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Research Article

Physicochemical and antioxidant properties of *Coffea arabica* honey from Western Oromia, Ethiopia

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Abstract

Coffea arabica is one of the most widely consumed and marketed commodities in the world. The study was designed to characterize *C.arabica* honey for botanical composition, physicochemical parameters, and antioxidant properties of honey. Twelve honey samples of *C. arabica* honey were collected during the flowering period of coffee flowers from the Zander hive. The physicochemical properties of honey and the Botanical origin of honey were determined based on Harmonized methods of the International Honey Commission. The antioxidant power of the coffee monofloral honey samples was determined by dissolving 1.5 gm of honey with 25 ml distilled water and mixing it with 25ml methanol and placed at 25°C for sixty minutes of maceration using a temperature shaker. The pollen count percentage from honey indicated that all honey samples collected from Gera, Gomma, Yayu, and Manna districts were identified as coffee monofloral honey representing 84%, 93%, 75%, and 73 % of pollen count respectively. The mean moisture, ash, HMF, EC, FA, pH, fructose, glucose and sucrose content of *Coffea arabica* honey were 22.48%, 0.21%, 11.88, 0.49 mS/cm, 13.44 meq/Kg, 3.32, 32.77%, 32.9%, and 3.57% respectively. The total phenol and flavonoid content range from 42.1-82.1 and 21.7-59.7 mg/100 g of GAE/g respectively while the radical scavenging activity ranges from 60.2- 66.3%. The pollen analysis of honey from the area is coffee monofloral honey since its pollen count exceeds more than 45% and the honey quality also meets the Ethiopian and International standards. The antioxidant power of Coffee honey has a considerable amount of polyphenolics which have relevant antiradical activity.

Introduction

Honey is a complex substance and a source of nutrition that has been used by people since ancient times. It is the ordinary sugary substance processed by honey bees from the floral reward or from secretions of existing parts of flora which honey bees gather and mix with their specific enzyme, deposit, and put in the honeycomb to mature [1,2]. There are nearly 181 constituents have been reported in honey which include sugars and organic acids [3]. Moreover, honey is also a source of secondary metabolites such as antioxidants and phenols which has medicinal value against various diseases including anti-aging, cancer, cardiovascular disease, and gastrointestinal [4]. The main cause of the antioxidant power of honey is owing to the occurrence of biologically active ingredients such as phenol and flavonoids [5,6]. The constitutes of honey differ by its plant type and agro-ecological condition of the area [7].

Coffee (*C. arabica*) is a perennial crop that belongs to the family Rubiaceae and originated in Ethiopia [8,9]. It is the most important cash crop for trading and exports since the 18th century, providing jobs for millions of families worldwide.

The coffee flowers are extremely aromatic with mass flowering yields copious nectar and pollen making the plant highly attractive to honeybees [10]. Coffee honey is an important product and contains some basic nutritional composition [11]. Coffee honey is produced in areas where coffee is intensively grown particularly in Brazil and Indonesia and Ethiopia. In the country, coffee is cultivated in vast areas of Ethiopia primarily from altitudes ranging from 1200 to 3000 m [12]. Coffee is a major source of bee plants in southwestern and southeastern parts of Ethiopia [13,14]. The production of coffee honey is not adequately known in Ethiopia, although the country with its center of origin and one of the biggest coffee producers in the world due to a lack of knowledge of the flowering calendar and management of colonies for coffee honey production. *Coffea arabica* honey is usually harvested during the harvesting period

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of *Vernonia amygdalina* hence, the honey is usually mixed with Vernonia honey since there is an overlap in the flowering period of both species [15].

Currently, there are different coffee cultivation practices in Ethiopia such as conserving forest coffee production, cultivating coffee in the forest which is named, semi-forest coffee, and cultivating around the home garden for home consumption [16]. These production systems may provide a great opportunity for producing coffee honey by smallholder farmers and investors if it is supported with appropriate management of honeybee colonies for coffee honey production.

