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Physicochemical and antioxidant characterization of commercially available honey sample from Addis Ababa market, Ethiopia

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ABSTRACT

High-quality and genuine honey is crucial to provide consumers with natural honey and prevent any potential health issues. This study aimed to examine the quality of commercial honey available in the Addis Ababa market. A total of 30 honey samples were randomly collected from eight sub-cities of Addis Ababa city. Both High-Performance Liquid Chromatography (HPLC) and UV–Vis spectroscopic methods were used to determine 12 physicochemical and three antioxidant activity parameters in the honey samples according to internationally recognized standards. The findings of this study showed that the hydroxymethylfurfural (HMF), free acidity, and ash content of all commercial honey samples conformed to honey standards. However, except for honey samples collected from processors (19.48 ± 0.4 %) and retail outlets (20.49 ± 0.13 %), all other commercial honey samples failed to meet the moisture content criteria (≤21 %). Proline levels in honey samples taken from the street (67.1 ± 0.52 mg/kg) were also found to be below the required standard. The commercial honey samples contained fructose, glucose, sucrose, and maltose within a range of 33.85 ± 0.65 to 48.61 ± 0.51 %, 33.07 ± 1.58 to 44.3 ± 0.82 %, 0.91 ± 0.05 to 6.23 ± 2.49 %, and 0.51 ± 0.14 to 2.4 ± 0.44 %, respectively. Furthermore, honey samples from market areas showed good Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and antioxidant activity. Overall, the results revealed that all physicochemical parameters, except for proline, moisture, and sucrose content, complied with approved standards (Codex Alimentarius, European Union (EU), and Ethiopia Standard Agency (ESA). Accordingly, it is recommended that stakeholders receive regular training on how to manage honey quality issues and detect adulteration techniques to prevent contaminated honey from reaching the markets.

1. Introduction

Honey refers to the sweet natural substance produced by *Apis mellifera* Linnaeus bees from the nectar of plants, secretions of living parts of plants, or excretions of plant-sucking insects [1]. It is a highly nutritious food containing saccharides, amino acids, minerals, vitamins, enzymes, phenols, organic acids, pigments, volatile oils, and aromatic substances [2,3]. The quality of honey is dependent on its sensorial, chemical, and physical characteristics [4,5]. Standards outlined by both the Codex Alimentarius Commission [6] and the

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Ethiopian Standard [7], including parameters such as water content, ash, pH, electrical conductivity, HMF, reducing sugar, and sucrose, are used to characterize honey. Commercially available honey samples differ in quality due to factors like geographical location, floral source, storage, and processing conditions [8].

Ethiopia possesses diverse ecological and climatic conditions suitable to a plethora of flora and fauna. While the country is known for its honey production and export, most of the honey products available in the market are crude and poorly managed. Addis Ababa is the capital city of Ethiopia, and several companies and individuals are involved in honey production, processing, and exportation [9]. Unfortunately, the honey market in Addis Ababa faces several challenges, including low quality and adulteration. The Markato market, one of the largest open marketplaces in Africa, suffers from low-quality honey. Other areas of the sub-city, such as supermarkets, small individual open markets, and retail stores, also serve as places for honey markets, yet most of these honey samples are traded without any quality standards or traceability to their origins. Therefore, the quality of honey and its adulteration are a real concern. Recent evidence [10] indicated that honey value chain actors have unhygienically handled the honey, resulting in contamination, unsafe products, and reduced quality.

While research on Ethiopian honey has mostly concentrated on physicochemical and botanical origins [11–22], there is a lack of information on commercially available honey in terms of physicochemical and antioxidant characteristics. Customers, buyers, beekeepers, and honey processors rely on experience and observation to determine honey quality. Therefore, this study aimed to characterize the quality of commercially available honey in Addis Ababa, Ethiopia, using physicochemical and biochemical markers. The study aimed to authenticate the quality of honey and provide a more comprehensive understanding of the characteristics of honey available in the market of Addis Ababa, Ethiopia.

2. Methodology

2.1. Honey samples and sampling techniques

This study was conducted in Addis Ababa, the capital city of Ethiopia. Honey samples were randomly collected from various locations in the city where consumers can purchase honey, including supermarkets, individual open markets, small shops, street vendors, and big honey verandas. A total of 500 g of honey was purchased for each sample. Samples were collected in triplicate from each market type and sub-categorized based on the source for analysis. Table 1 provides information about the collection area and source of each sample. The samples were collected from supermarkets, small shops, street vendors, retail stores (veranda), honey traders, processors, and open markets. As a control, a sample of raw, fresh honey was collected from Holeta Bee Research Center (HBRC) bee farm colonies, which had not been fed and was carefully processed without heating or additives. The honey samples were taken immediately to the HBRC bee product laboratory and stored at room temperature until analysis.

Table 1
A description of sources of studied honey samples and collection area.

Sample code	Area	Sub-city	Sample source
1	Gojjam beranda	Addis Ketema	Street areas
2	Gojjam beranda	Addis Ketema	Retail store(veranda)
3	Gojjam beranda	Addis Ketema	Retail store(veranda)
4	Gojjam beranda	Addis Ketema	Retail store(veranda)
5	Gojjam beranda	Addis Ketema	Honey trader/retailer
6	Gojjam beranda	Addis Ketema	Honey trader/retailer
7	Amade gebeya	Addis Ketema	Local market
8	Piasa	Areda	Supermarket
9	Piasa	Areda	Supermarket
10	Churchill road	Areda	Supermarket
11	Atlas	Kirkos	Supermarket
12	Bole madinalem	Bole	Supermarket
13	Bole madinalem	Bole	Supermarket
14	Bole (Japan Embassy)	Bole	Supermarket
15	Bole rewanda	Bole	Honey processors
16	Walo safer	Kirkos	Honey processors
17	Walo safer	Kirkos	Honey processors
18	Sarbet	Nefassilklafto	Supermarket
19	Sarbet	Nefassilklafto	Supermarket
20	Bisrat Gabriel	Nefassilklafto	Supermarket
21	Lideta	Lideta	Supermarket
22	Kolfe keraniyo	Kolfe keraniyo	Supermarket
23	Kolfe keraniyo	Kolfe keraniyo	Supermarket
24	Kebena	Yeka	Small shop
25	Shola market	Yeka	Honey trader/retailer
26	Shola market	Yeka	Honey trader/retailer
27	Maganagna	Yeka	Small shop
28	Guard shola	Bole	Supermarket
29	Guard shola	Bole	Supermarket
30	Guard shola	Bole	Supermarket

