

Research Article

Physicochemical Properties and Botanical Sources of Honey from Different Areas of Ethiopia: An Implication for Quality Control

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Honey is one of the important food commodities due to its nutritional and medicinal values. However, the issue of its quality and authenticity remain as important factors in consumption and marketing. This study was aimed at determining the botanical sources and quality parameters of honey samples collected from different areas of Ethiopia. The botanical sources of honey were analyzed using the melissopalynological method. Sugar profiles were analyzed using HPLC, and physicochemical properties were determined following the harmonized methods of honey analysis. Diverse plant species, including *Schefflera abyssinica*, *Eucalyptus* spp., *Guizotia abyssinica*, *Echinops* spp., *Coffee arabica*, *Bersama abyssinica*, and *Rumex* spp., were identified as dominant sources of honey. However, honey from the Gimbo district contains no pollen fingerprints, and its source has remained unknown. The average values of honey sugar compositions ranged from 30.3–46.7%, 29.9–40.3%, 15.0–22.2%, and 0.28–4.4% for fructose, glucose, sucrose, and maltose, respectively. Although the quality parameter values of most honeys fit the acceptable range of national and international honey quality standards, honeys collected from Guassa district revealed some abnormal characteristics. This abnormality may be related with poor handling, processing, or suspected for honey adulteration. Thus, regular testing and monitoring of honey quality are crucial in order to maintain its natural properties as well as control the current widespread practice of honey adulteration in Ethiopia.

1. Introduction

Honey is a sweet natural food that is well-known for having a wide range of uses and applications. It is a delicious, sticky, and viscous liquid that various bee species make from the nectar of flowers or from the secretions of living plant components [1]. Globally, honey is known to be used for medicinal, nutritional, and industrial purposes due to its diverse composition. Owing to its main energy source that helps to prolong nitrogen retention in the digestive system, honey is specifically recommended for elderly, pregnant women, and ill people [2].

Naturally, honey is a supersaturated solution of sugars that accounts for approximately 95% carbohydrates of its dry weight, in which fructose and glucose are the principal sugar components [3, 4]. Though honey contains other several substances, the nutritional characteristics of honey are mostly determined by its sugar composition [5]. Besides these, there are other constituents such as proteins, enzymes, amino acids, minerals, vitamins, organic acids, and phenolic compounds that contribute towards the quality and health benefits of honey [6]. Most volatile compounds found in honey include alcohols, ketones, aldehydes, acids, and esters, which are also responsible for its flavor and aroma characteristics [7]. Moreover, natural honey is rich in flavonoids and phenolic acids that exhibit a wide range of biological effects and which also act as natural antioxidants [8].

In Africa, a variety of beekeeping techniques used for honey production in which traditional way of honey production still dominantly practiced. Traditionally, honey is produced either by hunting from tree holes or through hanging different kinds of hives on tree branches. In this case, the hives are made from locally available materials and placed in forest trees and inhabited by swarms of wild bees that are genetically identical to the wild population [9, 10]. On the other hand, honey is produced by the modern beekeeping system by using moveable framed hives and topbar hives [11]. As a result, the quality and composition of honey was also determined by the honey production system and postharvest processing techniques.

Apart from production and processing techniques, the physical and chemical compositions of honey in general are affected by different factors and honeys from different sources have varied characteristics. Several factors including floral sources, geographic and environmental origin, season, processing and storage methods, and other factors have an impact on the composition and quality of honey [5, 12, 13]. Moreover, honey-making processes by different bee species can also influence the composition of honey as the amount of enzymes added by bees throughout honey-making is quite important for its quality. For instance, harvesting unripe honey at its nectar stage reduces the required amount of enzymes, which in turn reduces the honey quality and minimizes its market demand [14]. According to Azeredo et al. [15], honeys harvested at different seasons of the year from different areas could have different compositions depending on the nectar types and foraging sources of the bees. Moreover, honey from the same region but harvested at various seasons of the year may have different qualities. This suggests that different climatic and seasonal conditions, as well as pre- and postharvest beekeeping practices, might potentially have an impact on the honey quality [16, 17].

In Ethiopia, some studies identified the physicochemical properties and geographical origins of Apis mellifera honeys which were harvested from specific areas through various seasons of the year [18-21]. However, these studies suggested the regular testing and evaluation of honey based on its botanical and geographic sources. Also, the current honey adulterations with foreign substances are significantly altering the natural composition of honey and becoming a risk factor for marketing and consumption. A review by Damto [22] has explored the widespread practices of honey adulteration in Ethiopia that have been causing quality deterioration as well as affecting the current domestic and international honey markets. As a result, regular monitoring and determining the major properties of honey such as moisture content, sucrose and reducing sugars, pH value, electrical conductivity, ash content, free acidity, diastase activity, and hydroxymethyl furfural (HMF) against the established standard quality parameters [23, 24] are very crucial to maintain the natural honey quality and its origin. Thus, our aim was to analyze the major quality parameters of honey collected from ten potential beekeeping areas of Ethiopia and to identify their specific botanical origins. This will provide information to monitor the widespread practice of honey adulteration in the country and aid in determining the natural composition and the quality of honey.

