Evaluation of the physicochemical properties of honey produced in Doyogena and Kachabira Districts of Kembata Tambaro zone, Southern Ethiopia

Teshale Tigistua a, Zemene Workub b, Abdo Mohammed b, *

a Animal Feed Production and Utilization Expert, Doyogena district, Doyogena Ethiopia
b Department of Animal Science, College of Agriculture, Jimma University, Jimma Ethiopia

ABSTRACT

This study was conducted to evaluate the physicochemical properties of honey produced in Doyogena and Kachabira districts, of Kembata Tambaro Zone, Southern Ethiopia. For the laboratory evaluation a total of eighteen (18) honey samples were collected. Of the total of 18 honey samples, 12 (6 from highland and 6 from midland) were purposively taken directly from the beekeepers from frame beehives and 6 honey samples were randomly taken from six retailor shops. The main parameters analyzed were color, moisture, electrical conductivity, ash, reducing sugar, fructose to glucose ratio, glucose to water ratio, fructose, glucose, fructose plus glucose, sucrose, Hydroxyl methyl furfural, power of hydrogen and free acidity. These parameters were evaluated in the Holetta bee research center laboratory and results were analyzed by Statistical analysis system software. The result of laboratory work indicated that the mean value of moisture, electrical conductivity, ash, reducing sugar, fructose to glucose ratio, glucose to water ratio, fructose, glucose, fructose plus glucose, sucrose, Hydroxyl methyl furfural, power of hydrogen and free acidity was, 18.83/ C6 0.69 g/100g, 0.58/ C6 0.03 mS/cm, 0.25/ C6 0.02 g/100g, 68.55/ C6 0.56 g/100g, 1.05/ C6 0.03, 1.78/ C6 0.08, 34.22/ C6 0.55 g/100g, 32.61/ C6 0.70 g/100g, 66.83/ C6 0.44 g/100g, 2.54/ C6 0.40 g/100g, 3.42/ C6 1.95 mg/kg, 4.03/ C6 0.21 and 13.39/ C6 1.43 meq/kg respectively. There was a significant difference (P < 0.05) between districts in terms of moisture and power of hydrogen. Significant difference (P < 0.01) was declared between agro-ecologies in terms of the moisture, glucose to water ratio and free acidity. Moreover, a significant difference (P < 0.05) was seen between agro-ecologies concerning electrical conductivity and ash. There was a statistically significant difference (P < 0.01) between the honeys of the retailer shop and farm gate sources concerning moisture content, electrical conductivity, ash, reducing sugar, glucose to water ratio, sucrose, Hydroxyl methyl furfural and free acidity. The result of the study indicated that all the physicochemical parameters of honey evaluated lie within the range of national and international standards. Because of the good quality of honey of the study area, it is advised to exploit the potential for export market with better intervention.

1. Introduction

In nearly all countries of the world bees and their products are not only well known and have wider consumer preferences, but provide sustainable livelihoods to many small-scale farmers, other rural and non-rural peoples (FAO, 2012). Having suitable environmental conditions for the growth of varied natural vegetation’s and cultivated crops, Ethiopia is one of the best areas in the world for beekeeping activities (Nuru, 2007; EIAR, 2017). Ethiopia is believed to have the largest honeybee population in Africa. The country’s honeybee colony population is estimated to be about 10 million, of which 7.5 million are kept in beehives by about 2 million smallholder beekeepers, and the remaining is existent in the forest as a wild honeybee colonies (CSA, 2018; Haftey et al., 2018; “APIMONDIA International symposium, 2018”). The estimated honey and wax production potential of the country is 500,000 and 50,000 tons correspondingly (MoARD, 2008). But, in the last year report, 66,221.82 tons of honey and 6,000 tons of wax was produced (CSA, 2018) per year which is 13.24% and 12% of the estimated potential of honey and beeswax respectively.

* Corresponding author.
E-mail address: abdo2009misku@gmail.com (A. Mohammed).

https://doi.org/10.1016/j.heliyon.2021.e06803
Received 2 October 2020; Received in revised form 14 December 2020; Accepted 10 April 2021
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This indicates that both honey and beeswax production the country has not used the existing potential excellently.

