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Determination Of Quality And Adulteration Effects Of Honey From Adigrat And Its Surrounding Areas

Tadesse Gebremariam, Gebregziabher Brhane

Department of Chemistry, College of Natural and Computational Sciences, Adigrat University, Ethiopia
Tadesse Gebremariam; E-mail: tgmg21@gmail.Com

ABSTRACT: The aims of the present study were to assess the quality of local honey samples and discriminate pure and adulterated ones. The samples were analyzed for physicochemical parameters including pH, free acidity, moisture content, ash content, electrical conductivity, reducing sugars, sucrose content, and HMF content. The pH values ranged from 3.40 to 4.65 while the free acidity varied from 3.99 to 45.17 meq/kg of honey. The electrical conductivity (0.13 - 0.56 mS/cm), ash content (0.09 - 0.54 %), reducing sugars (50.31 – 79.56 %), sucrose content (2.24 – 12.21 %), moisture content (17.56 – 22.57 %), and HMF content (8.32-45.26 mg/kg of honey) were detected. During preliminary assessment, the commonly added adulterants by honey traders were identified and quick test methods suitable for local honey samples have been developed. Results obtained from this study showed that all honey samples obtained from apiary sites and many of commercial samples collected from local markets in the study area are of good quality and met the national and international standard limits. However, the physicochemical test results for some honey samples collected from local markets had higher level of certain parameters than recommended suggesting some level of adulteration is practiced by few honey traders.

Keywords: Adigrat, Adulteration, Determination, Honey, Quality

1 INTRODUCTION

Honey is the natural sweet substance produced by honey bees 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 the honey comb to ripen and mature [1]. It is a natural food produced by bees from nectar or secretion of flowers. Honey has a content of 80-85 % carbohydrates, 15-17 % water, 0.3 % proteins, 0.2 % ashes, and minor quantities of amino-acids and vitamins as well as other components in low levels of concentration [2]. It is a complex mixture and presents very great variations in composition and characteristics due to its geographical and botanical origin and its main features depending on the floral origin or the nectar utilized by bees [3-4]. Ethiopia is the largest honey- producing country in Africa and one of the top ten countries in the world [5]. Many parts of the Tigray National Regional State in general and the Eastern parts of the region in particular are known for their production of high quality of honey in Ethiopia. The amount of honey produced in the Tigray Region is almost 15% of the global production of Ethiopian honey. An attempt was made to assess the physicochemical properties of some Tigray honey samples for their electrical conductivity, ash content, moisture content, pH value and elemental composition of some selected metals [4]. The physiochemical characteristics of honey obtained from traditional and modern hive production systems in Tigray region have been also compared [6]. Nevertheless, there is no sufficient work on quality determination as well as effect and extent of adulteration for locally produced natural honey. Nowadays, adulteration of different natural food items becomes a common problem in many parts of the world regardless of economic status of the nation. One of the susceptible food materials for intentional or unintentional adulteration or contamination is honey. The most common adulteration practiced with honey is the addition of sucrose, corn syrup, molasses, banana or other harmless or harmful materials. The act of honey adulteration is causing severe impact on the domestic and international market opportunities of the product and may result nutritional and health problems on consumers [7-8]. Honey is declared adulterated if cheaper or inferior substances are substituted wholly or in part. It may also contain some added substances injurious to health and for whatever reasons its quality is below the standard. In this work, honey samples from apiary sites and selected household beekeepers as well as from local markets were collected and assessed for their quality parameters. Moreover, susceptible adulterants were checked for their effect on the test parameters and simple methods were suggested to screen adulterated honey samples from the pure ones.

Description of the Study Area

Adigrat town is located in the Northern part of Ethiopia, Tigray National Regional State. It is found between $14^{o}16.453'$N latitude and $39^{o}27.654'$E longitude with altitude of 2457 meters above sea level. The samples considered for investigation were collected from Adigrat market and its surrounding districts namely, Hawzen, Gantafeshum, Saesie-tsaedaemba,Gulomekada, and Irob.

