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Original Research

Evaluation of Agro-Ecological Variation in Honey Quality of Amuru, Horro Guduru Wollega, Ethiopia

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Abstract

Research on honey's physical and chemical properties was carried out in various agro-ecologies in Western Ethiopia's Amuru region, Horro Guduru Wollega Zone. To choose the agro-ecologies, a multi-stage stratified sampling method was employed. The Holetta Bee Research Centre collected 27 honey samples from beekeepers in different agro-ecologies (highland, lowland, and mid-land) and tested them for pH, hydroxy-methylfurfural, sucrose, glucose, fructose, electrical conductivity, and moisture content. The average amount of honey produced was determined by the following parameters: hydroxy-methylfurfural, sucrose, glucose, fructose, free acidity content, pH value, 3.267±1.1359, 2.6756±.1611, 30.6633±1.008, 32.3011±.516, 22.22±.805, and so on. Across different agroecologies, there was a substantial variation in moisture, ash, and electrical conductivity (P < 0.05). All of the samples were less contaminated and can be used for human consumption, thus the relevant office should take steps to maximize honey production capacity and improve the commodity's sale market share.

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INTRODUCTION

A cozy, well-run sector, beekeeping contributes significantly to Ethiopian agriculture. Since it has long been recognized as a workable solution to achieving food security and reducing poverty in Ethiopia, Ethiopian apiculture is starting to play a significant role in plans for rural and agricultural development. Ethiopia's abundant apicultural resources have made it the continent's greatest producer of honey and beeswax (Fikru, 2015). There are several benefits to beekeeping for food security and environmental stability. In Ethiopia, around 10% of rural households maintain bees, making honeybee keeping a major economic endeavor for the rural populace (Sebsib &

Yibrah, 2018). Approximately 1.7 million farm households manage these colonies as a way to supplement their income (CSA, 2016/17). Apiculture encourages plant reproduction, environmental conservation, and a source of income, according to Wubie et al. (2014). Although apiculture has the capacity to produce honey, it currently contributes very little and is not expected to contribute much to the state GDP (Berhe et al., 2016). The Amuru district is frequently covered in natural vegetation, including bushes and annual and perennial plants suitable for beekeeping, honey production their byproducts. Beekeeping in the district is believed to greatly enhance rural livelihood. Naturally, the quantity and caliber of bee goods dictate their capacity to bring in money as well as their noteworthy role in lowering poverty and enhancing standard of living. Additionally, effective management of honey bee colonies, maximizing productivity percentages, and wise application of advanced technologies are necessary for this (Amuru Agricultural and Natural Resource Office, 2020). Many studies have looked on the chemical makeup of honey in the Zone (Desalegn and Alemayehu, 2022). Empirical research indicates that agroecologies' biological makeup and changes can impact the honey's quality. Further, the district's great potential for honey production, nothing is known about the physicochemical properties of the honey produced in Amuru District. Because of this, the current study was initiated with the intention of evaluating the physicochemical properties of honey produced in different agroecologies within the Amuru district's Horro Guduru Wollega Zone.

MATERIAL AND METHODS

Description of the Study Area The analysis was directed in the Amuru area of the Horro Guduru Wollega Zone in Oromia, Ethiopia. In the district, there are twenty-one administrative kebeles. There are two urban kebeles among these, and the other nineteen are rural. The limits of a district are the Jardaga Jarte district in the east and south, the Blue Nile river and Amhara regional state in the north, and the Kiramu district in the East Wollega zone in the west. The district is located 68 miles from Shambu, the seat of Horro Guduru Wollega Zone, and 385 km west of Finfinnee, the capital of Ethiopia's regional state of Oromia. A geographical plot of the selected region is displayed in Figure 1.

