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Research article

Physico-chemical characteristics of honey produced from northeastern Ethiopia

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ABSTRACT

The research was carried out to determine the physico-chemical quality aspects of honey harvested from northeastern Ethiopia. Twenty four honey samples were collected from four locations and two hive types. R software was used to analyze the data. The average values were 14.47%, 0.28%, 28.22 meq/kg, 4.28, 48.48 mg/kg, 13.75 Goth scale, 2.56%, and 52.43% for moisture, ash, acidity, pH value, hydroxyl-methyl-furfural (HMF), diastase, sucrose, and reducing sugars, respectively. The honey of highlands (17.43%) was higher (P < 0.05) in moisture than lowland (14.48%) implicating highland lower in quality. Highland honey has a higher acidity (P < 0.05) probably due to soil nature. Honey from lowland, midland, and market were higher (P < 0.05) than highland in HMF. Diastase from traditional (16.32 Goth scale) was higher (P < 0.05) than mixet (1.68%) although both were within the acceptable limits. Generally, the moisture, ash, acidity, diastase, sucrose, and pH contents of the honey were within the limits set for honey quality standards. However, in HMF and reducing sugars, it had not met the European standard. To make the honey exportable to international market, HMF and reducing sugars should be improved to the required level.

1. Introduction

Ethiopia has diversified flowering plant species and is home to huge honeybee colonies [1]. Due to this, northeastern Ethiopia is expected to have a good potential for honeybee production practices. Despite the potential and opportunities for honeybee production in the area, the quality and the produced quantity of honey are found relatively low (MoARD (2007) as cited in Refs. [2,3].

Among the different beehive products, honey is found the major product which is harvested and marketed significantly which is one of Ethiopia's image products [1]. The main focus of the government of Ethiopia is to increase the productivity of honeybees by applying modern technologies to increase honey production. While increasing honey production, keeping the quality of honey and amending it to European standard is a very important issue to maintain the country's image of the product and enhance its marketability [4].

Honeys having quality characteristics like unfamiliar taste, started fermentation, and being heated intensively is not desired in the international honey market [5]. Controlling these quality parameters is vital to make the honey suitable in the global market by satisfying the acceptable limits set for honey quality standards [6]. Besides, determining the major quality characteristics of honey

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produced in an area and comparing with the limits set by the national and international standards is important to know the drawbacks encountered during pre-harvest and post-harvest management practices of the honey so that to improve and increase the marketability. This in turn enhances the income from honeybee production practices to the country in general and beekeeping households in particular.

However, documented information related to the quality characteristics of honey harvested from eastern Amhara, particularly from Kallu district, is not available so far [7]. Conversely, such information obtained from the study is very useful to know the quality standard of the honey produced in the study area in which findings would be used as input for policymakers and concerned stake-holders for possible development interventions in the sector. The study was, therefore, aimed to determine and compare the major physico-chemical aspects of honey collected from eastern Amhara in general and Kallu district in particular.

2. Methodology

2.1. Description of study area and honeybee species

Kallu district is situated roughly from West $39^{\circ}40'4''$, East $40^{\circ}6'72''$ longitude; and South $10^{\circ}51'4''$, North $11^{\circ}19'24''$ latitude. The district had 35 rural and 5 urban *Kebeles* (smallest local administrative unit). The honeybee species of the study area are identified as *A. mellifera* and the race is categorized under Jementica. So, the honeybee under this study is *A. mellifera* Jementica.

2.2. Sampling techniques and sample size

Stratified random sampling technique was used to select the sampling sites. To select the sampling sites, the *Kebeles* were stratified by agro-ecological locations and random sampling technique was applied. Six representative *Kebeles* were selected: two from highland, two from midland, and two from lowland; and three market areas (*Ancharo, Gerba, Harbu*) which are located one in each agro-ecology.

The total number of samples collected for the study was twenty four. From these, twelve samples from the beekeeping farm gates located in each agro-ecology, i.e., highland, midland, and lowland (two from traditional and two from frame hive from each *Kebeles*), and the other twelve samples were from the market areas (four per a given market area, viz., two from each hive type). The collected honey samples were put into plastic containers of clean food-grade and held at room temperature until analysis.

2.3. Analysis of honey quality parameters

The parameters (moisture, total reducing sugars, pH, total acidity, sucrose, HMF, diastase activity, and ash contents) were determined using the procedures of [8] for honey quality standards in the laboratory of food and chemical faculty at Bahirdar University.

