PHYSICOCHEMICAL ANALYSIS OF TIGRAY HONEY: AN ATTEMPT TO DETERMINE MAJOR QUALITY MARKERS OF HONEY

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ABSTRACT. The objective of this study was to assess quality of Tigray honey and content of selected metals (Cu, Co, Cr, Ni, Fe, Zn, Cd, Mn). Thirty honey samples from various areas of Tigray Region, Ethiopia were collected and analyzed for major quality parameters (pH, electrical conductivity, moisture content, ash content and levels of selected metals). Moisture content had a value of 18.6 (minimum) and 18.8 (maximum) indicating optimum harvesting and good degree of maturity. All honeys analyzed in this work had ash contents less than 0.6%, indicating that they were more likely to be floral than honeydew origin. Electrical conductivity varies from 8.27 ± 0.02 µS/cm (Hawze) to 33.5 ± 0.2 µS/cm (Abiy Adi). Values recorded for honey pH ranged from 3.82 ± 0.01 (Hawzen) and 4.45 ± 0.01 (Abiy Adi). In general the Tigray honey shows relatively acidic behavior which is similar to other honeys in the world. The concentration of trace metals in honey was more or less different depending on the sampling area. The highest concentration was observed for iron, copper, nickel, manganese, zinc, chromium, cobalt and cadmium in that order. The highest concentration of metals was observed in Adigrat honey and then Hagereselam, Abiy Adi, Hawzezen and the least for Atsbi.

KEY WORDS: Honey, Moisture content, Electrical conductivity, Tigray, Ash content, pH, Mineral content, Bioindicator

INTRODUCTION

Honey is the natural sweet substance produced by Apis mellifera bees from the nectar of blossoms or from the secretion of living parts of plants or excretions of plant-sucking insects living on parts of plants, which honey bees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature [1-3]. It is essentially composed of a complex mixture of carbohydrates (of which fructose and glucose are major ones) and other minor substances, such as organic acids, amino acids, proteins, minerals, vitamins and lipids [2-10].

Honey is a complex mixture and presents very great variations in composition and characteristics due to its geographical and botanical origin, its main features depending on the floral origin or the nectar foraged by bees [10-13]. The composition and quality of honey also depend on several environmental factors during production such as weather and humidity inside the hive, nectar conditions and treatment of honey during extraction and storage. The composition of honey varies with the feeding of the bees [14]. Honey has been reported to contain more than 180 substances and is considered as an important part of traditional medicine [8, 15]. Honey has numerous uses and functional applications worldwide such as in food systems, religious and magical ceremonies as well as in human and veterinary medicine [16]. It is a very important energy food and is used as an ingredient in hundreds of manufactured foods, mainly in cereal-based products, for its sweetness, color, flavor, caramelization, pumpability and viscosity [17].

Honey characterization is based on the determination of its chemical, physical or biological properties [7]. Physicochemical parameters such as electrical conductivity, ash content, water

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content, free acid and pH have all been suggested as criteria for the characterization of honeys [7, 14]. Electrical conductivity is a good criterion of the botanical origin of honey. Blossom honeys and mixtures of blossom and honeydew honeys should have less than 0.8 mS/cm and honeydew honeys should have more than 0.8 mS/cm [6]. The electrical conductivity of the honey is closely related to the concentration of mineral salts, organic acids and proteins and proved useful for discriminating honeys of different floral origins [18]. The ash content in honey is generally small and depends on nectar composition of predominant plants in their formation [8]. As such, the variability in ash contents has been associated in a qualitative way with different botanical and geographical origins of honeys [19]. The total content of elements or ash must be lower than 0.6% for nectar honey and lower than 1.0% for honeydew honey [20, 21].

