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Botanical Origin and Physicochemical Composition of *Apis mellifera* L. Honey from Western Oromia, Ethiopia

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ABSTRACT

Background and Objective: Honey is a natural sweetener synthesized by bees, from the nectar of living parts of the plant. Its composition varies based on botanical, geographical, entomological and seasonal honey types. This study compared the physicochemical properties of seasonal fresh honey types. **Materials and Methods:** The pollen grain was analyzed by the Methods of Melissopalynology while physicochemical analyses of honey were determined using the Harmonized Method of the International Honey Commission and analyzed by SAS Version 9.1.3 computer package. **Results:** From the First Season Honey Sample (FSHS), 84% of the total pollen count was that of *Guizotia* species (*Guizotia* monofloral honey), while 46% of *Coffee arabica* L. pollen (*Coffee* monofloral honey) was recorded from the Second Season Honey Sample (SSHS). A significant reducing sugar difference ($p<0.05$) was observed between SSHS and FSHS, the highs being in SSHS (80.00±5.71). The FSHS is significantly more acidic with a pH of 3.34±0.12 compared to SSHS (3.59±0.05). The Hydroxymethylfurfural (HMF) sucrose, moisture, free acidity and ash contents between honey types were not shown statistical differences. **Conclusion:** The physicochemical parameters of honey produced in the study area were found within the acceptable international standards and hence, had an acceptable physicochemical composition.

KEYWORDS

Coffee arabica, *Guizotia*, honey, physicochemical properties, fermentation, granulation, shrubs forages

INTRODUCTION

Honey is the natural sweetener food synthesized by bees, from the nectar of living parts of the plant. The bee collect nectar from the plant, deposit and reduce the water content and store and leave it in honeycombs or honey pots to ripen and mature for their consumption[1]. Honey production has gone from traditional hunting to a more elaborate production stage. This has led to an increase in the availability of honey stock. According to FAO[2] Report, 45,300 metric tons of honey are produced per annum in Ethiopia, making the country rank the first honey producer in Africa and ninth in the world. It is a complex mixture and presents very great variations in composition and characteristics based on geographical and floral types or the nectar foraged by bees[3].

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The composition and quality of honey primarily rely on its botanical, geographical and environmental conditions[4]. They depend on the maturity of honey, the mode of production, climatic conditions and treatment and storage conditions as well as the source of plant from which nectar is collected[5]. At the market, level getting good quality honey by consumers able the country to earn foreign currency to increase the national economy[6].

Worldwide, the quality of honey is interpreted by its physicochemical composition. To say a honey sample has good physicochemical quality, it should contain ≤20% water content, <0.6 water activity, ≤40 mg kg^{-1} Hydroxymethylfurfural (HMF), 50 meg kg^{-1} of free acidity, ≤0.6 g/100 g ash, ≥60% reducing sugar and ≤5% sucrose[7]. Higher water content may enhance honey spoilage through fermentation and HMF in levels higher than 40 mg kg^{-1} is a sign of honey degradation through heating or long storage in hot coniditons[8]. Furthermore, acidity influence the texture, stability and shelf life of honey[9].

Because of the multiple importance of honey from food to medicine, it is of great interest to carry out a complete analysis of honey and to formulate values ranges of various honey constituents and characteristics. Honey industries have shown great interest in these constituents as they influence the storage quality, granulation, texture and flavour, nutritional and medicinal values of the honey. Honey is generally evaluated by a physicochemical analysis of its constituents. Acceptability of honey depends on its quality which can be assessed by among other things its physicochemical characteristics. Most of these studies were reported by Belay *et al.*[10], Melaku and Tefera[11] and Tesfaye *et al.*[12], in the Northern and Southern parts of Ethiopia and no study has been done in the study area.

The objective of this study was therefore, to evaluate the physicochemical qualities and botanical sources between different types of honey harvesting seasons in Kellem and West Wollega Zone of Western Oromia, Ethiopia.

MATERIALS AND METHODS

Honey season and study site: In Kellem and West Wollega Zone, honey is harvested two or three times annually depending on the availability of bee forages. In late September or early November, herbaceous bee plants such as *Bidens* spp. (Meskel flower or Adey Abeba in Amharic), *Trifolium* spp. and *Plantago lanceolate* L. (Yebeglat in Amharic whereas literally Qorxobbii in Afaan Oromoo) are the dominant species which release both pollen and nectar sources[13]. This makes the main honey harvesting season to be practised on the first of December and the honey type is said to be Meskel flower honey which is Monofloral (Literally Damma Tuufoo in Afaan Oromoo) in all studies areas. Besides this, another honey harvesting season is practised in February dominantly from *V. amygdalina* L. and *C. Arabica*. Plant in all the study areas. Moreover, in some places, there is a possibility of harvesting honey from *Eucalyptus* spp. and *Croton macrostachyus* L., at the end of May through June although it's not well practised by farmers' beekeepers since a dearth period is expected after harvesting. Then, the study was carried out at Holota Apiculture Research Center, Bee Botany and Product Laboratory, Ethiopia from September, 2018 to June, 2020.