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2. Materials and Methods

2.1. Honey Sample Collection and Preparation. Honey samples were collected from different areas of Ethiopia including Jimma zone (Gomma, Gera, Manna, Dedo, and Kersa districts), Bale zone (Rira and Dollo-Mana districts), Kefa zone (Gimbo and Gesha district), and North Shewa zone (Anaz-Guassa highland, Afroalpine district). A total of 30 honey samples with three replications were collected and stored in the laboratory at room temperature (Figure 1) until processing for quality parameters.

For the sugar and physicochemical property analysis, standard chemicals and reagents were purchased from Addis Ababa chemical importers. Then, all the collected honey samples were prepared and analyzed at Holeta Bee Research Center laboratory following the harmonized method of the International Honey Commission [25].

2.2. Botanical Origin Identification. The botanical origins of honey samples were characterized by melissopalynology using the method of Louveaux et al. [26]. For this, 10 gram of honey was dissolved in 20 ml of warm distilled water and stored at a temperature range of 20-40°C. The solution was then centrifuged at 3800 rpm for 10 minutes and the supernatant was decanted. Again, 20 ml of distilled water was added to completely dissolve the remaining sugar crystals and recentrifuged at 3800 rpm for 5 minutes and the supernatant was removed. The remaining precipitate was spread evenly on a microscope slide and the sample was exposed to air dry. Finally, one drop of glycerin jelly was added to the cover slip and examined under the light microscope (Zeiss AxioVert, Mg. Power 40x), and the morphological structure of selected pollen pictures were taken from each slide (Figure2). The source of dominant pollen plants was then identified using reference slides and pollen atlas [11]. The percentage of each pollen type was calculated in every honey sample using total pollen counts, and honey samples with more than 45% pollen dominance of single flora was considered as monofloral honey.

2.3. Physicochemical Property Analysis. The major physicochemical properties such as moisture content (MC), ash content (Ash), pH, free acidity (FA), and hydroxylmethylfurfuraldhyde (HMF) were investigated following the standard protocols [27]. In brief, the moisture content of the honey samples was determined using an Abbe refractometer (ABBE-5 Bellingham Stanley. Ltd, United Kingdom) that was thermostatic at 20°C and regularly calibrated with distilled water. After homogenization of honey samples, the prism refractometer surface was covered with honey and the value for the refractive index was determined using a standard table [28].

The pH value was directly measured using a pH meter (Mettler Toledo, China) and free acidity was determined by titrating the sample solution with 0.1 M sodium hydroxide solution (NaOH) to pH 8.30 [27], then expressed as mill equivalents or a mill mole of acid/kg and was equivalent to ml of $0.1 \text{ M NaOH} \times 10$.

The ash content was determined by incinerating honey samples at 600°C in a muffle furnace (BioBase JKKZ.5.12GJ, Shandong, China) to reach the constant weight [28]. Then, the ash value was calculated in percent in g/100 g mass as described in [27].

HMF value of the honey was measured using UV-vis spectrophotometer absorbance at 284 nm and 336 nm wavelength against the reference solution following the harmonized method of International Honey Commission [29]. Subsequently, a spectrophotometer working in a wavelength range of 284 nm-336 nm was employed and HMF expressed in mg/kg.

2.4. Sugar Profile Analysis. Honey samples were analyzed for sugar profiles using high performance liquid chromatography (HPLC-1260 Infinity Series Agilent Technologies, Germany). Five grams of honey was dissolved in 40 ml distilled water and the honey solution was transferred into a 100 ml volumetric flask containing 25 ml of acetonitrile. Then, the solution of each honey sample was filtered using syringe filter (0.45 μ m) before chromatographic analysis. Using the HPLC separation system at a flow rate of 1.3 ml/ min, mobile phase Acetonitrile: water (80:20, v/v) and sample injection volume $10 \,\mu$ l, the sugars were detected by a refractive index detector thermostat at 30°C temperature regulated column oven at 30°C. The identification of honey sugars was obtained by comparison of their retention times with those of the standard sugars [28]. Five series serial dilution standards of fructose, glucose, sucrose, and maltose mixture were dissolved in 40 ml distilled water and 25 ml acetonitrile following the International Honey Commission [28] to draw a calibration curve.

2.5. Statistical Analysis. One-way ANOVA was employed to compare the variations between the means of each variable in every location of the honey samples. SPSS version 20 statistical software was used, and tests were performed at 5% level of significance. Moreover, principal component analysis (PCA) was employed to categorize the botanical sources and honey sample collection areas based on pollen count data (Figure 2).