Moisture content (MC): MC was measured using the Abbe Refractometer. The relationship between the refract index and the water content reading at 20 °C done using [8]. The MC was determined by reference to a standard table using the honey refractive index. The refractive index reading was fixed to 20 °C. The refractive index was converted to moisture content by "(-log¹⁰ (Corrected Refractive Index - 1) - 0.2681)/0.002243" [9].

Reducing sugar content (RSC): RSC was calculated by Ref. [10] modified process, which involved reducing the Soxlet modification of Fehling solutions by titrating at 60 °C against a solution for reducing honey sugars using methylene blue as an internal indicator [11]. The result was calculated as: $C = (25/W) \times (1000/Y)$ [11]; Where, C = gram of invert sugar per 100 g honey, W = weight of honey sample, and Y = volume of diluted honey solution consumed.

Apparent sucrose content (ASC): ASC was determined using Pearson's procedures [11]. The result was calculated as: ASC = (invert sugar content after inversion - invert sugar content before inversion) \times 0.95 [11]. It was expressed as gram apparent sucrose per 100 g honey.

Ash content (AC): AC was calculated using [8] procedures. A dish has been weighed (M₂). Five grams of the honey (M₂) was added to the ash dish. Two drops of olive oil was added to the dish, and placed in preheated furnace and heated at a temperature of 600 °C for 1 and a half hours. The ashing process continued until attaining constant weight (M1). The ash (%) was calculated using the following formula: Ash (%) = (M₁ - M₂)/M_o × 100; Where, M₂ = weight of empty crucible, M₁ = weight of the ash and crucible, and M_o = mass of the sample taken.

Free acidity (FA): FA was calculated using [8] procedures. The result was expressed in meq of acid per kg of honey using the following equation: Acidity = 10V [8]; Where V = the volume of 0.1 M NaOH used and 10 is the amount of honey used.

pH value: Ten grams of the honey samples was dissolved in 75 ml of distilled and stirred with magnetic stirrer. It was measured using pH meter [8].

Hydroxy-methyl-furfural content (HMF): HMF was determined using 6800 UV–Vis spectrophotometer [12]. The absorbance was recorded by subtracting the absorbance measured at 284 nm for HMF in the honey sample solution against the absorbance of reference at 336 nm and the result was calculated as: HMF per 100 g honey = $[(A284 - A336) \times (14.97 \times 5)]$ per g sample [12]; Where A284 = absorbance at 284, A336 = absorbance at 336, 14.97 = constant, 5 = theoretical nominal sample weight and g = mass of honey.

Diastase activity (DA): DA was determined using 10 g of honey, 5 ml of acetate and 20 ml of water. Three ml of sodium chloride 0.5 M was added and diluted to 50 ml with water. A starch solution was standardized using an iodine solution. Both solutions were warmed at 40 °C. Five ml of starch solution were added into ten ml of honey solution. An aliquot was taken every 5 min and was added to 10 ml of iodine solution. The number 300 was divided by the time needed to reach the absorbance value of 0.235 and expressed as diastase

number [12].

2.4. Statistical analysis

To analyze the collected data, analysis of variance and principal component analysis were used using R software. Whenever a significant difference was shown among means, means were separated by least significant difference at 5% level of significance.

3. Results

The average water content was 14.47% varying from 13.2% to 17.6% (Tables 1–3). Honey produced from the highland (17.43%) was higher in moisture (P < 0.05) than honey produced from the lowland (14.48%). However, difference was not observed (P > 0.05) between hive types.

The average content of ash was 0.28% varying 0.13%–0.60% (Tables 1 and 2). The result was within the acceptable limits of QSAE (0.6 max), EU (\leq 0.6) and CAC (\leq 0.6) standards (Table 3). The content of ash showed difference among the locations (P < 0.05) but not between hive types (P > 0.05).

Acidity was ranged from 20.85 to 39.14 meq/kg with average value of 28.22 meq/kg (Tables 1 and 2). Acidity had met the standard limits set by CAC (\leq 50 meq/kg) and QSAE (<40 meq/kg). Honey collected from the highland was higher in acid (P < 0.05) than the other locations but difference (P>0.05) was not found between hive types.

The pH was ranged from 3.35 to 4.62 with average of 4.28 (Tables 1 and 2) which is within the acceptable quality limits. Differences was not observed among locations (P > 0.05) and between hive types (P > 0.05) in pH.

HMF was ranged from 22.66 to 65.94 mg/kg, and had an average value of 48.48 mg/kg (Tables 1 and 2). HMF was within the acceptable range of CAC quality standards (\leq 60 mg/kg) (Table 3). Honey from lowland, midland and market locations were higher (*P* < 0.05) in HMF value than highland. However, no difference (*P* > 0.05) between hive types.

