Research article

Comb honey and processed honey of *Croton macrostachyus* and *Schefflera abyssinica* honey differentiated by enzymes and antioxidant properties, and botanical origin

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ABSTRACT

In this study, comb and industrially processed honey samples collected from Bonga forest were investigated in relation to Melissopalynology and enzyme content, antioxidant and physicochemical properties. Melissopalynology categorized honey samples as *Croton macrostachyus* and *Schefflera abyssinica* honey. The physicochemical properties of the honey were determined using the Association of Official Analytical Chemists (AOAC) and International Honey Commission (IHC) methods. The enzymatic and antioxidant properties of honey were evaluated using the spectrophotometric method. The highest enzyme activity was observed in *Croton macrostachyus* comb honey (diastase = $7.44 \pm 0.13°$Schade and invertase = 13.97 ± 0.2 Invertase number (IN). *Croton macrostachyus* processed honey exhibited the highest values in flavonoids (83.36 ± 1.65 mg Catechin equivalents (CEQ)/100g)), Ferric reducing antioxidant power (FRAP) (69.94 ± 1.0 mg Ascorbic acid equivalents (AAE)/100g), and Inhibitory Concentration (IC_{50}) (136.3 ± 0.00 mg/ml), while *Schefflera abyssinica* comb honey had stronger 1,1-diphenyl-2-picrylhydrazyl radical scavenging (DPPH) ($49.47 \pm 0.00\%$) activity. The principal component analysis revealed that enzymes can be associated with comb honey, and antioxidants with processed honey. Thus, comb and processed honey can be differentiated based on the enzyme level, and *Croton macrostachyus* and *Schefflera abyssinica* honey can be identified using pollen analysis.

1. Introduction

Honey is a very complex natural product that contains sugars, organic acids, amino acids, enzymes, minerals, vitamins, lipids, phenols, flavonoids, pigments, waxes, pollen grains, and other phytochemicals (Machado et al., 2018; Belay et al., 2017a, b). Raw honey could also own probiotic potential, mainly coming from the *Bacillus subtilis* species (Paşca et al., 2021). Depending on the contribution of the primary honey plants, honey can be classified as monofloral or polyfloral. Honey can be called monofloral honey, if it primarily originated from a dominant floral source and show the typical flowing property of the corresponding type of honey (Ohe et al., 2004). The botanical designation and processing of honey is allowed, and can use in the therapeutic and technological interventions, which relate to the honey collection, extraction, straining, filling, packaging, labeling, blending, and branding of the product (Zarei et al., 2019; Belay et al., 2017a). The extraordinary flavor, biofunctional

benefit, and economic advantage of honey are governed by its origin and production methods. Accordingly, branding and labeling of honey can be used to notify consumers about the special merit of the honey (Mădas et al., 2020).

Croton macrostachyus is a deciduous tree. The showy light-yellow flowers of *Croton macrostachyus*, together with their fragrance, are attractive to honeybees used for bee foraging. It flowers from April to July, which makes the tree a very important source of honey as it flowers profuse, when most annual honeybee plants cease flowering (Fichtl and Admasu 1994). *Schefflera abyssinica* is an indigenous tree, sometimes growing as an epiphyte. It produces creamy-yellowish or creamy white flowers from March to May. *Schefflera abyssinica* is one of the most important honey trees in Ethiopia. It has abundant nectar and pollen. Honeybees produce large quantities of light and pure white honey from the plant (Fichtl and Admasu 1994; Belay et al., 2015).

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The production of honey in Ethiopia plays a great role in the economic, environmental, social, and cultural benefit of the citizens; and greatly benefits the forest dwellers (Belay et al., 2017a). Among the many honey producers in the country, the Bonga forest is the major honey producing area. Bonga forest is one of the Regional Forest Priority Areas (RFPA) and is endowed with a large number of honey plants (Bekele 2003).

According to Belay et al. (2013), physicochemical properties of Harrena forest honey from traditional and frame hives were investigated. The physicochemical property of honey is influenced by the nectar types that the honeybee used, geographical ecology, and honey handling practices. Nowadays, honey is one of the important world commodities and a large volume of honey is traded, annually (FAO 2010). In 2016, 587,389 tons of honey were imported by the top 25 honey importing countries. Since 2010, the net global honey demand has grown at a rate of 19,504 tons per year (García 2018). However, the issue of its quality, handling practices (raw and processed honey), and the authenticity of its geographical and botanical origins remain important factors in the marketing and consumption of honey. In addition, the composition of honey largely depends on the types of source, environmental factors, processing, and storage conditions. Hence, this study was designed to investigate the Melissopalynology and enzymes content, and physicochemical and antioxidant properties of comb and processed monofloral (*Croton macrostachyus* and *Schefflera abyssinica*) honey collected from Bonga forest, Ethiopia.

2. Materials and methods

2.1. Study area

The study was conducted in Bonga forest, Ethiopia, 460 km southwest of Addis Ababa (Figure 1). Bonga forest is registered as Regional Forest Priority Area (RFPA), covers 59,447 ha. It is situated between $35°30'0''E$ to $36°30'0''E$ and $7°0'0''N$ to $8°0'0''N$. The area experiences a long rainy season of 8 months, lasting from March/April to October (Bekele 2003).

2.2. Sample collection

Forty-eight (48) honey samples were collected from Bonga forest, Ethiopia, and these were from the farm gate (Comb honey collected from the local beekeepers) and processors/exporters (Industrially processed honey collected from processors/exporters) in the Bonga forest from June to August, 2019. Beekeepers at the farm gate were selected using randomized lottery sampling methods. The industrially processed honey is separated from the comb and other debris using straining and settling. These processes are quickened by making honey flow through special buffer tanks, prior to filling into the settling tank. In the buffer tank, the honey is heated through a water jacket, in which impurities remain at the surface. Both the comb and industrially processed honey were strained, settled, and later poured in 500 g food-grade honey containers, and stored at $<4\,°C$ until analysis (Belay et al., 2015; 2017a).

2.3. Melissopalynology

In a pointed glass centrifuge tube (capacity ca. 50 mL), the honey sample (10 g) was measured and dissolved in 20 mL of distilled water (20–40 °C). After centrifuging the solution for 10 min, the supernatant was decanted. Once more, the supernatant was decanted after centrifuging for 5 min with 20 mL of distilled water. On a microscope slide, the residue was spread evenly with a micro spatula and left to dry. The coverslip was coated with a drop of glycerin jelly, and the sample was evaluated under the microscope. A pollen atlas was used to identify the pollen source plant. Counting 500 pollens from a single slide was used to determine the frequency of occurrences. The pollen count was then translated to a percentage to determine relative dominance (Belay et al., 2015; Ohe et al., 2004).