2.2. Analysis of physicochemical properties

The following physicochemical properties of commercially available honey were analyzed based on International Honey Commission methods (IHC) [23] compared with pure honey (control) and standard requirements.

2.2.1. Determination of moisture content

The refractive index was used to determine the moisture content of the honey samples. To determine the moisture content (MC), an Abbe refractometer (ABBE-5 Bellingham Stanley, Ltd, United Kingdom) was used following the guidelines IHC [23]. Firstly, the honey samples were homogenized and placed in a water bath until all the sugar crystals were dissolved. Then, the surface of the refractometer's prism was covered with honey sample, and the refractive index was determined after 2 min. The measured refractive index of the honey sample was converted into the moisture content using a standard table recommended by the IHC [23].

2.2.2. Determination of pH and free acidity

A pH meter (METTLER TOLEDO, CHINA) was used to analyze the pH and free acidity of the honey samples. The pH value was directly measured using a calibrated pH meter that had been calibrated at pH 4.00, 7.00, and 9.00 using buffer solutions. To prepare the sample, 10 g of honey was dissolved in 75 mL of distilled water in a 250 mL beaker and stirred using a magnetic stirrer following the guidelines of IHC [23]. To measure the free acidity, the honey sample solution was further titrated with 0.1 M sodium hydroxide (NaOH) solution to a pH of 8.30. The free acidity is expressed as mill equivalents or a mill mole of acid/kg honey, which is calculated as ml of 0.1 M NaOH x 10. The result is expressed to one decimal place as per the procedure of IHC [23], where acidity = 10 V, V is the volume of 0.1 N NaOH in 10 g of honey.

2.2.3. Determination of ash content

To determine the ash content of the honey samples, they were incinerated at 600 °C in a Muffle furnace (BioBase JKKZ.5.12 GJ, Shandong, China) until a constant mass was achieved, according to the guidelines set by IHC [23]. Initially, the empty crucible was heated in an electrical muffle furnace and then cooled in a desiccator before being weighed to 0.001 g (M2). Afterward, each honey sample weighing 10 g (M0) was weighed to the nearest 0.001 g and taken into a platinum crucible, with two drops of olive oil added to prevent foaming during the ashing process. The crucible was then placed in the preheated furnace and ashed for at least 1 h, and the ashing process was continued until a constant weight was reached (M1). The weight of ash in g/100 g honey was calculated using the following formula:

$$WA = \frac{M1 - M2}{M0}$$

where M0 = Weight of honey taken.

M1 = Weight of ash + crucible; M2 = Weight of a crucible.

2.2.4. Determination of sugar profile

To determine the honey sugars present in the samples, high-performance liquid chromatography (HPLC) (HPLC-1260 Infinity Series Agilent Technologies, USA) was utilized in accordance with the guidelines set forth by IHC [23]. Five grams of honey were dissolved in 40 mL of distilled water and filtered using a syringe filter (0.45 μm) before chromatographic analysis. The sugar profile was analyzed using an analytical stainless-steel column containing amine-modified silica gel (250 mm × 4.6 mm, 5–7 μm particle size). A mobile phase consisting of 80 % acetonitrile and 20 % water was employed using the isocratic method, and the flow rate was 1.3 mL/min. The amount of each sugar was detected by a Refractive Index Detector maintained at a temperature of 30 °C following injection of 10 μL into the column.

In preparation for calibration, sugar standards (fructose, glucose, sucrose, maltose) mixture which contain 20 mg/mL, 15 mg/mL, 10 mg/mL, 5 mg/mL, and 1.5 mg/mL were weighed, and five-level serial dilutions were prepared in accordance with IHC procedures. Each standard solution was dissolved in 40 mL of HPLC-grade water and mixed with 25 mL of methanol (HPLC grade) in a calibrated 100 mL flask. The standard solution was then filtered through a 0.45-μm nylon membrane filter (syringe filter), and the filtrate was poured into an injection vial. Calibration sugar standard solutions were prepared by pipetting 1.0 mL mixed standard stock solution into five 1.5 mL amber glass vials. Identification of honey sugars was obtained by comparing their retention times with those of the standard sugars [23], and triplicate injections were performed. Average peak areas were used for the peak quantification.

2.2.5. Determination of proline level

The proline concentration was determined using a modified version of the method developed by Meda et al. [24]. Briefly, a 0.5 mL solution of honey (5 g/100 mL of distilled water) was combined with 1 mL of 80 % formic acid and 1 mL of 3 % ninhydrin solution in ethylene glycol monomethyl ether. The mixture was then continuously vortexed for 15 min using a Vortex mixer. Next, the mixture was placed in a 70 °C water bath for 15 min. Following this, 5 mL of a 50 % v/v solution of 2-propanol was added. The mixture was allowed to cool for 45 min at room temperature, and the absorbance at 510 nm was measured using a UV–Vis spectrophotometer (JENWAY, United Kingdom). For comparison, deionized water and a 0.032 mg/mL solution of proline were used as the blank and standard solutions, respectively. The proline concentration in mg/kg of honey was calculated to one decimal place using the following equation:

$$\text{Proline (mg / kg)} = \frac{ES}{EA} * \frac{E1}{E2} * 80$$

where, ES = Absorbance of the sample solution; EA = Absorbance of the proline standard solution (average of two readings); E1 = mg proline taken for the standard solution; E2 = Weight of honey in grams; 80 = Dilution factor.

