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Mineral Content and Physicochemical Characteristics of Honey Samples Obtained From Districts of Hadiya and Kembata Zones, SNNPR, Ethiopia

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ABSTRACT: The aim of this study was to determine mineral (Fe, Zn, Cu, Ca, Mg, Na and K) contents and physicochemical properties of honey samples obtained from districts of Hadiya zone and Kembatea Tembaro zone. The physicochemical properties considered in the study are (moisture content, ash content, titarable acidity, pH, Hydromethylfurfural, Total sugar, Reducing sugar, Non-Reducing sugar, density, electrical conductivity and Total soluble Solids. The finding of the present study showed that the mineral contents and physicochemical parameters are in accordance with limits set by International standards and Quality Standards Authority of Ethiopia suggesting that honey products of the study areas have acceptable qualities. The findings of study would serve as base line information about honey qualities of the study areas. However, further studies are recommended on multiple honey samples obtained from local markets and farmers to get more reliable information about qualities of honey products of the study areas.

KEY WORDS: physicochemical characteristics, honey, mineral content, moisture content, ash content, Electrical conductivity, pH

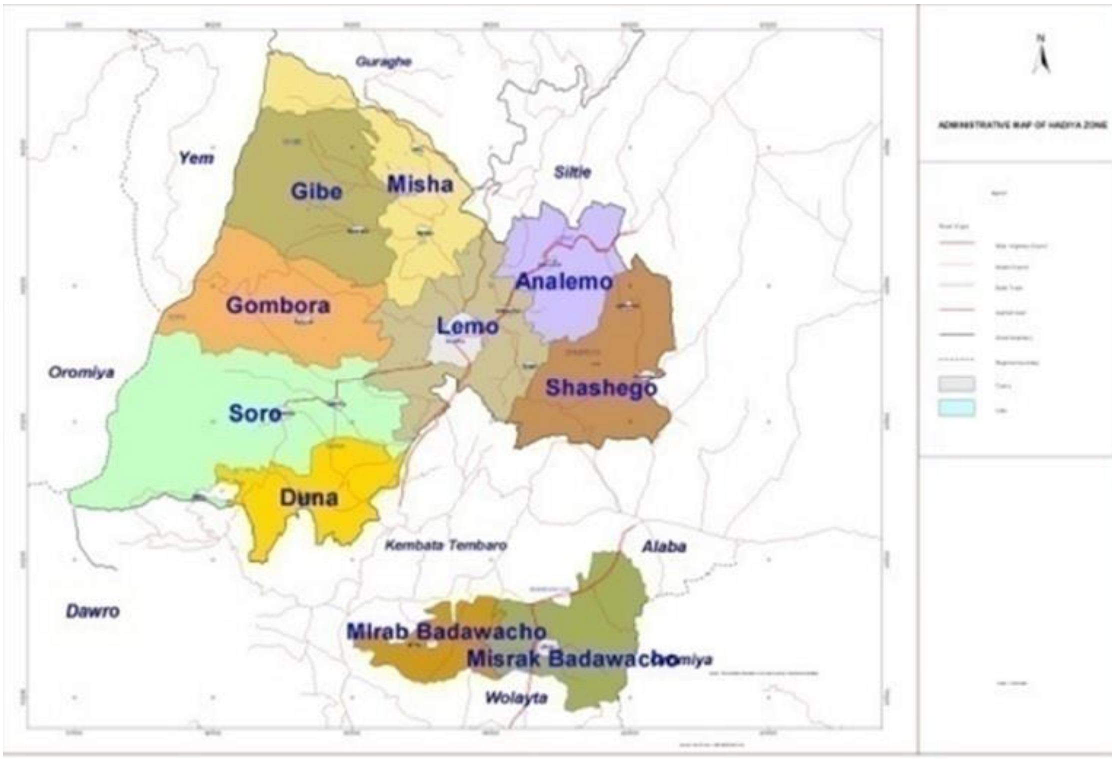
I. INTRODUCTION

Honey is a sweet food made by bees using nectar from flowers. The variety of honey produced by honey bees is consumed by humans. Honey gets its sweetness from the monosaccharides such as fructose and glucose, and has approximately the same relative sweetness as that of granulated sugar [1]. It has attractive chemical properties for baking and a distinctive flavor that leads some people to prefer it over sugar and other sweetners and most micro-organisms do not grow in honey because of it slow water activity (0.6%) [2]. It is a complex mixture that consists of carbohydrates, especially glucose and fructose, organic acids, amino acids, minerals, vitamins (e.g., Vitamin C and many forms of Vitamin B complex), enzymes, pollens, and pigments [3-6]. Its nutritional quality, medicinal and sensory properties have attracted thousands of consumers throughout the world [7,8]. It is used in various foods and beverages as a sweetness enhancer and flavoring agent [9,10]. It is also known that flavors and qualities of honey vary based on the nectar (floral) sources, climatic condition, geographic origin, the environmental temperature, the type of botanical plant used to produce it, the honey bees species, the sugar composition and methods of treatment of honey during extraction, processing and subsequent storage conditions [11-15]. All these facts make it mandatory to determine qualities and/or physicochemical properties of honey products before they are supplied to consumers.