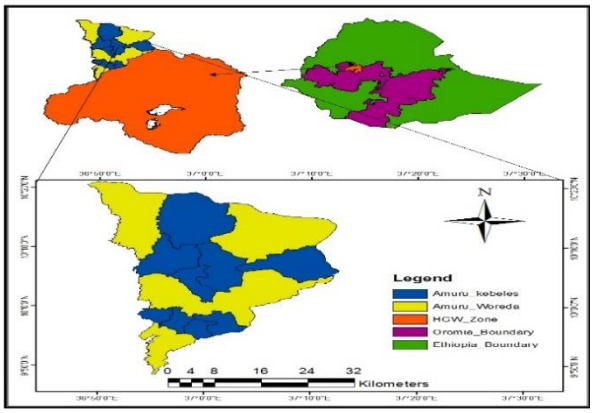

Figure 1. *Map of Study Area*

Total area, demography, Climate and Vegetation Cover Topographic feature

The Amuru district, which spans 129, 452.0805 hectares of land, is primarily distinguished by its generally soft and steep topography. There are 75,539 people living in the Amuru district overall, with 37,014 men and 38,525 women. There are three distinct climate zones in the district: lowland, midland, and highland. Within the district, the lower altitude varies from 760 m.a.s. l. to 2502 m.a.s. l. The district's geographical mass has divided into roughly 50% midland, 43% lowland, and 7% highland areas. The average temperature is 15 degrees lower and 25 degrees higher, with a maximum rainfall of 1100 mm and a minimum rainfall of 900 mm. As stated in the Oromia Sustainable Land Management program (SLMP, 2014), the study region covered latitudes 9° to 10° North and longitudes 36° to 37° East. The months of June through August get the most rainfall, April through May the most moderate, and March, September, and October the shortest. The districts are home to a wide variety of plant species, including annual and perennial crops that supply honeybees with pollen and nectar.

Selection of the study site and sampling methods

To choose the agro-ecologies, a multi-stage stratified sampling technique was utilized. The smallest administrative unit in Ethiopia, the rural kebeles, were divided into lowland (kola), mid-altitude (woina dega), and highland (dega). The kebeles within each stratum were purposefully chosen based on factors such as the quantity of honey bee colonies, the number of beekeepers, and the accessibility of common bee flora. Therefore, among the 21 kebeles, the following were chosen as representations of each agro-ecology: highland (Ejere Goromti, Walage, Wara Bera, and Haro Guddina), midland (Gulufa, Xombe dangab, and Agamsa), and lowland (jawaj and migir). To gather pertinent data regarding the potential for honey production, the main plants that provide honey, the main varieties of honey, and the times when honey is harvested, focus groups with specialists, development agents, and beekeepers were arranged. To identify the plant, field observations were conducted regarding the bee flora in the vicinity. Nine samples of pure honey were taken from each agro-ecology, for a total of 27 samples. After being gathered and kept at 4Oc for 500 g of honey, the honey was transferred to the Holetta Bee Research Center for additional examination (Tura and Admassu, 2019).

Honey Quality Analysis

The obtained honey samples' physico-chemical characteristics were examined in accordance with the (IHC, 2009). At the Holetta Bee Research Center, a laboratory analysis was carried out on a selected set of honey samples. The following is an explanation of the methodology, materials, tools, and equipment used in the physico-chemical examination of honey samples:

Color of honey

The Pfund (color grader) classifier was secondhand to examine the color of the honey samples. Samples of homogenous honey that are bubble-free are placed into a transparent cuvette. The cuvette was placed into a color

photometer Pfund honey color grader (No. 0061, manufactured in USA) in accordance with the protocol of (IHC, 2009), and the grades were articulated in millimeters (mm) Pfund Grades in comparison to an analytical grade glycerol reference.

Electrical conductivity

Honey's electrical conductivity is expressed as the weight-in-volume solution at 20˚C in water, where the 20% represents the dry matter content of honey. The milli Siemens per centimeter ($mS.cm^{-1}$) result was given. An electrical conductivity cell was used to evaluate the electrical conductivity of a solution containing 20g dry matter honey in 100 ml distilled water. Electrical resistance measurements serve as the foundation for determining electrical conductivity (IHC, 2009). The 0.1M potassium chloride solution was one of the reagents utilized. In a 1000 ml flask, dissolve 7.4557g of dried at 130 °C potassium chloride (KCl) in newly distilled water, then fill the flask to the brim with distilled water. Tools such as a lower range 10-7 S conductivity meter, a double electrode platinized conductivity cell, a thermostatically controlled water bath at 20°C ± 0.5°C, volumetric flasks (100 and 1000 ml), and beakers were employed. The electrical conductivity of honey was then examined using precise methods.

Moisture content

The refract metric method (Abbe Refractometer) was founded on the idea that honey's refractive index rises as its solid content does. The Abbe Refract meter's prism surface was cleaned and allowed to dry. The homogenized honey sample was applied to the prism immediately following homogenization, covering all of its surfaces equally. Read the refractive index with the Abbe Refract meter after two minutes. The border between the white and black areas is marked by the Abbe Refractometer's calibration. The refractive index measurement was modified to account for the 20℃ temperatures. The moisture contend can be determined as,

$$W = \frac{\bar{1}.73190 - log(RI - 1)}{0.002243}$$

Ash content

After the sample was burned in the conventional method, the amount of ash was ascertained. Ash-free olive oil was utilized as a reagent. The equipment used to assess the mineral ash content of honey included a platinum or quartz ash dish of appropriate size, a hotplate, an electric furnace that could be adjusted to 600°C (± 25°C), desiccators with the right drying material, and forceps.