2.4. Enzyme activity

2.4.1. Diastase

Honey (1g), weighed into a 100 ml volumetric flask, dissolved in the acetate buffer solution, and filled to the mark. Five milliliters of the solution were poured into a test tube and placed in a water bath at 40 degrees Celsius. A blank was made by placing a 5 mL aliquot of the acetate buffer in a separate test tube and preparing it the same way as the sample solution. The timer was started after adding the Phadebas tablet to both solutions. The reagent mixer's solutions were mixed until the tablets dissolved before being returned to the water bath. After exactly 15 min, the reaction was stopped by adding 1 mL of sodium hydroxide solution. For around 5 s, the mixture was mixed again in the reagent mixer. The solutions were immediately filtered through filter papers (Whatman® Grade 541), and the absorbance was measured using a UV

Figure 1. The Study area.

spectrophotometer at 620 nm. (CECIL, CE 7500, 7000 series) (Bogdanov, 2009).

2.4.2. Invertase

The honey (5 g) was dissolved in a 0.1 M buffer solution, then transferred to a 25-ml flask and filled to the desired volume. Before adding the honey solution, 5 mL of substrate solution (p-nitrophenyl-D-glucopyranoside (pNPG), 0.02 M) was poured into a test tube and placed in the water bath at 40 °C for 5 min. The contents were combined briefly in a mixer and incubated at 40 °C after adding 0.5 mL of honey solution (beginning time). 0.5 mL of the reaction-terminating solution (3 M, pH = 9.5 tris- (hydroxymethyl) Aminomethane) was added and mixed again in a mixer after exactly 20 min (sample solution). A blank was created by incubating 5 ml of substrate solution at 40 °C at the same time. A reaction-terminating solution of 0.5 mL was added after five minutes. The test tube was stopped, thoroughly mixed, and 0.5 mL of honey solution was added. The solutions were promptly cooled to room temperature, and their absorbance was measured using a UV Spectrophotometer at 400 nm (CECIL, CE 7500, 7000 series). The absorbance of the blank was subtracted from that of the sample solution (ΔA_{400}). Invertase activity was expressed as the invertase number (Bogdanov, 2009).

2.5. Antioxidant property

2.5.1. Total phenolic content

The Folin-Ciocalteu method was used to determine the total phenolic content (TPC) using gallic acid as standard (Singleton et al., 1999). Briefly, the honey sample was extracted by dissolving 5 g of honey in 50 mL of 25% methanol in a water solution. 1 mL of Folin-Ciocalteu reagent (diluted ten times) was added to 0.1 mL of the honey extract (100 mg/mL) and the mixture was left for 5 min, and then 1 mL (75 g/L) of sodium carbonate was added. After incubation in the dark (nearly 25 °C) for 90 min, the absorbance of the resulting blue color was measured at 765 nm with a UV- visible spectrophotometer (CECIL, CE7500, 7000 series). The total phenol content was estimated from gallic acid (1–100 µg/mL) and results were expressed as milligram gallic acid equivalent/100g of the extract.

2.5.2. Total flavonoids content

The honey sample was extracted by dissolving 5 g of honey in 50mL of 25% methanol in a water solution. 1mL of honey extract (100 mg/mL) was mixed with distilled water (1.25 mL) and a 5% $NaNO_2$ solution (75 µL). The solution was incubated (nearly 25 °C) for 6 min, then 150 µL of a 10% $AlCl_3 \cdot 6H_2O$ solution was added, and incubated for a further 5 min before 0.5 mL of 1 M NaOH was added. Distilled water was added to give a final volume of 2.5 mL and the absorbance was determined spectrophotometrically (CECIL, CE7500, 7000 series) at 510 nm against methanol as a blank. The total flavonoid content is expressed as (+)-catechin equivalents (CE) per 100g of honey (mg of CE/100g of honey) as described in Chang et al. (2002).

2.5.3. 1,1-Diphenyl-2-picrylhydrazyl radical scavenging (DPPH) assay

The scavenging activity against DPPH radical was determined as described by Saxena et al. (2010). Different concentrations (10–140 mg/mL) of the honey solutions were taken in different test tubes. Freshly prepared DPPH solution (2 mL, 0.006%, w/v) in methanol was added to each of the test tubes containing 1 ml of the extract. The reaction mixture and the reference standard (ascorbic acid) were vortex mixed and left to stand at room temperature in the dark for 30 min. The absorbance of the resulting solution was then taken at 520 nm using UV Spectrophotometer (CECIL, CE7500, 7000 series). Methanol was used as a blank. The ability to scavenge the DPPH radical was calculated using the following equation (Equation 1).

$$DPPH \quad scavenging \quad (\%) = \frac{(Ac - As)}{Ac} X \, 100 \tag{1}$$

Where A_c is the absorbance of the control and A_s is the absorbance of the sample.

2.5.4. Ferric reducing antioxidant power assay

The ferric reducing power of honey samples was determined based on the method described by Oyaizu (1986). One ml (100 mg/mL) honey sample was mixed with 2.5 mL sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. Then, the mixture was incubated at 50 °C for 20 min. Trichloroacetic acid (2.5 mL, 10%) was added to the mixture, which was then centrifuged (Centurion, 1000 series, UK) at 1008 g (3000 rpm) for 5 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 mL of distilled water and 0.5 mL FeCl$_3$ (0.1%), and absorbance was measured at 700 nm using UV Spectrophotometer (CECIL, CE7500, 7000 series). FRAP assay expressed as milligram ascorbic acid equivalents/100g of honey (mg AAE/100g).

2.5.5. Inhibitory concentration (IC$_{50}$)

The inhibitory concentration (IC$_{50}$) is a measure of a substance's efficacy in inhibiting a given biochemical function by 50%, and it's calculated using Al-Farsi et al. (2018). The IC$_{50}$ of honey extract was estimated by mapping the proportion of inhibition against concentration on dose-response curves.

2.6. Physicochemical analysis

2.6.1. Sugar profile

Determination of sugars was performed with high-performance liquid chromatography (HPLC- 1260 Infinity Series Agilent Technologies, Germany) equipped with a refractive index (RI) detector using AOAC (1990). Honey (5g) was taken from a properly homogenized sample and dissolved in Acetonitrile: water (70:30, v/v). Each honey sample's solution was filtered using a 0.45 m syringe filter before being transferred to auto-sampler vials. To determine sugars, HPLC chromatogram peaks were detected by comparing retention times obtained from standards.