Hadiya and Kambata zones are two of the zones found in Southern Nations and Nationalities People Regional (SNNPR) state [16]. The areas are well known for availabilities of variety of forest and plant species that are important for honey production. The zones are known for honey production. Moreover, large part of the population uses it as source of income to support their livelihoods However; there are no study reports about the mineral content and physicochemical properties of honey products from these zones [17-20]. This study, therefore, was focused on the determination of mineral contents and physicochemical properties such as mineral content, hydroxymethylfurfural (HMF) values, moisture content, Ash content, titrable acidity, total soluble solids (TSS), pH, Total sugar (TS), specific gravity or density (ρ) and electrical conductivities of the honey samples collected from aforementioned zones.

II. MATERIALS AND METHODS

Description of the study areas: Hadiya and Kambata zones are two of the 14 zones found in SNNPR, Ethiopia (Figure 1a and 1b). Hadiya zone is administratively divided in to 11 woredas (districts) [21] whereas Kembata Tembaro zone is divided in 7 woredas (districts) [19,22]. Hadiya zone and Kembata Tembaro zone are found at distances of 235 Km and 306 Km from Addis Ababa, in the southern Ethiopia, respectively [23,24].



(a)

(b)

Figure 1. The administrative map of Hadiya zone (a) and Kambta Tembaro zone (b).

Collection of honey samples : Honey samples were selected from purposively selected two districts of Hadiya zone (Gibe and Gomibora districts) and two districts of Kembata Tembaro zone (Hadaro and Mudula districts) (Figure 1)[26]. The samples were collected from September 2021 to December 2021**.** Three representative sample sites were selected by random sampling technique from each district. 3kg of honey samples were collected from traditional beehive owners from each site. The collected samples were stored in clean glass bottles and were then sealed. The tightly sealed bottles containing the samples were taken to Analytical Chemistry Research Laboratories of Jimma University and Addis Ababa University, Ethiopia, for analysis. Prior to analyses, the bottles containing crystallized honey were placed in a water bath for 30 minutes. The bath was set at 53°C. This was done to liquefy the crystalized honey samples for easy handling and analyses.

Data collection and analyses : In the present study, focus was made to determine mineral contents, HMF levels, moisture content, ash content, titrable acidity, total soluble solids, pH, sugars (reducing and non-reducing), density and electrical conductivity values of the collected honey samples. All the measurements were done in triplicates by employing literature reported procedures. The mean ± standard deviation values are used to discuss the results of the study.

Determination of mineral content : Determination of mineral contents was carried out in JIJE Analytical Testing Service laboratory in Addis Ababa, Ethiopia. The mineral (Fe, Ca, Cu, Mg and Zn) compositions of the honey samples were analyzed using Atomic Absorption spectroscopy (AAS) (Model AA320N, 085221030321060001). The sodium (Na) and potassium (K) contents were also determined by emission photometry following literature reported procedures [12].