The physicochemical properties of honey are considerably influenced by the nectar types that the honeybees used, climate, soil type and post-harvest handling practices (Gomes et al., 2016; Kebede et al., 2012). Harvesting not fully matured honey, unsuitable honey storing containers and storage places ascribed to inferior honey quality (Awarris et al., 2012). Southern Nation Nationalities and Peoples Regional State (SNNPRS) is one of the potential beekeeping regions of the country, which has enormous honeybee colonies and suitable conditions for the beekeeping activities. It includes 20.73% and 14.27% of traditional beehive colonies and honey production of the country respectively (CSA, 2018). Kembata Tambaro Zone (KTZ) is one of the potential beekeeping zones of the SNNPRS encompassing a total of eight districts. Two of the study districts (Doyogena and Kachabira) are located in the zone, where special concern was provided in this study. The two study districts Doyogena and Kachabira encompass 15.72% and 17.5% of honey production of the Kembata Tambaro Zone respectively (KDDOANR, 2018).

Even though there are certain studies conducted on honey production and honey quality in Guji Zone (Birhanu, 2016), physicochemical characteristics of honey produced from traditional and frame bee hives in Tigray region (Gebreziabber et al., 2013), physicochemical analysis of honey and major honey production challenges around the Gondor (Addis and Malede, 2014) and honeybee production practices and Honey quality in silti district (Alemayehu and Nuru, 2011), they are not all-inclusive enough and some of them did not compare physicochemical quality properties of honey with farm gate and retailer shop sources. In Doyogena and Kachabira districts there was no research information on physicochemical properties of honey based on the national and international honey quality standards. Therefore, this study was conducted to evaluate the physicochemical properties of honey produced in Doyogena and Kachabira districts, Kembata Tambaro zone, Southern Ethiopia.

2. Materials and methods

2.1. Sample collection and sampling technique

Eighteen fresh ripe honey samples were collected from the beekeepers and retailer shops. Twelve out of eighteen honey samples were directly collected from twelve beekeepers purposively and six honey samples were collected from six retailer shops randomly. The honey samples were harvested in the same period in the year 2019 during the main honey harvesting season of the study area (March to June). The samples were collected by using tightly closed chemical-free plastic containers having a capacity of one kilogram (IHC, 2009).

2.2. Sample preparation

Honey samples were made free from any foreign materials and strained freshly as harvested with great precautions not to be contaminated and exposed for heat (Pavelkova et al., 2013). The honey samples were prepared according to the “COMESA 002 (2004) standard for honey” protocol for quality analysis and labeled with numbers, site of collection. Collected honey samples were stored at room temperature, in a dry room, free of odors, ants and other unwanted materials, until they were evaluated at the Holetta bee research center laboratory.

2.3. Physico-chemical properties analysis

The physicochemical quality properties (color, electrical conductivity, moisture, ash, pH and free acidity, sugars and HMF) of collected honey samples were evaluated according to the principles and procedures of the international honey commission (IHC) at the Holetta bee research center laboratory (Bogdanov, 2009).

2.4. Color of honey

Color of honey samples was assessed according to the Pfund (color grader) classifier (Bogdanov, 2009).

2.5. Electrical conductivity

The determination of the electrical conductivity is based on the measurement of the electrical resistance of which the electrical conductivity is the reciprocal (IHC, 2009). The conductivity cell was connected to conductivity meter: - the cells were thoroughly rinsed with potassium chloride solution and immersed the cell in the solution, together with a thermometer. The electrical conductance of the solution was read in mS/cm after the temperature has equilibrated to 20 °C. The electrical conductivity of the honey solution was calculated by using the following formula: SH = K* G, Where, SH = electrical conductivity of the honey solution in mS.cm-1; K = cell constant in cm-1; G = conductance in mS. K = 11.691*1/G in which, 11.691 = is the sum of the mean value of the electrical conductivity of the freshly distilled water in mS per cm and electrical conductivity of a 0.1M potassium chloride solution (Bogdanov, 2009).

2.6. Moisture content

The moisture content was evaluated by the refractometric method by Abbe Refractometer (Bogdanov, 2009). The refractive index read from the instrument was converted into honey moisture content based on previously established relationship (Wedmore, 1955).

2.7. Ash

Ash content of the sampled honey was evaluated following incineration of the sample in electrical muffle furnace. Ash (% by mass) = (m1-m2)/Mo*100 where; m0 = weight of honey sample taken (10 g); m1 = weight of crucible + ash; m2 = weight of crucible.

2.8. pH and free acidity

Solution of honey sample and distilled water was prepared based on scientific procedure for subsequent step. The solution was titrated with0.1M sodium hydroxide (NaOH) solution to pH 8.30 consequently pH was evaluated with the help of pH meter after calibration. Acidity was determined as follow:

Acidity = 10V

Where V = the volume of 0.1N NaOH used and 10 is the amount of sample.