Diastase activity was ranged 9.44–18.27 Goth scale and average of 13.75 Goth scale (Tables 1 and 2). Diastase had met limits set by CAC and EU (Table 3). Diastase activity of traditional hives (16.32 Goth scale) was found higher than (P < 0.05) frame hives (11.19 Goth scale). However, locations were not revealed difference (P > 0.05).

Sucrose was varied from 0.29 to 4.3% with the mean of 2.56% (Tables 1 and 2). Sucrose was within the acceptable limits of QSAE (max.10%), CAC (\leq 5) and EU (\leq 5) (Table 3). Sucrose from highland (3.88%) was higher (P < 0.05) than those collected from local vendors (1.68%). However, difference was not found between hive types (P > 0.05).

Reducing sugars ranged from 47.1 to 60.44 g/100g with the mean of 52.43 (Tables 1 and 2). Reducing sugars were within the acceptable limits of CAC standard (Table 3). Locations and hive types did not shown differences in the content of reducing sugars (P > 0.05).

The eight physicochemical parameters on the twenty four locations of honey samples were subjected to Principal Component Analysis (PCA) to observe the leading vectors that contain most of the variances. The first two principal components explained about 35% & 19%, respectively, accounting 54% of the total variations (Fig. 1).

4. Discussion

Honey moisture content of Kallu district was lower than honeys of Harenna Forest, Bale Natural Forest, Belete-Gera Forest, Sekota district and Tigray region [13–17]. Moisture content (14.47%) has met the limit set by Codex Alimentarius Commission [18]. The

Table 1

Physicochemical properties of honey samples collected from different locations.

Variable	Location								
	Lowland (N = 4)		Midland (N = 4)		Highland (N = 4)		Market (N = 12)		
	Range	$\text{Mean} \pm \text{SD}$	Range	$\text{Mean} \pm \text{SD}$	Range	$\text{Mean} \pm \text{SD}$	Range	$\text{Mean} \pm \text{SD}$	
Moisture (%)	13.5–15.3	$\begin{array}{c} 14.48 \pm \\ 0.87^{ba} \end{array}$	14.1–16.1	$\begin{array}{c} 15.05 \pm \\ 0.85^{ba} \end{array}$	17.31–17.6	17.43 ± 0.12^{aa}	13.2–16.2	14.58 ± 0.93^{ba}	
Ash (%)	0.13-0.22	0.15 ± 0.04^{ba}	0.21-0.36	0.32 ± 0.07^{aba}	0.17-0.60	0.48 ± 0.21^{aa}	0.16-0.54	0.25 ± 0.11^{ba}	
pН	4.1-4.56	4.38 ± 0.22	4.3-4.48	$\textbf{4.43} \pm \textbf{0.08}$	3.35-4.29	$\textbf{4.02} \pm \textbf{0.45}$	3.4-4.62	$\textbf{4.28} \pm \textbf{0.36}$	
Acidity (meq acid/ kg)	20.85-29.7	23.81 ± 4.01^{ba}	21.86-32.84	26.44 ± 5.10^{aba}	31.78–39.14	36.30 ± 3.16^{aa}	21.86-35.54	$\textbf{27.60} \pm \textbf{5.19}$	
HMF (mg/kg)	37.76–65.94	54.36 ± 11.6 ^{aa}	47.47–53.4	50.40 ± 3.32^{aa}	22.66-37.88	$29.17 \pm 6.37^{\mathrm{ba}}$	29.32-65.93	52.31 ± 12.22^{aa}	
DA (Goth scale)	13.56-18.17	16.04 ± 2.23	9.44-17.04	14.26 ± 3.43	9.54–16	12.95 ± 3.28	9.74-18.27	13.09 ± 3.12	
RS (%)	49.64-54.59	50.94 ± 2.44	47.2-60.44	54.04 ± 5.72	47.1-59.62	52.13 ± 6.10	48.64-59.52	52.50 ± 3.74	
Sucrose (%)	1.15–4.3	$\begin{array}{c} 3.26 \pm \\ 1.42^{aba} \end{array}$	1.2-4.0	3.17 ± 1.32^{aba}	3.69-4.12	$3.88\pm0.18^{\text{aa}}$	0.29–3.97	1.68 ± 1.36^{ba}	

abMeans with different superscripts within a row are significantly different; N = Number of sample; SD = standard deviation; HMF = hydroxyl methyl furfural; DA = diastase activity; RS = reducing sugar.

^a There is a significant difference at 95% confidence level.

Table 2

Physicochemical properties of honey samples collected from hive types.