Determination of Hydroxymethylfurfural (HMF) : The HMF values of the samples were determined according to the European commission honey method [27]. From each honey sample, 20 g of honey sample was dissolved in 100 ml Milli-Q water, filtered through a 0.22 mL nylon filter into an HPLC vial, capped and injected (20 µl) into the HPLC. The HMF values of the samples were expressed as mg/kg honey, and were calculated by comparing the peak areas to that of the standard staking into account the dilutions.

Determination of moisture content : The moisture contents of the samples were determined following procedures reported in literature [28]. 20 g of the samples were taken in a flat-bottom dish (pre-weighed) and were kept overnight in an oven at 100 -110 °C. The heated samples were weighed, and the loss in weight was regarded as a measure of moisture content as calculated by the following formula:

$$Moisture\ \% = \frac{Weight\ fresh\ sample - Weight\ of\ dry\ sample}{weight\ of\ fresh\ samples}\ X\ 100$$

Determination of ash content : Literature reported method was used to determine the ash contents of the honey samples [28]. In doing so, 10 g of each sample was weighed in a silica crucible. The crucible was heated in a muffle furnace for about 3-5 hours at 500 ^{0}C. The contents of the heated crucibles were cooled in desiccators and then weighed. To ensure completion of ashing, the samples were reheated again for half an hour. The contents of the heated crucibles were cooled and weighed again. The procedures were repeated consequently till the weights of the contents of the crucible became constant. The weights of ash contents of the samples were calculated by the following formula:

$$Ash(\%) = \frac{Weight\ of\ sample\ after\ ashing}{Weight\ of\ fresh\ sample\ taken}\ X\ 100$$

Determination of titrable acidity : Titrable acidity as tartaric acid was determined according to the method reported in literature [28]. A sample from each site was treated with 0.1N NaOH solution using titration kit. 3 to 5 drops of phenolphthalein indicator was also added as indicator of this titration experiment. The volume of alkali (NaOH) used in the experiment was recorded and calculated using the following formula:

$$Titrable\ acidity\ (\%) = \frac{Eq\ Wt\ of\ acid\ X\ Normality\ of\ NaOH\ X\ titer}{10\ Wt\ of\ sample\ in\ gram}\ X\ 100$$

Determination of total soluble solids : The total soluble solids (TSS) was determined using the method reported in literature [28,29] using Digital-Bench-Refrectometer (Model DRBO-45). Prior to the measurements, the instrument was cleaned and adjusted to zero at 20°C using distilled water. An appropriate quantity of each honey sample was placed on the prism-plate of the Refrectometer with the help of a glass rod and folding back the cover. For each sample, the instrument was calibrated using distilled water. The reading that appeared on the screen was directly recorded as total soluble solids.

Determination of pH and and titrable acidity : For determination of pH of the honey samples, literature reported method [28,29] and digital pH meter (Model 3510) was used. The pH meter was calibrated with buffers at pH 4 and 10. The sample solutions were taken in the beaker and inserted into the pH meter. After taking the first reading, the electrodes were washed with distilled water and dried-up with tissue paper. Similar procedures were for consecutive measurements of all other samples.Titrable acidity as tartaric acid will be determined according to the method reported in literature [28]. Each sample of the honey will be treated with 0.1N NaOH solution using titration kit, where 3 to 5 drops of phenolphthalein indicator will be used. The volume of alkali used will be noted and the results are expressed as milliequivalents per kilogram using the following equation

$$Titrable\ acidity\ (\%) = \frac{Eq\ Wt\ of\ acid\ X\ Normality\ of\ NaOH\ X\ titer}{10\ Wt\ of\ sample\ in\ gram}\ X\ 100$$

Determination of sugars : Literature reported procedures were used to determine total sugar (reducing and non-reducing sugares0 in the honey samples [29].