2.6.2. Moisture content

The moisture content of the honey was determined using the refractive index of the honey, by a digital refractometer (Abbe refractometer, Leica Mark II Plus) thermostated at 20 °C based on AOAC (1990). Table 969.38 was used to convert the refractive index reading to moisture content (g/100 g) (AOAC, 1990 method number 969.38).

2.6.3. Ash content

Ash content was determined based on AOAC (1990) method number 920.181. The samples (5 g) were incinerated at high temperature (550 °C) in a burning muffle (THERMO CONCEPT, KLS 45/13, Germany) for 1 h. After cooling at room temperature, the obtained ash was weighed. The following formula was used to compute the amount of ash (AC) in g/100g of honey (Equation 2).

$$AC = \frac{(m1 - m2)}{m3} \times 100 \tag{2}$$

Where AC = Ash content, m3 = weight of honey, m1 = weight of dish + ash, m2 = weight of dish.

2.6.4. Free acidity and pH

Free acidity and pH measurement was done by AOAC (1990) method number 962.19 using a pH meter (HANNA, HI 2550, PH/ORP). Free acid (meq of acid/1000 g) was determined by dissolving a honey sample (10 g/75 mL distilled water) and titrating with standardised 0.1 M NaOH to

pH 8.3 using a pH glass electrode attached to pH meter (Consort C861, Belgium) as end point indicator.

2.6.5. Electrical conductivity

A conductivity meter (HANNA, HI 2550, EC/TDS/NaCl meter) was used to determine electrical conductivity. The honey solution (20% w/v) was suspended in deionized water (Bogdanov, 2009). The electrical conductance of this solution was read in mS after the temperature has been equilibrated to 20 °C (AOAC 1990). Anhydrous honey (20 g) was dissolved in distilled water. The solution was transferred quantitatively to a 100ml volumetric flask and made up to volume with distilled water. After the temperature had been equilibrated to 20 °C, the electrical conductance of this solution was measured in milliseconds. The honey solution's electrical conductivity was measured using the formula below (Equation 3).

$$SH = K \times G \qquad (3)$$

Where SH = electrical conductivity of the honey solution in mS.cm^{-1}, K = cell constant in cm^{-1}, G = conductance in mS.

2.6.6. Hydroxymethylfurfural

Hydroxymethylfurfural (HMF) content was determined by high-performance liquid chromatography (Agilent, Germany) at an absorbance of 285 nm using DAD (UV detector) (Bogdanov, 2009). Accordingly, 10 g of the honey sample was taken into a beaker (50 ml), and dissolved into 25 ml of water. The dissolved honey sample transfer quantitatively to a 50 ml volumetric flask, and makeup using distilled water, and filter through a membrane filter (0.45 μm). By comparing the corresponding peak regions of the sample, the HMF content of the sample was estimated.

2.6.7. Specific rotation

The specific rotation was determined using Bogdanov (2009) by reading the angular rotation (α) in the polarimeter (KRUSS, A. KRUSS OPTRONIC) at 20 °C. The specific rotation was calculated by Eq. (4).

$$\text{Specific rotation } [\alpha]^{20}D = \frac{\alpha}{L \ (dm)^* g} * 100 \qquad (4)$$

where α = angular rotation found, L = length in decimeters of polarimeter tube, g = grams of dry matter sample mass taken, and D = sodium D line.

2.6.8. Color

The color of honey samples was measured using a UV/Vis spectrophotometer (Labomed Inc, USA) as described in Hailu and Belay (2020). Honey samples were warmed in a water bath at 50 °C to dissolve sugar crystals. The samples were rapidly cooled to room temperature and the absorbance was read from honey solution (50% w/v) at 635 nm. The absorbance was converted and classified according to the Pfund scale (White, 1984). The conversion of the absorbance values (ΔA$_{635}$) was done using the following equation (Equation 5).

$$(mm \ Pfund) = -38.70 + (371.39*Abs) \qquad (5)$$

2.6.9. Viscosity

The viscosities of honey samples were determined based on Belay et al. (2017a), using Rapid Visco Analyzer (Perten RVA 4500, Australia) at a constant time, temperature, and speed of 8 min, at 25–50 °C and 960 rpm, respectively. Accordingly, the honey sample was heated to 45 °C for 3 h in a thermostatically controlled water bath to dissolve any crystals present in the honey sample. Sequentially, the honey sample was poured in a sample holder cup and measured at 25, 30, 35, 40, 45, and 50 °C; and

the Arrhenius model is predicted from the logarithmic value of viscosity and the reciprocal value of temperature.

2.7. Statistical analysis

Data was generated from multiple runs of samples with minimum duplicate measurements, and analyzed by SPSS, Version 20, using a one-way analysis of variance (ANOVA). The PCA (principal component analysis) was analyzed using XLSTAT 2020 statistical software. Correlations were done using the Pearson correlation analysis.

3. Result and discussion

3.1. Melissopalynology

Honey samples subjected to a Melissopalynological investigation revealed differences in floral origins. The relative dominancy of *Croton macrostachyus* pollen ranged from 49-69.6%, and *Schefflera abyssinica* was 45–79.8%. This indicated that *Croton macrostachyus* and *Schefflera abyssinica* were found as dominant monofloral honey (Table 1). As stated by Belay et al. (2015) and Ohe et al. (2004), honey can be considered monofloral, if the dominance is more than 45%. Some other honey plant pollen, which is not dominant; and found in the honey include *Coffea Arabica* (1–26.8%), *Guizotia scabra* (1.2–21.2%), *Eucalyptus spp* (1–9.8%), *Vernonia amygdalina* (0.6–9.4%), *Grass spp* (0.8–13%), *Syzygium spp* (0.2–11%) and *Rumex spp* (0.1–3.5%) pollen species (Table 1).

3.2. Enzyme activity

3.2.1. Invertase

The mean ± SD of invertase activity is presented in Table 2. *Croton macrostachyus* comb and processed honey had 13.97 ± 0.2 and 13.37 ± 0.15 IN, respectively. Whereas, *Schefflera abyssinica* honey had 5.55 ± 0.2 and 4.79 ± 0.49 IN for comb and processed honey, respectively. There was a significant difference ($p < 0.05$) in invertase activity amongst both comb and processed monofloral honey (ANOVA Table S7, Please find the additional tables and figures in 'Revised Supplementary material'.), and the results of this study complied with the report Belay et al. (2017b). The monofloral honey was classified as low (10IN) (*Schefflera abyssinica*) and medium to high (10-20IN) (*Croton macrostachyus*) based on the invertase classification of honey (Oddo et al., 1999).