Honey sample collection: The honey samples were collected from the honey production potential of Sedi Chanka and Haro Sebu Districts of Kellem Wollega Zone and while Nedjo and Guliso Districts of the West Wollega Zone. From each district, three representative kebeles (peasant association) and a total of 12 kebeles (peasant association) were selected for honey sample collection. For this work, two different sources of honey samples of *A. mellifera* was used to determine the physicochemical composition between honey harvesting season in the study area. The first source was the honey samples that were harvested in October through November and labelled as the First Honey Harvesting Season (FHHS), while the second source was the honey samples that were harvested in January through February and labelled as the

Second Honey Harvesting Season (SHHS). For each source, 24 ripen honey samples (two samples from each kebele) from each season and a total of 48 ripened honey samples from both sources of the honey season were collected. The collected honey samples were brought to Holota Bee Research Centre Laboratory, using sterile glass cup honey containers for analysis.

Analyzing the botanical origin of honey: The standard procedure by Louveaux *et al*.[14] was used to analyze the botanical origin honey samples. A total of 10 g of honey was dissolved in 20 mL of warm distilled water in a centrifuge tube at temperatures that ranged from 20-40°C and centrifuged at 3800 rpm for 10 min and the supernatant was decanted. Again 20 mL distilled water was added to completely dissolve the remaining sugar crystals and centrifuged at 3800 rpm again for 5 min and the supernatant was removed completely. The sediment was spread evenly using a sterile micro spatula on a microscope slide and the sample was dried for a while. Then, the plant type was identified under a light microscope (Swift instrument international, serial number 8750038, Japan, high power 400x) and linked to a computer using a pollen atlas. Then after counting, the percentage of pollen grain in the sample was calculated based on the total number of different pollen grains that occurred and categorized as predominant pollen (monofloral honey), if pollen counted is >45%, secondary pollen (16-45%), important minor pollen (3-15%) and minor pollen (<3%). According to the standard procedure by Louveaux *et al*.[14].

Determination of physicochemical composition

Sugars: High-performance liquid chromatography (HPLC-1260 Infinity Series Agilent Technologies, Germany) was used. Forty milliliters of water was dissolved with 5 g of honey sample. Then, 25 mL of acetonitrile was pipetted into a flask and the honey solution was transferred to a flask and filtered using a syringe filter (0.45 µm) before chromatographic analysis.

The HPLC separation system was composed of an analytical stainless steel column, 4.6 mm in diameter and 250 mm in length, containing amine-modified silica gel with 5-7 µm particle size. Flow rate 1.3 mL min^{-1}, mobile phase acetonitrile:water (80:20, v/v) and sample volume 10 µL. The sugars were detected by a Refractive Index Detector Thermostated at 30°C temperature regulated column oven at 300°C. The identification of honey sugars was obtained by comparing their retention times with those of the standard sugars[7].

Moisture content: Abbérefractometer (ABBE-5 Bellingham Stanley Ltd., United Kingdom) material was used. This material was thermostated at 20°C and regularly calibrated with distilled water. Honey samples were placed in a water bath till the sugar crystals were homogenized and dissolved. Then, the surface of the prism of the refractometer was covered with honey and after 2 min refractive index for moisture was recorded and its value was determined using a standard table[7].

pH and free acidity: Ten grams of the sample were mixed in 75 mL of distilled water and stirred using a magnetic stirrer. The electrode of the pH meter (Mettler Toledo, China) was immersed in the solution and its pH was recorded. Then, the solution was further titrated with 0.1 M Sodium Hydroxide (NaOH) solution to pH 8.30 for measurement of free acidity. For precision, the reading to the nearest 0.2 mL was recorded using a 10 mL burette. Free acidity is defined as mill equivalents or a mill mole of acid/kg honey and is equal to mL of 0.1 M NaOH×10 g of honey[7].

Total ash: Honey samples were incinerated at 600°C in a muffle furnace (BioBase JKKZ.5.12GJ, Shandong. Ltd., China) to constant mass. First, the ash dish was heated in an electrical muffle furnace at an ashing temperature and subsequently cooled in desiccators to room temperature and weighed 0.001 g (M2). Then 5 g (M0) of each honey sample was weighed to the nearest 0.001 g and taken into a platinum dish

and two drops of olive oil were added to prevent foaming. Water was removed and started ashing without loss at a low heat rising to 350-400 using electrical devices. After the preliminary ashing, the dish was placed in the preheated furnace and heated for at least 1 hr. The ash dish was cooled in desiccators and weighed. The ashing procedure was continued until a constant weight was reached (M1). Lastly, percentage of the weight of ash in g/100 g honey was calculated using the following formula[7]:

$$WA = \frac{M_1 - M_2}{M_0}$$

Where:

M_0 = Weight of honey taken
M_1 = Weight of ash+dish
M_2 = Weight of dish

Hydroxylmethylfurfural (HMF): The UV-Vis spectrophotometer (JENWAY, United Kingdom) was used[7]. A 5 g honey sample was mixed in 25 mL distilled water and transferred into a 50 mL volumetric flask. A 0.5 mL carrez solution I (15 g K_4Fe (CN) 6. $3H_2O$/100 mL distilled water) was added and mixed into 0.5 mL carrez solution II (30 g Zn acetate/100 mL distilled water). The solution was mixed into the honey solution. A droplet of alcohol was added to the solution. The solution was filtered through a filter paper and the filtrate (10 mL) was discarded. A 5 mL filtrate was added to each of the two test tubes and 5 mL distilled water was added to the first test tube (sample solution), while 5 mL Sodium Bisulfite Solution (0.20% of 0.20 g $NaHSO_3$/100 mL distilled water) was added into the other test tube. The contents of both test tubes were well mixed by vortex mixer and their absorbance was recorded spectrophotometrically by subtracting the absorbance measured at 284 nm for HMF in the honey sample solution against the absorbance of reference (the same honey solution treated with sodium bisulfite, 0.2%) at 336 nm and the result was calculated and expressed according to International Honey Commission[7], which is:

$$\frac{\text{Hydroxymethylfurfural (HMF)}}{\text{100 g honey}} = (A284 - A336) \times 14.97 \times 5 / \text{g sample}$$

Where:

A284 = Absorbance at 284
A336 = Absorbance at 336
14.97 = Constant
5 = Theoretical nominal sample weight
g = Mass of honey sample

Colour: Homogeneous honey that was free of air bubbles was pipetted into a 10 mm light path cuvette till the cuvette was approximately half full. Then after the cuvette was inserted into a colour photometer Pfund honey colour grader (No. 0061, made of USA). The colour grades were expressed in millimetre Pfund grades compared to an analytical grade glycerol standard following the procedure of Codex Alimentarius Commission Standards[8].

Data collection and analysis: Data Mean±SD of physicochemical parameters of each honey type were calculated using SAS Software (SAS Institute, 2003, 14). For botanical origin analysis, pollen grain morphology between the honey types was counted from the slide microscopically and their percentage was calculated by dividing the single plant species pollen grain morphology over the total different plant pollen grain morphology and then multiplying by 100.

RESULTS AND DISCUSSION

Botanical origin of honey types: The characteristics of honey plant species identified from FSHS and SSHS were listed in Table 1 which shows that seven herbaceous species and one tree species, a total of eight plant species from five families were identified from FSHS. On the other hand, SSHS was synthesized from ten plant species under six families including herbs, shrubs and tree life forms. All of the identified plant species (eighteen in number plant species) provide both pollen and nectar sources for the bees[13], even though their pollen frequency class varies.

The microscopic-based pollen grain morphology from FSHS and SSHS was demonstrated in Fig. 1 and 2, respectively. The predominant pollen source for FSHS and SSHS were *Guizotia* species (Fig. 3) and *C. arabica* (Fig. 4), respectively. *Sesamum indicum* and *Trifolium* spp., were secondary pollen sources for FSHS while *Vernonia amygdalina* and *Vernonia auriculifera* were secondary pollen sources for SSHS. Pollen of a particular plant species is said to be predominant (monofloral honey type) if its occurrence in the honey sample is more than 45% of the total pollen count, secondary pollen (16-45%), important minor pollen (3-16%) and minor pollen (<3%)[13]. Therefore, the FSHS of this study could be called *Guizotia* (monofloral) honey (similarly, the *C. arabica* species was a dominant plant and its pollen grain counted for 46% of the total pollen count from SSHS and could be said that *C. arabica* (monofloral) honey after its pollen grain morphology is counted from the honey sample.

Fechner *et al.*[15] investigated that the collection of pollen and nectar by bee foragers depends on the availability of botanical resources within their foraging ranges which are affected by environmental and seasonal factors. *Guizotia* species flowered from mid-October to November in the study area (field observation and personnel communication). It was further verified based on the microscopic pollen grain morphology from its honey samples during this period it grows in abundance in a very wide range of habitats and is available everywhere like in cultivated fields, forest margins and open grasslands which might be a reason for its pollen grain dominancy from FSHS.

The flowering period of *C. arabica* in the study area depends on the rain condition. Mostly it flowers either in January or February following the rain soon. This plant is widely cultivated for its fruits and is a much-known cash crop in the study area. When flowered it is abundantly available for the forager bees and releases plenty of nectar and pollen.