2.9. Sugars

Honey sugars were determined by HPLC (High Pressure Liquid Chromatography) method that determines different sugars in honey. It is based on the method of (Bogdanov and Baumann, 1988). After filtration of the solution, the sugar content is determined by HPLC with RI-detection. Peaks are identified on the basis of their retention times. Quantitation is performed according to the external standard method on peak areas or peak heights. Regarding standard substances fructose, glucose, sucrose, turanose and maltose were dissolved with water by the following ratio: - fructose: 2.0 g, glucose: 1.50 g, sucrose: 0.25 g, turanose: 0.15 g and maltose: 0.15 g.

HPLC separation was performed on an analytical column containing amino-modified silica gel using Acetonitrile: - water (80:20, v/v) mobile phase. The flow rate was 1.3 ml/min. Membrane filter for aqueous solutions, pore size 0.45 μm. Column and detector temperature: - 30 °C. Injection volume: 5μl. Analytical stainless-steel column (4.6 mm in
diameter and 250 mm length) containing amine modified silica gel with 5–7 μm particle size was used. Analysis time was 6 min. Prior to injection, solvents and diluents were filtered via 0.45 μm filters.

The result of sugar analysis by HPLC method was expressed by using the following formula (Bogdanov, 2009); 
\[
W = A_1 \times V_1 \times m_1 \times 100/A_2 \times V_2 \times m_0,
\]
Where; 
- \(A_1\) = Peak areas or peak heights of sugar compound in the sample solution, expressed as units of area, length or integration; 
- \(A_2\) = Peak heights of the given sugar compound in the standard solution, expressed as units of area, length or integration; 
- \(V_1\) = Total volume of the sample solution in ml; 
- \(V_2\) = Total volume of the standard solution in ml; 
- \(m_1\) = Mass amount of the sugar in grams in the total volume of the standard (V2); 
- \(m_0\) = sample weight in grams.

### 2.10. Hydroxyl methyl furfural (HMF)

Principle of UV absorbance of HMF at a wavelength of 284nm was utilized to estimate HMF of the sampled honey (Bogdanov, 2009). Reagents used were, Carrrez solution I: 15 g of potassium hexacyanoferrate, K4Fe(CN)6•3H2O was dissolved in distilled water and make up to 100 ml. Carrrez solution II: 30 g of zinc acetate, Zn (CH3COO) 2.2H2O was diluted and make up to 100 ml. Sodium bisulphate solution 0.20 g/100g: dissolve 0.20 g of solid sodium hydrogen sulphite NaHSO3, (meta-bisulphite, Na2S2O5) in water and diluted to 100 ml. Spectrophotometer operating in a wavelength range including 284 and 336 nm, vortex mixer, filter paper, and 1 cm quartz cells (cuvettes) were used to evaluate HMF. The result was calculated by using the following formula.

**Hydroxyl methyl furfural determination**

HMF in mg/kg = \((A284 – A336) \times 149.7 \times 5 \times D/W, \)  
\(eij = \text{random error term}\)

Model statement to study the effect of source of honey on the physicochemical properties of honey.

**Statistical model for physicochemical properties**

\[ Y_{ij} = \mu + A_i + B_j + A_{ij} + e_{ij} \]  
Where, 
- \(Y_{ij}\) = the value of the honey quality parameter for the jth honey sample in the ith honey source 
- \(\mu\) = the overall (grand) mean 
- \(A_i\) = the effect of sources (i = 2; Farm gates and the retailer shop sources) 
- \(B_j\) = district and agro-ecology interaction effect 
- \(e_{ij}\) = random error

### 3. Results and discussion

#### 3.1. Honey color

The color result of evaluated honey samples is indicated in Table 1. In the current study, the color of honey varied from extra light amber to amber. Among the assessed honey samples, 66.7%, 27.8% and 5.5% were categorized under extra light amber, light amber and amber color, respectively. Concerning the districts, almost all honey samples collected from the Kachabira district were extra light amber colored. But, 83.3% and 16.7% of honey of the Doyogena district was extra light amber and light amber colored respectively.

As far as the source of honey is concerned, the honey samples collected from the farm gate sources were mainly (91.7%) of extra light amber colored. Nevertheless, the honey samples collected from the retailer shop sources were predominantly (66.7%) of light amber color. The color of the retailer shop source honey autonomously was characterized under three color categories, amber (16.7%), light amber (66.6%) and extra-light amber (16.7%). Moreover, all honey samples collected from the midland agro-ecology were extra-light amber-colored. The highland agro-ecology honey was predominantly (83.3%) of extra-light amber-colored, whereas, 16.7% were categorized under light amber color.