3.2.2. Diastase

The diastase activity *Croton macrostachyus* and *Schefflera abyssinica* honey are presented in Table 2. The mean ± SD of diastase activity for *Croton macrostachyus* comb and processed honey were 7.44 ± 0.13 and 6.68 ± 0.18 °Schade, respectively. Whereas, *Schefflera abyssinica* honey had 4.49 ± 0.11 and 3.78 ± 0.11 °Schade for comb and processed honey, respectively. The comb monofloral honey was significantly different ($p < 0.05$) in diastase activity with processed monofloral honey (ANOVA Table S8, Please find the additional tables and figures in 'Revised Supplementary material'.), and is in agreement with the report of Belay et al. (2017b). When honey is heated, there is a relative reduction in enzyme level.

3.3. Antioxidant property

3.3.1. Total phenolic content

The mean ± SD of total phenol content for *Croton macrostachyus* and *Schefflera abyssinica* honey samples are presented in Table 2. Total phenol content for *Croton macrostachyus* comb and processed honey was 53.36 ± 9 and 68.83 ± 2.43 mg GAE/100g, respectively; and for *Schefflera abyssinica* comb and processed honey samples were 19.32 ± 0.86 and 69.64 ± 2.56 mg GAE/100g, respectively. There was a significant

Table 1. Relative pollen distribution of the honey samples from different places (Percentage from 500 pollen counts).

Honey sample	Croton macrostachyus	Schefflera abyssinica	Coffea arabica	Guizotia scabra	Eucalyptus spp	Vernonia amygdalina	Grass spp	Syzygium spp	Rumex spp
CC	69.60	24.20	0.00	2.00	0.00	1.00	0.80	2.00	0.40
CC	55.40	41.80	0.00	1.20	0.00	0.00	1.00	0.60	0.00
CC	63.00	27.80	0.00	2.50	0.00	1.00	4.00	0.70	1.00
CC	67.40	10.20	13.20	2.00	1.80	1.20	2.00	0.20	2.00
CC	53.00	14.80	26.80	0.60	0.00	0.00	1.80	3.00	0.00
CP	60.20	18.00	10.80	2.70	1.30	0.60	4.00	2.00	0.40
CP	55.80	20.60	13.40	0.00	2.00	1.00	0.20	0.00	7.00
CP	54.20	37.20	2.4.00	0.00	1.00	0.00	3.00	0.00	2.20
CP	51.40	2.00	1.00	19.20	9.80	6.40	9.00	1.00	0.20
CP	49.00	12.60	19.80	0.00	6.40	0.80	1.40	7.00	3.00
SC	0.00	79.80	0.00	6.70	1.00	0.00	5.00	4.00	3.50
SC	8.20	62.60	15.80	0.40	0.00	0.40	0.00	11.00	1.60
SC	29.40	61.00	9.60	0.00	0.00	0.00	0.00	0.00	0.00
SC	22.20	51.40	13.80	2.20	8.60	0.00	1.00	0.80	0.00
SC	0.00	47.20	24.20	7.50	4.20	3.60	130	0.00	0.30
SP	20.20	69.00	5.20	2.00	0.00	1.00	0.00	0.60	2.00
SP	17.20	59.60	13.80	6.60	0.40	0.00	1.00	1.40	0.00
SP	4.60	50.40	15.20	10.40	6.20	2.10	9.00	2.00	0.10
SP	16.80	47.80	1.80	21.20	0.00	9.40	0.00	0.00	3.00
SP	44.00	45.00	0.00	4.20	3.00	0.60	2.20	1.00	0.00

CC = *Croton macrostachyus* comb, CP = *Croton macrostachyus* processed, SC = *Schefflera abyssinica* comb, SP = *Schefflera abyssinica* processed.

difference (p < 0.05) in the total phenolic content of *Croton macrostachyus* and *Schefflera abyssinica* comb and processed honey (ANOVA Table S9, Please find the additional tables and figures in 'Revised Supplementary material'.), which could be due to the variation in botanical origin. The finding of this study was in agreement with the report of Mahmoodi- Khaledi et al. (2017) of the Iranian honey, which stated that the variation of the total phenolic content (12.51 ± 0.77 to 74.49 ± 6.02 mg GAE/100 g) correlated with the botanical origin of honey.

3.3.2. Total flavonoid content

The total flavonoid content (mean ± SD) for *Croton macrostachyus* and *Schefflera abyssinica* honey samples are presented in Table 2. The total flavonoid content for *Croton macrostachyus* comb and processed honey were 67.49 ± 1.43 and 83.36 ± 1.65 mg CEQ/100g, respectively; and for *Schefflera abyssinica* comb and processed honey samples were 48.21 ± 1.55 and 46.63 ± 2 mg CEQ/100g, respectively. There was a significant difference (p < 0.05) in the total flavonoid content of the monofloral honey (ANOVA Table S10, Please find the additional tables and figures in 'Revised Supplementary material'.), which may be linked to their floral source. The results reported in this study were higher than those reported by Hailu and Belay (2020) (42.03 ± 1.49 mg CEQ/100g). However, it was in line with the report of Saxena et al. (2010).

3.3.3. DPPH scavenging activities

The DPPH scavenging activity of *Croton macrostachyus* and *Schefflera abyssinica* honey are presented in Table 2. The mean ± SD of DPPH values for *Croton macrostachyus* comb and processed honey were 46.61 ± 0.01 and 47.35 ± 0.00 % inhibition, respectively. Similarly, *Schefflera abyssinica* comb and processed honey had 49.47 ± 0.00 and 49.31 ± 0.00 % inhibition, respectively. There was a significant difference (p < 0.05) (ANOVA Table S11, Please find the additional tables and figures in 'Revised Supplementary material'.) in percentage inhibition among the monofloral honey at a concentration of 120 mg/ml, and the results in this study were relatively higher than the report of Hailu and Belay (2020) (44.43 ± 0.97 %).

3.3.4. Ferric Reducing Antioxidant Power assay

Ferric Reducing Antioxidant Power assay (FRAP) values for *Croton macrostachyus* and *Schefflera abyssinica* honey are presented in Table 2. The mean ± SD of FRAP values for *Croton macrostachyus* comb and processed honey were 42.33 ± 3.53 and 69.94 ± 1.04 mg AAE/100g, respectively; whereas, *Schefflera abyssinica* comb and processed honey were 24.14 ± 0.86 and 23.9 ± 2.72 mg AAE/100 g, respectively. There was a significant difference (p < 0.05) in FRAP among the monofloral honey (ANOVA Table S12, Please find the additional tables and figures in 'Revised Supplementary material'.), which may be due to of their botanical origin. The results of this study were in line with the report of Mahmoodi- Khaledi et al. (2017) (28–182 µmol Fe(II)/100g).