2.2.6. Determination of hydroxyl methyl furfural (HMF) content

The determination of hydroxymethylfurfural was carried out using a UV–Vis spectrophotometer (JENWAY, United Kingdom). A small beaker was used to weigh five (5) grams of honey sample, which was then mixed with 25 mL of distilled water before being transferred to a 50 mL volumetric flask [23]. Carrezz solution I, which consisted of 15 g K_4Fe (CN) $_6.3H_20$/100 mL distilled water, was added to the mixture, followed by 0.5 mL of carrezz solution II (30 g Zn acetate/100 mL distilled water). This was mixed with the honey solution and a droplet of alcohol was added before filtering the solution through a filter paper. The first filtrate (10 mL) was then discarded, and five (5) mL of the filtrate was added to each of the two test tubes. In the first test tube (sample solution), 5 mL of distilled water was added, while 5 mL of sodium bisulfite solution (0.20 % of 0.20 g $NaHSO_3$/100 mL in distilled water) was added to the other test tube (reference). Both test tubes were mixed well using a vortex mixer, and their absorbance was recorded spectrophotometrically. The results were calculated according to the IHC [23] and expressed as HMF/100g honey = (A 284 – A 336) × 14.97 × 5/g sample, where A 284 represents the absorbance at 284, A336 represents the absorbance at 336, 14.97 represents the constant, 5 represents the theoretical nominal sample weight, and g represents the mass of the honey sample.

2.3. Analysis of antioxidant activities

2.3.1. Total phenolic content (TPC)

The Folin-Ciocalteu method with slight modification [25] was used to analyze the total phenol content of the honey samples. A stock solution of honey was prepared by mixing 5g of the honey sample with 50 mL of distilled water and filtering the mixture through Whatman no.1 filter paper. Next, 1 mL (1 mg/mL concentration) of ethanolic extract solution was treated with 1 mL (2°N) FC reagent followed by the addition of 5 mL distilled water and shaken well for 5 min. After that, 1 mL of 10 %, Na2CO3 was poured and incubated for 1 h. In the same manner, a blank solution was prepared without a sample. Finally, after the solution was incubated for 2 h at 25 °C, the absorbance of the reaction mixture was measured at 765 nm using a UV–Vis spectrophotometer (PerkinElmer Lambda 950 UV/VIS/NIR spectrophotometer). Gallic acid was used as a standard. In this study, different concentrations of gallic acid (0.02 *mg*/mL, 0.04 *mg*/mL, 0.06 *mg*/mL, 0.08 *mg*/mL, and 0.1 *mg*/mL) were prepared by serial dilution technique to produce a calibration curve. All the measurements were evaluated in triplicate [26]. The total phenol content was reported as mean ± standard error and expressed as milligrams of gallic acid equivalent (GE) in 100 g of honey.

2.3.2. Total flavonoid content (TFC)

Total flavonoid content (TFC) was determined by adopting an established method with slight modification [27]. The aluminum chloride ($AlCl_3$) method was used to estimate the total flavonoid content of honey. To determine the total flavonoid content of each honey sample, a stock solution was prepared by diluting 5 g of honey sample in 50 mL of distilled water and filtering the mixture through Whatman no.1 filter paper. Next, 5 mL from the honey stock solution were pipetted and mixed in 5 mL of 2 % aluminum chloride ($AlCl_3$) solution. Similarly, a blank solution was prepared without a sample. After incubation for 30 min at room temperature the absorbance of the reaction mixture was measured at 415 nm by using a spectrophotometer (PerkinElmer Lambda 950 UV/VIS/NIR spectrophotometer) against the blank. A standard flavonoid compound was quercetin. Quercetin (0.025 mg/L, 0.075 mg/L, 0.125 mg/L, 0.175 mg/L, and 0.25 mg/L) was prepared from the stock solution (using ethanol as a solvent to produce a calibration curve. All the measurements were examined in triplicate [25]. The total flavonoid content was expressed as a milligram of Quercetin equivalent (QE) per 100 g of honey.

2.3.3. Determination of radical scavenging activity

The radical scavenging activity of the honey sample was determined using 2,2′-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay according to the established method with slight modification [25]. The DPPH was prepared by dissolving 0.5 mg of DPPH in 25 mL of methanol. Antioxidant compounds in honey samples were evaluated by measuring the ascorbic acid equivalent antioxidant capacity (AAEAC). A reference solution of ascorbic acid at a concentration of 10 mg/mL was used. The honey solution was prepared by mixing 30 mg honey in 1 mL methanol and 0.75 mL of methanolic honey solution was added to 1.5 mL of DPPH solution. The decrease in the absorption of the DPPH solution after the dilution of an antioxidant was measured at 517 nm by using a UV–Vis spectrophotometer. The blank was composed of 0.75 mL of a methanolic honey solution mixed with 1.5 mL of methanol. Ascorbic acid (0–200 mg/L) was used as a standard chemical to produce a calibration curve. Finally, the mean value was expressed as milligrams of ascorbic acid equivalent antioxidant content per 100 g of honey. The measurements were performed in triplicate [25].

2.4. Statistical analysis

All measurements of each honey sample were determined in triplicates. SPSS for Windows Version 20 software package was used for analyzing the data. Determination of the significant differences between honey samples was done using one-way ANOVA and,

Independent-Sample T-Tests. Means and standard errors of the recorded data were calculated.

3. Results and discussion

3.1. Physicochemical characteristics analysis

Table 2 displays the findings of the study of the physicochemical characteristics of commercially available honey samples collected from various market sources in Addis Ababa.