Preliminary pollen analysis of honey from Yayu in the Illuabore zone of Oromia indicated that over 75% of the pollen count of Coffee pollen was found in honey samples. This indicated that it is possible to produce coffee monofloral honey by integrating beekeeping with coffee production. Thus the characterization of the monofloral honey from *Coffea arabica* may benefit beekeepers to exploit the niche market opportunities for the commercialization of coffee honey. Therefore, it is important to authenticate the botanical source of honey through pollen analysis and characterization of physicochemical properties for identification of adulterants and branding of Coffee honey. In this investigation, an attempt was made to analyze the quality parameters of Coffee honey including physicochemical and antioxidant properties and the botanical origin of honey.

Materials and methods

Site description

This study was carried out in one of the potential coffee belts of Gera, Goma, Mana, and Yayu districts in the Jimma and Illubabore zones of Oromia regional states Figure 1. The area is dominated by a small holder's coffee production system, including coffee agroforestry, larger remnants of continuous forest dominated by forest, and a semi forest coffee production system. Honey production is common practice in the area and it is one of the income-generating activities after coffee production.

Collection of honey sample

Honey samples were collected from established honeybees kept in zander hives from Gera, Yayu Goma, and Manna districts following the flowering period of coffee (March 2019). The samples were collected after the flower shedding. The samples of honey 500 gm were collected from each district and kept in a sample bottle and stored at -20 °C until analysis.

Melisso palynological analysis of honey

To determine the botanical origins, ten grams (10 g) of the honey sample was placed in a test tube and added 20ml of distilled water. Subsequently, the samples were centrifuged and the supernatant solution was decanted following the methods described by [17]. The extracted pollen was placed on a glass slide and added with a drop of glycerin jelly and observed under light stereomicroscope Zeiss 2010 and pollen grains were identified using pollen atlas [18] and frequency occurrences of pollen were determined according to [19] Figure 2.

Physicochemical analysis of honey

The physicochemical analysis of honey was done using Harmonized methods [20]. The MC of honey was determined using an Abbé refractometer which was adjusted at 20 °C, and standardized with distilled water. The honey samples were properly stirred until the honey was properly liquefied. After proper mixing of the sample, the surface of the refractometer was smeared and covered with honey and then the reading was

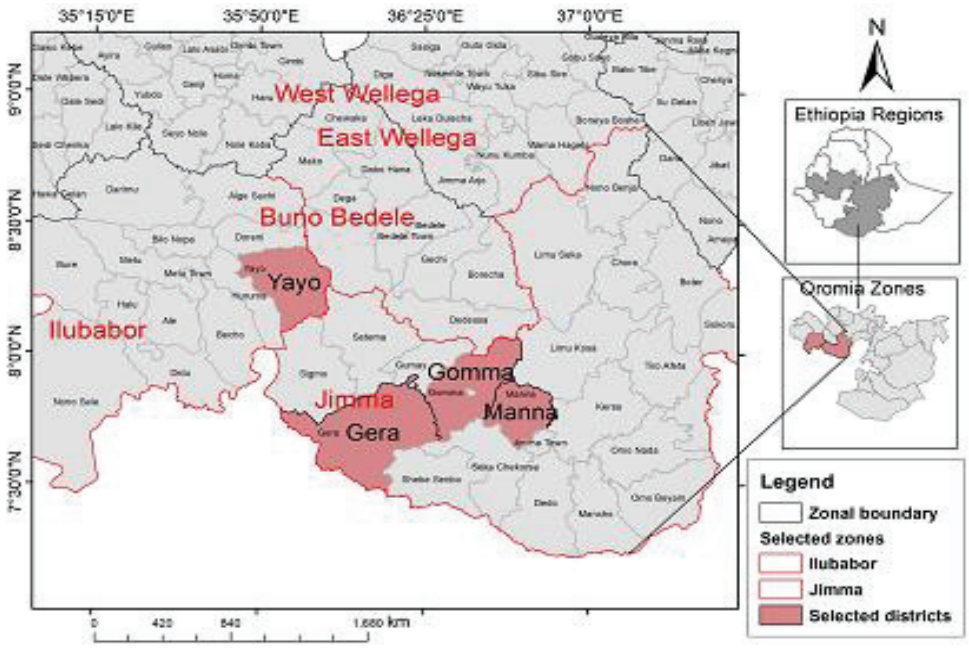

Figure 1: District map of the study area.