The steps taken to analyze the amount of ash in honey were: preparing the ash dish (heating it to an ashing temperature in an electrical furnace), cooling it to room temperature in a desiccator, and then weighing it to the nearest 0.001g (M_2); preparing the sample (10g) was weighed to the nearest 0. 001g into an ash plate that has been ready as previously said (M_0). Two drops of olive oil were then added. Next, the water was removed, and the ashing process was started without loss at a low heat that was raised to 350–400°C using one of the appliances. Following the initial ashing, the dish was heated for at least an hour in the preheated furnace. After that, the dish was weighed after cooling in the desiccators. Until consistent

weights were attained, the ashing process was continued (M_1).

$$Ash(\%) = \left(\frac{M_1 - M_2}{M_0}\right) \times 100$$

PH and free acidity

The amount of all free acids in honey, measured in meq/kg, is its free acidity. After dissolving the sample in distilled water and measuring the pH, the solution was titrated to a pH of 8.30 using a 0.1M sodium hydroxide solution. The sample's PH and free acidity were assessed using the guidelines and methods of (IHC, 2009). The reagents used in the determination of PH and free acidity included distilled water, buffer solutions for calibrating the PH meter at PH 3.7 (or 4.0), 9.0, and precisely calibrated 0.1M sodium hydroxide solution. The tools used to measure the PH and free acidity of honey were beakers, a magnetic stirrer with a hot palate, a PH meter accurate to 0.01 units, and burettes with a capacity of 10 milliliters.

Determination of sugars by HPLC

This technique measures the sugars in honey: fructose, glucose, and sucrose. This approach is based on Bogdanov and Baumann's (1988) first published method. Following solution filtering, RI-detection HPLC (High Pressure Liquid Chromatography) was used to measure the sugar content. The retention times of the peaks were used to identify them. The external standard procedure for quantization of peak areas was followed. The primary reagents utilized in the HPLC analysis of honey's sugar content were: HPLC grade water, acetonitrile for HPLC, fluent solution for HPLC; 80 volumes of acetonitrile were mixed with 20 volumes of water and allowed to degas before use; the standard substances (fructose, glucose, and sucrose) were pipetted into a 100 ml calibrated flask; subsequently, fructose, glucose, and sucrose in various ratios were dissolved in 40 ml of water, transferred to the flask, and filled to the appropriate level using water and a syringe; finally, the solution was transferred to sample vials using a membrane filter that was pre-mounted.

The equipment used for sugar analysis by HPLC included sample vials, an ultrasonic bath, a calibrated fask, a 25 ml pipette, membrane filters with pore sizes of o.45 µm for the aqueous solution, a filter holder for membrane filters with a suitable syringe, and High-Performance Liquid Chromatography, which included a pump, sample applicator, temperature-regulated column oven at 300 °C, a temperature-regulated thermometer, and an integrator. The procedures that were taken were to weigh 5g of honey in a beaker and dissolve it in 40 ml of distilled water. A 100 ml volumetric flask was pipetted with 25 ml of acetonitrile, then the honey solution was quantitatively added and the flask was filled with water to the designated level. Similar to the standard solution, the liquid is poured through a membrane filter, collected in sample vials, and then stored. Subsequently, the HPLC was calibrated in accordance with its operational principles. For this reason, the HPLC's standard solution and honey sample solution are both set up and adjusted. For a total of 8.8 hours, the HPLC was run out by giving each sample, including the standard solution, 22 minutes and can be determined as,

$$W = \left(\frac{A_1 V_1 m_1}{A_2 V_2 m_0}\right) \times 100$$

Hydroxyi methyl furfural (HMF)

The 5-(hydroxymethyl-) furan-2-carbaldehyde concentration is ascertained using this approach. The milligrams per kilogram result was given. Following the subtraction of the background absorbance at 336 nm, the HMF content was determined. Hydroxy-methylfurfural (HMF) concentration was calculated using the HMF's UV absorbance at 284 nm as a foundation. Following the subtraction of the background absorbance at 336 nm, the HMF content was determined.

White's original study served as the basis for the analysis (IHC, 2009). The Carrez solution I, which contained 15 g of potassium hexacyanoferrate (K4Fe (CN) 6•3H2O) diluted in 100 ml of distilled water, was the reagent used. Carrez solution II: To make 100 ml, 30 g of zinc acetate, Zn (CH3.COO) 2.2H2O, was diluted. Sodium bisulphate solution 0.20 g/100 g: 100 ml of water was used to dissolve 0.20 g of solid sodium hydrogen sulphate (NaHSO3), also known as meta bisulphate, Na2S2O5. The equipment used includes a vortex mixer, filter paper, 1 cm quartz cells (cuvettes), and a spectrophotometer that operates in the wavelength range of 284 and 336 nm.