2. METHODOLOGY

Equipments

UV-Visible spectrophotometer (UV-1700, Shimadzu, Japan), pH meter (Hanna Instruments, Switzerland), Conductivity meter (ME 976C, MAX Electronics, India), Hand held Refractometer , Analytical balance (CPA124S, Sartorius AG Germany),Thermostatic Blast Oven (DHG-9140, Shanghai Medical Apparatus, China) water bath (memmert), Muffle Furnace , Alumina Crucibles, different sized volumetric flasks, pipettes, beakers, and test tubes.

Chemicals and Reagents

During the entire research work, analytical grade chemicals and distilled water were used. The following are some of the chemicals, reagents and solvents that has been utilized for sample preparation and analysis purposes: Sodium hydroxide (NaOH), Hydrochloric acid (HCl), Sulphuric acid (H_2SO_4), Nitric acid ($HNO_{3)}$, Sodium bisulphate ($NaHSO_{3)}$, methylene blue, Zinc acetate ($Zn(CH_{3.}COO)_2$),Potassium haxacyanoferrate(II)trihydrate, Sodium potassium tartarate ($COOK(CHOH)_2COONa$), Coppersulphate ($CuSO_4.5H_2O$), Phenolphthalein indicator, methanol (HPLC grade), aniline and Barium Chloride.

Collection of Honey Samples

A total of ten types market samples were randomly purchased from local markets of various locations in surrounding districts. Additional four types of pure honey samples were also purchased from different Beekeeping centers and representative households within Eastern zone of Tigray Regional state. These honey samples to be analyzed were homogenized by stirring thoroughly to prepare representative sample. Crystallized honey samples in a hard and compact mass were softened by heating using hot plate at temperature less than 40^0C and were used for analysis of all test parameters except determination of HMF content (HMF content for all samples were determined without heat treatment).

Analytical Methods

Honey samples were collected from households, bee keeping centers and local markets of the study area. These samples were assessed for their compositional properties when fresh and after deliberate addition of some susceptible adulterants. All honey samples were analyzed using the same methods during the same time period to ensure uniform conditions and comparability. The following common test parameters as quality markers were analyzed for pure and adulterated honey samples

Free Acidity and pH

Free acidity of honey is defined as the content of all free acids expressed in meq./kg of honey [9]. To determine these test parameters, 10 grams of sample was dissolved in 75 mL of CO_2-free water in a 250 mL beaker with the help of magnetic stirrer. The Electrode of calibrated pH meter (Hanna Instruments, Switzerland) was immersed into the solution and the pH value was recorded. To determine the free acidity, the solution was then titrated with 0.10M NaOH to pH= 8.30 and amount of 0.10M NaOH solution was recorded. Free acidity (meq/kg) = Volume of 0.10M NaOH (mL) x 10

Determination of Mineral (Ash) Content

To determine the mineral (ash) content, 5 g of each honey sample was transferred into ignited and pre-weighed platinum crucibles. The contents were charred on Bunsen burner until the sample is dry and smokeless. The sample is then ignited in a muffle furnace at 600 ^{0}C for about 4 hours. After complete ignition to constant weight, the sample is cooled in a desiccator and weighed immediately.

$$Ash\ Content\ (\%) = \left(\frac{W_2 - W_1}{M}\right) x100$$

Where, W_1 = weight of empty crucible; W_2 = weight of the ash + crucible after ignition; M = mass of the sample taken for test

Moisture Content

The water or moisture content of honey is a measure of its stability and resistance to fermentation. To determine the moisture content of the samples using gravimetric method, five grams of honey was weighed and placed into pre-weighed drying dish. The sample was dried to constant weight in a vacuum oven at 105 ^{0}C for four hours. The moisture content of each sample was then calculated as follows:

Moisture (%) = (M_1-M_2) x100/(M_1-M_0)

where, M_0= weight of empty dish; M_1= weight of fresh sample + dish ; M_2= weight of dried sample + dish

Electrical Conductivity

The electrical conductivity of honey is defined as that of a 20% w/v solution in water at 20^0C, where 20% refers to honey dry matter [9]. 20g of dry matter of honey was weighed and dissolved in 100 mL distilled water. Electrical conductance of the specified solution was measured using previously calibrated digital conductivity meter (ME976C, MAX Electronics, and India).