3.1.1. Proline

Table 2 shows the results of the analysis of Proline content in various commercially available honey types. A statistically significant difference ($P < 0.05$) in Proline content was observed among honey samples collected from different sources such as the open market, processors, supermarkets, stores, and street areas. The open-market honey types recorded the highest Proline content (423.05 ± 11.9 mg/kg) while the street samples recorded the lowest (67.1 ± 0.52 mg/kg). These findings are consistent with previous research conducted on commercial Portuguese honey samples, which reported similar Proline values ranging from 453.09 to 470.54 mg/kg [28]. In contrast, Tunisian honey was found to have Proline concentrations less than 180 mg/kg [29]. Higher values of Proline above 2000 mg/kg were reported in Burkina Faso [24] and Algerian [30] honey. The mean Proline content of all samples was generally in agreement with international parameters recommended for *Apis mellifera* honey, which should be above 180 %, except for the street honey types. The low amounts of Proline in the street honey samples indicate that the honey is unripe, and there is a high probability of sugar adulteration. Previous studies have shown that the addition of sugar to honey leads to low Proline values, while honey from bees fed on sugar water has similarly low Proline levels [31]. Proline concentration is an indicator of the quality and authenticity of honey, and it also reflects its antioxidant activity [24,32]. Furthermore, it can be used to determine the honey's botanical origin [33]; [34]. Variations in Proline content can be attributed to beekeeping practices such as feeding bees with more syrup, plant species, and environmental factor [35].

3.1.2. Moisture content

Moisture content is a crucial factor in determining the freshness and prevention of fermentation in honey [36,37]. In our study, the highest moisture content was found in honey collected from honey traders (23.04 ± 0.62 %), while the lowest was observed in honey obtained from processors (19.48 ± 0.4 %). These findings align with previous studies conducted by Gebreegziabher et al. [38], Tesfaye et al. [14], and Fikru et al. [39], which reported moisture content values of 18.4 ± 0.8 %, 18.80 ± 0.36 %, and 17.2 ± 0.86 %, respectively in honey from processors. Our results also correspond with the study by Adgaba [40], who reported a mean moisture content of 20.5 % in Ethiopian honey. However, our findings differ from Tewodros et al. [41], who measured a moisture content of 16.0 ± 1.25 % in honey directly obtained from hives in Ethiopia, and from Latif et al. [42], who found moisture content ranging from 14.3 to 18.6 % in Pakistani honey. A moisture content of more than 20 g/100g makes honey susceptible to fermentation and microbial spoilage [43,44]. High moisture content could be attributed to improper packaging, premature harvesting, extraction in a damp environment, or a combination of these factors. Finola et al. [5] suggested that low moisture content might result from harvesting honey when it is fully mature, which helps extend its shelf life during storage [45]. The moisture content of honey is influenced by the temperature and relative humidity of its geographical origin [46].

The mean moisture content of honey obtained from processors and retail stores (veranda) complied with national standards set by the Ethiopian Standard Agency (ESA) [7], as well as the international Codex [6] and European Union (EU) [1] parameters, which prescribe a maximum moisture content of 20 %. However, honey from honey traders, small shops, supermarkets, and street areas showed slightly higher moisture content, indicating a deviation from national and international standards. This variation in moisture content can be attributed to improper handling practices throughout the honey supply chain, from harvesters to retailers and merchants, within the same ecological zone. The fluctuations in the moisture content of honey are mainly influenced by harvest practices, handling procedures, processing methods, and potential adulteration [47,48].

3.1.3. Ash content

Ash content is an important quality measure for identifying the botanical and geographical origin of honey. In our study, significant differences ($P < 0.05$) were observed in ash content between honey samples collected from supermarkets, processors, and small shops,

Table 2
The mean and standard error (mean ± SE) values for the physicochemical content of commercially available honey collected from study areas.

Source of the sample	Proline(mg/kg)	Moisture content (g/100g)	pH	HMF (mg/kg)	Free acidity(meq/kg)	Ash(g/100g)
Supermarket	234.45 ± 16.9^{c}	21.36 ± 0.31^{ab}	3.62 ± 0.07^{ab}	20.15 ± 2.94^{a}	29.28 ± 3.1^{ab}	0.26 ± 0.03^{a}
Small shops	349.79 ± 53.4^{abc}	22.56 ± 1.19^{a}	3.44 ± 0.04^{ab}	14.72 ± 1.91^{a}	26 ± 1.71^{ab}	0.24 ± 0.04^{a}
Street honey	67.1 ± 0.52^{d}	21.77 ± 0.48^{ab}	3.46 ± 0.02^{ab}	16.26 ± 3.63^{a}	12 ± 1.15^{c}	0.15 ± 0.1^{ab}
Retail store(veranda)	281.98 ± 36.01^{bc}	20.49 ± 0.13^{ab}	3.32 ± 0.04^{ab}	14.95 ± 0.44^{a}	16.5 ± 1.3^{bc}	0.13 ± 0.03^{bc}
Honey trader	398.27 ± 29.73^{ab}	23.04 ± 0.62^{a}	3.38 ± 0.04^{ab}	21.23 ± 3.67^{a}	28.13 ± 1.1^{ab}	0.21 ± 0.1^{ab}
Processors	236.18 ± 44.69^{c}	19.48 ± 0.4^{b}	3.77 ± 0.05^{a}	17.5 ± 5.95^{a}	33.83 ± 5.15^{a}	0.26 ± 0.05^{a}
Open market	423.05 ± 11.91^{a}	22.3 ± 0.53^{a}	3.22 ± 0.00^{b}	10.66 ± 1.24^{a}	17.5 ± 0.29^{abc}	0.07 ± 0.01^{c}

Means with different superscript (a, b, c) columns are significantly different at $P < 0.05$ assessed by Duncan's multiple ranges.