Total sugars and reducing sugars : In this experiment, 5 g of sample was taken in a beaker and 100 ml of warm water was added into the beaker. The solution was stirred until all the soluble matters were dissolved and filtered through Whatman filter paper into a 250 volumetric flask. Of the prepared solution, 100 ml of was pipetted and transferred into a conical flask. Then 10 ml of diluted hydrogen chloride (HCl) was added and boiled for 5 minutes. On cooling, the solution was neutralized with 10% NaOH in the presence of phenolphthalein indicator in a 250 volumetric flask. The solution was then used for titration against Fehling's solution and the reading calculated using the following formula:

$$Total\ sugar\ (\%) = \frac{\text{Factor } (4.95) \times \text{dilution } (250) \times 2.5}{\text{Titre} \times \text{wt of sample} \times 10}$$

$$\text{Reducing sugar } (\%) = \frac{\text{Factor } (4.95) \times \text{dilution } (250)}{\text{Titre} \times \text{wt of sample} \times 10}$$

Non-reducing sugar : Non-reducing sugars (NRS) in the honey samples were estimated as the mass differences between the total sugar content and reducing sugar content.

Determination of the density : The density (ρ) of the honey samples were obtained by comparing the mass (m) and the volume (v) of honey measured in a beaker of 250 ml. The beakers were filled with the previously weighed honey samples and then weighed on a precise scale. Three measures on each honey sample were carried out. The density values of the honey samples were determined using the formula: ρ = m/v.

Electrical conductivity: The electrical conductivity of a solution of 20 g dry matter of honey samples in 100 mL of distilled water was measured using an electrical conductivity cell (Model EC350). This analysis was based on the measurement of the electrical resistance, of which the electrical conductivity is the reciprocal. The results are reported as μScm^{-1}.

III. RESULTS AND DISCUSSION

As discussed in Experimental Section, the four sampling areas (districts) namely Gibe, Hadaro, Gomibora and Mudula districts were selected for availability of high number of beehive colonies [17-20]. The results are presented in the following sub-sections along with appropriate discussions.

Determination of mineral content: It is known that the metal content is a key indicator of the geographical origin of particular honey and in turn, can be used as a quality criterion [30]. Moreover, the presence of a high concentration of metals in honeys causes health several risks on consumers [31,32]. Thus, the mineral content/compositions of honey product should be determined (quantified) in order to reduce health related risks. In this study, the mineral contents of the honey samples were carried out by measuring the levels (mg/Kg) of eight metals (Table 1). The results showed that Gibe district honey samples contain highest K (594.71 ± 0.163 mg/kg) followed by Mg (372.73 ± 0.116 mg/kg), Ca (306.76 ± 0.112 mg/kg), Na (11.83 ± 0.161 mg/kg), Fe (3.67 ± 0.016 mg/kg), Zn (0.953 ± 0.015 mg/kg) and Cu (0.28 ± 0.02 mg/kg) (Table 1). The data from the other district of Hadiya zone (Gomibora district) showed that the order of Ca (421.67±0.07 mg/kg) > Mg (384.16±0.042 mg/kg) > K (245.28±0.272 mg/kg) > Cu (0.83±0.049 mg/kg) > Zn (0.48±0.036 mg/kg) > Fe (0.163±0.025 mg/kg) (Table 1). The element Na was not detected (ND) in the honey sample obtained from Gomibora district. Analyses of honey samples obtained from districts of Kembata Tembaro zone (Hadero and Modula) also showed similar trends. That is the honey samples were rich in K (Table 1). The levels of minerals (metals) of honey samples from Hadero district was found to have K (449.74±0.021mg/kg), Mg (293.74±0.048 mg/kg), Ca (290.11±0.048 mg/kg), Na (22.52±0.075 mg/kg), Cu (0.63±0.016 mg/kg,) and Zn (0.46±0.017 mg/kg). However, Fe was not detected during the course measurements. In the case of Mudula honey, the levels of the metals were found to contain highest level of K (901.31±0.592 mg/kg) followed by Mg (668.07±0.042 mg/kg) and Ca (300.28±0.651 mg/kg). The levels of the other metals analyzed in the present study were 24.43±0.027 mg/kg, 6.76±0.027 mg/kg, 1.16±0.025 mg/kg and 0.81±0.044 mg/kg for Na, Fe, Zn and Cu, respectively (Table 1).