This difference in honey color between districts (Doyogena and Kachabira), sources (farm gate and retailer shop) and agro-ecologies (highland and midland) might be due to the difference in flora type processing procedures such as heating, time and storage conditions, and processing procedures.

#### 3.2. Moisture

The average honey moisture content of the study area was 18.83 ± 0.69 g/100g (Tables 2 and 3) which is within acceptable standard range of QSAE (17.5–21g/100g), CAC (<21g/100g) and EU (<21g/100g). There was statistically significant difference (\(P = 0.0143\)) between districts in moisture content of the honey. The mean moisture content of the Doyogena district honey (18.67 ± 0.61 g/100g) was statistically higher than the mean moisture content of the Kachabira district honey (15.98 ± 0.99 g/100g). The difference between districts in moisture content could be ascribed to the moisture content of the original plant, geographic and environmental factors (Gomes et al., 2010). There was also statistically significant difference (\(P =<.0001\)) between agro-ecologies concerning the honey moisture. The mean moisture content of the highland agro-ecology honey (19.33 ± 0.42g/100g) was statistically higher than that of the midland agro-ecology honey (15.32 ± 0.53g/100g).

The higher moisture content of the highland honey samples, as opposed to the midland agro-ecology, might be due to the prevailing higher relative humidity in the highland agro-ecology which might have
The high moisture content of the retailer predisposed the honey to an increased moisture content of the honey by absorption, following the hygroscopic nature of honey.

There was significant difference (P = 0.0004) in moisture content between sources of honey. The mean moisture content of the retailer shop source of honey (21.83 ± 0.31 g/100g) was statistically higher than the honey samples collected from the farm gate sources (17.33 ± 0.68 g/100g).

The mean moisture content of the honey from retailer shop indicates that it was beyond the acceptable standard set by QSAE (2005), CAC (2001) and Council (2002). The high moisture content of the retailer shop honey could be due to poor handling practices and addition of water and other adulterants to the honey. In honey retailing shops, most of the time the honey handling and storage equipment were poor. Furthermore, frequent opening and closing during retailing of honey might have exposed to the environmental humidity to be absorbed resulting in a higher moisture content.

According to the moisture content, QSAE (2005) categorized honey into three grades (A, B, C). Honey with the moisture content of 17.5–19 g/100g is categorized under grade A. Honey with a moisture content of 19–20 g/100g is categorized under grade B and honey with the moisture content of 20–21 g/100g is identified as grade C. Therefore, Doyogena and Kachabira district honey could be characterized as grade (A) honey. Furthermore, the farm gate-source of honey is also grouped as grade A honey. But, the shop source honey was out of the indicated grade levels.

The current finding is higher than Alemayehu and Nuru (2011) who reported a mean moisture content of 15.94 ± 1.15g/100g in Silte district, Southern Ethiopia. But, Awaris et al. (2014) reported higher mean moisture content of honey (22.86 ± 1.03 g/100g) from Gesha, Mash and Sheko districts of Southern Ethiopia, than the current finding. In line with the current result, Chala et al. (2011) reported a mean moisture content of 18.52 ± 0.33g/100g from Gomma district of Southwestern Ethiopia. Other authors also reported a similar result from different locations of the

### Table 1. Classification of honey color based on color grade classifier.

| Parameter | Moisture | EC in mS-1cm | Ash g/100g | RS g/100g | F/G | G/W | Fructose g/100g | Glucose g/100g | Sucrose g/100g | HMF mg/kg | pH | Acidity meq/kg |
|-----------|----------|--------------|-----------|----------|-----|-----|----------------|--------------|--------------|-----------|-----|-------------|
| Overall Mean | 18.83 ± 0.69 | 0.58 ± 0.03 | 0.25 ± 0.02 | 68.55 ± 0.56 | 1.05 ± 0.03 | 1.78 ± 0.08 | 34.22 ± 0.55 | 32.61 ± 0.70 | 66.83 ± 0.44 | 2.54 ± 0.40 | 3.42 ± 1.95 | 0.40 ± 0.21 | 13.39 ± 1.43 |
| Minimum | 14.4 | 0.31 | 0.1 | 64.4 | 0.75 | 1.2 | 30 | 29 | 62 | 0.3 | 0 | 2.6 | 8 |
| Maximum | 23 | 0.8 | 0.38 | 72.1 | 1.27 | 2.35 | 37 | 40 | 70 | 5.9 | 25.6 | 5.8 | 28 |