3.3.5. Inhibitory concentration (IC_{50}) against DPPH assay

The concentration of the material necessary to inhibit 50% of free radical (IC_{50}) is important to determine the scavenging activity against the free radical (Hailu and Belay 2020). The mean ± SD values for IC_{50} of *Croton macrostachyus* and *Schefflera abyssinica* honey, in relation to scavenging the DPPH free radical, were presented in Table 2. *Croton macrostachyus* comb and processed honey had 131.62 ± 0.00 and 136.3 ± 0.00 mg/ml IC_{50}, respectively; while *Schefflera abyssinica* comb and processed honey had 126.6 ± 0.00 and 133.84 ± 0.00 mg/ml IC_{50}, respectively. There was a significant difference (p < 0.05) in IC_{50} between the comb and processed honey (ANOVA Table S13, Please find the additional tables and figures in 'Revised Supplementary material'.). When the honey is heated and processed, some minor compounds are generated, and can be used as antioxidant agents (Šarić et al., 2013). The increasing temperature in Jujube honey of Iran caused an increase in total phenolic content and Maillard reaction products (Molaveisi et al., 2019). According to Brudzynski and Miotto (2011), minor compounds like Melanoidin are generated during honey heating. A lower IC_{50} concentration in honey indicates a higher ability to neutralize free radicals (Al-Farsi et al., 2018). The result of this study was in agreement with the report of Hailu and Belay (2020) (134.60 ± 8.66 mg/ml) IC_{50} for *Schefflera abyssinica* honey.

Table 2. Mean ± SD value for enzyme activity, antioxidant, and physicochemical –properties of honey samples.

Parameters	*Croton macrostachyus* honey		*Schefflera abyssinica* honey	
	Comb honey	Processed honey	Comb honey	Processed honey
Fructose (g/100g)	36.02 ± 2.5[a]	38 ± 3[a]	37.92 ± 2.91[a]	36.53 ± 2.33[a]
Glucose (g/100g)	26.85 ± 2.2[b]	30.02 ± 1[a]	28.08 ± 1.9[ab]	27.4 ± 1.4[b]
Sucrose (g/100g)	1.15 ± 0.4[a]	0.72 ± 0.35[a]	1.08 ± 0.2[a]	0.81 ± 0.5[a]
Turanose (g/100g)	1.46 ± 0.6[ab]	2.18 ± 0.64[a]	1.96 ± 0.42[a]	1.13 ± 0.43[b]
Maltose (g/100g)	5.94 ± 0.67[a]	5.32 ± 1.24[a]	5.62 ± 1.73[a]	5.51 ± 1.6[a]
Moisture (g/100g)	18.67 ± 0.15[b]	20.36 ± 0.47[a]	18.89 ± 0.06[b]	18.75 ± 0.16[b]
Refractive Index	1.4899 ± 0.00[a]	1.4857 ± 0.00[b]	1.4894 ± 0.00[b]	1.4897 ± 0.00[b]
Ash (g/100g)	0.19 ± 0.01[a]	0.19 ± 0.01[a]	0.20 ± 0.01[a]	0.19 ± 0.01[a]
pH	4.31 ± 0.02[ab]	4.44 ± 0.43[a]	4.12 ± 0.00[b]	4.11 ± 0.00[b]
Free Acidity (meq/kg)	34.55 ± 0.1[a]	33.58 ± 1.14[a]	30.22 ± 0.91[b]	26.00 ± 2.75[c]
Electrical Conductivity(ms/cm)	0.49 ± 0.04[b]	0.55 ± 0.05[a]	0.47 ± 0.02[b]	0.23 ± 0.01[c]
HMF (mg/kg)	3.91 ± 0.33[b]	8.63 ± 0.72[a]	2.81 ± 0.09[c]	3.56 ± 0.31[b]
Specific rotation $[\alpha]^D_{20}$	-71.83 ± 0.06[c]	-62.64 ± 0.65[a]	-68.72 ± 0.47[b]	-76.52 ± 0.1[d]
Color (Pfund mm)	91.8 ± 6[b]	111.80 ± 3.5[a]	15.20 ± 1.6[d]	28.3 ± 1.7[c]
Diastase (˚Schade)	7.44 ± 0.13[a]	6.68 ± 0.18[b]	4.49 ± 0.11[c]	3.78 ± 0.11[d]
Invertase (IN)	13.97 ± 0.2[a]	13.37 ± 0.15[b]	5.55 ± 0.2[c]	4.79 ± 0.49[d]
Phenols (mg GAE/100g)	53.36 ± 9[b]	68.83 ± 2.43[a]	19.32 ± 0.86[c]	69.64 ± 2.56[a]
Flavonoids (mgCEQ/100g)	67.49 ± 1.43[b]	83.36 ± 1.65[a]	48.21 ± 1.55[c]	46.63 ± 2[c]
DPPH (%I)	46.61 ± 0.01[d]	47.35 ± 0.00[c]	49.47 ± 0.00[a]	49.31 ± 0.00[b]
FRAP (mg AAE/100g)	42.33 ± 3.53[b]	69.94 ± 1.04[a]	24.14 ± 0.86[c]	23.9 ± 2.72[c]
IC$_{50}$ for DPPH (mg/mL)	131.62 ± 0.00[c]	136.3 ± 0.00[a]	126.6 ± 0.00[d]	133.84 ± 0.00[b]

Values are expressed as mean ± SD from replicated experiment. Means with different letters in a row were significantly different at the level of P < 0.05. GAE = Gallic acid equivalents, CEQ = Catechin equivalents, AAE = Ascorbic acid equivalents, %I = Percent inhibition.