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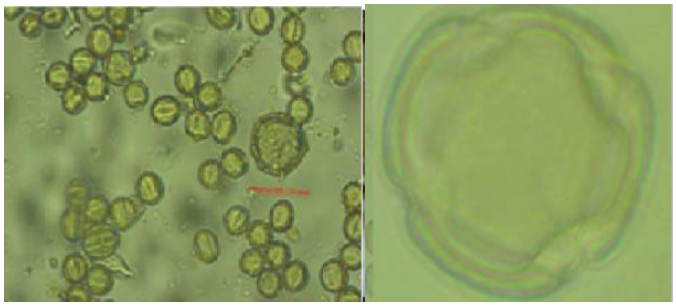

Figure 2: Pollen grain morphology of *Coffea arabica*.

taken from the Refractive index. E.C was determined according to [20] using a conductivity meter. For the measurement of the pH of honey, 10gm of honey was weighed and dissolved in 75 ml of distilled water and a magnetic stirrer was immersed and the pH of the honey was recorded. The HMF was determined following the methods [21] and for the purpose, 5 gm of honey samples were taken and liquefied into a 50 ml of beaker in 25 ml of distilled water. The reading was made using HPLC equipped with RID detection.

Determination of mineral content (Ash)

The total ash level was identified using the method QSAE [22]. The dish was placed in an oven at 600 °C and cooled in a desiccator and then the weight of the dish was taken again (M_2). Five grams of honey sample was measured using a weighing balance to the nearest 0.001 g and added to the ash dish (M_0). The dish was put in a heated furnace and stayed for one and half an hour at a temperature of 600 °C. The ash sample was taken out when constant weight was achieved (M1) and % ash content was determined using the following formula.

$WA = (M_3-M_1)/M2*100$

Where: M_1= weight of dish,

M_2 = weight honey taken

M_3 = weight of dish + ash

Determination of sugars by HPLC

The standard substances, for common sugars (fructose, glucose, sucrose, and maltose) were prepared according to International Honey Commission [20]. The standard was prepared and 5 gm of honey was taken and mixed in 40 ml distilled water. A 25 ml of methanol was placed into 100 ml of volumetric flask and then filtered using filter paper and poured into vials. Peaks were determined based on the holding times of the glucose, fructose, and sucrose.

Determination of total Phenol

The phenolic compounds concentration in honey samples was estimated with the Folin-Ciocalteu reagent according to the methods described by [23]. The solution of the coffee honey sample was prepared by dissolving 2.5 g honey in 50 mL distilled water and filtered through Whatman no.1 filter paper and then one ml of Folin-Ciocalteu reagent was put into the mix and shaken. After 3 minutes 1 ml of saturated sodium carbonate (20%) solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction was placed in a dark room for nighty minutes and then the absorbance was read at 725 nm. The total phenolic content of the samples was expressed in milligram per Gallic acid equivalents (GAE). The total phenolic content was calculated as Gallic acid equivalent (GAE).

Determination of flavonoid

The flavonoid level of coffee monofloral honey was determined by $AlCl^3$; Quercetin was used as the reference which was expressed as QE [24]. The stock solution was prepared by diluting five grams of honey sample in fifty milliliters of distilled water and strained through filter paper. Five ml of stock solution was pipetted and dissolved with 5 ml of 2% aluminum chloride ($AlCl_3$) solution. After incubation for ten min, the absorbance was measured at 415 nm by using a spectrophotometer (Lambda 950 UV/VIS/NIR spectrophotometer). The total flavonoid content was expressed as milligram of Quercetin equivalent (QE) per 100 gram of honey from the mean value of triplicate data using the calibration equation.