3. Result and Discussion

3.1. Botanical Origin of Honey. One of the fundamental aspects that influence the commercial value of honey is its botanical and geographical origin which is principally different based on plant species coverage. One of the main methods for establishment and confirmation of honey botanical and geographical origin is pollen analysis (melissopalynology) [30]. In this result, the melissopalynological analysis of honey samples indicated the dominant pollen representatives of plant species including *Guizotia abyssinica*, *Brassica spp.*, *Schefflera abyssinica*, *Echinops* spp., *Coffee arabica*, *Bersama abyssinica*, *Rumex* spp., *Satureja paradoxa*, and *Eucalyptus* spp. (Figure 3). These plant species are also known as major honey sources for *A. mellifera* bees in different areas of Ethiopia [31, 32].



FIGURE 1: Honey samples collected from ten different areas of Ethiopia for quality analysis.

However, the relative pollen frequency analysis of honey samples showed that Sh. abyssinica was a predominant honey plant in samples collected from Jimma and Kefa zones with total percentage frequency 41.4%. This confirmed that Sh. abyssinica is a known bee forage plant in forest highlands of southwestern Ethiopia and it has been providing white monofloral honey in these particular areas [33, 34]. While the honey samples collected from northwest Shewa zone of Anaz-Guassa Afroalpine was uniquely represented by Thymes spp. (T. vulgaris) which is commonly known as medicinal and aromatic plant herbs in Ethiopia. Phoenix spp. and Syzygium guineense were predominantly found in honey samples collected from Rira and Dollo-menna districts of Bale zone. Similarly, a study conducted by Bareke and Addi [32] also indicated that Syzygium guineense is the predominant plant species in Guji zone of Oromia region, which is a neighboring area to this sampling site. Pollen dominance for Eucalyptus spp. was identified in honey samples collected from Gesha, Guassa, and Manna districts. Guizota scarba and Trifolium spp. were another honey source plants detected in honey samples collected from different areas including Gera and Manna districts. Indeed, Guizota spp. is known for its golden-yellow flowers abundantly covering the highlands and mid-highland areas of Ethiopia during the spring season [31]. In addition, honey samples from the Rira, Gesha, and Gera districts were found to contain unidentified pollen types in this study, providing an evidence for further in-depth honey's botanical identification. Surprisingly, there was no pollen types found in honey samples collected from Gimbo which might suggest that the honey is either adulterated with foreign substance or it may not be a natural honey.

Moreover, despite the fact that numerous forage plant species were discovered in the honey samples, PCA of the dominating pollen data was used to briefly identify the botanical origin of the honey samples. Thus, the sources of honey samples were categorized into four clusters having similar pollen types prevailing dominant plant species in the samples of honey. As a result, Cluster-1 contains samples from Jimma and Kefa zones which were dominated by *Schefflera abyssinica* honey due to its abundance and major source of nectar in these areas. Cluster-2 includes samples from Jimma and Bale zones which were dominated by bee forages of *Guizotia schara* that is known to adapt in the highlands and mid-highlands of the country. Cluster-3

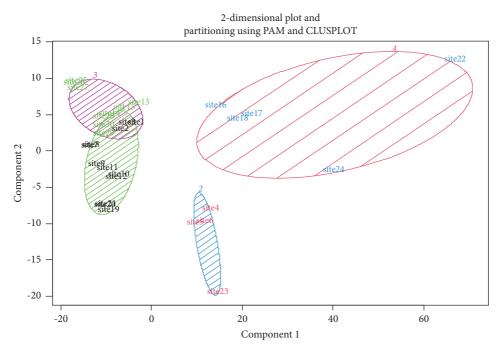


FIGURE 2: Clusters of honey samples using PCA component plot analysis. Component 1, 2, 3, and 4 are PCA axis. A number in the clusters represents the sampled sites with replications ($10 \times 3 = 30$ sites) containing dominant plant species and the diagram in the figure shows a level of similarity among one another.

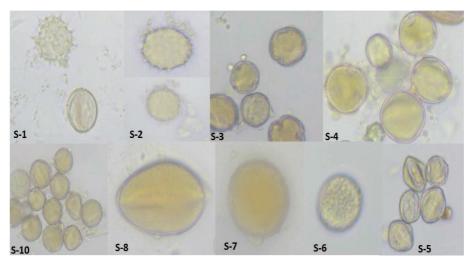


FIGURE 3: Pollen fingerprints showing dominant plant origins of honey samples where S-1 *Guizotia abyssinica* and *Brassica* spp., S-2 *Echinops* spp., S-3 *Schefflera abyssinica*, S-4 *Coffee arabica*, S-5 *Bersama abyssinica*, S-6 *Rumex* spp., S-7 *Satureja paradoxa*, S-8 *Thymes* spp., and S-9 *Eucalyptus* spp.

includes samples from all sites which were dominated by the *Eucalyptus globulus* plant.Cluster-4 represents honey samples from Bale zone which was predominated by honey of *Phoenix* spp. and *Syzygium guineense* (Figure 2).