compared to those gathered from open markets and retail stores (honey veranda). Honey from supermarkets (0.26 ± 0.03 g/100g) and processors (0.26 ± 0.05 g/100g) showed the highest mean ash contents, while samples from the open market had the lowest (0.07 ± 0.01 g/100g). Our ash content results were lower than those reported by Alvarez-Suarez et al. [49] (0.46 ± 0.03), but comparable to the findings of Kayode & Oyeyemi [50], who measured ash content values ranging from 0.004 to 0.440 g/100g in Nigerian honey. Soil and plant characteristics are natural contributors to variance and fluctuations in ash content in honey products [51]. High levels of ash content could potentially indicate environmental or handling and equipment-related contamination [5]. Thus, honey produced from bees fed with sugar syrup typically shows lower levels of ash content [52,53]. Honey's ash content is an essential quality criterion, with variations potentially influenced by beekeeping practices and harvesting procedures [5]. The maximum permissible limit for honey's ash content is set at 0.6 %, according to guidelines established by the EU [1], Codex Alimentarius [6], and ESA [7]. None of the honey samples tested in our investigation exceeded this permissible limit.

3.1.4. Hydroxylmethylfurfural (HMF)

HMF, an aldehyde generated by the acidic decomposition of fructose via a non-enzymatic browning reaction called the Maillard reaction, is an important quality criterion for evaluating the freshness of honey. This study showed that the HMF levels of honey samples collected from commercial areas ranged from 21.23 ± 3.67 mg/kg mean value for the honey trader to 10.66 ± 1.24 mg/kg mean value for the individual open market (Table 2). However, these values were not statistically different from one another ($P > 0.05$). None of the honey samples tested exceeded the national (ESA) [7] and international Codex [6] and EU [1] quality standards of 40 mg/kg. Studies in the Jimma Zone [20], Tigrai Region [15], and Gonder [54] reported similar HMF levels of 6.3 mg/kg, 15 mg/kg, and 6.3 mg/kg, respectively. However, other studies from Kenya and Uganda reported higher HMF values, such as 85.4 ± 0.15 mg/kg, 3.7–389.4 mg/kg, and 103.2 ± 40.5 mg/kg in honey samples from supermarkets [55–57].

Higher HMF values in honey may suggest poor storage conditions, aging, excessive heating during processing, adulteration, and inferior quality [58,59]. Fresh honey typically has no trace levels of HMF [60]. Various factors influence HMF levels in honey, including the temperature and time of extraction and processing, storage conditions, aging, pH, and floral sources [61]. Processes like liquefaction and pasteurization, as well as handling, extracting, conditioning, and storage conditions, may raise HMF levels above the natural levels of 10 mg/kg [62,63]. Thus, HMF is an essential indicator of honey freshness and quality.

3.1.5. pH

The pH values of honey samples from commercial markets ranged from 3.22 to 3.62. The mean pH values of honey samples collected from processors were significantly different ($P < 0.05$) from those gathered from the individual open market. The pH range in this study (3.22 ± 0.00 to 3.77 ± 0.05) was sufficiently low to prevent microbial growth [64,65] and was comparable to a prior study in Malaysia (3.78 ± 0.21) [66]. However, it was lower than the pH value of honey samples from the Istanbul market (4.32) [67]. Honey from Burkina Faso, on the other hand, had pH values ranging from 3.5 to 4.7 [24], while Nigerian honey had pH values ranging from 3.1 to 6.1 [68]. A decline in pH values may indicate honey fermentation [69,70] and may limit and inhibit microbial growth, contributing to honey stability. Honey pH can also indicate its origin and predict honey degradation during storage [71].

Low pH values can prolong shelf life and enhance taste, making honey more compatible with various food products for domestic and international markets [19]. Organic acids, including gluconic acid, formic acid, oxalic acid, and lactic acid, contribute to honey's pH values [72]. The EU and the Codex have set a range of 3.6–4.3 for honey pH values. The honey samples collected from Addis Ababa markets are within these standards [1,6]. Differences in honey source, processing techniques, and botanicals may contribute to variations in pH values [45], as enzymatic processes, fermentative conversion of raw materials, and foraged plants all influence pH variation in honey samples [73]. Therefore, pH values are an important parameter for determining honey origin and quality.

3.1.6. Free acidity

The free acidity range of honey samples in this study was from 12 ± 1.15 (Street honey) to 33.83 ± 5.15 meq/kg for processor's honey types. The average free acidity of each honey sample was in line with national (40 meq/kg), international Codex [6], and EU [1] quality requirements, which should be 50 meq/kg or below. Comparable free acidity levels were reported in Kamal et al. [74] study of various honey samples (6.7–22.9 meq/kg). Meanwhile, close findings were reported in Tigray (29.895 meq/kg; Gebreegziabher et al. [38] and Amhara (27.34 meq/kg; Alemu et al. [21]). However, the free acidity of honey from our study was higher than that of honey from Nigeria (18.67 0.64 meq/kg) [75] and the Polish market (14.40 ± 0.58 meq/kg) [76]. Muli et al. [77] also reported free acidity ranging from 8 to 71.9 meq/kg in samples of honey collected from traditional processors, beekeepers, and honey traders in Kenya. Gebremariam & Brhane [16] found that market samples had higher free acidity than those recommended for authentic (pure) honey. Additionally, Fredrick et al. [55] demonstrated that the free acidity of the local market (supermarket) honey samples and different honey brands was 56.7 meq/kg, significantly higher than our results.

High honey acidity results from the fermentation of sugars in honey into organic acids, which contribute to important characteristics like flavor and stability against microbial spoilage [78]. Additionally, high acidity in honey may indicate high mineral concentrations [79]. With increasing acidity, the sour taste of honey makes it less acceptable [57,80], whereas low acidity values indicate freshness [64]. The significant variation in free acid amounts in different honey samples may be due to factors like the amount of time nectar takes to transform completely into honey, colony strength, and nectar sugar concentrations. Diverse management, harvesting, and processing techniques can also affect free acid levels in honey and, consequently, its final quality [81].