Compared to other honey samples, Mudula honey was found to contain the highest amount of the corresponding metals. But generally, the level of metals followed the same trend as the Gibe and Hadaro honey samples (Table 1). The observed slight variation in metal content is probably due to the floral type, the botanical origin, storage conditions anthropogenic factor, season of the year, rain fall and so on [33]. The metals such as Fe and Na were not detected in Hadaro and Gomibora in both districts but in very small minute quantities in the honey samples of the other site (Table 1). Consistent with literature reports, the K content was found to be the highest followed by Ca and Mg (Table 1). The levels of the other five metals were found in small quantities [33-35]. The data obtained in the present study indicated that the mineral contents of all honey samples to be in the ranges allowed limit by FAO and WHO[36] and are also below their maximum tolerable limits [37,38]. These suggest that honey products in the study areas are safe for human consumption.

Determination of Hydroxymethylfurfural (HMF) : HMF is a natural constituent of honey, and it needs to be determined for two main reasons (a) it is an indicator of the quality (appearance) and storages and processing conditions of honey and (b) it causes toxicity (mutagenicity and carcinogenic effects) in humans when it exists in higher amounts in a given honey product [39,40]**.** In fresh honey, HMF is found in small amount (less than 15 mg/kg) but increases in the high temperature (25°C - 50°C), low pH (pH=5), prolonged storage, acid hydrolysis of sugar molecules in honey and high moisture contents [5,11,12, 41]. So, a honey product with high level of HMF is of poor quality product that could be due to poor storage conditions (such as storing it in higher temperature > 25^{o}C) and/or excess heating of honey [41,42]. The data from the present study showed that the mean values of HMF of the honey samples from Gibe, Hadaro, Gomibora and Mudula to be 10.28±0.018 mg/Kg, 0.641±0.47 mg/Kg, 6.67±0.014 mg/Kg and 8.871±0.025 mg/Kg, respectively (Table 1). Moreover, the HMF values of the honey samples of the study areas were found to be in good agreement with the national and international (WHO/FAO) honey quality standards (< 40 mg/Kg), respectively [36]. The slight higher values for honey sample from Gibe and Gomibora sites could be due to either poor storage condition (such as containers that are not tightly closed or storage temperature > 25^{o}C [41] or one of the factors mentioned above [43] that increase the level of HMF in honey products. However, the exact reasons are not determined as this was not our intention in this study. The values could indicate the qualities of the honey samples obtained from the areas are in safe ranges for human consumption. Moreover, the data indicated that the honey produced in the study areas could be used human consumption.

Determination of ash content : It is a common practice to determine ash contents of honey samples in order to assess the qualities and/mineral contents (indirectly). This can also be used as quality criteria to determine the botanical and geographical sources of honey samples [44]. The data in this study indicated that the ash contents to be 0.19%, 0.12%, 0.17 % and 0.35 %, for honey samples from Gibe, Hadaro, Gomibora and Mudula districts, respectively (Table 2). The values are below the maximum allowable ash contents values of honey samples set by Ethiopian standards (<0.6 %), European standard (<0.6%) and Codex Alimnetarius standards (< 0.8%) [45]. Thus, the honey samples of the study areas possess acceptable qualities as their ash contents were below the allowed limit (0.6 %) by different standards [45]. As reported in literature reports, the high ash content in some honey samples could be due to high mineral contents (Table 1 and 2).