3.2. Sugar Composition of Honey. Sugars are the major constituents of honey, with a predominance of the reducing monosaccharides including glucose and fructose. Sugar composition depends mainly on the honey's botanical origin, geographical origin, and can be affected by climate, processing, and storage [35, 36]. In this study, four major

sugar types (fructose, glucose, sucrose, and maltose) were identified in collected honey samples with different percentage concentration (Table 1). The mean percentage composition values ranged for fructose (30.33%–46.68%), glucose (29.9%–40.3%), sucrose (15%–22.2%), and maltose (0.28%–4.35%) (Table 1). The higher percentage concentration of fructose (46.68%) was recorded in honey collected from Gera and the lowest percentage concentration 30.33% was recorded in honey sample from Jimma. High sucrose values in honeys are related to its botanical origin, honey immaturity, high nectar flux, or artificial feeding of bees [35]. The highest glucose value (40.3%) was recorded in honey

TABLE 1: Percentage of sugar concentration in honey from different areas of Ethiopia.

Sample location	Percentage of sugar concentration (conc. area)					
	Fructose	Glucose	Sucrose	Maltose		
Gomma	46.2 ^a	34.7 ^{ab}	17.9 ^a	0.4^{b}		
Gimbo	36.9 ^{ab}	40.3 ^a	22.2 ^a	1.7 ^{ab}		
Gesha	40.7 ^{ab}	32.7 ^b	16.6 ^a	1.7 ^{ab}		
Dollo menna	37.1 ^{ab}	29.9 ^b	15.4^{a}	2.6 ^{ab}		
Gera	43.3 ^{ab}	36.4 ^{ab}	15.0 ^a	1.5 ^{ab}		
Dedo	46.7 ^a	34.2 ^{ab}	17.8 ^a	1.2^{ab}		
Guassa	39.8 ^{ab}	32.6 ^b	16.7 ^a	4.4 ^a		
Kersa	39.6 ^{ab}	34.8 ^{ab}	19.5 ^a	0.3 ^b		
Manna	30.3 ^b	33.6 ^{ab}	17.2 ^a	0.6^{b}		
Rira	39.4 ^{ab}	31.5 ^b	16.3 ^a	3.4 ^{ab}		

*Mean values percentage of sugar concentration followed by different superscripts within the column is indicating significant differences (P < 0.05).

samples collected from Bonga district and the lowest glucose value (29.9%) was measured in honey samples collected from Dollo mena district. Apart from this, there was no significance difference of glucose mean value recorded among honey samples collected from different areas (Table 1). Interestingly, there is no significant difference for the value of sucrose for all the honey samples collected from all the sampling areas and the average value of sucrose (18.6%) ranged within the international standard value of honey quality measure [27]. However, the mean value of maltose in honey samples varied among the locations that the highest was registered for honey from Guassa (4.35%) and the lowest was recorded for honey from Jimma area (0.28%) (Table 1).

Apparently, the sugar percentage concentration values of honey samples were found to be in the normal range of the standard honey composition, except for honey from Anaz-Guassa area according to the International Standard Honey Commission [27]. These sugar constituents are known to determine the honey properties such as energy value, viscosity, hygroscopicity, granulation, and antibacterial activity of honey [37].

3.3. Physicochemical Properties of Honey. Honey's physical and chemical properties are decisive to determine its quality for consumers' choice, as well as for the commercialization management. In this study, five principal properties of honey (MC, Ash, PH, FA, and HMF) were analyzed for honey samples collected from different geographical origins of the country.

The value of moisture contents ranged from 17.07 to 25.0% with a mean value of 21.87% (Table 2). It was observed that there was statistical difference (P > 0.05) in the value of moisture contents among the study areas. The highest mean value of moisture content (25.0 ± 1.20) was recorded for the honey sample collected from the Gomma district of Jimma zone, while the lowest MC (17.07 ± 1.01) was recorded for honey collected from Guassa (Table 2). However, the overall mean value of the MC of honey samples in the present study (21.87%) was slightly higher than the standard value of national [38] and international standard [27] moisture

content (20%) for *A. mellifera* honey. Honey moisture is the quality criterion that determines the capability of honey to remain stable and to resist spoilage by yeast fermentation: the higher the moisture, the higher the probability that honey will ferment upon storage [29].

The ash content of the honey samples ranged from 0.03 ± 1.6 to $2.38 \pm 3.2\%$ with an overall mean value of 0.421%. Significantly, the highest mean ash content (2.38%) was recorded for honey collected from the Anaz-Guassa area ($2.38 \pm 3.2 \text{ g/100 g}$) (Table 2). However, the overall mean value of the ash content (0.421 g/100 g) is similar to our previous finding for stingless bee honey ash content measured 0.41% in West Shoa zone of Oromia region [39]. In general, the ash content demonstrates the abundance of minerals in honey sources, which is mainly influenced by the nectar botanical origin, location, species of the bee, and processing and handling. With this respect, Biluca et al. [40] also indicated that the mineral content in honey depends on nectar composition of major bee forages during the honey harvesting.