### Table 2. Ranges of Physicochemical properties of honey in the study area.

| Parameter | Moisture g/100g | EC in mS-1cm | Ash g/100g | RS g/100g | F/G | G/W | Fructose g/100g | Glucose g/100g | Fructose + Glucose g/100g | Sucrose g/100g | HMF mg/kg | pH | Acidity meq/kg |
|-----------|----------------|--------------|-----------|----------|-----|-----|----------------|--------------|-----------------------------|--------------|-----------|-----|-------------|
| Overall Mean | 18.83 ± 0.69 | 0.58 ± 0.03 | 0.25 ± 0.02 | 68.55 ± 0.56 | 1.05 ± 0.03 | 1.78 ± 0.08 | 34.22 ± 0.55 | 32.61 ± 0.70 | 66.83 ± 0.44 | 2.54 ± 0.40 | 3.42 ± 1.95 | 0.40 ± 0.21 | 13.39 ± 1.43 |
| Minimum | 14.4 | 0.31 | 0.1 | 64.4 | 0.75 | 1.2 | 30 | 29 | 62 | 0.3 | 0 | 2.6 | 8 |
| Maximum | 23 | 0.8 | 0.38 | 72.1 | 1.27 | 2.35 | 37 | 40 | 70 | 5.9 | 25.6 | 5.8 | 28 |

### Table 3. Physicochemical properties of honey as affected by District, Agro-ecology and sources of honey.

| Parameters | Doyogena | Kachabira | p-value | Highland | Midland | p-value | Farm gate | Shop | p-value |
|-----------|----------|-----------|---------|----------|---------|---------|-----------|------|---------|
| Moisture % | 18.67±0.61 | 15.98±0.99 | 0.0143 | 19.33±0.42 | 15.32±0.53 | <.0001 | 17.33±0.68 | 21.83±0.31 | 0.0004 |
| EC mS-1cm | 0.65±0.04 | 0.63±0.03 | 0.6901 | 0.69±0.03 | 0.59±0.01 | 0.0037 | 0.6±0.02 | 0.46±0.05 | 0.0014 |
| Ash g/100g | 0.29±0.02 | 0.28±0.01 | 0.7299 | 0.32±0.02 | 0.26±0.01 | 0.0368 | 0.29±0.01 | 0.19±0.03 | 0.0014 |
| RS g/100g | 70.10±0.73 | 69.42±0.85 | 0.7003 | 70.20±0.71 | 69.32±0.85 | 0.5201 | 69.76±0.55 | 66.13±0.43 | 0.0005 |
| F/G ratio | 0.96±0.06 | 1.06±0.03 | 0.1499 | 1.01±0.05 | 1.01±0.06 | 0.5952 | 1.01±0.04 | 1.14±0.06 | 0.0701 |
| G/W ratio | 1.89±1.05 | 2.07±1.09 | 0.6866 | 1.77±0.06 | 2.19±0.04 | 0.0017 | 1.98±0.07 | 1.38±0.04 | <.0001 |
| Fructose | 33.00±1.18 | 34.67±0.67 | 0.2213 | 34±1.09 | 33.67±0.95 | 0.5285 | 33.83±0.69 | 35.0±0.89 | 0.3337 |
| Glucose | 34.83±1.22 | 32.67±0.67 | 0.1161 | 33.83±0.83 | 33.67±1.31 | 0.6552 | 33.75±0.74 | 30.33±1.02 | 0.0162 |
| Fru + Glu | 67.83±0.50 | 67.34±0.66 | 0.6996 | 67.83±0.48 | 67.34±0.76 | 0.6996 | 67.58±0.43 | 65.33±0.71 | 0.0119 |
| Sucrose | 1.83±0.49 | 1.40±0.47 | 0.4254 | 1.83±0.49 | 1.40±0.47 | 0.4431 | 1.62±0.33 | 4.40±0.37 | <.0001 |
| HMF | 0.00±0.00 | 0.00±0.00 | - | 0.00±0.00 | 0.00±0.00 | - | 0.00±0.00 | 10.25±4.99 | 0.0058 |
| pH | 3.67±0.12 | 4.30±0.46 | 0.0180 | 3.42±0.13 | 4.55±0.35 | 0.2597 | 3.98±0.25 | 4.12±0.45 | 0.4006 |
| Acidity | 9.50±1.52 | 11.33±2.64 | 0.5067 | 13.17±2.53 | 7.67±0.42 | 0.0084 | 10.42±1.48 | 19.33±0.71 | <.0001 |

N = Observation; SE = Standard error of the mean; mS-1cm = millisiemens; Means in the same row with different superscripts are significantly different at (P < 0.05); (P < 0.01); EC = Electrical conductivity; RS = Reducing sugar; F/G = Fructose to glucose; G/W = Glucose to water ratio. Fru + Glu = Fructose plus Glucose.
country (Bekele et al., 2016; Eyobel and Miresa, 2017; Mekuanint and Meareg, 2019).