Table 1: Characteristics of identified honey plants with their pollen frequency class from honey

Honey type	Scientific name	Family name	Vernacular name (Afan Oromo)	Life form	Resources for bees	Frequency class	Honey type
FSHS	*Guizotia* species	Asteraceae	Tufu/hada/nuugii	Herb	Pollen and nectar	PP	Monofloral
	Sesamum indicum	Pedaliaceae	Saalixa	Herb	Pollen and nectar	SP	honey-
	Trifolium species	Fabaceae	Siddisa	Herb	Pollen and nectar	SP	*Guizotia* species
	Grass species	Poaceae	Gosamargaa	Herb	Pollen and nectar	MP	honey type
	Parkinsonia aculeata	Fabaceae		Tree	Pollen and nectar	MP	
	Vicia faba	Fabaceae	Baaqelaa	Herb	Pollen and nectar	MP	
	Plectranthus assurgens	Lamiaceae	Ajooftuu	Herb	Pollen and nectar	MP	
	Andropogen species	Poaceae	Marga	Herb	Pollen and nectar	MP	
SSHS	*Coffee arabica*	Rubiaceae	Buna	Shrub	Pollen and nectar	PP	Monofloral
	Vernonia amygdalina	Asteraceae	Eebicha	Shrub	Pollen and nectar	SP	honey-
	Vernonia auriculifera	Asteraceae	Reejjii	Shrub	Pollen and nectar	SP	*Coffee arabica*
	Syzygium guineense	Myrtaceae	Baddeessaa	Tree	Pollen and nectar	IMP	honey type
	Terminalia species	Combretaceae	Dabaqqaa	Tree	Pollen and nectar	IMP	
	Cirsium species	Asteraceae	Qoraattiiharree	Herb	Pollen and nectar	MP	
	Vernonia leopoldii	Asteraceae	Sooyyama	Shrub	Pollen and nectar	MP	
	Pterolobium stellatum	Fabaceae	Harangamaa	Shrub	Pollen and nectar	MP	
	Grevillea robusta	Proteaceae	Botoroo	Tree	Pollen and nectar	MP	
	Guizotia species	Asteraceae	Tufo/hada/nuugii	herb	Pollen and nectar	MP	

PP: Predominant pollen (>45% of the pollen grains counted), SP: Secondary pollen (16-45%), IMP: Important minor pollen (3-15%) and MP: Minor pollen (<3%)

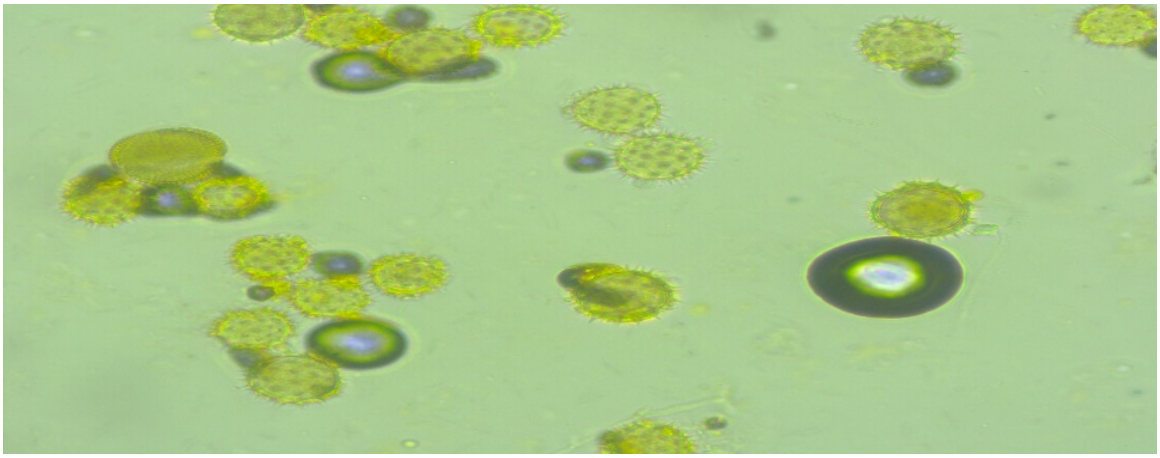

Fig. 1: *Guizotia* species pollen grain morphology identified from First Season Honey Sample (FSHS)