Determination of Total Sugar (TS) content : Honey is composed different sugar types (80-85%, w/w)[46]. A recent report by Solayman et al. (2015) stated the presence of 39.44% fructose, 28.15% glucose and 3.19% sucrose in honey [47]. The experimental finding of the present study indicated that the concentrations of TS to be 76.61+0.32%, 90.33±0.764%, 87.97±0.43% and 86.54±0452% in the honey samples of Gibe, Hadaro, Gomibora and Mudula, respectively (Table 2). These results are comparable with literature reports on TS contents of different honey varieties. Such reports mentioned that TS levels of honey could be in the range of 53.3-80.73 % [46-48]. With regard to reducing sugar (RS), the findings showed that the RS level to be 73.37% in Gibe, 85.73% in Hadaro, 84.33% in Gomibora and 83.81% in Mudula honey samples (Table 2). The results of this study on RS are in good agreement with findings of similar study reported in literatures. The reported RS values of different honey varieties to be in the range of 65-86% [49-51,52]. The values are higher than the RS limit set by QSAE (> 65%) [53]. Moreover, the level of non-reducing sugar (NRS) of the honey samples (Table 2) were found to be in good agreement with literature reported data for NRS values of different honey varieties (1.115-12.135) [45,46].

Determination of moisture content : The water content is the most important measure and related to honey quality as it is reported to cause spoilage due to fermentation. Fermentation of sugar in honey caused by osmophilic yeasts and it occurs if moisture content of higher (>18%) [41,52]. Some other reports also suggested that water (moisture) content of honey related to climatic conditions and level of maturity of the honey product that, in turn, depends on the origin of honey nectar, harvesting season, storage condition and water content of the nectar source plants [47]. The moisture contents of honey samples obtained from Gibe, Hadaro, Gomibora and Mudula were found to be 16.63±0.824, 14.1±1.51, 19.61±0.21 and 15.42±1.51, respectively (Table 2). The data also showed that the moisture contents of the honey samples of the study areas to be in the range of 14.1±1.51% - 19.61±0.21. The values are lower/comparable with similar data reported from honey varieties from Tigray region [52] and Harar region of Ethiopia [55]. Such reports indicate moisture contents of honey samples to be 18.4% and 20.5% from Tigray and Harari regions, respectively. The data are in agreement with literature reported water content of honey (15-20%) [41,56]. Moreover, they are in good agreement with Quality Standard Agency of Ethiopia (QSAE) (i.e., 21%) [53]. If moisture content of honey is > 20%, it could be considered as index of adulterations. This is because due to high abundance and low cost of water it is the most frequently added to get unfair economic advantage by sellers or producers [54].

Determination of pH and titrable acidity : pH is one the commonly used parameters to assess quality of honey products. Literature reports showed that honey contains acids that belong to amino acids (0.05-0.1%) and organic acids [45]. The pH values of all honey samples used in the study were found to be acidic ranges. The results showed the pH of honey sample from Gibe, Hadaro, Gomibora and Mudula to be 4.12±0.334, 4.16±0.017, 3.84±0.033 and 4.14±0.016, respectively. The lower pH values are expected to inhibit growth of microorganisms that spoil the honey product as most microorganisms grow at pH around 7. Moreover, the values are also comparable with each other (Table 1). The values are in the accepted ranges of literature reported standards for acidity of honey (pH= 3.5-5.5) [45,52]. Titrable acidity as tartaric acid was determined using the procedures reported in literature [28]. The acidities of the honey samples were found to be in the range of 18.33 meq/kg to 33.93 meq/kg (Table 2). Though the acidities of Mudula and Gibe honey varieties are slightly higher than the corresponding values of Gomibora and Hadaro honey varieties, all the data are in good agreement with literature reported acidity limits of honey (12.5meq/kg to 38.9 meq/kg) [45,52]. The values are also within the limits set (40 meq/kg) by QSAE [2005] [53]. It is concluded that the investigated honey samples were acidic but within recommended limits. These data suggest that the honey products of study areas to be safe for human consumption.

Determination of Electrical Conductance (EC) : Electrical conductance (EC) honey is closely related to the concentration of mineral salts, organic acids and proteins [57]. The EC values of the four honey samples used in the study were found to be 545.67 ± .53 μScm^{-1}, 354.3±1.53 μScm^{-1}, 359.33±1.53 μScm^{-1} and 698.3±1.53 μScm^{-1} for honey samples of Gibe, Hadaro, Gomibora and Mudula, respectively (Table 1). Except for honey sample from

Modula (EC=698.3 μScm^{-1}), the EC values of the rest honey samples were lower than literature reported standards (EC < 645μScm^{-1}) [35]. The slightly higher EC value could be attributed to higher mineral content (Table 1 and Table 2) as there is direct correlation between EC and mineral content of honey [35,58]. The results suggest that the honey samples have acceptable quality.