Hydroximethylfurfural (HMF) Content

The amount of HMF in honey is one of the important indicators of honey quality. In fresh honey, HMF is present only in trace amounts and its concentration increases with storage and prolonged heating of honey. The analysis of the hydroxymethylfurfural (HMF) content was done based on the determination of UV absorbance of HMF at 284 nm. In order to avoid the interference of other components at that wavelength, the difference between the absorbance of a clear aqueous honey solution and the same solution after addition of bisulphite was determined. The HMF content was calculated after subtraction of the background absorbance at 336 nm [10]. The HMF content of the sample was then calculated by the following formula:

HMF (mg/Kg) **= (A_{284} – A_{336}) x149.7x5/W**

Where, A_{284}= absorbance at 284 nm; A_{336}= absorbance at 336 nm; W= Weight of sample taken

Reducing Sugars and Sucrose

Reduction of sugar was carried out using the Layne- Enyon method. About 2.6 g of honey sample was weighed and then transferred to a 500 mL volumetric flask. Five milliliters of standardized Fehling A and B solutions were transferred to a 250 ml Erlenmeyer, with 7 mL of water and 15 mL of honey solution. The Erlenmeyer was heated and 1 mL methylene blue 0.2 % was added. Titration was carried out adding the diluted honey solution until the indicator was decolorized. Determining sucrose content was carried out by inversion, adding 10 mL of diluted HCl, 50 mL diluted honey solution and water to a 100 mL volumetric flask, heating in water bath, then cooling and diluting to mark. Finally the Lane-Enyon method was applied and sucrose content was obtained by difference.

Quick Test for Adulteration

The collected honey samples were analyzed for various adulterants. All of the collected representative honey samples were evaluated at regular intervals of the adulterants. Physicochemical tests will be carried out and the samples will be characterized when pure and after addition of susceptible adulterants (preliminary assessment has been made to identify the commonly used local adulterants).

3. RESULTS AND DISCUSSION

The quality parameters of honey requirements were determined for use as food or other applications such as in medicinal purpose. These quality parameters which include pH, free acidity, electrical conductivity, sugar content, moisture content, ash content, and hydroxymethyl furfural (HMF) content were determined for ten honey samples collected from local markets in Eastern zone of Tigray region and other four

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pure (authentic) honey samples directly obtained apiary sites and household beekeepers. Table 1, below, summarizes all physico-chemical analysis results of fourteen honey samples collected from the described study area.

PH and Free Acidity

The acid content of honey is relatively low but it is important for the honey taste, stability and resistance to micro-organisms [9]. All honey samples collected from the described study area were found to be acidic irrespective of their different geographic origin and purity. The pH values ranged from 3.4 to 4.65 with a mean result of 4.09 $\pm$ 0.36 (Table 1) for all samples collected from local markets and from authentic honey providers. Similar results were reported by for pH values of Tigray honey ranged from 3.82 to 4.45 [4]. The acidity of honey is due to the presence of organic acids, particularly the gluconic acid (a product of glucose oxidation by glucose oxidase) and inorganic ions such as phosphate and chloride[10-11]. Honey is a buffer that means its pH does not change by the addition of small quantities of acids and bases. The buffer capacity is due to the content of phosphates, carbonates and other mineral salts [9]. Variations observed in the honey samples may be attributed to the presence of different acids found in different floral types [6]. Free acidity analysis results ranged from 3.99 meq to 45.17 meq per kg of honey samples. The mean value was found to be 12.66 $\pm$ 10.61 meq/kg of honey (Table 1). This free acidity value represents the organic acids content in honey. As per the Ethiopian Standard specifications for honey [13], the maximum permissible limit for free acidity value is 40 meq/kg of honey. Thus, one sample among the market samples was found to have higher free acidity value than recommended For the authentic (pure) honey samples, pH values ranged from 3.66 to 3.97 with overall mean of 3.83 $\pm$ 0.14. Similarly, the average free acidity was found to be 15.44 $\pm$ 4.93 meq/kg of honey sample. The comparative study of pH values for pure (authentic) and adulterated honey samples with deliberate addition of sugar, common adulterant, was performed and results showed significant differences. These results revealed that pH value increases where as free acidity decreases up on addition of commercial sugar (Table 2).