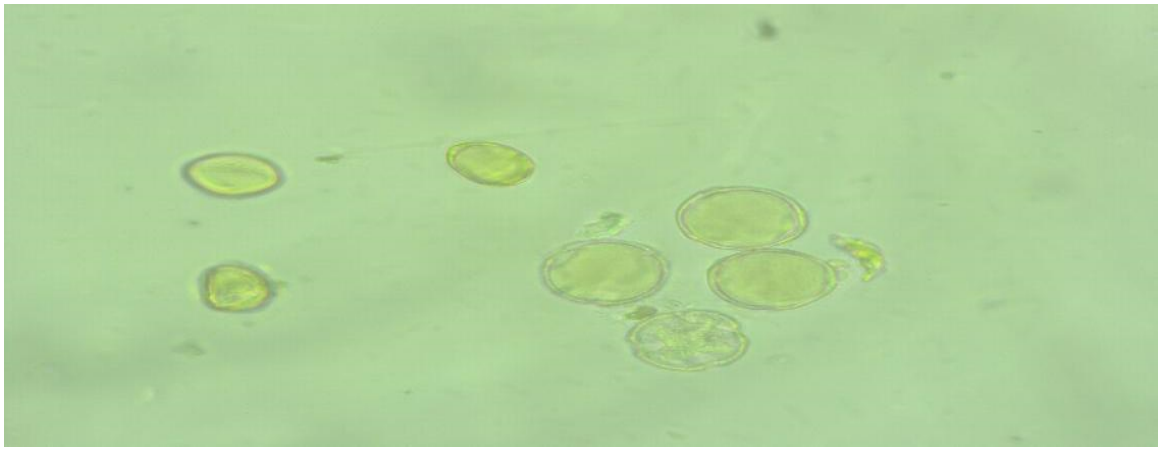

Fig. 2: *Coffee arabica* pollen grain morphology identified from Second Season Honey Sample (SSHS)