3.1.7. The sugar profile of commercially available honey samples

Honey's sugar content is mainly fructose, glucose, and sucrose [82]. The sugar content was determined using high-performance

liquid chromatography (HPLC). Figs. 1 and 2 show the chromatogram of the samples and standard sugar, respectively.

The lowest fructose content was found in small shops (33.85 ± 0.65 %), while the individual open market had the highest fructose content (Table 3). Fructose is the primary sugar responsible for honey's sweetness [63], and honey from the individual open market (48.61 ± 0.51) had particularly high fructose content, likely a result of nectar-rich fructose [73]. These results are comparable with Al-Arrify [83] (43.19 %), Joshi et al. [84] (45.93 %), Erejuwa et al. [85] (21–43.5 %), Pascual-Maté et al. [86] (38 %), Kucuk et al. [87] (7.7–43.9 %), Amri et al. [88] (30.6–45.1 %), Amri [89] (27.2–44.3 %), and Ethiopian monofloral honey by Belay et al. [90] (43.1 + 0.4 g/100g) indicating fructose is the dominant sugar in natural-quality honey.

The glucose content in our study ranged from 33.07 ± 1.58 to 44.3 ± 0.82 (Table 3), with the highest mean glucose value found in honey from a veranda store. This could be due to the honey being made from specific plant species, feeding syrup to bees, or honey contaminated with artificial ingredients with high glucose levels [56]. Comparable studies by Kucuk et al. [87] and Amri et al. [88] found glucose levels of 34.9–40.2 % and 20.3–40.2 %, respectively, which are consistent with our results. Glucose content was found to be between 22.0 and 40.8 % in the study by Jeanne [91], and Belay et al. [90] reported glucose levels of 37.2 + 0.4g/100g in Ethiopian monofloral honey. However, some of our samples had glucose content above the level stated by the Codex [6], with samples from veranda stores (44.3 ± 0.82 %) and honey traders (41.53 ± 2.65 %) exceeding 40 %. Honey with high glucose concentration tends to crystallize faster than those with high fructose content [92]. Therefore, the extremely high glucose values in our study suggest the addition of additives [56]. The glucose content in honey is influenced by its botanical and geographical origin, climate, processing, and storage conditions [81].

Table 3 displays the reducing sugars content of commercially available honey, where veranda store honey samples had the highest reducing sugar content (87.88 ± 1.22 %), while small stores had the lowest (68.01 ± 3.64 %). These findings are consistent with previous studies on different types of honey [53,79,93], as well as the study done by Birhanu [94], who reported a 60.5 % rate for honey samples collected from farmers' hives and local honey markets. Low levels of reducing sugar in honey may be a result of adulteration with other products, as the addition of substances reduces the ratio (%) of reducing sugar from the honey's components. Low levels can also indicate poor honey quality due to insufficient ripening periods or harvesting in hot conditions during honey collection [82]. The sum of fructose and glucose values (F + G) corresponded to the value (>60 g/100 g) given by Codex [6].

Street honey samples had the highest sucrose content (13.4 ± 0.77 %), followed by veranda store honey samples (6.23 ± 2.49 %) and honey traders (5.74 ± 1.52 %). The high sucrose content may be a result of overfeeding bees with sugar, harvesting unripe honey, or adding sugar and syrups [80]. This finding aligns with previous studies on honey samples from local markets, pure honey, contaminated honey, or gathered from forest areas [15,19,54,95,96]. However, the sucrose content in this study was higher than those reported for samples of honey from Malaysia, Bangladesh, and Nigeria. The honey samples obtained from street areas, retail stores (veranda), and honey traders had sucrose values above the 5 g/100g limit proposed by the EHC and Ethiopia standards, indicating possible adulteration by the direct addition of sugar to honey [15,97]. High sucrose content leads to poor honey quality [98].

Street honey samples had the highest maltose content, while processor honey samples had the lowest (3.04 ± 0.97 and 0.73 ± 0.14, respectively). Kaskonienė [99] found that natural honey could be distinguished from artificial honey by its high maltose content, and compared to honey from other parts of the world; commercially collected honey samples from this study had a low maltose level.

The fructose to glucose (F/G) ratio in commercially available honey samples ranged from 1.43 ± 0.08 to 0.99 ± 0.07, with street honey showing a significant difference ($P < 0.05$) from other types of honey. The F/G ratio is a good indicator of honey quality, and its ability to crystallize in particular [53]. It ranged from 0.84 to 1.89 in different types of honey [100], and ratios less than one (1) may indicate adulteration with syrup [101]. The F/G ratio is used to predict and manage granulation tendencies in honey, being slower