Most honeys are supersaturated solutions of fructose and glucose with low pH between 3.2 and 4.5. This relatively acidic pH level prevents the growth of many bacteria. This value could be due to the presence of some weak organic acids primarily gluconic acids, ascorbic acid and even acetic acid [11, 12, 22]. Moisture content of honey depends on harvest season, along with the degree of maturity reached in the hive. This parameter is highly important for the shelf-life of the honey and its stability against fermentation and granulation during storage [23, 24]. The moisture content of samples in permitted levels was found in the range of 13.6-19.4% [17]. Moisture content of honey depends on the environmental conditions and the manipulation from beekeepers at the period of harvest [25].

Minerals seem to be good candidates for a classification system of honey, mainly because they are stable and can be associated to the soil where melliferous flora grows. Bees are estimated to forage on plants growing in a relatively large area of more than 7 km$^2$. It is because of this large surface area that honey bees and their products have been proposed as suitable bioindicators of chemical pollution [26]. Minerals can be highly indicative of the geographical origin of honey and can be used as environmental indicators [18, 27, 28]. Bee honey can be a good source of major and trace elements needed by humans [29, 30]. The general features and elemental composition of honey depend on its botanical and geographical origin [5, 15, 30]. The concentration of mineral compounds ranges from 0.1% to 1.0% that varies widely depending on the particular botanical origin, pedoclimatic conditions and extraction technique [31]. Different researchers used various methods for the determinations of mineral contents of honey, such as flame atomic absorption spectrometry (FAAS), electrothermal atomic absorption spectrometry (ETAAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), ion chromatography and voltammetry [22, 32].

There are about 10 million bee colonies and over 800 identified honey-source plants in Ethiopia. The annual honey and beeswax production are estimated at 24,700 tons and 3,200 tons respectively. More than 90% of the honey produced is used in the country for domestic consumption. Ethiopia is the first honey-producing country in Africa and the fourth beeswax-producing country in the world, after China, Mexico and Turkey. Studies show that, under modern management, the traditional yield of 5 kg of honey in one harvesting season can be improved to 15-20 kg [33]. The major honey and beeswax producing regions in Ethiopia are Oromia Region, the Southern Nations, Nationalities, and Peoples Region (SNNPR) and Tigray Region [34]. The amount of honey produced in the Tigray Region is almost 15% of the global production of Ethiopian honey. The Tigray Region can be divided into two honey production areas: North and South with two seasons of harvest in a year. The first one is called the prime season and runs from September to December [35]. The main purpose of this study was to assess physicochemical properties of Tigray honeys (electrical conductivity, ash content, moisture content and pH) and elemental composition of some selected metals (iron, manganese, zinc, copper, nickel, chromium, cobalt and cadmium). In addition to this, the research would

give the current quality of Tigray honey and also would initiate other researchers to perform more research activities.

EXPERIMENTAL

Chemicals and reagents. Nitric acid (HNO₃, Spectrosol®, England) was used for digesting honey samples. Distilled water was used for all dilutions. All plastic and glassware were cleaned by soaking in dilute HNO₃ (1:9) and rinsed with distilled water prior to use.

Apparatus and/or instruments. The apparatus used included automatic pipettes, oven ("Industrial Furnace" Supreme Enterprises, India), PHZ11 pH meter (Hanna, USA), Jenway 4200 electrical conductivity meter (Chelmsford, England), Abbe refractometer (Analytik Jena, Belgium), Spectra AA – 20 plus flame atomic absorption spectrometer (Varian Australia Pty Ltd. Mulgrave Victoria, Australia in Ezana Mining Development PLC, Mekelle, Ethiopia) and analytical balance (0.1 mg accuracy, Lark, China).

Sample area identification and sample collection. Honey samples were collected from the known apiculture areas in Tigray Region, Ethiopia, namely Atsbi (13°52'N 39°44'E), Adigrat (14°16'N 39°27'E), Hawzene (13°58'N 39°26'E), Hagereselam (13°39'N 39°10'E) and Abiy Adi (13°37'N 39°00'E). A total of 30 samples of honey of different types were collected two times from the five selected sampling areas. Preliminary information was collected from the local peoples on honey collection. Before being analyzed, all the honey samples were kept at room temperature and protected from light.