A 50 ml beaker was precisely filled with a 5 gram sample after the proper processes were followed. After dissolving the weighted honey sample in 25 milliliters of distilled water, it was put into a 50 milliliter volumetric flask. To make up the mark with water, 0.5 cc of Carrez solution was then added and stirred. Filter paper was used to filter the solutions, and the first 10 milliliters of the filtrate were discarded. Subsequently, one test tube was filled with 5.0 ml of water and thoroughly mixed (the sample solution). Subsequently, 5.0 milliliters of 0.2% sodium bisulphate solution were introduced into the second test tube and thoroughly mixed. The sample solution's absorbance was then measured in an hour using 10 mm quartz cells at 284 and 336 nm in comparison to the reference solution. The HMF in mg/kg was determined as,

$$HMF = \frac{[(A284 - A336) \times 149.7 \times 5]}{Weight\ of\ the\ sample}$$

Data Analysis

SPSS version 24 and MS Excel were utilized to compute the quantitative and qualitative variables. Three copies of each analysis were done, and the findings were reported as mean standard errors (±). When utilizing one-way analysis of variance (ANOVA) and an ANOVA that revealed a statistically significant difference, the means were separated using the least significance difference at the $P<0.05$ level. The position of the highland, midland, and lowland agro-ecologies was compared using the following statistical model.

$Y_{ijk} = \mu + T_{ijk} + e_{ijk}$, Where,

RESULTS AND DISCUSSION

Physico-chemical Property and Quality of Honey

The marketing of honey both domestically and internationally is heavily influenced by the quality of the honey. Three of the hive types are found in more densely inhabited locations, where samples were gathered for analysis of several honey physio-chemical quality characteristics. To evaluate the quality and purity of the honey samples, their physicochemical characteristics were ascertained. The color, moisture content, ash

content, free acidity, pH value, electrical conductivity, sugar content, and hydroxy methyl furfural (HMF) were the quality indices that were utilized to assess the honey samples. The quality of a European Union and Standards Authority of Ethiopia (QSAE) Codex Alimentarius Commission (CAC) and (QSAE) was compared with the outcome of laboratory examination of honey samples in Table 1.

Table 1

Physico-chemical properties result as compared to QSAE, EU and CAC

Parameters	Number of sample	Mean± S E	Minimum	Maximum	National and international institutions and their standard				
					QSAE	EU	CAC	World	FAO/WHO
Moisture content g/100g	27	18.956±.1405	18.2	19.6	< 21	< 21	18-23	21-23	< 21
Electrical conductivity $mS^{-1}cm$	27	.7478±.07365	.49	1.10	<0.6	<0.8	<0.8		
PH value	27	4.889±.0611	4.6	5.3		-	-	3.2-4.5	-
Ash (g/100g)	27	.3722±.04789	.20	.60	<0.6	< 0.6	0.25 – 1	0.6 – 1	<0.6
Free Acidity (meqkg-1)	27	22.22±1.451	18	31	40	< 40	< 50	5-54	40
HMF (mg/kg)	27	3.267±2.0478	.0	17.7	40	< 40	< 60	40-80	80 maximum
Fructose content g/100g	27	32.3011±.93118	26.94	35.70			60-70%		
Glucose content/100g	27	30.6633±1.818	23.8	40.4					
Sucrose g/100g	27	2.6756±.29046	1.68	3.77	10	< 5	< 5	3-10	5-10

Color of honey

The kind of flora that bees feed on, the minerals in the soil, the age of the honey, storage conditions, and the processing of the honey are just a few of the variables that affect the color of the honey. Based on the findings from the color grade classifier reading, the honey color of the study region was divided into three color groups. Light amber was the research area's predominant honey color. Of the twenty-seven honey samples that were evaluated, around twelve (12) (44.44 %) were classified as light amber, and nine (9) (33.33 %) were classified as amber. The remaining excess pale amber, dark amber, and (11.11%), or 11.11%), were classified as belonging to a certain color category. The mineral concentration of honey affects its color; the higher the mineral proportion in the honey, the darker the color.