3.4. Physicochemical properties

3.4.1. Sugar profile

The sugar components have the largest share in the honey composition (Belay et al., 2013). This is also true for this study. The sugar compositions in *Croton macrostachyus and Schefflera abyssinica* honey are presented in Table 2. Fructose content for *Croton macrostachyus* and *Schefflera abyssinica* comb honey was 36.02 ± 2.5 and 37.92 ± 2.91 g/100g, respectively, and the processed honey fructose content for *Croton macrostachyus* and *Schefflera abyssinica* honey was 38 ± 3 and 36.53 ± 2.33, respectively. There was no significant difference (p > 0.05) in fructose content among the honey samples (ANOVA Table S14, Please find the additional tables and figures in 'Revised Supplementary material'.). The fructose content in the current study was in agreement with the report of Chakir et al. (2011) and Adgaba et al. (2017). The glucose content in *Croton macrostachyus* comb and processed honey was 26.85 ± 2.2 and 30.02 ± 1 g/100g, respectively. On the other hand, *Schefflera abyssinica* contained 28.08 ± 1.9 and 27.4 ± 1.4 g/100g of glucose for comb and processed honey, respectively (Table 2). There was a significant difference (p < 0.05) in glucose content between the comb and processed honey (ANOVA Table S15, Please find the additional tables and figures in 'Revised Supplementary material'.). This could be due to the difference in floral source, which was in agreement with the report of Belay et al. (2017c). The fructose plus glucose value for *Croton macrostachyus* comb and processed honey was 62.87 ± 4.7 and 68.2 ± 4 g/100g, respectively. Similarly, *Schefflera abyssinica* had 66 ± 4.81 and 63.93 ± 3.73 g/100g for comb and processed honey, respectively. The sum of fructose and glucose, for both comb and processed monofloral honey, corresponds to the limits required by Codex Alimentarius Commission, which is not less than 60 g/100 g (Codex Alimentarius 2001).

The sucrose content of *Croton macrostachyus* and *Schefflera abyssinica* honey is presented in Table 2. *Croton macrostachyus* comb and processed honey had 1.15 ± 0.4 and 0.72 ± 0.35 g/100g sucrose, respectively.

Whilst, 1.08 ± 0.2 and 0.81 ± 0.5 g/100g values of sucrose were reported for *Schefflera abyssinica* comb and processed honey, respectively. There was no significant difference (p > 0.05) in sucrose content among the honey samples (ANOVA Table S16, Please find the additional tables and figures in 'Revised Supplementary material'.). The sucrose content in this study was in agreement with the reports of Saxena et al. (2010) and Habib et al. (2014). All of the monofloral honeys, both comb and processed, were under the Codex Alimentarius' permissible limit (5 g/100g) (Codex Alimentarius 2001).

3.4.2. Moisture content

The mean ± SD of moisture content for *Croton macrostachyus and Schefflera abyssinica* honey is presented in Table 2. *Croton macrostachyus* comb and processed honey were 18.67 ± 0.15 and 20.36 ± 0.47 g/100g, respectively. Whereas, *Schefflera abyssinica* had 18.89 ± 0.06 and 18.75 ± 0.16 g/100g moisture for comb and processed honey, respectively. There was a significant difference (p < 0.05) in moisture content among the *Croton macrostachyus* processed and the rest of the honey samples (ANOVA Table S19, Please find the additional tables and figures in 'Revised Supplementary material'.). Belay et al. (2013) reported that moisture content mostly depends on the botanical origin, harvesting techniques, and extraction from the comb. All of the honey samples were under the Codex Alimentarius' permissible level of 20 g per 100 g (Codex Alimentarius 2001).

3.4.3. Ash

The mean ± SD of ash for *Croton macrostachyus* and *Schefflera abyssinica* honey is presented in Table 2. The ash content of *Croton macrostachyus* comb and processed honey had the same value (0.19 ± 0.01 g/100g). While, *Schefflera abyssinica* comb and processed honey had 0.2 ± 0.01 and 0.19 ± 0.01 g/100g, respectively. There was no significant difference (p > 0.05) in ash content among the honey samples (ANOVA Table S21, Please find the additional tables and figures in 'Revised

Figure 2. (a) Effect of temperature on viscosity of *Croton macrostachyus* and *Schefflera abyssinica* honeys; (b) The Arrhenius logarithmic value of viscosity (in cp) versus the reciprocal value of temperature (1/T) of *Croton macrostachyus* and *Schefflera abyssinica* honey.

Supplementary material'.). All of the honey was within the Codex Alimentarius allowed limit (0.6g/100g) (Codex Alimentarius 2001).

3.4.4. Free acidity and pH

The free acidity for *Croton macrostachyus* comb and processed honey was 34.55 ± 0.1 and 33.58 ± 1.14 meq/kg, respectively. On the other hand, *Schefflera abyssinica* comb and processed honey free acidity were 30.22 ± 0.91 and 26.00 ± 2.75 meq/kg, respectively. There was a significant difference ($p < 0.05$) in free acidity between the comb and processed honey of *Croton macrostachyus* and *Schefflera abyssinica* honey (ANOVA Tables S22, S23, Please find the additional tables and figures in 'Revised Supplementary material'.). The free acidity of *Croton macrostachyus* honey was lower than the report of Belay et al. (2017c) (55 ± 7 meq/kg), while higher values were observed in *Schefflera abyssinica* (23.90 ± 1.85 meq/kg) for the same report. Variation in free acidity among the monofloral honey could be due to the differences in honey harvesting conditions and season. All of the honey samples were under the Codex Alimentarius standard of 40 meq acid/kg (Codex Alimentarius 2001). The pH for *Croton macrostachyus* comb and processed honey were 4.31 ± 0.02 and 4.44 ± 0.43, respectively. Similarly, 4.12 ± 0.00 and 4.11 ± 0.00 were the pH values for *Schefflera abyssinica* comb and processed honey, respectively. There was a significant difference ($p < 0.05$) in pH among the honey. This could be due to the influence of extraction and storage conditions. The pH of both *Croton macrostachyus* and *Schefflera abyssinica* were higher than the report of Belay et al. (2017c) (3.61–3.77) and Adgaba et al. (2020) (3.5–3.7), and agreed with the report of Saxena et al. (2010) (3.7–4.4).

3.4.5. Electrical conductivity

The mean $\pm$ SD of electrical conductivity (EC) in honey samples is presented in Table 2. *Croton macrostachyus* comb and processed honey had EC values of 0.49 ± 0.04 and 0.55 ± 0.05 ms/cm, respectively. Whereas, *Schefflera abyssinica* had 0.47 ± 0.02 and 0.23 ± 0.01 ms/cm values for comb and processed honey, respectively. There was a significant difference ($p < 0.05$) in electrical conductivity among the monofloral honey (ANOVA Table S24, Please find the additional tables and figures in 'Revised Supplementary material'.). This could be due to the dependency of electrical conductivity on ash, organic acids, some complex sugars, and polyols contents, and varies with botanical origin (Belay et al., 2013). EC is used to differentiate between floral and honeydew honey. The electrical conductivity of floral honey must not exceed 0.8 mS/cm, according to Codex Alimentarius (Codex Alimentarius 2001). As

a result, all of the honey samples were classified as floral honey, and the results of this investigation were consistent with the report of Belay et al. (2017c) (0.04–0.58 ms/cm).