Procedure. Analysis of each sample was carried out in triplicate for each test for determining the physicochemical properties (average moisture content, total ash content, electrical conductivity, pH and mineral content) of Tigray honey.

Determination of pH. The pH of a 10% (w/v) solution of homogenized honey prepared in boiled warm water was measured by a pH-meter [6, 7, 18]. The pH meter was calibrated using standard buffers of pH 4.0, 7.0 and 9.0 prior to measuring the pH of the samples.

Determination of electrical conductivity. A 10% w/v (about 1 g of honey and about 9 mL of boiled warm water) solution of honey in freshly warm distilled water was dissolved using glass rod in a 50 mL beaker [18]. The conductivity meter was calibrated using standard calibration solution at 20 °C.

Determination of moisture content. Moisture contents were determined using a refractometric method reading at 20 °C. About 20 g of honey samples were placed in high density double cap plastic bottles, labeled and closed firmly so as no water or moisture enter into it. The samples were kept in oven at 60 °C for about half an hour so as to get liquid samples [7, 13]. Then the refractive index values were measured and obtaining the corresponding % moisture (g/100 g honey) from the refractive index of the honey sample by consulting a standard conversion table (Chataway table or Wedmore’s table) [10].

Determination of total ash content. Stainless steel crucibles were washed, rinsed with distilled water and oven dried at 105 °C. About 5-10 g of honey samples were weighed and placed in a furnace first at 110 °C for half an hour and then at 550 °C for two hours to constant weight. Care was taken during heating so that no excess foaming took place. Then the honey samples were kept in open air for cooling and the masses after heating were measured. Finally ash content was calculated as g ash/100 g of honey.
Optimization of the procedure for digestion of honey samples. The honey samples were treated with different acids for the acid-digestion methods. After performing repeated trials the best digestion procedure was obtained using concentrated nitric acid aided by heat. Then the procedure was optimized for amount of acid, amount of honey sample, temperature, digestion time and level of dilution. A 2.0 g portion of honey samples was measured with analytical balance with 50 mL-volume Pyrex beaker and then 4.0 mL conc. HNO₃ was added using automatic pipette. The beaker stood for 10 min to complete the digestion and then first kept at 50 °C for 15 min and next for another 15 min at 90 °C. Care was taken during heating so that no excessive foaming took place. After cooling the samples, each sample was poured into 25 mL-volumetric flask and diluted to the mark with distilled water making ready for analysis. A blank solution was prepared following similar procedure. Moreover one sample was deliberately repeated to check consistency throughout the entire activity.

Determination of mineral content. The content of selected metals (copper, nickel, iron, manganese, zinc, chromium, cobalt and cadmium) was determined at 324.8, 232.0, 248.3, 279.5, 213.9, 357.9, 240.7 and 228.8 nm, respectively, and using air-acetylene flow where the acetylene flow was made 1.5) was done in triplicate using flame atomic absorption spectroscopy.

RESULTS AND DISCUSSION

Based on the above procedure results summarized in Table 1 were obtained for the major honey quality indicator parameters: pH, electrical conductivity, moisture content and total ash content.

The pH of Tigray honey. All the Tigray honeys analyzed were found to be acidic in character irrespective of their variable geographical origin. Their pH values ranged from 3.82 (Hawzene) to 4.45 (Abiy Adi) (Table 1), which are more or less similar to the pH values of the honeys of Algeria (3.49 to 4.53) [6, 10], Brazil (3.10 to 4.05) [10], India (3.70 to 4.40) [10], Portugal (3.70 to 4.30) [7], Spain (3.63 to 5.01) [10] and Turkey (3.67 to 4.57) [10]. Honey pH values are of great importance during extraction and storage as they influence texture, stability and shelf-life [13]. The low pH of honey inhibits the presence and growth of microorganisms [7]. The acidity of honey is due to the presence of organic acids, particularly the gluconic acid and inorganic ions such as phosphate and chloride [6, 36].