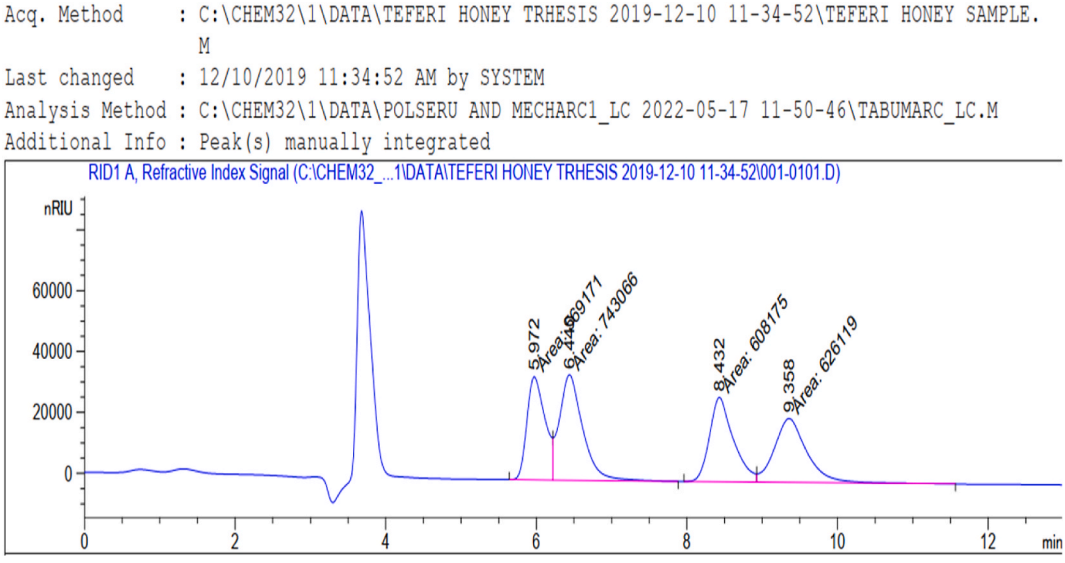

Fig. 1. Chromatogram of sugar standards.

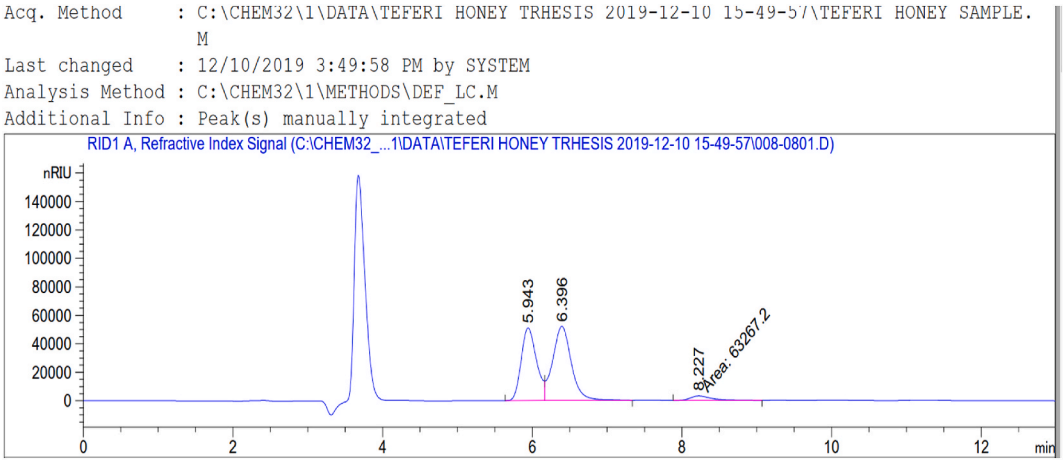

Fig. 2. Chromatogram of sugars of commercially available honey.

Table 3
The mean and standard error (mean ± SE) of the sugar profile of commercially collected honey.

Source of the sample	Fructose (%)	Glucose (%)	Sucrose (%)	Maltose (%)	Reducing Sugar (%)	F/G ratio
Supermarket	39.13 ± 0.98^{cd}	37.9 ± 0.9^{abc}	2.87 ± 0.4^{bc}	1.17 ± 0.18^{b}	77.02 ± 1.1^{bcd}	1.07 ± 0.04^{b}
Small shops	33.85 ± 0.65^{d}	34.2 ± 3.34^{bc}	1.51 ± 0.1^{bc}	0.84 ± 0.08^{b}	68.01 ± 3.64^{d}	1.04 ± 0.10^{b}
Street honey	47.2 ± 0.24^{ab}	33.07 ± 1.58^{c}	13.4 ± 0.77^{a}	0.51 ± 0.14^{b}	80.3 ± 1.34^{abc}	1.43 ± 0.08^{a}
Retail store (veranda)	43.58 ± 1.9^{abc}	44.3 ± 0.82^{a}	6.23 ± 2.49^{b}	0.56 ± 0.15^{b}	87.88 ± 1.22^{a}	0.99 ± 0.06^{b}
Honey traders	39.42 ± 1.0^{bcd}	41.53 ± 2.7^{ab}	5.74 ± 1.5^{bc}	0.57 ± 0.10^{b}	80.95 ± 3.4^{abc}	0.99 ± 0.07^{b}
Processors	41.45 ± 1.6^{abc}	33.64 ± 1.7^{bc}	2.08 ± 0.5^{bc}	2.4 ± 0.44^{a}	75.10 ± 1.4^{cd}	1.27 ± 0.09^{ab}
Open market	48.61 ± 0.51^{a}	37.1 ± 0.04^{abc}	0.91 ± 0.05^{c}	0.79 ± 0.26^{b}	85.65 ± 0.55^{ab}	1.31 ± 0.01^{ab}

Note: Means with different superscripts (a, b, c, d) within the columns are statistically different at $P < 0.05$, F/G = Fructose to Glucose ratio.

when the ratio is more than 1.0 and quicker when it is less than 1.0 [102,103]. In this study, the F/G ratio is lower than in previous studies in Spain, Nepalese, and African honey, but higher than those in Polish honey.

3.2. Levels of antioxidant activity in commercially available honey

The contents of total phenols, total flavonoids, and antioxidant activities in this study are discussed in the following sections.

3.2.1. Total phenolic content (TPC)

The processor's honey sample had the highest total phenolic content (TPC), with a mean of 533.39 ± 28.9 μg GAE/100 g, followed by the supermarket sample (343.27 ± 33.45 μg GAE/100 g) and honey traders (263.02 ± 10.06 μg GAE/100 g). The findings of Sime et al. [103] align with this study, as honey samples from the Southern region of Ethiopia also had a total polyphenol level ranging from 330 ± 38 to 610 ± 5 mg GAE/100g. It is consistent with the findings of many researchers who state that honey with a darker color

Table 4
Mean and standard error (mean ± SE) values for total flavonoids, total phenolics, and antioxidant concentrations in honey samples collected from the market.