Free acidity is related to the source of nectar, bee species, and the action of enzymes or bacteria [41]. It indicates one of the quality parameters of honey and reveals whether the honey is fermented or not [42]. International regulations specify a free acidity not higher than 50 meq/kg honey for A. mellifera honey [1, 27]. In this study, there was a significant difference (P > 0.05) for free acidity value between the honey samples among honey source locations, in which it ranged from 13.33 ± 1.7 to 51.67 ± 4.0 meq/kg with a total mean value of 28.83 ± 2.85 meq/kg (Table 2). The highest free acidity was recorded for honey sample collected from Anaz-Guassa (51.67 ± 4.0^{a}) and the lowest was recorded for honey from Bonga area. This variation of free acidity may be related to the harvest season, the maturity of honey, floral sources, locations, storage condition, and/or climatic factors, which would favor chemical, enzymatic, and microbiological reactions able to release acidic compounds in honey [23]. However, the overall mean value of free acidity in the current honey samples lies within the international standard value of free acidity that permits below 50 meq/kg.

Most of the pH value analyzed for honey samples collected from different areas showed differences (P > 0.05)with maximum value registered for honey collected from Dollo Menna (5.34 ± 3.0) and minimum value for honey sample from Jimma area (3.49 ± 2.5) . Such pH value variation could be attributed to the geographical origin of honey, floral sources, as well as the bee's species type [43]. The low pH value of any honey may contribute to the antibacterial activity of honey as it acts against microbial growth inhibition so that honey is commonly used as a potential substitute in reducing some infectious diseases such as coughs and wounds [44, 45]. In general, the Ethiopian honey quality standard allows pH values in nectar honeys in the range of 3.2-4.5 [38]. The higher mean pH value recorded in honey from Dollo Menna area (5.34 ± 3.0) might be due to higher mineral contents existed in this particular honey [29].

HMF is the major honey quality factor indicating the honey freshness and overheating. In fresh honeys, there is practically no or very low HMF, but it increases upon storage, depending on the pH of the honey and on the

Honey source $(N=30)$	Parameters (mean ± SE)						
	MC (%)	Ash (g/100 g)	pН	FA (meq/kg)	HMF (mg/kg)		
Gomma	25.0 ± 1.2^{a}	0.12 ± 2.0^{bc}	3.7 ± 0.9^{e}	23 ± 2.3^{e}	$3.3 \pm 3.2^{\circ}$		
Gimbo	23.7 ± 0.5^{bc}	$0.05 \pm .1^{bc}$	3.9 ± 2.0^{cde}	13.3 ± 1.7^{f}	$4.5 \pm 1.5^{\circ}$		
Gesha	20.8 ± 1.0^{e}	$0.03 \pm 1.6^{\circ}$	3.79 ± 1.0^{cde}	21 ± 3.4^{e}	6.9 ± 2.6^{bc}		
Dallo menna	22.4 ± 0.7^{d}	$0.3 \pm 2.3^{\mathrm{bc}}$	5.3 ± 3.0^{a}	21 ± 2.5^{e}	12.2 ± 0.8^{bc}		
Gera	23.8 ± 0.2^{b}	0.3 ± 2.0^{bc}	4.1 ± 2.1^{cd}	23.3 ± 1.3^{e}	$12.0 \pm 2.0^{\circ}$		
Dedo	19.6 ± 0.5^{f}	0.5 ± 1.3^{b}	$4.2 \pm 2.3^{\circ}$	42.3 ± 0.6^{b}	$20.0 \pm 0.6^{\mathrm{abc}}$		
Guassa	17.1 ± 1.0^{g}	2.4 ± 3.2^{a}	$4.7\pm0.9^{ m b}$	51.7 ± 4.0^{a}	48.1 ± 3.8^{a}		
Kersa	$23.4 \pm 0.6^{\circ}$	0.1 ± 0.9^{bc}	$3.6 \pm .0.6^{de}$	$34 \pm 3.1^{\circ}$	33.2 ± 0.6^{b}		
Manna	$23.4 \pm 2.0^{\circ}$	0.1 ± 0.6^{bc}	3.5 ± 2.5^{e}	$31.3 \pm 0.7^{\circ}$	$2.1 \pm 1.0^{\circ}$		
Rira	$19.6 \pm 2.0^{\rm f}$	$0.4 \pm 1.0^{\mathrm{bc}}$	$4.2 \pm 4.0^{\circ}$	27.3 ± 3.1^{d}	$5.9 \pm 4.2^{\circ}$		
Overall mean	21.9	0.42	4.1	28.8	14.8		
Standard value	18-23	0.14-0.30	3.2-4.5	<50	<40		

TABLE 2: Physicochemical parameters of honey samples collected from different areas.