The moisture content

Honey's moisture content determines how long it will last on the shelf. When honey's moisture content rises above a certain point, it becomes susceptible to microbial fermentation. According to Gebreegziabher et al. (2013), the more moisture a honey contains, the higher the likelihood that it may ferment on storage molds and yeasts, degrading the honey's quality until it reaches the maximum amount. Table 2 shows the moisture contents of honey. In highland, lowland, and midland agro-ecology, respectively, the mean moisture content of honey was 18.600±.1155, 19.133±.0882, and 19.133±.1202 g/100g in the current study. The honey samples obtained from various agro-ecologies in the current study area had a mean moisture content of 18.956±.0780, falling within the permissible range of QSAE (17.5-21), CAC (<21), and EU (<21). The season of harvest, the

level of maturity at harvest, the kind of hive utilized, and the surrounding temperature all affect the moisture content of honey.

Table 2

Physico-chemical parameters of honey samples across three agro-ecologies

Agro ecology		Moisture content (%)	Electrical con(mS)	PHvalue	Free acidity(meq)	Ash(g))	HMF(mg)	% of fructose	% of glucose	%sucrose
	Mean	18.600	.7200	4.900	20.00	.333	3.90	33.70	30.650	3.0433
	Std. Error of Mean	.1155	.07654	.0000	.764	.044	1.27	.7747	2.5042	.22606
Highland	Minimum	18.2	.49	4.9	18	.20	.0	30.65	23.85	2.14
	Maximum	19.0	1.01	4.9	23	.50	8.7	35.70	40.42	3.53
Lowland	Mean	19.133	.9133	4.867	24.67	.513	.000	32.59	27.533	2.7967
	Std. Error of Mean	.0882	.06566	.0167	1.740	.029	.000	.1138	.59324	.29868
	Minimum	18.8	.66	4.8	19	.40	.0	32.24	25.35	1.71
	Maximum	19.4	1.10	4.9	31	.60	.0	33.02	29.43	3.77
Midland	Mean	19.133	.6100	4.900	22.00	.270	5.90	30.60	33.806	2.1867
	Std. Error of Mean	.1202	.01443	.1041	1.155	.008	2.95	1.172	.91333	.25333
	Minimum	18.8	.56	4.6	18	.24	.0	26.94	31.98	1.68
	Maximum	19.6	.66	5.3	26	.30	17.7	34.97	37.46	3.20
Total	Mean	18.956	.7478	4.889	22.22	.372	3.26	32.30	30.663	2.6756
	Std. Error of Mean	.0780	.04085	.0339	.805	.026	1.13	.5165	1.0085	.16112
	Minimum	18.2	.49	4.6	18	.20	.0	26.94	23.85	1.68
	Maximum	19.6	1.10	5.3	31	.60	17.7	35.70	40.42	3.77

Fructose content of honey in g/100gm

Table 2 illustrates the fructose content result. The present study's examined honey samples had a fructose concentration of 32.30±.5165 g/00g, ranging from a minimum of 26.94g/100g to a maximum of 35.70g/100g. In the highland, lowland, and midland agro ecologies, the mean fructose concentration of the honey samples was 33.70±.7747, 32.59±.1138, and 30.60±1.172meq/kg, respectively. Regarding the fructose content, there was no significant variation ($P > 0.05$) across agro-ecologies (Table 3). The results of this study are similar to those of Aregay et al. (2018) and Teshale (2017), who found (34.22g/100g) and (34.22± 0.55g/100g) in the southern Ethiopian districts of Doyogena and Kachabira in the Kembata Tambaro zone and the Godere district of the Gambella region, respectively.

Glucose content of honey in g/100g

Table 2 shows the outcome of the glucose content. The research area's examined honey samples had a glucose content of 30.66±1.0085g/100g, ranging from a low of 23.85g/100g to a maximum of 40.42g/100g. In the highland, lowland, and midland agro-ecologies, the mean glucose concentration of the honey samples was 30.650±2.5042, 27.533±0.593, and 33.806±.91333meq/kg,

respectively. Regarding the glucose content, there was no discernible variation (P > 0.05) throughout the agro ecologies (Table 3). The latest result is less than a report by Aregay et al. (2018) that showed 36.37±2.14 g/100g in Godere district, Gambella area, and comparable with findings of Teshale (2017), which recorded 30.6633±1.81817 g/100g.