Variable	Traditional hive		Modern hive		
	Range	Mean \pm SD	Range	$\text{Mean}\pm\text{SD}$	
Moisture (%)	13.2-17.39	14.82 ± 1.43	14.0-17.6	15.42 ± 1.20	
Ash (%)	0.13-0.59	0.27 ± 0.15	0.13-0.6	0.30 ± 0.16	
pH	3.35-4.6	4.27 ± 0.36	3.4-4.62	4.29 ± 0.32	
Acidity (meq acid/kg)	20.9-39.1	$\textbf{28.46} \pm \textbf{6.23}$	21.9-37.1	27.99 ± 5.89	
HMF(mg/kg)	27.4-65.9	49.5 ± 13.7	22.7-65.1	47.5 ± 13.3	
DA (Goth scale)	13.7–18.3	$16.3\pm1.4a^{a}$	9.4–14.8	$11.2\pm1.9\mathrm{b}^{\mathrm{a}}$	
Reducing sugar (%)	47.2-60.44	52.3 ± 4.5	47.1–59.6	52.6 ± 4.02	
Sucrose (%)	0.43-4.3	$\textbf{2.94} \pm \textbf{1.44}$	0.29-3.97	2.2 ± 1.5	

abmeans with different superscripts within a row are significantly different; N = Number of sample; SD = standard deviation; HMF = hydroxyl methyl furfural; DA = diastase activity.

^a There is a significant difference at 95% confidence level.

Table 3

Honey quality comparison of Kallu district with national and international standards.

Characteristics	Overall Range (Kallu)	Overall Mean (Kallu)	Standards			
			CAC	EU	QSAE	
Moisture (%)	11.87-16.70	15.12	≤ 21	≤ 21	21 max.	
Total ash (%)	0.12-0.49	0.28	≤ 0.6	≤ 0.6	0.60 max.	
Reducing sugar (%)	39.95-59.04	52.43	≥45	≥ 60	65 min.	
Sucrose (%)	0.98-4.11	2.56	≤ 5	≤ 5	10 max.	
Acidity (meqkg ⁻¹)	19.55-35.57	28.22		<u>≤</u> 40	40 max.	
HMF (mg/kg)	23.31-59.03	48.48	$\leq 60 \text{ mg/kg}$	\leq 40 mg/kg	40 max.	
Diastase (Goth scale)	9.32-17.10	13.75	≥8	≥8	3 min.	
pH	2.93-4.53	4.28	3.4–6.1	_	-	

CAC = Codex Alimentarius Commission; HMF = Hydrixymethylfurfural; EU = European Union; meq = milliequivalent; QSAE = Quality and Standards Authority of Ethiopia.

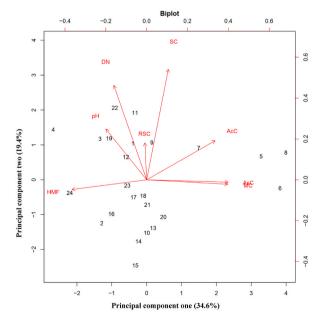


Fig. 1. Principal component analysis of the honeys and their physicochemical attributes.

moisture content was within the acceptable quality limits. According to Ref. [15], low moisture content indicates a good quality honey. On other hand, honey with high moisture implies a poor quality which is more likely to be fermented [19]. In the global trade of honey, moisture content is considered as the most important honey quality parameter. Moisture of honey of the study area became higher as the altitude was increasing. This might be because in tropical countries like Ethiopia with increasing altitude the weather condition is

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becoming cooler which in turn is a factor for high honey moisture as explained by Ref. [20] who reported climatic as one of the factors for honey moisture variability.

Ash (0.28%) was higher than the report of [13,17,21] for honeys collected from Belete-Gera Forest, Sekota and Tigray, respectively. However, it is lower than [22] who reported 0.34% honey ash content for honey produced in Godere district. Ash content was found to the acceptable quality standards. The difference in the ash content of honeys might be due to the nature of soil and minerals level of content found in the nectars.

Acidity (28.22 meq/kg) of the honey of Kallu district was found more than the findings of [13,23] who reported 22.3 meq/kg and 23.5 meq/kg for honey produced in Tehulederie district and Sekota district, respectively. According to Ref. [24], differences of acidity in honeys might be due to floras or to differences in season of honey harvesting. Honey fermentation causes an increase in acidity value, i.e., as the honey acidity increases, sourness of honey becomes higher.