Means with different superscript (a, b, c, d, e, and f) columns are significantly different at P < 0.05 assessed by Duncan's multiple ranges. MC (%) = moisture content of honey in percent, FA (meq/kg) = free acidity in mill equivalents per kilogram of honey, HMF (mg/kg) = hydroxymethyl furfural in mg/kg of honey, and Ash (g/100 g) = ash content of honey in g/100 g. Bold represents mean significance level for P value at "P < 0.005."

storage temperature [46]. The present study indicated that significantly varied HMF value recorded for honey samples collected from most study locations. Relatively, the lowest HMF value $(2.06 \pm 1.03 \text{ mg/kg})$ was registered for honey collected from the Jimma zone. The highest concentration of HMF was recorded for honey collected from Anaz-Guassa district of the North Shoa zone $(48.05 \pm 3.8 \text{ mg} \cdot \text{kg}^{-1})$ (Table 2), showing higher than 40 mg·kg⁻¹, a maximum value showed unsatisfactory in international trade [46].

Generally, the quality parameter values of most honevs in this study fit within the acceptable range of national and international honey quality standards, except for the honey samples collected from the Anaz-Guassa area, North Shewa zone. The honey sample collected from this specific area showed a lower moisture content and significantly higher HMF, ash, and free acidity. This abnormality may be related to the dehydration or crystallization of the honey due to a longer shelf life, improper handling, or some contaminates in the honey [47-49]. These conditions may directly affect the other honey quality parameters, such as the HMF and ash contents [50]. Belay et al. [51] also suggested that HMF concentration increases during honey processed by heat treatment, and also through adulteration of honey with commercial sugars and throughout the storage. Therefore, the abnormal properties of the honey samples collected from the Anaz-Guassa area suggest improper handling, long storage life, or contamination with some adulterants.

4. Conclusions

In this study, melissopalynological data revealed that *Schefflera abyssinica, Eucalyptus* spp., *Bersama Abyssinica, Syzygium guineense, Datura* spp., and *Thymes* spp. are major honey bee flora sources with greater than 45% pollen frequency. The sugar concentration values in our honey samples were found to be within an accepted range of the International Standard Honey Commission. However, the honey sample from Anaz Guassa district showed the highest mean values for HMF, ash, and FA and lower MC, and honey from Gimbo district has no pollen fingerprint under

melissopalynology analysis. These nonstandard quality parameters of honey from these two areas suggest suspected poor handling, storage, honey adulteration, or due to some other factors influencing the honey quality. Therefore, regular testing and monitoring of honey quality properties following the standard procedures and based on their geographical origins are very crucial to sustainably maintain the honey natural quality. This will help to control the widespread practice of honey adulteration in different parts of the country and increases the market demand of Ethiopian honey over the EU markets.

Data Availability

The research data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

- Eu Council, "Council directive 2001/110/EC of 20 december 2001 relating to honey," Official Journal of the European Communities - Legislation, vol. 10, pp. 47–52, 2002.
- [2] C. Kinati, T. Tolemariam, and K. Debele, "Assessment of honey production and marketing system in Gomma district, South Western Ethiopia," *Greener Journal of Business and Management Studies*, vol. 3, no. 3, pp. 99–107, 2013.

- [3] F. Buba, A. Gidado, and A. Shugaba, "Physicochemical and microbiological properties of honey from North East Nigeria," *Biochemistry & Analytical Biochemistry*, vol. 2, no. 142, pp. 61–67, 2013.
- [4] M. A. Cantarelli, R. G. Pellerano, E. J. Marchevsky, and J. M. Camiña, "Quality of honey from Argentina: study of chemical composition and trace elements," 2008, https:// www.researchgate.net/publication/262590217_Quality_of_ho ney_from_Argentina_Study_of_chemical_composition_and _trace_elements.
- [5] J. M. Alvarez-Suarez, F. Giampieri, A. Brenciani et al., "Apis mellifera vs Melipona beecheii Cuban poly-floral honeys: a comparison based on their physicochemical parameters, chemical composition and biological properties," Lebensmittel-Wissenschaft und -Technologie, vol. 87, pp. 272–279, 2018.
- [6] C. Santos-Buelga and A. M. González-Paramás, "Chemical composition of honey," in *Bee Products-Chemical and Biological Properties*, pp. 43–82, Springer, Heidelberg, Germany, 2017.
- [7] C. E. Manyi-Loh, R. N. Ndip, and A. M. Clarke, "Volatile compounds in honey: a review on their involvement in aroma, botanical origin determination and potential biomedical activities," *International Journal of Molecular Sciences*, vol. 12, no. 12, pp. 9514–9532, 2011.
- [8] A. S. Alqarni, H. M. Balhareth, and A. A. Owayss, "Performance evaluation of indigenous and exotic honey bee (*Apis mellifera* L.) races in Assir region, southwestern Saudi Arabia," *Saudi Journal of Biological Sciences*, vol. 21, no. 3, pp. 256–264, 2014.
- [9] E. Crane, Bees and Beekeeping: Science, Practice and World Resources, Heinemann Newness, Portsmouth, NH, USA, 1998.
- [10] N. Bradbear, "Bees and their role in forest livelihoods: a guide to the services provided by bees and the sustainable harvesting, processing and marketing of their products," *Non--wood Forest Products*, Vol. 19, Food and Agriculture Organization of the United Nations, Rome, Italy, 2009.
- [11] N. Adgaba, Geographical races of the honeybees (Apis mellifera L.) of the northern regions of Ethiopia, Ph.D. thesis, Rhodes University, Grahamstown, South Africa, 2002.
- [12] N. Gheldof, X. H. Wang, and N. J. Engeseth, "Identification and quantification of antioxidant components of honeys from various floral sources," *Journal of Agricultural and Food Chemistry*, vol. 50, no. 21, pp. 5870–5877, 2002.
- [13] I. C. Silva, D. Message, C. D. Cruz, L. A. O. Campos, and M. J. Sousa-Majer, "Rearing Africanized honey bee (*Apis mellifera* L.) brood under laboratory conditions," *Genetics and Molecular Research*, vol. 8, no. 2, pp. 623–629, 2009.
- [14] W. M. A. Alaerjani, S. Abu-Melha, R. M. H. Alshareef et al., "Biochemical reactions and their biological contributions in Honey," *Molecules*, vol. 27, no. 15, p. 4719, 2022.
- [15] L. Azeredo, M. A. A. Azeredo, S. R. De Souza, and V. M. L. Dutra, "Protein contents and physicochemical properties in honey samples of *Apis mellifera* of different floral origins," *Food Chemistry*, vol. 80, no. 2, pp. 249–254, 2003.
- [16] S. Gomes, L. G. Dias, L. L. Moreira, P. Rodrigues, and L. Estevinho, "Physicochemical, microbiological and antimicrobial properties of commercial honeys from Portugal," *Food and Chemical Toxicology*, vol. 48, no. 2, pp. 544–548, 2010.
- [17] K. Nigussie, P. A. Subramanian, and G. Mebrahtu, "Physicochemical analysis of Tigray honey: an attempt to determine