Determination of antioxidant

The antioxidant power of the coffee monofloral honey samples was determined by dissolving 1.5mgm of honey with 25 ml distilled water and mixing it with 25ml methanol and placing it at 25 °C for sixty min maceration using a temperature shaker (ZHWY103B). The residue was then re-extracted 25 ml portions of methanol and the combined methanol extracts were evaporated at 40 °C and re-dissolved in methanol at the concentration of 50 mg/ml. The antioxidant activity of methanol extracts was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl). A 0.004% solution of DPPH was prepared and then 2 ml of this solution was combined with honey extracts in methanol. Radical scavenging activity of honey solution was read spectro-photometrically at 517 nm. The scavenging power of the honey was obtained using the following formula:

$DPPH (\%) = (A_0-A_1)/A_0* 100$

Where:

A0 = absorbance of the control

A1 = absorbance of the sample

Statistical analysis

Statistical analysis was accomplished on SPSS version 20 for windows and analysis of variance (ANOVA) was performed for the significant difference using a post hoc test ($p < 0.05$). Correlation among the different parameters was computed by Pearson's correlation coefficient (r) in a bivariate linear correlation.

Results

Melissopalynological analysis of honey

Microscopic pollen determination of samples showed that all samples collected from four districts (Gera, Gomma, Yayu, and Manna) were monofloral since *Coffea arabica* honey pollen count constituted 84%, 93%, 75% and 73 respectively Figure 3.

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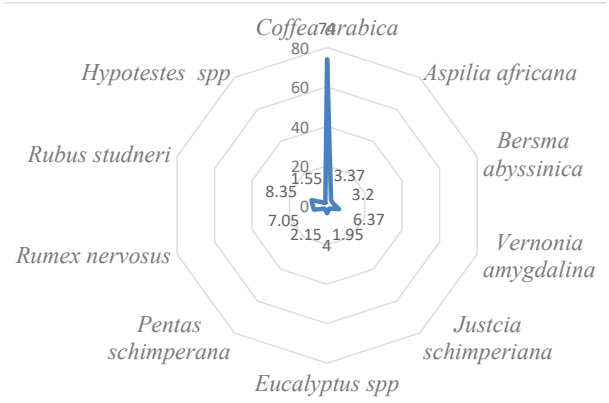

Figure 3: Contributing bee forages to Coffee monofloral honey in Gomma, Gera, You and Manna districts.

The secondary pollen source plants contributing to coffee honey from the study districts were *Aspilia Africana (8.6%), Bersama abyssinica* (8.86%), *Rumex nervosus* (21%), *Rubus studneri* (12.7). *Vernonia amygdalina* (9.7%). The minor pollen source plants *Caesalpina decaptella* (3%), *Eucalyptus spp* (3.8%), and *Hypoestes forskaolii* (0. 3%).

Physiochemical properties of honey

The results of the Physico-chemical analysis of the monofloral honey of *Coffea arabica* for different parameters were indicated in Table 1.

Moisture content

The moisture content (MC) of the Coffee monofloral honey of the study area is ranging from 21 to 24.05 % with a mean of 22.46 and there was a statistical difference ($p < 0.05$) between honey samples of the study districts for moisture Content. The moisture content of honey samples of the Gera and Manna are significantly different from Gomma and Yayu (Table 1).

Ash

Ash content is one of the important quality parameters in worldwide honey marketing. The result of coffee honey samples for Ash showed that it ranges from 0.15 to 0.23 g/100 g and a mean of 0.21 ± 0.3 g/100 g). The ash content of honey samples significantly varies between honey samples ($p < 0.05$). The ash value of the coffee honey from Manna and Gomma is significantly different ($p < 0.05$) from Gera and Yayu.

Hydroxymethylfurfural (HMF)

The level of hydroxymethyl Furfural (HMF) in honey is one of the major parameters of honey quality and it indicates whether honey is aged or overheated [25]. In this result, the HMF level was found to be between 6.67 to 16.74 mg/ kg and HMF content between districts was not significant ($p > 0.05$).

Free acidity

Acidity is an essential quality parameter measure, for its antimicrobial property. The level of Free acidity in honey is an indication of fermentation of the honey by yeasts. During the fermentation process, simple sugars such as glucose and fructose are transformed into CO_2 and alcohol. The acidity of tested honey varied from 32.96 to 38.90 mg/100 gm with a mean of 35.89 mg/100 gm of honey and there was no significant variation ($p > 0.05$) in free acidity among the honey samples.

pH

The pH of the honey plays a great role in keeping the quality of honey, as they impact the texture, stability, and shelf life of honey [26]. There was no significant difference ($p > 0.05$) in the pH of honey between honey samples obtained from four districts. The pH of coffee monofloral honey ranged from 3.31 to 3.48 with an average value of (3.41 ± 0.09).