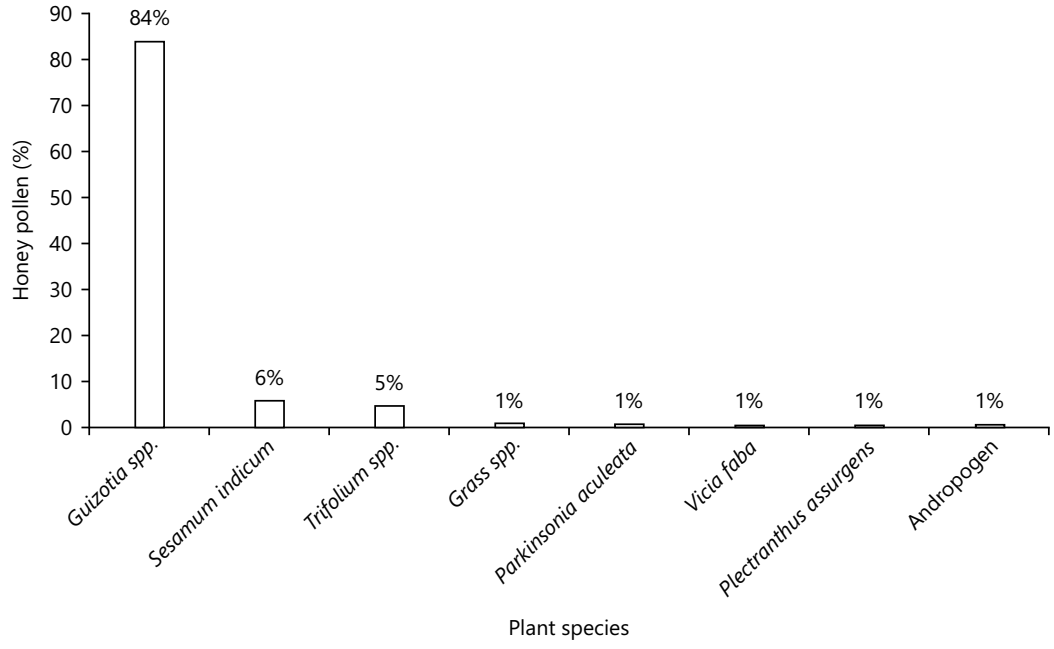

Fig. 3: Percentage relative frequency of nectariferous plant species from FSHS

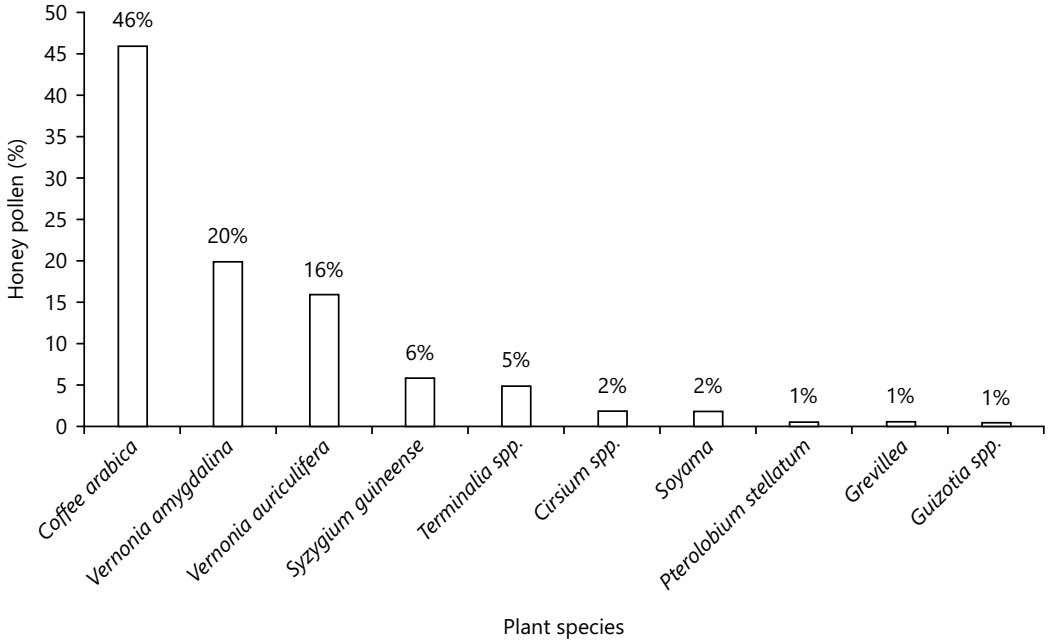

Fig. 4: Percentage relative frequency of nectariferous plant species from SSHS

Physiochemical composition between honey types

Sugar profile: The sugar composition results (%) of the analyzed honey types were indicated in Table 2. A significant difference ($p<0.05$) was observed in fructose content with a mean of 42.20 ± 0.74^{a} by *C. arabica* honey and 37.71 ± 3.65^{b} by *Guizotia* honey. However, the mean glucose and maltose content between honey samples didn't show significant variation ($p>0.05$). *Coffee arabica* and *Guizotia* had recorded glucose content of 36.10 ± 5.60^{a} and 33.95 ± 4.33^{a}, respectively while maltose content from *C. arabica* and *Guizotia* was 1.72 ± 0.90^{a} and 1.70 ± 1.20^{a}, respectively. A current study showed that fructose is the predominant monosaccharide sugar followed by glucose and maltose and is more recorded from *C. arabica* than *Guizotia*. The disparity between the honey types of this study might be due to the possible effects of plant species.

The predominance of fructose over glucose and glucose over maltose in our result was in line with the finding by Makarewicz *et al.*[16] from Poland Country. They encountered a mean fructose content of 41.36±1.42 9 (*Eucalyptus* honey type) to 47.18±0.78 (*Lime* honey type), a mean glucose content of 26.34±1.84 (*Thyme* honey type) to 37.93±1.42 (*Eucalyptus* honey type) and a mean maltose content of 1.88 ± 0.11^{b} (*Eucalyptus* honey) to 6.64 ± 0.15^{a} (*Coriander* honey). Moreover, Belay *et al.*[17] analyzed the sugar profile of Ethiopian monofloral honey and they obtained a mean fructose content of 35.30 ± 3.53^{d} (*Becium grandiflorum*) -43.07 ± 0.37^{a} (*Acacia*), a mean glucose content of 29.34 ± 2.75^{e} (*B. grandiflorum*) to 37.20 ± 0.35^{a} (*Leucas abyssinica)* and a mean maltose content of 0.55 ± 0.34^{f} (*Schefflera abyssinica*) to 2.04 ± 0.45^{a} (*Eucalyptus globules*). Although the concentration of both sugars varies depending on the botanical and entomological origin of the honey, it is generally expected that fructose will be found in a higher proportion than glucose and maltose[18].

A statistically significant difference ($p<0.05$) was seen in the mean of reducing sugar (a cumulative mean of fructose, glucose and maltose) and 80.00 ± 5.71^{a} and 73.35 ± 5.30^{b} were recorded from *C. arabica* and *Guizotia*, respectively (Table 2). They were within the acceptable limit of international standards as determined by Bogdanov[7] which should be ≥60%, The mean reducing sugar ours was exceedingly higher than what was observed for pineapple type (61.17 ± 0.17^{b}) to 63.89 ± 0.25^{a} (*Acacia* type) from the Malaysian honey (*A. mellifera*) sample[19]. Furthermore, Melaku and Tefera[11] from Eastern Ethiopia reported a mean reduction sugar of 71.2±2.5%, while Tigray, Ethiopia[20] informed a mean of 67.3±2.42%, which all are lower

Table 2: Percentage sugar composition between monofloral honey types

	Honey type (Mean±SD)					
Parameters	*Guizotia*	*C. arabica*	X	LSD	p-value	International standard
Fructose (%)	37.71±3.65[b]	42.20±0.74[a]	39.60	3.33	0.01	-
Glucose (%)	33.95±4.33[a]	36.10±5.60[a]	34.90	5.76	0.43	-
Maltose (%)	1.70±1.20[a]	1.72±0.90[a]	2.16	1.28	0.97	-
RS (Fru+Glu+Malt) (%)	73.35±5.30[b]	80.00±5.71[a]	76.20	6.44	0.04	≥60
Sucrose (%)	2.17±1.75[a]	2.15±0.61[a]	1.70	1.64	0.94	≤5