3.3. Electrical conductivity

The mean electrical conductivity of the honey sample of the study area was 0.58 ± 0.03 mS/cm (Tables 2 and 3) which is within the acceptable range of QSAE (2005) of <0.6mS/cm-1, Council (2002) of <0.8 mS-1cm and CAC (2001) of <0.8 mS-1cm. The minimum and maximum electrical conductivity result of the honey samples of the study area were 0.31 mS-1cm and 0.8 mS-1cm, respectively. According to CAC (2001), electrical conductivity values for floral honey should have a value of less than 0.8 mS cm-1, whereas honeydew honey should have values greater than 0.8 mS cm-1. All honey samples evaluated for the electrical conductivity had values below 0.8 mS/cm which shows that all samples from d/t districts; sources and agro-ecology are of floral origin.

There was no significant difference (P = 0.6901) between districts in the electrical conductivity of honey. Nevertheless, there was a statistically significant difference (P = 0.0347) between agro-ecologies concerning the electrical conductivity of honey. The mean electrical conductivity result of the highland agro-ecology honey (0.69 ± 0.03 mS/cm) was statistically higher than the midland agro-ecology honey (0.59 ± 0.01 mS/cm). The difference in the parameter may be ascribed to the fluctuations in the mineral salts, organic acids, and protein concentrations which in turn are influenced by soil type and flora origin (Terrab et al., 2003). Significant difference (P = 0.9089) was not noticed in interaction effects in terms of the electrical conductivity of honey. There was a significant difference (P = 0.0014) between sources of honey collected concerning the electrical conductivity. The mean electrical conductivity result of a honey sample collected from the farm gate-sources (0.64 ± 0.02 mS/cm) was statistically higher than the mean electrical conductivity of the retailer shop sources of honey (0.46 ± 0.05 mS/cm).

The difference might be due to the source of honey where honey from shop might be collected from the midland agro-ecology which has comparatively lower electrical conductivity than that of the highland agro-ecology honey (Terrab et al., 2003). The current finding is in a good agreement with the result of Eyobel and Miresa (2017) who reported the mean electrical conductivity of 0.55 ± 0.08 mS/cm from Ambo district. Abera et al. (2013) reported a mean of 0.70 ± 0.04 mS/cm from the Bale Harenna forest which is higher than the current result. Based on electrical conductivity it is possible to generalize that honey of the study area is of good quality and full fills the standards of the QSAE (2005), CAC (2001) and Council (2002).

According to CAC (2001), blossom and honey daw honey classification, Doyogena as well as Kachabira district honey is blossom honey because its mean electrical conductivity value is within the indicated range of blossom honey which is < 0.8 mS/cm.

3.4. Ash content

The overall mean ash content of honey of the study area was 0.25 ± 0.02 g/100g ranging between 0.1 g/100g to 0.38 g/100g (Tables 2 and 3) which is within the standard range of QSAE (2005) of, 0.6 g/100g and CAC (2001) of ≤0.6 g/100g. There was no statistically significant difference (P = 0.7299) between districts in the ash content of honey. Similarly significant difference (P = 0.8820) was not noticed in interaction effect. However, there was statistically significant difference (P = 0.0368) between agro-ecologies in honeys ash content. The mean ash content of highland agro-ecology honey (0.32 ± 0.02g/100g) was statistically higher than the midland agro-ecology (0.26 ± 0.01 g/100g). Also, significant difference (P = 0.0014) was noticed between sources of honey. The mean ash content of honey collected from farm gate source honey (0.29 ± 0.01g/100g) was statistically higher than the retailer shop honey (0.19 ± 0.03 g/100g).