Electric conductivity

The current results showed that the average electric conductivity of Coffee honey (0.44 $mS.cm^{-1}$) to 0.58 and with a mean value of 0.49 ± 0.08 and honey samples significantly differ between the districts ($p < 0.05$). The honey sample from the Gera district is significantly varied among the three districts.

Sugar profile

The fructose content of coffee honey ranges from 31.46 ± 5.6 to 35.31 ± 6.08 with a mean value of 32.77 ± 6.06. The amount of sucrose detected in the honey samples did not show significant differences ($P > 0.05$) between districts. The glucose content of coffee honey ranges from 31.09 to 32.8 with a mean value of 31.94. The fructose level of coffee honey is within the range of National and International ranges nearly close to reports [27]. The range of the sucrose content of the coffee honey was 2.72 to 4.75 with a mean value of 3.75 ± 1.52. The sucrose level of honey between honey was significantly different ($p < 0.05$). The average sucrose level of the coffee honey is less than the country's average of 3.6%, which was agreed with [28] and lower than the maximum limits of 10% set by QSAE (2009) and 5% set by [29]. Similarly, the maltose content of honey range from 0.27 to 0.8 with a mean value of 0.41 ± 0.39. The maltose content between honey samples was significantly different ($p < 0.05$). The maltose level of honey from the Yayu district is significantly varied from the rest of the honey.

Table 1: Physiochemical properties of *Coffea arabica* honey from different districts.

Sample locality	MC	ASH	EC	FA	pH	HMF	Fructose	Glucose	maltose	Sucrose
Yayu	21 ± 0.9b	0.2 ± 0.07 ab	0.5 ± 0.1 ab	35.9 ± 66 a	3. ± 0.18 a	17 ± 8 a	35 ± 6.08 a	33 ± 2.31 a	0.8 ± 0.8 a	3.6 ± 1.3 ab
Gomma	22 ± 0.3b	0.30 ± 0.15 a	0.63 ± 0.21 a	37.8 ± 9 a	3 ± 0.1 a	11 ± 7 ab	32 ± 6.2 a	32 ± 2 a	0.27 ± 0.21 b	3 ± 1.2 b
Manna	23 ± 0.1a	0.26 ± 0.09 a	0.59 ± 0.16 a	37 ± 8 a	3 ± 0.12 a	12 ± 18 ab	33 ± 6 a	32 ± 3 a	0.28 ± 0.11 b	2.72 ± 1.3 b
Gera	24. ± 1a	0.08 ± 0.009 b	0.28 ± 0.012 b	31. ± 4 a	3. ± 0.4 a	6 ± 2 b	31 ± 6 a	31 ± 2 a	0.32 ± 0.01 c	4.75 ± 0.9 4.75 ± 0.97 a
Mean	22.4	0.21	0.49	18	3	12	33	31.9	0.41	3.5

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Table 2: Pearson correlation coefficients among the analyzed parameters.

	MC	Ash	EC	HMF	PH	FA	Fr	Glu	Sucr	maltose
MC	1									
Ash	-0.17 ns	1								
EC	-0.157	0.99**	1							
HMF	-0.197 ns	0.16 ns	-0.17	1						
PH	-0.16 ns	-0.104 ns	-0.065	0.22ns	1					
FA	-0.07 ns	0.20	-0.182	0.021 ns	-0.054 ns	1				
Fr	-0.31 ns	-0.094	-0.09	-0.105 ns	0.10 ns	0.46*	1			
Glu	-0.34 ns	-0.38 ns	-0.36*	0.009 ns	-0.056	-0.09 ns	0.198 ns	1		
Sucr	0. 01 ns	0.003 ns	-0.19	-0.22 ns	0.047 ns	0.036 ns	-0.039 ns	-0.062 ns	1	
Malt	-0.43 ns	0.026 ns	0.001	-0.23 ns	0.23 ns	0.099 ns	0.241	0.022 ns	-0.087	1

*, **, ns = significant at 5 and 1%, and non-significant at 5%, respectively.MC: Moisture Content; Ash: Ash Content; HMF: Hydroxyl Methyl Furfural aldehyde, pH: pH of honey; FA: Free Acidity; Fr: Fructose; Glu: Glucose; Malt: Maltose; Su: Sucrose.