Sucrose content

Table 2 shows the outcome of the sucrose content. The amount of sucrose naturally present in a particular honey sample can be ascertained or the adulteration of honey with table sugar can be detected using the sucrose content analysis method. The sample from the research area had a mean sucrose level of 2.6756±.16112 g/100 g, ranging from 1.68 g/100 g to 3.77 g/100 g. In the highland, lowland, and midland agro ecologies, the mean sucrose concentration of the honey samples was 3.0433±.226, 2.7967±.29868, and 2.1867±.25333meq/kg, respectively. The samples' mean value fell within the 10g/100g permissible range established by the QSAE. Table 3 shows that there was no significant difference (P > 0.05) between the agro-ecologies. Honey contains a decreasing amount of sugar as ripeness increases. These show that as honey ages, its sugar content falls. The present result is similar to those of Gebreegziabher et al. (2013) and Teshale (2017), who found 2.71g/100g and 2.32±0.44g/100g in the northern Ethiopian Tigray area and the southern Ethiopian Kembata Tambaro zone's Doyogena and Kachabira districts, respectively. That being said, the sucrose content found in this investigation is lower than that found in studies conducted by Abebe et al. (2017), which measured 4.04g/100g, 7.55g/100g, 4.11.2g/100g, and 4.462.59g/100g in various climate conditions across the nation. The current study's low sugar content suggested that the honey extracted from the study regions was unadulterated and natural.

Regarding moisture content, there was a significant difference (p<0.05) between the agro-ecologies in the research area. (Table 3). The current honey moisture content value is higher than that of Mekuanint and Meareg (2019) and Abebe (2017), who recorded 16.7±1.42g/100g and 18.09±1.23g/100g, respectively, in selected districts of the Tigray Region and the Amahara Region.

Table 3

Physico-chemical properties of honey sample of the study area

Parameters	N	Mean± S E	Minimum	Maximum	P value	SL
Moisture content g/100g	27	18.956±.780	18.2	19.6	.002	**
Electrical conductivity	27	.7478±.04085	.49	1.10	.005	**
PH value	27	4.889±.0339	4.6	5.3	.905	*
Ash (g/100g)	27	.3722±.02657	.20	.60	000	***
Free Acidity (meqkg)	27	22.22±.805	18	31	.053	*
HMF (mg/kg)	27	3.267±1.1359	.0	17.7	0.94	*
Fructose content g/100g	27	32.3011±.516	26.94	35.70	0.39	*
Glucose content/100g	27	30.6633±1.008	23.85	40.42	0.33	*
Sucrose g/100g	27	2.6756±.1611	1.68	3.77	0.77	*

*Keynote:** Significant (P<0.05); meq = milli equivalent; ***highly Significant,* Significant (P>0.05); SL=Significance level*

However, the current honey moisture content results are comparable to those reported by Aregay et al. (2018), Gebreegziabher et al. (2013), and Addis and Malede (2014) in the Godere district of the Gambella region, the Tigray region, and the northwestern part of the Amhara region, respectively, at 18.76±1.09 g/100g, 18.60 g/100g, and 18.52 g/100g.

Table 4.

ANOVA for moisture analysis of honey from three agro-ecologies

Variables	Agro ecology	df	Mean Square	F value	Sig.	SL
Moisture content g/100g	Between Groups(Combined)	2	.853	8.000	.002	**
	Within Groups	24	.128			
	Total	26				

*** NonSignificant (P>0.05); SL=Significance level*

The research area's increased moisture content may have resulted from the area's relative humidity at the time the honey sample was gathered and harvested in mid-March, during the autumnal rainy season. The honey produced in the study region generally met the standards set by national and international authorities and was of excellent quality based on its moisture content (Table 4).

Electrical conductivity

Electrical conductivity varies with botanical origin and is dependent on ash, proteins, organic acids, and some complex carbohydrates. A common method for differentiating between blossom and honeydew honeys as well as characterizing unifloral honeys is electrical conductivity. Compared to honeydew, a lower limit has been suggested for blossom. The resulting conductivity increases with their content (Chefrour et al. 2009). Table 5 displays the electrical conductivity result. The average electrical conductivity of honey samples found in the current study region is 0.7478±.04085, falling between the permitted range specified by the Codex Alimentarius commission and the European Union (<0.8 $mS^{-1}cm$). The honey sample's electrical conductivity ranged from 0.49 $mS^{-1}cm$ to 1.10 $mS^{-1}cm$, at its lowest and maximum, respectively.