Honey pH is acceptable if it is between 3.4 and 6.1 [18]. The pH of the study area is higher than honeys of Sekota district [13] and Silte district [25]. However, it is lower than [26] reported 4.45 in Guji Zone. Honey pH of Kallu district was found within acceptable quality limits. pH has immense value during storing the honey. As the honey pH is low, it will act against the occurrence and development of microorganisms [27]. According to [21, 28), factors like nectar source, the substances added by the honeybees, method of processing and soil pH.

HMF (48.48 mg/kg) was far higher than the findings of [28,29]. Heating and storing of the honey for longer time increase the amount of honey HMF which indicates a lower quality [18,26]. Although HMF of honey from the highland was within the acceptable range, honey from the lowland, midland and market areas failed to meet the quality standard which could be due to exposure of honey to high temperature. Kallu's honey was not found within the acceptable quality limits.

Diastase activity (13.75 Goth scale) is in line with the report of [23] who indicated 14.4 Goth scale for honey collected from Tehulederie district. DA was found within acceptable quality limits. Unlike HMF, heating and storing of honeys for longer period of time decrease diastase activity [26]. Even though DA is variable depending on honey bee floral source, lower value from what is expected informs about its quality [27].

The sucrose content (2.56%) was less than the mean values of sucrose in the Sekota district (3.1%) and Silite district (4.1%) which were reported by Refs. [13,25], respectively. The sucrose content of Kallu district had met Ethiopian and global honey quality limits. As honey ripeness is enhanced, the amount of sucrose will be declined as the result of invertase enzymes that dissociate to its simplest form. Adulteration of honeys with syrups can be determined using the procedures of [30].

The reducing sugars content of Kallu district (52.43%) is lower than Sekota, Tehuledere and Silite districts as indicated by Refs. [13, 23,25] who reported 67.3%, 64.3%, and 69.04%, respectively. Reducing sugar of Kallu district was not within acceptable quality limits of the European Union. Amount of honey reducing sugars depend on time of storage and collection [31,32]. The content of reducing sugar in honey is affected by botanical origin (types of flowers as the source of nectar), geographical origin (for beekeeping or meliponiculture), climate, processing and storage [33,34,35]. It is composed mainly fructose (\sim 38% w/v) which is responsible for the sweetness; and glucose (\sim 31%) which depends upon the nectar source, and sucrose (\sim 1%) in lesser amount.

The first two principal components explained majority (54%) of the total variations (Fig. 1). The biplot graph identified three separate honey types (market honey, beekeepers' and highland honeys) although some honey samples did overlap. A similar result was indicated by Ref. [36]. However, honeys from lowland, midland, traditional hive and modern hive did not show separate honey type. Most of the honey samples collected from the markets positioned towards the negative principal components of one and two, and were connected vastly to HMF; whereas, most of the honey samples collected from farmers' gate were located at the positive parts of principal components of one and two, and was associated with sucrose content and diastase activity. Honeys collected from the highland location were more associated in acid content, moisture content and ash content (designated by 5, 6, 7 & 8 in the biplot) than the other honey quality parameters. Moisture content and ash content of the honeys were close to each other which had a vivid correlation (similar data patterns). Sucrose content, diastase activity, free acidity, moisture content, ash content comprised most of the variations as predicted from the length of the loading plot projection; however, pH and total reducing sugars contributed less for the variations of physicochemical characteristics of the honeys (Fig. 1).

5. Conclusions

Honey of Kallu district had met the national and international acceptable honey quality standards in most of the analyzed parameters (moisture, ash, acidity, diastase activity, sucrose, and pH). However, HMF and reducing sugars were not found within the acceptable ranges of European quality standard. The first two principal components explained majority of the total variations. Three separate honey types (market honey, beekeepers' and highland honeys) were identified through PCA. To export honey of Kallu district to the European markets and for increasing its marketability, concerned stakeholders should emphasize on improvements of HMF and reducing sugars to the specified levels of European honey quality standards.

Author contribution statement

Tilahun Abera: Conceived and designed the study; Performed the experiment; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Tewodros Alemu: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no competing interests.

1&2 = honey from modern hive from farmers' gate of lowland; 3&4 = honey from traditional hive from farmers' gate of lowland; 5&6 = honey from modern hive at farmers' gate of highland; 7&8 = honey from traditional hive at farmers' gate of highland; 9&10 = honey from modern hive at farmers' gate of midland; 11&12 = honey from traditional hive at farmers' gate of midland; 13-18 = honey from modern hive of market; 19-24 = honey from traditional hive of market; HMF = hydroxyl methyl furfural; DN = diastase activity; RSC = reducing sugar content; SC = sucrose content; AcC = acid content; MC = moisture content; AsC = ash content.

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