Electrical Conductivity

Electrical conductivity varies with botanical origin and the values depend on the ash, organic acids, proteins, some complex sugars and polyols content [4]. 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 [14]. Analysis results for electrical conductivity of all samples were within the acceptable limit (i.e. < 0.8 mS/cm) [1]. They ranged from 0.13 mS/cm to 0.56 mS/cm with average value of 0.31 $\pm$ 0.12 mS/cm (Table 1). The comparative study of pure and adulterated honey samples indicated that; mean values for electrical conductivity for pure sample was 0.28 $\pm$ 05 mS/cm which was found to decrease to 0.17 $\pm$ 11 mS/cm (Table 2) for mixture of honey and sugar (1:1 w/w). Thus, the concentration of mineral salts, organic acids and proteins is lower in commercial sugars than natural honey.

Table 1: Mean values of physicochemical analysis results

Sample	pH (Number)	Free acidity (meq/kg)	Conductivity (ms/cm)	Reducing sugars (%)	Sucrose (%)	Moisture content (%)	Ash Content (%)	HMF (mg/kg)
S1	4.11 ± 0.06	45.17 ± 0.16	0.35 ± 0.05	58.12 ± 0.87	9.24 ± 0.96	21.32 ± 0.76	0.54 ± 0.02	38.58 ± 0.89
S2*	3.97 ± 0.05	9.67 ± 0.76	0.22 ± 0.02	66.36 ± 0.09	3.26 ± 0.58	17.56 ± 0.15	0.24 ± 0.01	11.64 ± 0.75
S3*	3.80 ± 0.06	11.67± 1.21	0.34 ± 0.05	65.67± 1.02	4.32 ± 0.16	118.35 ± 0.95	0.42 ± 0.02	9.41 ± 1.02
S4	4.26 ± 0.02	10.26± 0.87	0.56 ± 0.02	62.77± 0.51	6.55 ± 0.08	21.75 ± 0.23	0.15 ± 0.11	45.26 ± 0.93
S5*	3.66 ± 0.05	18.17 ± 0.18	0.26 ± 0.03	71.25 ± 0.12	2.24 ± 0.19	17.83 ± 0.85	0.09 ± 0.26	10.54± 0.92
S6	3.94 ± 0.12	12.33 ± 0.89	0.33 ± 0.14	64.24 ± 1.09	7.34 ± 0.54	19.26 ± 0.65	0.19 ± 0.08	13.42 ± 0.75
S7	4.32 ± 0.02	15.32 ± 0.59	0.26 ± 0.14	54.31 ± 0.02	4.66 ± 0.54	18.15 ± 0.99	0.24 ± 0.12	12.35 ± 0.95
S8	4.65 ± 0.04	18.07 ± 0.04	0.28 ± 0.31	79.585 ± 0.09	8.98 ± 1.13	20.75 ± 0.54	0.13 ± 0.15	21.15 ± 0.35
S9*	3.892± 0.21	13.28 ± 0.20	0.31 ± 0.15	75.96 ± 0.27	5.42 ± 1.11	18.54 ± 0.45	0.14 ± 0.09	8.32 ± 1.76
S10	4.58 ± 0.21	4.58 ± 0.21	0.13 ± 0.14	60.81 ± 1.21	6.22 ± 0.76	18.88 ± 0.08	0.10 ± 0.08	30.52± 0.15
S11	4.59 ± 0.03	4.59 ± 0.03	0.53 ± 0.27	50.31 ± 0.53	12.21 ± 0.46	22.58 ± 0.74	0.43 ± 0.33	23.26 ± 0.66
S12	3.99 ± 0.41	3.99 ± 0.41	0.16 ± 0.02	71.52 ± 0.83	3.36 ± 0.98	17.93 ± 0.99	0.34 ± 0.12	26.54 ± 0.53
S13	3.4 ± 0.04	6.01 ± 0.02	0.2± 0.51	63.17 ± 0.93	2.42 ± 0.08	18.28 ± 0.52	0.14 ± 0.14	10.49 ± 0.76
S14	4.07 ± 0.12	4.15 ± 0.11	0.33 ± 0.06	65.32 ± 0.64	5.98 ± 0.88	18.96 ± 0.89	0.36 ± 0.14	26.33 ± 0.82
Average	4.08 ± 0.36	12.66 ± 10.61	0.31 ± 0.12	64.96 ± 7.95	5.87± 2.85	19.29 ± 1.62	0.25 ± 0.14	20.56± 11.71
Range	3.40 - 4.65	3.99-45.17	0.13-0.56	50.31-79.56	2.24-12.21	17.56-22.57	0.09-0.54	8.32-45.26