3.4.6. Hydroxymethylfurfural

The HMF content of *Croton macrostachyus* and *Schefflera abyssinica* is presented in Table 2. The HMF content for *Croton macrostachyus* comb and processed honey samples were 3.91 ± 0.33 and 8.63 ± 0.72 mg/kg, respectively. *Schefflera abyssinica* honey had 2.81 ± 0.09 and 3.56 ± 0.31 mg/kg for comb and processed honey, respectively. There was a significant difference ($p < 0.05$) in HMF content among the comb and processed honey samples for both *Croton macrostachyus* and *Schefflera abyssinica* honey (ANOVA Table S25, Please find the additional tables and figures in 'Revised Supplementary material'.). This could be because of processing, which caused a relative increase in HMF values for the processed honey. The results of this finding were higher than Belay et al. (2017b) (0–3.37 mg/kg), and in line with Chakir et al. (2011) (0.09–53.38 mg/kg). Honey should have an HMF concentration of less than 40 mg g^{-1}, according to Codex Alimentarius international regulations. All of the honey samples were found to be in compliance with the requirements (Codex Alimentarius 2001).

3.4.7. Specific rotation

Specific rotation is useful for the differentiation between honeydew (dextrorotatory) and blossom honey (laevorotatory). The specific rotation for *Croton macrostachyus* comb and processed honey was -71.83 ± 0.06 and -62.64 ± 0.65 α^{20}_D, respectively. Similarly, *Schefflera abyssinica* comb and processed honey had -68.72 ± 0.47 and $-76.52 \pm 0.1 \alpha^{20}_D$, respectively (Table 2). There was a significant difference ($p < 0.05$) in specific rotation among the honey samples (ANOVA Table S26, Please find the additional tables and figures in 'Revised Supplementary material'.). The results of this study indicated that honey samples were from blossom honey, rotating negative (laevorotatory), and were higher than the report of Belay et al. (2013) (-160 to $-120 \alpha^{20}_D$).

3.4.8. Color

In addition to quality parameters stated by Codex and the European Union, color is an individual preference, and is the single most important factor determining import and wholesale prices (Belay et al., 2015). The mean $\pm$ SD of color for *Croton macrostachyus* and *Schefflera abyssinica* is presented in Table 2. *Croton macrostachyus* comb honey had 91.8 ± 6 and processed honey had 111.8 ± 3.5 mm Pfund. Whereas *Schefflera*

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Table 3. Pearson correlation matrix among enzyme activity, antioxidant and physicochemical property of *Croton macrostachyus* and *Schefflera abyssinica* honey.

Var	Moi	RI	Ash	SR	EC	pH	Acid	HMF	Fru	Glu	Suc	Color	Dias	Inv	Phe	Flav	FRAP	DPPH	IC$_{50}$
Moi	1																		
RI	-0.999*	1																	
Ash	0.282	-0.265	1																
SR	0.522	-0.804	0.708*	1															
EC	0.460	-0.436	0.891*	0.832*	1														
pH	0.530	-0.643	0.331	0.428	0.332	1													
Acid	0.336	-0.319	0.761*	0.401	0.828*	0.404	1												
HMF	0.117	-0.910	0.213	0.763*	0.478	0.519	0.438	1											
Fru	0.300	-0.303	0.169	0.258	0.101	0.225	-0.103	0.137	1										
Glu	0.582	-0.590	0.269	0.523	0.213	0.310	0.102	0.501	0.366	1									
Suc	-0.267	0.248	0.154	-0.143	0.080	0.148	0.049	-0.356	0.131	-0.257	1								
Color	0.594	-0.586	0.379	0.568	0.620	0.556	0.731*	0.782*	-0.01	0.259	-0.09	1							
Dias	0.338	-0.328	0.623	0.492	0.742	0.539	0.881*	0.499	-0.04	0.079	0.110	0.893*	1						
Inv	0.405	-0.396	0.543	0.510	0.702	0.529	0.822*	0.592	-0.05	0.142	0.037	0.947*	0.981*	1					
Phe	0.329	-0.338	-0.548	-0.085	-0.240	0.241	-0.042	0.545	-0.16	0.063	-0.32	0.525	0.196	0.323	1				
Flav	0.439	-0.429	0.475	0.249	0.311	0.570	0.534	0.476	0.053	0.413	-0.18	0.957*	0.833*	0.888*	0.436	1			
FRAP	0.226	-0.816	0.412	0.786*	0.657	0.580	0.642	0.336	0.116	0.474	-0.19	0.918*	0.732*	0.800*	0.45	0.973*	1		
DPPH	-0.287	0.279	-0.449	-0.359	-0.600	-0.50	-0.79	-0.502	0.108	-0.048	-0.05	-0.92	-0.97	-0.98	-0.37	-0.82	-0.72	1	
IC50	0.577	-0.583	-0.363	0.185	-0.056	0.366	0.089	0.761	-0.04	0.275	-0.37	0.670	0.303	0.437	0.441	0.627	0.679	-0.446	1

Moi = Moisture, RI = Refractive index, SR = Specific rotation, EC = Electrical conductivity, HMF = Hydroxymethylfurfural, Fru = Fructose, Glu = Glucose, Suc = Sucrose, Dias = Diastase, Inv = Invertase, Phe = Phenols, Flav = Flavonoids, FRAP = Ferric Reducing Antioxidant Power assay, DPPH = 1,1-diphenyl-2-picrylhydrazyl radical scavenging.

  * Correlation is significant at $p < 0.05$.

Figure 3. Principal component analysis predictive biplots of enzyme activity, antioxidant and physicochemical properties.

abyssinica had 15.2 ± 1.6 and 28.3 ± 1.7 mm Pfund values for comb and processed honey, respectively. There was a significant difference (p < 0.05) among the honey samples (ANOVA Table S27, Please find the additional tables and figures in 'Revised Supplementary material'.). The data showed that the mm Pfund scale for honey increased through processing. The color of Bonga forest honey ranged from 12.92-114.68 mm Pfund scale, which was grouped as extra white to amber (Table 2). *Schefflera abyssinica* comb honey was found to be extra white, *Schefflera abyssinica* processed honey was within the white range. Contrarily, *Croton macrostachyus* comb honey was light amber to amber and the processed honey samples were amber color. The results of this finding were in line with the report of Adgaba et al. (2020) (6.1–115.5 mm Pfund).