- [18] E. Mulugeta and M. Tadese, "Physicochemical characterization and pesticide residue analysis of honey produced in West Shewa zone, Oromia," *American Journal of Applied Chemistry*, vol. 5, no. 6, pp. 101–109, 2017.
- [19] A. Belay, G. D. Haki, M. Birringer et al., "Sugar profile and physicochemical properties of Ethiopian mono-floral honey," *International Journal of Food Properties*, vol. 20, no. 11, pp. 2855–2866, 2017.
- [20] B. Tesfaye, D. Begna, and M. Eshetu, "Evaluation of physicochemical properties of honey produced in Bale natural forest, southeastern Ethiopia," *International Journal of Agricultural Science and Food Technology*, vol. 2, no. 1, pp. 21–27, 2016.
- [21] Y. G. Gebru, Characterization of beekeeping systems and honey value chain, and effects of storage containers and durations on physicochemical properties of honey in kiltie awlaelo district, eastern tigray, Ethiopia, Ph.D. thesis, Department of Animal Production Study, Kilte Awulaelo, Ethiopia, 2015.
- [22] T. Damto, "A review on status of honey adulteration and their detection techniques in Ethiopia," *Journal of Nutrition & Food Sciences*, vol. 11, p. p180, 2021.
- [23] M. Ahmed, B. Khiati, A. Meslem, S. Aissat, and N. Djebli, "Evaluation of physicochemical and antioxidant properties of raw honey from Algeria," *Journal of Microbial & Biochemical Technology*, vol. 4, p. 6, 2014.
- [24] S. Bogdanov, K. Ruoff, and L. P. Oddo, "Physicochemical methods for the characterization of uni-floral honeys: a review," *Apidologie*, vol. 35, no. 1, pp. S4–S17, 2004.
- [25] S. Bogdanov, P. Martin, and C. Lullmann, "Harmonized methods of the international honey commission," Swiss Bee Research Centre, FAM, Liebefeld, vol. 5, pp. 1–62, 2002.
- [26] J. Louveaux, A. Maurizio, and G. Vorwohl, "Methods of melissopalynology," *Bee World*, vol. 59, no. 4, pp. 139–157, 1978.
- [27] A. Codex, "Draft revised standard for standard for honey (at step 10 of the Codex procedure)," *Alinorm*, vol. 1, no. 25, pp. 19–26, 2001.
- [28] International Honey Commission, "Harmonized methods of the international honey commission," 2009, https://www.beehexagon.net/en/network.htm.
- [29] S. Bogdanov, "Physical properties of honey," *Honey: Composition and Health Benefits, First*, Wiley, Hoboken, NJ, USA, 2009.
- [30] L. M. Estevinho, X. Feás, J. A. Seijas, and M. P. Vázquez-Tato, "Organic honey from Trás-Os-Montes region (Portugal): chemical, palynological, microbiological and bioactive compounds characterization," *Food and Chemical Toxicology*, vol. 50, no. 2, pp. 258–264, 2012.
- [31] R. Fichtl and A. Adi, *Honeybee flora of Ethiopia*, Margraf, Chiampo, Italy, 1994.
- [32] T. Bareke and A. Addi, "Honeybee flora resources of Guji zone, Ethiopia," *Journal of Biology, Agriculture and Healthcare*, vol. 8, no. 21, pp. 1–9, 2018.
- [33] T. Bareke and A. Addi, "Bee flora resources and honey production calendar of Gera Forest in Ethiopia," Asian Journal of Forestry, vol. 3, no. 2, 2019.
- [34] A. H. Degaga, "Identification of honey source bee floras during major and minor honey harvesting seasons in Jimma Zone, Southwest Ethiopia," *Journal of Environment and Earth Science*, vol. 7, no. 3, pp. 25–32, 2017.
- [35] O. Escuredo, I. Dobre, M. Fernández-González, and M. C. Seijo, "Contribution of botanical origin and sugar