Means with different superscripts (a, b, c) within the rows are statistically different at p≤0.05, SD: Standard deviation, X: Overall mean, LSD: Least significant difference at alpha = 0.05 and RS (%): Percent of reducing sugar in honey

Table 3: Physicochemical composition between monofloral honey types

	Honey type (Mean±SD)					
Parameters	*Guizotia*	*C. arabica*	X	LSD	p-value	International standard
Moisture content (%)	19.70±1.43[a]	18.80±0.54[a]	19.30	1.35	0.16	≤20
FA (mEq kg^{-1})	27.18±3.70[a]	31.43±1.52[a]	29.30	6.52	0.88	≤50
pH (pH units)	3.34±0.12[b]	3.59±0.05[a]	3.40	0.11	0.00	
HMF (mg kg^{-1})	14.85±6.72[a]	12.50±4.91[a]	13.90	7.10	0.49	≤40
ASH (g/100 g)	0.11±0.04[a]	0.27±0.06[a]	0.20	0.15	0.05	≤0.6
Color (mm pfund)	91.38±27.17[a]	114.70±5.27[a]	101.30	24.74	0.06	

Means with different superscripts (a, b, c) within the rows are statistically different at p≤0.05, SD: Standard deviation, X: Overall mean, LSD: Least significant difference at alpha = 0.05, FA (mEq kg^{-1}): Free acidity and HMF (mg kg^{-1}): Hydroxymethylfurfural

than ours. From our study, *C. arabica* produced more sugar than *Guizotia* honey which might be due to the more availability of enzymes and sugar in *C. arabica* nectar. This was in line with the observations of Cavian[21] that the presence of enzymes in bees and nectar as well as the presence of sugar in the nectar of plants is the main factor in the sugar production of any honey. The difference in reducing sugar between honey samples might be due to the conversion of sugars into organic acids[22].

Both of the analyzed honey types yielded the sucrose content within the accepted international standard[7], which should be ≤5. This study yielded sucrose content of 2.17±1.75[a] from *Guizotia* and 2.15±0.61[a] from *C. arabica* and no significant variation (p>0.05) was observed between them (Table 2). A comparable observation was made from Nigerian *A. mellifera* honey with a mean of 2.32±0.01-2.42±0.02[23]. The amount of sucrose is determined by the degree of maturity and origin of the nectar compound of the honey and is used to detect adulteration of honey by the addition of cane or other sugars. A related result between our honey types might be the effect of the harvested material. Even though the season between honey samples was different with plant species, both honey types were harvested by the bee technician and only a sealed honeycomb was selected. The sucrose content in matured honey could be low, due to the invertase enzyme, which degrades the disaccharide (sucrose) into two simple sugars (glucose and fructose)[24]. According to Scripcă *et al.*[25], the sucrose content is an important parameter of the authentication of honey and the presence of a high level of sucrose in honey indicates adulteration with different syrups and also harvesting of the product before maturation. The current study distinguished the maturity (ripeness), natural and free from foreign material (adulteration) of the tested honey samples.

Moisture Content (MC), Free Acidity (FA) and pH: The mean results of Moisture Content (MC), Free Acidity (FA) and pH of the analyzed honey types were indicated in Table 3. Statistically, no variation (p>0.05) was observed between honey types with 19.70±1.43[a] and 18.80±0.54[a] for *Guizotia* and *C. arabica*, respectively. The recorded MC results are within the limits (should be not more than 20%) set by international standards[7]. Both of our honey types fell within the range of the finding by Belay *et al.*[17], who investigated the highest MC by *S. abyssinica* (20.54±1.28) whilst the lowest MC by *E. globules* (14.14±0.19) from Ethiopian *A. mellifera* honey sample. However, these results were observed in Romania Country with the lowest water content in the polyfloral (16.78±0.65) sample and the highest content in the acacia (17.13±0.48) honey sample[25].

Moisture is the second most important constituent of honey following sugar from our study in particular and as a world in general. The comparability between our honey types might be the harvesting procedures. The honey samples were collected by the bee technician following the standard procedures and waiting for the proper level of maturity within the modern hive. Generally, uncapped honey that contains more water is not recommended for harvesting. Scripcă *et al*.[25] have claimed high water content indicates extraction of a product in high humidity conditions or premature extraction. Moreover, Acquarone *et al*.[26] have observed that the variation of MC in honey samples is fundamentally influenced by (a) Geographical position from where the nectar and pollen-producing plant and the bee colony were found, (b) Level of honey maturity in the hive, (c) Botanical origin of honey and (d) Harvesting and post-harvesting manipulation. When the MC is high in honey it increases the honey water activity and influences the shelf-life of honey. According to Bogdanov *et al*.[7], the water content should not exceed 20% in honey to ensure safety against fermentation caused by the action of osmotolerant yeasts during storage.

