationship between free acidity and ash content of the samples. The linear relationship may be due to the presence of some inorganic ions like phosphate. Sulfate and chloride in ash, which can contribute to a rise in free acidity (Mehryar *et al.*, 2013). Several studies have reported on mean ash contents in agreement with EC 2001 (Ahmed *et al.*, 2007; Gomes *et al.*, 2010; Kahraman *et al.*, 2010; Ojeda de Rodríguez *et al.*, 2004; Saxena *et al.*, 2010).

The free acidity of honey is a result of fermentation of sugar into organic acids, especially gluconic acid, in balance with the corresponding lactones or internal esters and inorganic ions such as phosphate and chloride (Al-Khalifa and Al-Arify, 1999). The mean total acidity of the samples varied from 8.55 to 42.25 meq/kg, which meets EC standards of <50 meq/kg (EC, 2001). Similar results have been detected by previous studies (Anupama *et al.*, 2003; Azeredo *et al.*, 2003; Gomes *et al.*, 2010; Iurlina and Fritz, 2005; Kahraman *et al.*, 2010; Kirs *et al.*, 2011). Diastase activity and HMF content are parameters used to evaluate the freshness of honey (Sancho *et al.*, 1992; Terrab *et al.*, 2002). Multiple factors influence HMF, including temperature, duration of heating, storage conditions, pH and floral source (Fallico *et al.*, 2006). HMF is formed during acid-catalysed dehydration of hexoses and is found to be present even in fresh honeys. The concentration of HMF increases with storage and prolonged heating of honey, although even storage at ambient temperatures increases HMF concentration in honey. HMF value is virtually absent or very low in fresh honey and is high in honey that has been heated. Stored in non-adequate conditions, or adulterated with invert syrup (Ajlouni and Sujirapinyokul, 2010; Mehryar *et al.*, 2013). The mean HMF for all samples except for those from Isfahan were <40 mg/kg. The mean diastase activity for all samples except for those from Zanjan and Ardebil were >8 DN. These results indicate that the samples (except for those listed) were below the maximum limit of 40 mg/kg for HMF and 8 DN for diastase (EC, 2001). Mean diastase activity of 23.1, 19.7, 17.9 and 39.1% have been reported by Esti *et al.* (1997), Şahinler and Gül (2004), Cantarelli *et al.* (2008), and Kirs *et al.* (2011), respectively. Significantly lower mean HMF values have been reported in previous studies. Kirs *et al.* (2011) reported a mean of 3.8 mg/kg, Devillers *et al.* (2004) reported 3.28 mg/kg and Esti *et al.* (1997) reported 7.80 mg/kg. Azeredo *et al.* (2003) reported low HMF values of between 3.06 and 43.81 mg/kg.

The mean sucrose content in the samples was 0.13 to 5.53%. Except for the sample from Mazandaran, all other samples had sucrose levels below 5%, which is the maximum prescribed limit according to Codex and EC standards (Codex, 2001; EC, 2001). High sucrose content can result from early harvest, overfeeding honeybees with sucrose syrup, and sucrose that did not convert to fructose and glucose (Azeredo *et al.*, 2003; Guler *et al.*, 2007). Anupama *et al.* (2003) reported mean sucrose contents of 1.2 to 5.7%. Mateo and Bosch-Reig (1998) reported mean sucrose contents of 0.062 to 4.24%. Saxena *et al.* (2010) reported mean sucrose contents of 0.4 to 8.8%.

Previous studies have reported free amino acid contents of 12% to 21% for different types of honey. Proline is the main component (50-80%) of the total amino acid content of honey. It is produced primarily from salivary secretions during the conversion of nectar into honey (Hermosín *et al.*, 2003; White and Doner, 1980). The samples of the present study had mean proline concentrations of 77.83 to 354.17 mg/kg. Five of the 10 regions had mean proline levels above 180 mg/kg, which complies with the accepted value from EC (2001). Moniruzzaman *et al.* (2013) reported mean proline concentrations of 184.75 to 564.91 mg/kg. Islam *et al.* (2012) found mean proline contents of 106.9 to 2932.8 mg/kg.

4. Conclusions

The physicochemical properties of 60 honey samples from 10 geographical regions of Iran were investigated. The study showed that the environmental and geographical locations of the hives affected the physicochemical properties. The honey sample from Markazi province had the highest proline content and the highest diastase activity was observed in honey from Isfahan. Free acidity and HMF were in accordance with EC standards. In 5 of 10 regions, the samples exceeded EC standards for mean ash content. Honey samples from Kurdistan, followed by samples from Kermanshah, showed the best values for moisture content, proline, HMF, and diastase activity. The variation in values appears to be the result of variations in environmental conditions; especially variety of climate, floral source, and storage conditions.

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