Sample site/Metal	Fe	Zn	Cu	Ca	Mg	Na	K
Gibe	3.67±0.016	0.953±0.015	0.28±0.02	306.76±0.112	372.73±0.116	11.83±0.161	594.71±0.163
Gomibora	0.163±0.025	0.48±0.036	0.83±0.049	421.67±0.07	384.16±0.042	0	245.28±0.272
Hadaro	0	0.46±0.017	0.63±0.016	290.11±0.048	293.74±0.048	22.52±0.075	449.74±0.021
Mudula	6.76±0.027	1.16±0.025	0.81±0.044	300.28±0.651	668.07±0.042	24.43±0.027	901.31±0.592

Determination of TSS : TSS is a parameter used to assess adulteration of honey products [59]. In this study, the findings showed that TSS values to be in the range of 78.7 ±0.265-84.56 ±0.105% (Table 2). These values are in agreement previous literature reported data (73%- 85%) of honey products [60, 61].

Determination of density (specific gravity) : It is a well-known fact that there is a direct correlation between relative density (specific gravity) and water content of a honey product [62]. Moreover, these two are used as parameters to evaluate adulteration in honey products. The specific gravity values of the honey samples used in this study were found to be in the range of 1.409±0.004 - 1.444±0.72 (Table 2). These data are similar to those values reported in reports of previous studies in Ethiopia [52] and in other parts of the world [62-64]. These reports showed that specific gravity values of honey to be 1.4± 0.1 [52] and 1.4224 - 1.4303 [62-64]. Moreover, the data are within the range of establish codex standard (1.38 - 1.45) [65].

IV. CONCLUSION

The finding of the study showed that regardless of their sources, the honey samples from all sites (Gibe, Hadaro, Gomibora and Mudula) were found to have acceptable mineral contents and physicochemical characteristics that are in good agreement with similar data reported in literatures. Almost all the data obtained from the honey samples of the study area meet the standards set by national and international authorities. However, further studies are recommended on multiple honey samples obtained from farmers and local markets in order to get information on the qualities of honey products of the study areas. The results of such studies would help individuals or institutions to invest on honey production and distribution from these areas. It also would help o increase incomes of farmers who involved in honey production.

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Conflicts of Interest: The authors declare no conflict of interest.

Table 1: The mineral contents of honey samples used in the study

Table 2. The physicochemical properties of the honey samples used in the study

	HMF	Ash %	TS%	R.S %	TTA	Moisture %	pH	E.C(μS/cm)	Density	TSS
Gibe	10.28 ±0.018	0.19± 0.027	76.61 ±0.32	73.37 ±0.144	32.06	16.63 ±0.824	4.12 ±0.334	545.67 ±1.53	1.4253+±0.01	82.2± 0.42

Hadaro	0.641 ±0.47	0.12± 0.1	90.33 ±0.764	85.73 ±0.93	18.33	14.1± 1.51	4.16 ±0.017	354.3 ±1.53	1.4441±0.72	84.56 ±0.105
Gomibora	6.67± 0.014	0.17± 0.021	87.97 ±0.43	84.33 ±0.29	30.17	19.61 ±0.21	3.84 ±0.033	359.33 ±1.53	1.409 ±0.004	78.7± 0.265
Mudula	8.871 ±0.025	0.35± 0.0265	86.54 ±0452	83.81 ±0.166	33.93	15.42 ±1.51	4.14 ±0.016	698.3 ±1.53	1.4333±0.005	83.13 ±0.412

TS =Total Sugar; **R.S =** Reducing sugar**; TSS =**Total Soluble Solids; **TTA=**titrable acidity**, EC.=**Electrical Conductivity; HMF: Hydromethylfurfural

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