Source of the honey	TPC (μg GAE/100g)	TFC (μg QE/100g)	DPPH (μg AEAC) /100 g)
Supermarket	343.27 ± 33.45^{ab}	1252.15 ± 142.35^{abc}	836.07 ± 146.10^{a}
Small shops	158.26 ± 1.23^{b}	303.89 ± 9.27^{bc}	959.65 ± 9.79^{a}
Street honey	176.24 ± 0.09^{b}	256.91 ± 60.50^{c}	130.54 ± 9.68^{b}
Retail store (big veranda)	201.05 ± 14.49^{b}	881.22 ± 26.89^{bc}	290.04 ± 13.21^{ab}
Honey traders/collectors	263.02 ± 10.06^{b}	295.40 ± 111.37^{bc}	380.11 ± 44.69^{ab}
Processors	533.39 ± 28.9^{a}	2013.63 ± 136.89^{a}	766.52 ± 22.39^{a}
Open market	212.08 ± 2.25^{b}	1420.07 ± 77.46^{ab}	1327.17 ± 909.29^{a}

Means with the same letter (a, b, c, d) within the columns are not statistically significant ($P \leq 0.05$). Notice: SE: Standard Error, TPC (μg GAE/100g of honey): Total phenolic content in milligram of GE per *one hundred gram of honey sample, TFC (μg QE/100g of honey): Total flavonoid content in milligram of Quercetin equivalent (QE)* per *one hundred gram of honey sample, AOC (μg AAE/100g of honey): Antioxidant content in mg of Ascorbic acid equivalent in one hundred of a honey sample.*

tends to have a greater concentration of total phenolic compounds [104]. The TPC of the honey used in this study was higher than those reported for Malaysian honey, but lower than Slovenian honey [66,105]. Ouchemoukh et al. [69] reported a high TPC range of 79–1304 mg GAE/100g, while Rebiai et al. [106] found a TPC range of 179.2–1831.8 mg kg^{-1}. According to Alvarez-Suarez et al. [107], the phenolic content of honey is influenced by various factors such as geographic origin, bee species, and bee forage. Phenolic compounds are derived from pollen and propolis constituents [108]. Natural antioxidants present in honey play an essential role in preserving food and promoting human health. Phenolic components such as phenolic acids are responsible for counteracting oxidative damage, lowering the risk of heart disease, cancer, cataracts, immune system decline, and inflammation [87].

3.2.1.1. Total flavonoid content (TFC). Table 4 shows the total flavonoid contents of the honey samples collected from the Addis Ababa honey market. The total flavonoid content ranged from 256.91 ± 60.50 for Street honey to 2013.63 ± 136.89 for Processors. The TFC from this study was higher than that reported for Turkish honey (1.73 ± 0.80 mg QE/100 g) [109] and commercial Portuguese honey (1.12–9.24 mg QE/100 g). However, the TFC results obtained in this study are comparable to those reported by Rebiai et al. [106] for Algerian honey, which ranged from 159.42 to 497.56 mg QE/100g. Polyphenols commonly found in honey include phenolic acid, flavonoid, and tannins, which have wide structural differences [110]. The variation in total flavonoid content observed in this study can be attributed to various factors, including geographical location, environmental factors, and treatment used [111]. Due to its antioxidant properties, honey has been regarded as a therapeutic food, with polyphenols and flavonoids being significant contributors to its nutritional benefits [112,113].

3.2.1.2. DPPH assay. As stated above the antioxidant activity of the honey samples was measured using the DPPH assay - a commonly used method for evaluating radical scavengers in natural foods due to its stability as a free radical. The total antioxidant content was determined through an ascorbic acid equivalent antioxidant capacity (AEAC) assay. The open market samples had the highest AEAC concentration (1327.17 ± 909.29 μg AAE/100g), while street honey had the lowest (130.54 ± 9.68 μg AAE/100 g) (Table 4). The mean values of antioxidants of the current study findings were above the results reported from Burkina Faso honey sample 11.27 ± 0.02 to 65.86 ± 0.1 mg AAE/100g [24], a honey sample from Bangladesh 18.4 ± 0.7 to 34.1 ± 1.4 mg AAE/100g [106] and Indian honey samples ranging from 15 to 30 mg AAE/100g [32]. However, the AEAC levels found in this investigation were comparable to those reported from Malaysian honey samples. (276.96–324.47 mg AEAC/kg) [66], Indian honey (151–295 mgAEAC/kg) [32], and Burkina Faso honey samples (270.40 ± 146.8 mg/kg) [24]. The finding of the study indicated that commercially collected honey samples in the study area exhibited higher antioxidant activity, which could be attributed to the variation in geographical flora used to produce the honey samples. The DPPH scavenging test, which measures the free radical-scavenging activity of natural products and other substances, is widely used to assess the antioxidant potential of honey [114].

4. Conclusion

This study aimed to determine the basic physicochemical and antioxidant characteristics of various commercial honey samples. Overall, the honey sold in Addis Ababa city met the acceptable range set by the Codex Alimentarius Commission and the Ethiopian standard agency for most of the parameters. However, some marketed honey samples deviated from the recommended range for moisture content, proline, and sucrose content, possibly due to unripe harvesting, improper handling, storage conditions, and adulteration. The study also revealed that commercial honey is a good source of natural phenolic, flavonoids, and antioxidant activity. Monitoring and testing honey quality characteristics in different market channels across the country are necessary to sustain the natural quality of honey and reduce honey adulteration. Therefore, future studies should combine physicochemical and quality criteria with multivariate approaches to validate honey quality and authenticity. Such studies will contribute to raising the demand for Ethiopian honey in EU markets.

Compliance with ethics requirements

The article does not contain any studies with human participants or animal subjects.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Teferi Damto: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Ashagrie Zewdu:** Formal analysis, Software, Supervision, Writing – review & editing. **Tarekegn Birhanu:** Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

influence the work reported in this paper.

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