It is known that the ash content of honey depends on the material contained in the pollen collected by the bees during foraging on the flora, geographical area, botanical origin of the honey and soil type where the nectar-producing plants are located (Kumar et al., 2018). Based on this information, the difference in ash content might be due to the difference in flora type, geographical area, and soil type and physiology of each plant. The current finding is in line with the finding of Chala et al. (2011) who reported the mean of 0.23 ± 0.05 g/100g ash content from Gomma district of Southwestern Ethiopia. Awarris et al. (2014) reported comparable result of 0.22 ± 0.16 g/100g honey ash content from Masha, Gesha and Sheko districts. Mekuanint and Meareg (2019) also reported comparable findings. It is possible to generalize that honey of the study area is of good quality and met national and international standards in its ash content.

3.5. Reducing sugar

The overall mean reducing sugars content of honey of the study area was 68.55 ± 0.56 g/100g with minimum and maximum of 64.4 g/100g and 72.1 g/100g respectively (Tables 2 and 3). The mean value of the reducing sugar content indicated that honey of the study area was within the acceptable range of QSAE (2005) and CAC (2001), i.e.65g/100g, and ≥65g/100g, respectively. Significant difference was not noticed between districts (P = 0.7003), agro-ecologies (P = 0.5201) and districts by agro-ecologies interaction effect (P = 0.1037). However, there was statistically significant difference (P = 0.0005) between the sources of honey. The mean reducing sugar content of the farm gate honey source (69.76 ± 0.55 g/100g) was statistically higher than that of the honey samples collected from the retailer shop sources (66.13 ± 0.43 g/100g). The lower reducing sugar content of the honey from retailer shop as compared to farm gate honey sources could be due to the higher moisture content of the retailer shop honey, since higher moisture content decreases the solid component of the honey which resulted in the decline of reducing sugar (Abera et al., 2013).

Moreover, the difference between sources of honey in terms of the reducing sugar content might be due to variation in the plant sources from which the honey was produced. The finding is in line with Alemayehu and Nuru (2011) who reported the mean reducing sugar content of 69.04 ± 1.49 g/100g from silte district. Chala et al. (2011) also reported a mean reducing sugar content of 67.92 ± 0.96 g/100g from Gomma districts, Southwestern Ethiopia. But, Awarris et al. (2014) reported the lower reducing sugar content of 66.79 ± 6.96g/100g from Masha, Gesha and Sheko districts, of Southern Ethiopia.

3.6. Fructose to glucose ratio

The mean fructose to glucose ratio of the study area was 1.05 ± 0.03 ranging from 0.75 to 1.27, respectively (Tables 2 and 3). Statistically significant difference was not observed in fructose to glucose ratio between districts (P = 0.1499), agro-ecologies (P = 0.5952), interaction of districts and agro-ecologies (P = 0.1919) and sources of honey (P = 0.0701).

Crystallization results from the creation of monohydrate glucose crystals, which vary in number, shape, dimension, and quality with the honey composition and storage condition. Fructose to glucose ratio is the standard quality measurement for honeys crystallization. In addition to fructose to glucose ratio, crystallization of honey depends on the sugar content, water-insoluble material, temperature, and storage condition (Biba et al., 2013). When the ratio of fructose to glucose is less than one it displays that the constituent of glucose is high and dominant, as a result, honey is susceptible to crystallization (Amir et al., 2015; Draiaia et al., 2015).

Based on this information the study area honey is less rapidly susceptible to crystallization because its glucose to fructose ratio is above one. In line with the current result, Aregay et al. (2018) reported the
mean fructose-glucose ratio of 1.06 ± 0.06 from Godere district located in Gambella region.

3.7. Glucose to water ratio

The mean glucose to water ratio of the study area was 1.78 ± 0.08 (Tables 2 and 3). A significant difference was not observed for districts (P = 0.6866) and interaction of districts and agro-ecologies (P = 0.8925) in terms of the glucose to water ratio. Nevertheless, there was significant difference (P = 0.0017) between agro-ecologies in terms of glucose to water ratio of honey. The mean glucose to water ratio of the midland agro-ecology honey (2.19 ± 0.04) was statistically higher than the highland agro-ecology (1.77 ± 0.06). Similarly, there was statistically significant difference (P = <.0001) between sources of honey. The mean glucose to water ratio of the farm gate honey (1.98 ± 0.07) was statistically higher than shop honey (1.38 ± 0.04).

The difference in glucose to water ratio between sources and agro-ecologies might be due to the difference in sugar and moisture content of the honey. In honey when the moisture content increases its crystallization rate drops because honey moisture reduces the solid component of honey. In the study area, the higher moisture was mainly obtained in the honey samples taken from the retailer shop honey and hence the smallest glucose to water ratio was obtained in a honey samples taken from the retailer shop sources as opposed to the honey samples collected from the farm gate.