composition of honeys on the crystallization phenomenon," *Food Chemistry*, vol. 149, pp. 84–90, 2014.

- [36] F. Tornuk, S. Karaman, I. Ozturk et al., "Quality characterization of artisanal and retail Turkish blossom honeys: determination of physicochemical, microbiological, bioactive properties and aroma profile," *Industrial Crops and Products*, vol. 46, pp. 124–131, 2013.
- [37] M. A. Kamal and P. Klein, "Determination of sugars in honey by liquid chromatography," *Saudi Journal of Biological Sciences*, vol. 18, no. 1, pp. 17–21, 2011.
- [38] Ethiopia Standard, Quality and Standards Authority of Ethiopia (QSAE), Honey Specification: Ethiopian Standard, ES 1202, Addis Ababa, Ethiopia, 2005.
- [39] A. Gela, Z. A. Hora, D. Kebebe, and A. Gebresilassie, "Physicochemical characteristics of honey produced by stingless bees (Meliponula beccarii) from West Showa zone of Oromia Region, Ethiopia," *Heliyon*, vol. 7, no. 1, p. 5875, 2021.
- [40] F. C. Biluca, F. Braghini, L. V. Gonzaga, A. C. O. Costa, and R. Fett, "Physicochemical profiles, minerals and bioactive compounds of stingless bee honey (Meliponinae)," *Journal of Food Composition and Analysis*, vol. 50, pp. 61–69, 2016.
- [41] A. A. M. De-Melo, L. Bicudo de Almeida-Muradian, and M. Teresa Sancho & Ana Pascual-Maté, "Composition and properties of *Apis mellifera* honey: a review," *Journal of Apicultural Research*, vol. 57, 2017.
- [42] M. F. Silvano, M. S. Varela, M. A. Palacio, S. Ruffinengo, and D. K. Yamul, "Physicochemical parameters and sensory properties of honeys from Buenos Aires region," *Food Chemistry*, vol. 152, pp. 500–507, 2014.
- [43] J. M. B. Sousa, E. L. de Souza, G. Marques et al., "Sugar profile, physicochemical and sensory aspects of mono-floral honeys produced by different stingless bee species in Brazilian semiarid region," *LWT--Food Science and Technology*, vol. 65, pp. 645–651, 2016.
- [44] S. A. Meo, S. A. Al-Asiri, A. L. Mahesar, and M. J. Ansari, "Role of honey in modern medicine," *Saudi Journal of Biological Sciences*, vol. 24, no. 5, pp. 975–978, 2017.
- [45] N. A. M. A. N. Malika, F. C. E. A. Mohamed, and E. A. Chakib, "Microbiological and Physicochemical properties of Moroccan honey," *International Journal of Agriculture and Biology*, vol. 7, no. 5, pp. 773–776, 2005.
- [46] S. Bogdanov, C. Lüllmann, P. Martin et al., "Honey quality and international regulatory standards: review by the International Honey Commission," *Bee World*, vol. 80, no. 2, pp. 61-69, 1999.
- [47] G. Mairaj, S. Akhtar, A. R. Khan, Z. Ullah, S. Bibi, and S. Ali, "Quality evaluation of different honey samples produced in Peshawar valley," *Pakistan Journal of Biological Sciences*, vol. 11, no. 5, p. 797, 2008.
- [48] R. Subramanian, H. Umesh Hebbar, and N. K. Rastogi, "Processing of honey: a review," *International Journal of Food Properties*, vol. 10, no. 1, pp. 127–143, 2007.
- [49] I. Singh and S. Singh, "Honey moisture reduction and its quality," *Journal of Food Science and Technology*, vol. 55, pp. 3861–3871, 2018.
- [50] U. M. Shapla, M. Solayman, N. Alam, M. Khalil, and S. H. Gan, "5- Hydroxymethylfurfural (HMF) levels in honey

and other food products: effects on bees and human health," *Chemistry Central Journal*, vol. 12, pp. 1–18, 2018.

[51] A. Belay, W. K. Solomon, G. Bultossa, N. Adgaba, and S. Melaku, "Physicochemical properties of the Harenna forest honey, Bale, Ethiopia," *Food Chemistry*, vol. 141, no. 4, pp. 3386–3392, 2013.