The free acidity result was presented in Table 3. Statistically, no variation ($p>0.05$) in free acidity between honey types and 31.43±1.52^a by *C. arabica* while 27.18±3.70^a by *Guizotia* was observed. The mean free acidity of this study fits the international[7] quality standards which should be a maximum of 50 mEq kg^{-1} from *A. mellifera* honey. Comparably, this means the value was close to the result reported by Melaku and Tefera[11] (29.89±5 mEq kg^{-1}) and Alemu *et al*.[20] (27.34±5.06 mEq kg^{-1}). However, the mean free acidity of ours was by far higher than the honey (*A. mellifera*) obtained from Nigeria[23] (18.67±0.64 mEq kg^{-1}) and the Polish market[16] (14.40±0.58 mEq kg^{-1}). Free acidity indicates one of the quality parameters of honey samples and it reveals whether the honey is fermented or not[27] and corresponds to the presence or absence of organic acids in the product.

Statistically significant variation ($p<0.05$) between our honey types with a pH of 3.59±0.05 by *C. arabica* and 3.34±0.12 by *Guizotia* was recorded (Table 3). The mean pH result of honey (*A. mellifera*) observed in the present investigation was within the pH range of international standards (3.2-4.5). The mean pH value ours fell within the range of the finding by Belay *et al*.[17], who investigated the highest pH value ranging from 4.6±0.1 by *E. globulus* to 3.4±0.1 by Hypoestes and a significant difference was observed. The pH of honey affects its texture, stability and shelf life and a pH unit between 3.4 and 6.1 indicates the freshness of honey samples[28]. The variations in pH values of honey from different locations are due to the different geographic origins as the nectar's pH and soil conditions may influence honey's physicochemical properties[29].

Hydroxymethylfurfural (HMF), ash and colour: The mean results of Hydroxymethylfurfural (HMF), ash and colour of the analyzed honey types were indicated in Table 3. The enzymatic activity and concentration of Hydroxymethylfurfural (HMF) in honey samples are important indicators of honey's quality (freshness) indicating whether the honey is aged or over-heated[30]. Statistically similar ($p>0.05$) amount of HMF was observed with a mean of 14.85±6.72 by *Guizotia* and *C. arabica* by 12.50±4.91. None of the investigated samples exceeded the allowed limit of international quality standards[7] which should be not more than 40 (mg kg^{-1}). The HMF concentration which could be comparable with ours was reported from Nigerian[23] *A. mellifera* honey and ranged from 11.97±0.05-16.12±0.12 mg kg^{-1}. The low HMF concentration of our honey types indicated that they are fresh honey and not taken at a high temperature which made them have good quality.

The mean ash content of *Guizotia* and *C. arabica* was 0.11±0.04 and 0.27±0.06 (g/100 g), respectively. The current result was found within the acceptable limit of international standards[7] which should be a maximum of 0.6 (g/100 g). From Ethiopian monofloral honey samples, Moniruzzaman *et al*.[19] observed a range from 0.19±0.07 by hypoestes honey to 0.39±0.04 g/100 g from *C. macrostachyus* honey. Ash

content expresses the richness of honey in mineral content. The minerals Calcium (Ca), Magnesium (Mg), Iron (Fe), Copper (Cu), Cadmium (Cd) and Zinc (Zn) in the form of Sulfate (SO_4^{2-} and Chloride (Cl^-) are found in small amounts. The ash content result depends on the floral origin and soil features and honey with an ash content of ≤0.6% has the nectar source[31]. The blossom honey (nectar of plants) has lower ash content than the honeydew (secretions of living parts of plants or excretions of plant-sucking insects on plants)[8].

The colour of this study's honey types is indicated in Table 3. *Guizotia* and *C. arabica* exhibited a mean of 91.38±27.17 and 114.67±5.27 Pfund values, respectively. The colour of untreated honey can be used for its floral origin and is the single most important factor determining import and wholesale prices[32]. Furthermore, the use of an old comb, contamination with metals, exposure of honey to high temperature and long storage might change the colour of honey samples.

CONCLUSION

Based on the laboratory analysis obtained, honey harvested during the months of mid-November through December (FSHF) was predominantly produced from *Guizotia* spp., while honey cropped during February was dominantly produced from *C. arabica*. Thus the study confirmed that at least two different monofloral honey can be obtained from the study areas. *Guizotia* honey had more acidity whilst less sugar production compared to *C. arabica* honey. The physicochemical quality of honey produced from the study areas meets international standards.

SIGNIFICANCE STATEMENT

The study identified the physicochemical quality of fresh *A. mellifera* honey between seasons with their plant source. The flowering plants that produce nectar, pollen and major contributor plants were identified. From the analyzed result, the honey produced had an excellent physicochemical quality. This finding answered when, where and what type of honey (botanical source) is produced in the study area. Hence, people of the study area in particular and Ethiopia, in general, can get the honey type they need. Further research on the mineral content, antioxidant and medicinal value of monofloral honey will be expected from the concerned staff.

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