3.4.9. Viscosity

The viscosity of *Croton macrostachyus* comb and processed honey range 429–2538 and 467–2414 cP viscosity (25–50 °C), respectively. Besides, *Schefflera abyssinica* comb and processed honey had 1042-4165 and 745–4137 cP, respectively (25–50 °C). There was a significant difference between the comb and processed honey. The viscosity of honey was affected by a rise in temperature between 25 and 50 °C. When honey was heated, the viscosity decreased, and this was in agreement with the report of Belay et al. (2017a). *Schefflera abyssinica* comb honey appeared to be the highest viscous honey compared to *Croton macrostachyus*. According to Belay et al., (2017a), *Schefflera abyssinica* honey has a tendency to form crystals, which is used to increase viscosity. This was in agreement with the reports of Belay et al. (2015). Processed honey of both monofloral honeys showed a lower viscosity in comparison to comb honey. This indicated that the processing temperature affects the viscosity of the honey. Similarly, the viscosity of the honey was found to decrease with an increase in temperature (Figure 2a). According to

Saxena et al. (2014), the temperature reduction in the viscosity of honey is attributed to reduced molecular friction and hydrodynamic forces, mainly governed by the composition of honey. The temperature dependence of the viscosity of monofloral honey was assessed by applying the Arrhenius model, which was presented in Figure 2b. The fitting of the logarithmic viscosity in cP versus the reciprocal temperature was examined at a temperature range of 298.15–323.15 K and projected by the Arrhenius model. The monofloral honey, *Croton macrostachyus* and *Schefflera abyssinica,* both comb and processed, showed Newtonian behavior (Figure 2b). This was in agreement with the report of Nayik et al. (2019) on saffron, apple, cherry, and *Plectranthus rugosus* monofloral honey; and Kamboj et al. (2021) on Cotton, Coriander, Dalbergia, and Murraya monofloral honey, which all showed a Newtonian behavior. The highest viscosity was observed at 25 °C, and decreased with increasing temperature. This was in line with the report of Belay et al. (2017a).

3.4.10. Correlation between enzyme activity, physicochemical and antioxidant properties of honey

Pearson correlation for enzyme activity, physicochemical and antioxidant properties of the honey sample is stated in Table 3.

Moisture content and refractive index had a negative relationship (r = -0.999). This was in line with Hailu & Belay's (r = -1) findings, which showed that the refractive index rises with solid content based on the idea that light travels quicker through honey with fewer solids than honey with numerous solids. Ash showed significant positive correlations with electric conductivity (r = 0.891), free acidity (r = 0.761), and specific rotation (r = 0.708). 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. This was in agreement with the electrical conductivity and free acidity (r = 0.828)

report of Belay et al. (2017c). A significant correlation was also found between electrical conductivity and specific rotation (r = 0.832), which was in agreement with Belay et al. (2013) (r = 0.74).

A significant correlation was observed between the color of the honey and enzymes, (Diastase (r = 0.893) and Invertase (r = 0.947)) and flavonoid (r = 0.957). According to Moniruzzaman et al. (2013), the color of the honey contributed to the enzyme and antioxidant spectrophotometric reading of the honey. This was in agreement with the report of Bertoncelj et al. (2007) for Slovenian honey (color with FRAP, r = 0.850). The correlation of color and FRAP was also in line with Indian honey (r = 0.85) (Saxena et al., 2010).

The honey enzymes are correlated with antioxidants. Diastase correlated with flavonoid (r = 0.833) and FRAP (r = 0.732). Likewise, invertase correlated with flavonoid (r = 0.888) and FRAP (r = 0.800). This could be due to enzymes having a role in the alteration of antioxidant content (Wang et al., 2004). This was in agreement with Braghini et al. (2020) report. Diastase correlated with invertase (r = 0.981). A similar correlation (r = 0.835) was found by Oddo et al. (1999). Honey with a low invertase content generally has a low diastase content and vice versa (Oddo et al., 1999).

A significant correlation was seen between flavonoids and FRAP (r = 0.973). This was in line with Algerian honey (r = 0.893) (Khalil et al., 2012). According to Perna et al. (2012), the reducing power of honey could be due to flavonoid contents, which can reduce Fe^{+3} to Fe^{+2}. Previous studies on honey indicate that the presence of compounds such as polyphenols and flavonoids may function as potential natural antioxidants (Saxena et al., 2010).

For both *Croton macrostachyus* and *Schefflera abyssinica* honey, a multivariate analysis was used to seek for the primary data structures of the comb and processed honey, as well as possible trends and the degree of variation detected between variables (Figure 3). To carry out interpretations based on corresponding associations in the comb and processed monofloral honey, PCA with predictive biplots was employed. A straight line was drawn from a sample point to the variable axis; the narrower the angle between response variables, the stronger the relationship (Hailu and Belay 2020).

The association between enzyme activity, antioxidant, and physicochemical properties were drawn on the PCA biplots (Figure 3), and the principal components explained 70.64% of the variation in the data set. PC1 explained 52.91% of the variability and PC2 explained 17.73%. The PCA biplots in Figure 3 indicated the existence of two important data structures; comb honey at the bottom and processed honey at the top. Comb honey associated with enzymes (diastase and invertase) and some physicochemical properties for both *Croton macrostachyus* and *Schefflera abyssinica* honey, which is found at the bottom quadrant (Figure 3). Meanwhile, antioxidants (DPPH, FRAP, IC_{50}, phenol, and flavonoid) and some physicochemical properties associated with processed *Croton macrostachyus* and *Schefflera abyssinica* honey, and found at the upper quadrant (Figure 3). This showed that comb and processed honey can essentially be differentiated based on enzyme patterns that projected towards the comb honey; and antioxidants associated with processed honey. According to Kowalsk et al. (2012), comb honey had a relatively higher amount of enzymes, and the processing of honey possibly reduced the level of enzymes. Contrarily, the processing of honey is used to increase the antioxidant activity of honey, which is generated from the Maillard and caramelization reactions (Turkmen et al., 2005).

4. Conclusion

Melissopalynology, enzymes, antioxidants, and physicochemical properties of monofloral (*Croton macrostachyus* and *Schefflera abyssinica*) comb and processed honey samples from Ethiopia's Bonga forest were investigated in this research. The Melissopalynology results showed that *Croton macrostachyus* and *Schefflera abyssinica* are the dominant honey. All the honey samples satisfy the Codex Alimentarius standards. The principal component analysis showed that enzymes (diastase and

invertase) are associated with comb honey, while, antioxidants (DPPH, FRAP, IC_{50}, phenol, and flavonoid) and HMF are allied with processed honey. Thus, the comb and processed honey can essentially be distinguished by enzyme and antioxidant patterns. *Croton macrostachyus* and *Schefflera abyssinica* monofloral honey can be also identified based on pollen analysis.

Declarations

Data availability statement

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

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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