Correlation

There were significant strong correlations between ash content and electrical conductivity $r = 0.99$ ($p < 0.01$) Table 2. The high correlation coefficient of the ash content and Electrical conductivity indicates the possible influence of mineral component of honey on its electrical conductivity. The measurement of electrical conductivity depends on the ash and acid contents of the honey. The higher the ash and acid content, the higher the resulting conductivity.

The total phenol and flavonoid

There was a significant difference in the total phenol content of coffee honey between the different districts. The total phenol content of honey samples from Manna is significantly different from Gera, Gomma, and Yayu. The total phenol content of coffee honey samples ranged from 42.5 ± 0.94 mg/100g to 82.1 ± 6.48 and highest for Manna (82.1 ± 6.48) and lowest for Gomma (69.3 ± 3.8 mg/100g). On the other hand, total flavonoids content in coffee honey ranges from 21.57 ± 0.90 to 53.9 ± 4.4 and highest for Yayu (42.5 ± 0.94 mg/100 g) and lowest for Manna (21.57 ± 0.90 mg/100 g). There was a strongly significant difference ($p < 0.05$) between flavonoid content of the Manna and Yayu districts. The variation in the number of flavonoids in honey is due to floral origin [30].

The antioxidant content of honey

The antioxidant content of coffee honey ranges from 57.9 ± 5.86 to 6.4 ± 0.32% inhibition Table 3. There was no significant difference (P>0.05) between honey samples and the highest antioxidant level was found in Manna and Gera 66.3 ± 4.84 and 66.4 ± 0.32% and the lowest for Yayu and Gomma (609 ± 6.9 and 60.2 ± 2.27) respectively. The higher correlations were observed between the DPPH radical scavenging activity and the total polyphenol ($r = 0.755$, $p < 0.001$), and total flavonoids ($r = 0.167$, $p < 0.01$), and between total flavonoids and total polyphenols ($r = 0.81$, $p < 0.01$).

Table 3: Total phenol and antioxidant content of coffee honey samples obtained from four districts in western Oromia.

Honey sample location	TPC mg/100ml	T FC mg/100ml	Percentage of inhibition
Yayu	71.6 ± 4.2 ab	53.9 ± 4.4 a	60.9 ± 6.9 a
Gomma	69.3 ± 3.8 b	35.9 ± 0.99 b	60.2 ± 2.27 a
Mana	82.1 ± 6.4 a	21.57 ± 0.90 c	66.3 ± 4.84 a
Gera	42.5 ± 0.94 c	33.05 ± 5.05 b	66.4 ± 0.32 a

TPC (Total Phenol Content); TFC (Total Flavonoid Content)

Discussion

Melissopalynological analysis of coffee honey

During honey pollen analysis, if the honey is considered to be monofloral and its pollen frequency in honey pollen sediments should constitute more than 45% or more in pollen count [19]. This is in agreement with Tura and Admassu 2020, the monofloral honey of *C. arabica* was contributed by four plant species (*Vernonia amygdalina*, Rumex spp, and *Vernonia auriculifera* and *Hypoestes forskaoli* and C. *arabica* honey is a new emerging monofloral honey in the Gera forest in western Oromia, Ethiopia. The availability of nectar and level of sugar concentration can be used as an indicator of *Coffea arabica* honey [31].

Moisture content

The moisture content of honey samples from *Coffea arabica* was higher than the country's average 20.6% reported by [32,33]. The higher moisture content of Coffee honey in Gera and Manna is due to the prevailing atmospheric humidity and pre-and post-harvest management of honey in the area. Moreover, Gera and Manna districts are dominated by moist forest which might have resulted in increased MC in the area. The result is in agreement with [34] and the MC of the content of honey within International standards of honey quality.