<table>
<thead>
<tr>
<th>No.</th>
<th>Place and type of honey samples</th>
<th>pH</th>
<th>Electrical conductivity (µS/cm)</th>
<th>Moisture content</th>
<th>Total ash (g ash/100 g honey)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Atsbi, white</td>
<td>4.088</td>
<td>25.50 (20)</td>
<td>18.60 (0.1)</td>
<td>0.169 (0.1)</td>
</tr>
<tr>
<td>2</td>
<td>Hawzene, white</td>
<td>3.820</td>
<td>8.270 (1.8)</td>
<td>18.60 (0.2)</td>
<td>0.078 (0.2)</td>
</tr>
<tr>
<td>3</td>
<td>Abiy Adi, yellow</td>
<td>4.450</td>
<td>33.50 (19)</td>
<td>18.80 (0.1)</td>
<td>0.152 (0.2)</td>
</tr>
<tr>
<td>4</td>
<td>Adigrat, white</td>
<td>3.952</td>
<td>24.40 (16)</td>
<td>18.60 (0.5)</td>
<td>0.111 (0.1)</td>
</tr>
<tr>
<td>5</td>
<td>Hagereselam, white</td>
<td>3.855</td>
<td>15.50 (10)</td>
<td>18.80 (0.1)</td>
<td>0.078 (0.3)</td>
</tr>
</tbody>
</table>

The electrical conductivity of Tigray honey. Electrical conductivity varies with botanical origin and conductivity values recorded ranged from 8.27 µS/cm in Hawzene to 33.5 µS/cm in Abiy Adi, where the values are within the limits (lower than 0.8 mS/cm) [2, 7]. This parameter depends on the ash, organic acids, proteins, some complex sugars and polyols content, and varies with botanical origin. The value of electrical conductivity changes, when the amount of the plant pollen decreases [9]. The electrical conductivity of the honey is closely related to the
concentration of mineral salts, organic acids and proteins; it is a parameter that shows great variability according to the floral origin and is considered one of the best parameters for differentiating between honeys with different floral origins [4].

The moisture content of Tigray honey. The honey samples showed moisture content from 18.6 to 18.8%, which show optimum harvesting and proper degree of maturity as can been seen in Table 1. The moisture content of honey is an important factor contributing to its stability against fermentation and granulation during storage. Moisture content was affected by climate, season of the year, degree of maturity/ripeness, processing techniques, storage conditions, botanical origin of the sample and moisture content of original plant nectar [36-38]. None of the samples exceeded the 19% allowed by Codex Alimentarius [13]. The moisture content is an important criterion for evaluating the grade of ripeness of the honey and its shelf-life. In general high amount of water causes the honey to ferment, to spoil and to lose flavor, with ensuing honey-quality loss. Honey moisture content depends on the environmental conditions and the manipulation from beekeepers at the harvest period, and it can vary from year to year. High moisture content could accelerate crystallization in certain types of honey and increase its water activity to values where certain yeasts could grow. The undesirable honey fermentation during storage is caused by the action of osmotolerant yeasts resulting in the formation of ethyl alcohol and carbon dioxide. The alcohol can be further oxidized to acetic acid and water resulting in a sour taste [6, 10].

The ash content of Tigray honey. The ash content is mainly determined by soil and clime characteristics [18]. Honey normally has low ash content and it depends on the material collected by the bees during foraging on the flora [17]. The wide variability of honey composition is reflected also in the ash content; this parameter, used for the determination of the botanical origin (floral, mixed or honeydew) showed values between 0.16% and 0.60%. None of the samples surpassed 0.6%, the permitted limit for flower honeys [4, 39]. The ash content is a quality criterion for honey botanical origin; the blossom honeys have lower ash content than honeydew honeys [6]. The variability in the ash content of honeys could be due to harvesting processes, beekeeping techniques and the material collected by the bees during the foraging on the flora [6, 10].