* represents authenticate samples collected from Beekeeper/Apiary sites and all results are mean values of three determinations; **S_1**= Adigrat;

S_2= Azeba* **;S_3**= Bizet 1*; **S_4**= Bizet 2; **S_5**= Buket 1*; **S_6**= Buket 2; **S_7**= Dawhan; **S_8**= Edagahamus; **S_9**= Fatsi 1*; **S_{10}**= Fatsi 2; **S_{11}**= Fireweini; **S_{12}**= Hawzien; **S_{13}**= Mugulat ; **S_{14}**= Zalambessa

Table 2: Mean values of physicochemical composition of pure and adulterated honey samples

Sample	pH	Free acidity (meq/kg)	Conductivity (ms/cm)	Reducing sugars (%)	Sucrose (%)	Moisture content (%)	Ash Content (%)	HMF (mg/kg)
Pure honey	3.83 ± 0.14	15.44 ± 4.93	0.28 ± 0.05	69.81 ± 4.79	3.81 ± 1.37	17.92 ± 0.55	0.22 ± 0.14	8.48 ± 5.55
Range	3.66-3.97	9.67-20.67	0.22-0.34	65.68-75.96	2.24-5.42	16.56-17.98	0.09-0.42	4.32-20.54
Honey+Sugar (1:1)w/w	4.92 ± 0.67	10.54± 2.38	0.17 ± 0.11	42.38 ± 7.93	9.80 ± 2.02	19.39 ± 0.93	0.07 ± 0.05	43.12± 10.27
Range	4.56-5.87	7.58-13.12	0.09-0.34	32.90-51.65	7.53-12.25	17.23-22.01	0.008-0.13	38.91-60.45

Reducing Sugars and Sucrose

Determination of sugars in honey is also a quality criteria which is influenced by honey storage and heating and thus is an indicator of honey freshness and overheating . Reducing sugar analysis results were found to be in the range of 5.31 % to 79.56 % and the overall mean value was 64.96 ± 7.95 % for honey samples collected from local markets. The average result for sucrose content was 5.87 ± 2.86 % (Table 1) which is higher than results reported for Tigray honeys from traditional and Modern hives [6]. The result for sucrose content ranged from 2.24 to 12.21%. Addition of commercial sugar products to honey increases sucrose content from 3.81 % for pure honey to 9.80 % for mixture of honey and sugar (1:1 w/w) as shown in Table 2. This study, therefore, revealed that some of the honey samples collected from local markets had sucrose content above the acceptable permissible maximum value. The results in Table 1 and Table 2 revealed that only some honey samples purchased from local markets and the mixture of honey and sugar (1:1 w/w) had higher sucrose contents. While all of the pure honey samples obtained from apiary sites or house hold beekeepers of various areas were found to have sucrose content within the internationally acceptable range (<5g/100g honey). This implies that higher sucrose content of honey is an indication for addition of commercial sugar to honey and there was possible adulteration with sugar syrup in some of the samples randomly purchased from local markets.