Ash

The result of coffee honey samples for Ash showed that it ranged from 0.15 to 0.23/100 g and a mean value of 0.21 ± 0.3 g/100 g. The variation in ash content of coffee honey might be owing to the variability of soil types and the number of minerals found in the nectar of the flowers at a different locations. These results were in agreement [2,34]. Ash level of Coffee monofloral honey samples was within the range of (0.1 to 0.5 g/100 g) accepted by the codex range [35].

Electric conductivity

The average electric conductivity of Coffee honey (0.44 $mS.cm^{-1}$) to 0.58 and with a mean value of 0.49 ± 0.08 and honey samples significantly differ between the districts ($p <$

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0.05). The variation of electrical conductivity of honey between honey samples is due to the floral composition of honey samples and the result is in agreement with [2].

HMF

The HMF content of the *C. arabica* honey was relatively low as compared to national and international standards set by [35] indicating the freshness of honey. In Ethiopia, the acceptable HMF level is below 40 mg/kg of honey and the HMF value of this study area was less than 40 mg/kg [28]. This is agreeing with [16,32] in Gera and Yayu districts in the Oromia region.

Free acidity and pH

Variation in free acidity among coffee honey samples owing to the different floral types and harvesting seasons [36]. The mean value of pH (3.4) of honey in the study area is coherent with the report of [37] that showed that honey pH should be between 3.2 and 4.5 which ensures freshness of the honey. The low pH of honey hinders the growth of microorganisms. All honey samples are acidic, indicating the absence of undesirable fermentation and the acidity of honey is important for taste [38]. The maximum limit for free acid is set by the Codex as 50 meq/kg of honey, while the EU and Ethiopian standard is 40 meq/kg of honey [39].

Sugar of content honey

The fructose content of coffee honey ranges from 31.46 ± 5.6 to 35.31 ± 6.08 with a mean value of 32.77 ± 6.06. The result also shows that all of the honey types have low sucrose content which indicates the complete conversion of sucrose into glucose. These results were in agreement with [34,31]. The fructose level of coffee honey is within the range of National and International ranges nearly close to reports [27]. The correlation between electrical conductivity and total ash content found in this work is in agreement with the findings of [19,20]. There was also a correlation between Free acidity and sugars in honey due to the production of alcohol transforming into organic acids from the fermentation of sugar in honey.

The total phenol and flavonoid

The concentration and type of phenolic substances depend on the floral origin of the honey and are mainly responsible for its biological activities [41]. There was a significant difference in the total phenol content of coffee honey in different districts. These findings are in line with [42-44] which found that there is a positive correlation between DPPH and total polyphenols, and total flavonoids. The variation in the number of Flavonoids in honey is due to floral origin [30]. A similar result was reported by [19,13] for the phenolic content of Ethiopian monofloral honey.

The antioxidant content of honey

The, higher correlations were observed between the DPPH radical scavenging activity and the total polyphenol (r = 0.755, $p < 0.001$), and total flavonoids (r = 0.167, $p < 0.01$), and between total flavonoids and total polyphenols (r = 0.81, $p < 0.01$). Generally, the antioxidant power of coffee honey is associated with the presence of compounds, which exert their action by breaking the free radical chain through the donation of a hydrogen atom [45].

Conclusions and recommendations

Coffea arabica is a good producer of nectar and significantly contributes to monofloral honey production since its pollen count is greater than 45% in most honey samples. The Melissopalynological analysis of honey indicated the existence of different floral species contributing to *Coffea arabica* honey. The physicochemical property of *Coffea arabica* honey meets the International honey quality standards. The antioxidant power of Coffee honey has a considerable amount of polyphenolics which can protect the human body from damage caused by radicals.

Therefore, beekeepers should focus on the production of coffee monofloral honey to exploit the niche market opportunities such as organic honey, promotion, and commercialization of mono-floral honey from *Coffea arabica*. The producers should focus on appropriate management of honey bee colonies following the flowering calendar coffee plant since coffee has a short flowering period.

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