The metal content of Tigray honey. The metal content in honey from selected sampling areas of Tigray is compiled in Table 2. As can be observed in Table 2, the concentration/amount of trace metals in the honey is more or less different depending on the sampling area. The highest concentration is observed for iron, manganese, zinc, copper, nickel, chromium, cobalt, lead and cadmium in that order. There are some differences and similarities among the results. This observation can be related to various factors, such as industry, mining, emission of automobile exhaust gases, botanical origins and geographical conditions are expected to affect the mineral content. Also, honey that comes into contact with metal containers or equipments during storage or processing may have elevated levels of some metals, such as iron. In general in totality the highest concentration of the metals is observed in Adigrat white honey. The levels of metals are in general less than the minimum allowable limit by different organizations. Therefore Tigray honey is safe for metal contamination but awareness should be created for stakeholders so that the quality of the honey will be maintained for relatively longer period of time. Any heavy metals present in honey above admitted levels by pollution standards, are threats to human body through the possible negative effect of the contaminants. On the other hand, honey is an excellent indicator of environmental pollution [40]. Apart from the nutritional significance of minerals and the fact that they affect color of honey, mineral content is also an important indicator of possible environmental pollution and a potential indicator of geographical origin of honey. Thus, the analyzed honey did not present evidence of pollution with these metals [13].

Table 2. The metal content of Tigray honey in mg/kg (Mean (percent relative standard deviation, % RSD, in parenthesis), n = 18) detected in Tigray honey by FAAS.

<table>
<thead>
<tr>
<th>No.</th>
<th>Element</th>
<th>Adigrat, white</th>
<th>Atsbi, white</th>
<th>Hawzene, white</th>
<th>Hagereselam, white</th>
<th>Abiy Adi, yellow</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cd</td>
<td>0.025 (0.4)</td>
<td>0.029 (0.4)</td>
<td>0.023 (1.3)</td>
<td>0.022 (0.9)</td>
<td>0.022 (0.6)</td>
</tr>
<tr>
<td>2</td>
<td>Cu</td>
<td>13.99 (0.2)</td>
<td>0.621 (0.3)</td>
<td>0.370 (0.2)</td>
<td>0.624 (0.3)</td>
<td>0.498 (0.3)</td>
</tr>
<tr>
<td>3</td>
<td>Cr</td>
<td>0.624 (0.3)</td>
<td>0.621 (0.3)</td>
<td>0.493 (0.2)</td>
<td>0.749 (0.4)</td>
<td>0.622 (0.3)</td>
</tr>
<tr>
<td>4</td>
<td>Co</td>
<td>0.125 (0.1)</td>
<td>0.249 (0.1)</td>
<td>0.000 (0.0)</td>
<td>0.125 (0.1)</td>
<td>0.249 (0.1)</td>
</tr>
<tr>
<td>5</td>
<td>Ni</td>
<td>1.998 (0.1)</td>
<td>2.484 (0.4)</td>
<td>2.217 (0.4)</td>
<td>2.371 (0.4)</td>
<td>2.612 (0.4)</td>
</tr>
<tr>
<td>6</td>
<td>Fe</td>
<td>13.611 (0.1)</td>
<td>6.334 (0.4)</td>
<td>9.237 (0.4)</td>
<td>12.730 (0.4)</td>
<td>12.685 (0.4)</td>
</tr>
<tr>
<td>7</td>
<td>Mn</td>
<td>1.124 (0.4)</td>
<td>1.242 (0.4)</td>
<td>0.985 (0.4)</td>
<td>1.498 (0.4)</td>
<td>1.244 (0.4)</td>
</tr>
<tr>
<td>8</td>
<td>Zn</td>
<td>1.124 (0.4)</td>
<td>0.745 (0.4)</td>
<td>0.370 (0.2)</td>
<td>0.749 (0.4)</td>
<td>0.373 (0.2)</td>
</tr>
</tbody>
</table>

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REFERENCES