Hydroxymethylfurfuraldehyde (HMF) Content

Hydroxymethyl furfuraldehyde (HMF) is a decomposition product of fructose. In fresh honey it is present only in trace amounts and its concentration increases with storage and prolonged heating of honey. It is a major honey quality factor that indicates honey freshness and adulteration associated with overheating. The HMF contents of honey samples collected from the described study area were presented in Table 1. The results ranged from 8.32 to 45.26 mg/kg with mean value of 20.56 ± 11.71 mg/kg of honey. Among the honey samples

understudy, one market sample and the laboratory prepared mixture of honey and sugar 1:1 w/w had HMF contents higher than the maximum permissible limit of 40 mg/kg honey [13]. The high values indicate that the honey samples had been heated and /or adulterated with processed sugar.

Moisture Content of Honey Samples

The maximum value of moisture content in honey is 20% [1]. 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 [4]. The average moisture content of investigated honey samples was 19.29 $\pm$ 1.62 % (Table 1) and the range was 17.56 % to 22.58 %. Three samples randomly collected from local markets namely Adigrat (21.32 %), Bizet 2 (21.75%) and Fireweini (22.58%) had moisture content above the permissible limit. Whereas, the pure honey samples purchased from authenticate sources had mean moisture content of 17.92 $\pm$ 0.55 % that ranged from 17.52% to 18.45%. The analysis results (Table 1 and Table 2) revealed that some honey samples collected from local markets had higher moisture content than national and international standards. But all pure honey samples directly purchased from Beekeepers and household farmers comply with the national and international requirements and shall be graded as 'Grade A' Honey.

Ash Content of Honey Samples

The mean ash content of honey samples collected from various locations of the study area were ranged from 0.09 % to 0.54% with an average value of 0.23 $\pm$ 0.13 % (Table 1). All analysis results for ash contents were within the internationally acceptable range of < 0.6% [1]. Variations observed in the ash contents between different locations of sample origin can be explained by the differences in floral origin of the honey samples.

Quick Tests for Honey Adulteration

A preliminary assessment was conducted to know the types of adulterants commonly added to honey and sold at local markets. Interviews and physical observation results on adulteration of honey revealed that the common substances usually added to honey as adulterants are: Sugar syrup, maize and/or wheat flour syrup, banana, and sweet potato. These adulterants are usually added to honey individually or in combination by some honey traders to maximize their profit. The following observations of physical tests were found to be helpful to identify pure and adulterated honey samples.

A. Flame Test:
- Pure honey gave smokeless flame when ignited using candle flame or laboratory Bunsen burner.
- presence of adulterants was confirmed by observation of smoky flame and/or cracking sound during flame test

B. Heating Effect:
Upon gentle heating of samples to dissolve crystallized substances:
- Pure honey melts to clear transparent viscous solution (while wax materials floating on top)
- A mixture of honey with starch from potato, banana or wheat flour melts to form dispersed and non transparent liquid
- A mixture of honey with commercial sugar (more than 50 % w/w) melts to form thicker, relatively dispersed and partially transparent liquid

Microscopic Analysis for adulteration of honey with commercial sugar and other materials such as banana was also employed. The test was conducted on the pure honey and that of deliberately adulterated ones and results revealed that some fibers and plant tissue were observed in mixtures of honey.

4. CONCLUSION

A laboratory test would have to be performed on the sugars present in the honey and the results compared with the national or international honey standard. Evidence of a large proportion of sucrose would suggest sugar had been added. Checking for adulteration with water, honey with high water content may be unripe or may be a mixture of honey and water. All pure honey samples directly purchased from Beekeepers and household farmers comply with the national and international requirements and are of good quality, but some honey samples collected from local markets had higher moisture and hydroxymethyl furfural (HMF) contents than recommended level indicating some levels of adulteration and heat treatment.

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