Table 5

ANOVA for electrical conductivity analysis of honey from different agro-ecologies

Variables	Agro ecology	Df	Mean Square	F value	Sig.	SL
Electrical conductivity mS-1cm	Between Groups (Combined)	2	.212	6.817	.005	**
	Within Groups	24	.053			
	Total	26				

*** Non Significant (P<0.05); SL=Significance level*

In the highland, lowland, and midland agro ecologies, the mean electrical conductivity values of honey were 0.7200±.07654, 0.9133±.06566, and 0.6100±.01443 g/100g, respectively, within the study region. Nearly half of the samples with electrical conductivity values examined showed nectar honey characteristics (≤ 0.8 mS-1cm). Table 5 shows that there was a significant difference (P<0.05) in electrical conductivity between the agro-ecologies. The current study's conclusion is similar to that of Abera et al. (2013) and Addis and Malede (2014), who found values in the Amhara region surrounding Gondar (0.620±0.20 mS/cm) and the Bale Harenna forest (0.70±0.04 mS/cm), respectively. However, the current study's result is higher than that of Mekuanint and Meareg (2019), who reported a value in a few Amhara and Tigray Region districts of 0.40±0.02 mS/cm. It is feasible to draw broad conclusions about the honey in the study region based on electrical conductivity, such as its high quality and compliance with EU and CAC regulations.

Ash content

Table 6 displays the outcome of the present result's ash content. The ash content of honey is influenced by a variety of factors, including the plant's botanical origin, the type of soil in which it grew, the location of the plant, the surrounding environment, and the items that honeybees collect (Gobessa et al., 2012). According to Bogdanov (2011), the ash content of blossom honey ranges from 0.1 to 0.3%, while the overall mean ash content of honey in the current study was 0.372+0.026 g/100g (ranging between 0.1g/100g and 0.38 g/100g).

Table 6

ANOVA for Ash analysis of honey from three agro-ecologies

Variables	Agro ecology	Df	Mean Square	F value	Sig.	SL
Ash (g/100g)	Between Groups(Combined)	2	.143	16.502	.000	***
	Within Groups	24	.019			
	Total	26				

This falls within the acceptable range of QSAE, ≤0.6g/100g and 0.6g/100g set by both CAC and E U. In highland, lowland, and midland agro-ecologies, the mean mineral content of the honey was 0.333±0.044, 0.513±0.029, and 0.270±0.08 g/100g, respectively. Furthermore, the substance in the pollen that bees gather when foraging on the flora determines the amount of ash in honey. Compared to the highland agroclimate, the midland had a larger plant coverage, which could be explained by the soil's higher organic matter content. On the other hand, Table 6 indicates that there was a significant difference (P<0.05) in the mineral ash concentration of honey throughout agro ecologies. The current study's result is higher than that of Aregay et al. (2018), Awraris et al. (2014), Addis and Malede (2014), and Mekuanint and Meareg (2019), who reported

values of mineral content in Godere district, Masha, Gesha, and Sheko districts of Southern Ethiopia, in Gondor selected districts of the Amhara and Tigray Regions, respectively, of 0.34±0.05g/100g, (0.22±0.16%), (0.23%), and (0.21±0.01), respectively.

Hydroxy-methylfurfural (HMF)

Table 7 shows the outcome of the hydroxy-methylfurfural (HMF) content. According to Awraris et al. (2014), hydroxy-methylfurfural (HMF) is a naturally occurring breakdown product of fructose that forms gradually during honey storage and more quickly when honey is cooked. The HMF levels of honey are the most often observed criterion for assessing its freshness. The current study's mean HMF values for highland, midland, and lowland agroecologies were 3.90±1.27, 5.90±.2.95, and.000±000 mg/kg, respectively. The current study's shown result falls between the permissible ranges of 40, ≤ 40, and ≤ 60 established by the QSAE, CAC, and EU, respectively. The hydroxy-methylfurfural (HMF) content found in this study satisfies both national and international requirements. Approximately 66.6% of the honey samples had no HMF in them overall. Table 3 shows that there was no significant difference (P > 0.05) in the HMF result among agro-ecologies in the research area. HMF was absent from both types of hives in lowland locations, whereas honey samples from midland and highland agro-ecologies had a mean of 3.267±.000 mg/kg. According to Bogdanov et al. (1999), there is no hydroxy-methylfurfural (HMF) in fresh honey, but it may grow during storage based on the honey's pH and storage temperature. It is possible to produce HMF by losing two fructose molecules.

Table 7

ANOVA for HMF analysis of honey from three agro-ecologies

Variables	Agro ecology	Df	Mean Square	F value	Sig.	SL
HMF (mg/kg)	Between Groups(Combined)	2	81.030	2.615	.094	*
	Within Groups	24	32.618			
	Total	26				

The honey sample's freshness may have contributed to the lower HMF finding in the research area. The present outcome is consistent with the findings of (Teshale, 2017), which stated that in the Doyogena and Kachabira districts of the Kembata Tambaro zone, Southern Ethiopia, the mean HMF value of honey samples evaluated in the study region was 3.42±1.95 mg/kg. The value reported by Aregay et al. (2018) was likewise higher than the current finding from Godere district, Gambella area, coming in at 9.91±2.64 mg/kg. However, this is probably consistent with the results of Addis and Malede (2014), who found 6.32 mg/kg HMF in a honey sample taken from the Amahara district of Gondar. The present study's findings is also less than that of Yetmiwork et al. (2015), who found that the HMF level in Kilte Awlaelo district, Eastern Tigray, was 1.71 mg/kg on average. This outcome contradicts the results of Awraris et al. (2014) and Abebe, 2017, which found 19.52±9.41 mg/kg and 37.7

mg/kg in the Masha, Gesha, and Sheko districts of Southern Ethiopia and the Tehulederie district, South Wollo zone, respectively.

pH value

The pH result is displayed in Table 8. Regardless of its place of origin, honey has an inherent acidity that may be caused by the organic acids that provide it flavor and stability against microbial deterioration (Khalil et al., 2012). In the highland, lowland, and midland agro-ecologies of the current study, the mean pH value of honey was 4.900±.000, 4.867±.0167, and 4.900±.1041, respectively. Following midland agro ecology, highland agro ecology had a mean pH that was higher than that of lowland agro ecology.

Table 8

ANOVA for pH analysis of honey from three agro-ecologies

Variables	Agro ecology	Df	Mean Square	F value	Sig.	SL
PH value	Between Groups(Combined)	2	.003	.100	.905	*
	Within Groups	24	.031			
	Total	26				

** Significant (P>0.05); SL=Significance level*

The PH average was 4.889±.0339 overall. Table 8 shows that there was not a single agro-ecological difference (P>0.05). The varying acid contents detected in various flower varieties may be the cause of the pH differences. The pH value obtained in this study was greater than the values reported by Mekuanint and Meareg (2019) and Aregay et al. (2018), who reported mean pH values of 3.94±0.14 and 4.04, respectively. The pH value obtained in the present investigation is, however, lower than the values reported by Abebe (2017), Addis and Malede (2014), and Yetimwork et al. (2015), who reported mean pH values of 3.85±0.46, 3.86, and 3.81, respectively.

Free acidity

Table 9 displays the findings from the free acidity study region. One quality parameter that has been used to determine whether fermentation has occurred is free acidity. Fermentation of honey raises its acidity. High free acidity is a sign that yeasts are fermenting honey sugar (Moussa et al., 2012). Carbon dioxide and alcohol are produced during the fermentation process from glucose and fructose.

Table 9

ANOVA Table Free acidity of honey from agro-ecologies

Variables	Agro ecology	Df	Mean Square	F value	Sig.	S
Free Acidity ($meqkg^{-1}$)	Between Groups(Combined)	2	49.333	3.326	.053	
	Within Groups	24	16.000			
	Total	26				

** Significant (P>0.05); SL=Significance level*

Honey's degree of free acidity is increased by the further hydrolysis of alcohol in the presence of oxygen, which results in the production of acetic acid. In the highland, lowland, and midland agro ecologies, the mean free acidity measured in the current study was 20.00±.764, 24.67±1.740, and 22.00±1.155meq/kg, respectively. The greatest free acidity value ever measured in lowland agro ecological may be related to variations in handling techniques, harvesting methods, and geographic circumstances. In the current study region, the mean free acidity of the honey samples examined was 22.22±.805meq/kg. All honey samples had free acidity levels within the permissible range of 40 meq/kg for QSAE, 40 meq/kg for CAC, and 50 meq/kg for EU. Table 9 shows that there was no significant difference (P>0.05) in honey acidity between agro-ecologies. Overall, the honey in the study region is of high quality and satisfies national and international acidity standards established by the EU, CAC, and QSAE. The new result was less than the mean of 28.32±14.14 meq/kg reported by Awraris et al. (2014).

CONCLUSIONS

The study determined that the physicochemical parameters of honey, as assessed by the Quality and Standards Authority of Ethiopia, the EU Council, and the Codex Alimentarius Commission, fall within the permitted range of national and international standards. There was a significant difference ($p<0.05$) in the levels of moisture, ash, and electrical conductivity between the agro-ecological zones. However, the effects of agroecologies on hydroxy-methylfurfural, sucrose, glucose, fructose, free acidity content, and pH value were not statistically significant ($p > 0.05$). Since all of the samples were deemed safe for ingestion by humans, the concerned office must take action to maximize honey production potential of the area and raise the commodity's share of the export market.

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DATA AVAILABILITY STATEMENTS

The data of this study are available from the corresponding author upon request.

DECLARATION

The authors declare that there is no conflict of interest.

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APPENDICES

Picture: Honey Quality Examination