The higher moisture content was recorded in a sample collected from the highland agro-ecology; as a result of which the glucose to water ratio of higher than the midland agro-ecology honey. According to Amir et al. (2010), the least ability of honey crystallization is obtained when the glucose to water ratio is less than 1.0, while it crystallizes faster when that ratio is more than 2.0 (Amir et al., 2010). Based on this information the farm gate and midland agro-ecology honey crystallizes faster than the shop source and highland agro-ecology honey. This result is in line with the finding of Aregay et al. (2018) who reported 1.94 ± 0.21 from Godere district.

3.8. Fructose and glucose content

The fructose content of honey sample of the study area was 34.22 ± 0.55 g/100g, with a minimum and maximum of 30 g/100g and 37 g/100g respectively (Tables 2 and 3). A significant difference was not declared between districts (P = 0.2213), agro-ecologies (P = 0.5258), district by agro-ecology interaction effect (P = 0.4311) and sources (P = 0.3337) in terms of fructose content of the honey. The current finding is in line with Aregay et al. (2018) who reported the mean fructose content of 38.64 ± 0.61 g/100g from the Godere district.

The glucose content of the evaluated honey samples of the study area was 32.61 ± 0.70g/100g with a minimum and maximum of 29 g/100g and 40 g/100g respectively. There was no significant difference between districts (P = 0.1161), agro-ecologies (P = 0.6552) and district by agro-ecology interaction effect (P = 0.0873) in terms of honesy glucose content. A significant difference (P = 0.0162) was noticed between sources of honey. The mean glucose content of the farm gate sources of honey 33.75 ± 0.74 g/100g was statistically higher than the retailer shop source 30.33 ± 1.02 g/100g. The difference in the glucose content between the sources of the honey collection might be due to the difference in the flora of honeybee and honey handling practices. Aregay et al. (2018) reported similar finding of 36.37 ± 2.14g/100g glucose content from Godere district. The sugars of honey are responsible for several of the physicochemical properties such as viscosity, hygroscopic and granulation characteristics of honey.

The two principal sugars in honey are fructose and glucose. The content of fructose and glucose in honey varies from one type of honey to the other based on the origin of honey. According to Khalil et al. (2012), fructose content in honey ranges from 30-44% and glucose from 25-40%. The balance of these two major sugars is the principal cause that leads to honeys crystallization. The percentage of each sugar regulates whether it crystallizes rapidly or slowly. Based on its fructose and glucose content honey of the current study area is within the range of the finding indicated by (Khalil et al., 2012).

The mean fructose + glucose content of the study area honey was 66.83 ± 0.44 g/100g which is within the recommended standards of the national as well as international institutions. There was no significant difference between districts (P = 0.6996), agro-ecologies (P = 0.6996) and district by agro-ecology interaction effect (P = 0.1806) in fructose + glucose content of honey. However, there was a significant difference (P = 0.0119) between sources. The mean fructose + glucose content of farm gate source of honey 67.58 ± 0.43g/100g was statistically higher than retailer shop honey (65.33 ± 0.71g/100g). The difference in its content might be due to the difference in the honeybee flora and honey handling practices. The sum of fructose and glucose should not be less than 60 g/100g for blossom honey and not less than 45 g/100g for honeydew honey (CAC, 2001; Council, 2003). Therefore, the sum of fructose and glucose of the current study area was 66.83 g/100g implying that the honey produced in the study area is of blossom origin.

3.9. Sucrose content

The mean sucrose content of the sampled study area honey was 2.54 ± 0.40 g/100g with a minimum of 0.3 g/100g and a maximum of 5.9 g/100g (Tables 2 and 3). The result revealed that 100% of the samples were within the standard range of QSAE (2005) and 83.3% of honey samples were found within the range of CAC (2001) and Council (2002). But, of the total, nearly 16.7% of the samples surpassed the standard set by CAC (2001) and Council (2002). A significant difference was not noticed between districts (P = 0.4254), agro-ecologies (P = 0.4431) and district by agro-ecology interaction effect (P = 0.5186) in sucrose content of honey. However, there was statistically a significant difference (P = <.0001) between sources in sucrose content of honey. The mean sucrose content of the retailer shop sources (4.40 ± 0.37 g/100g) was statistically higher than the honey samples collected from the farm gate sources (1.62 ± 0.33 g/100g).

The higher sucrose content of honey from shop as opposed to the farm-gate source of honey might be due to the adulteration of honey by the addition of commercial sugar in honey to increase the volume of honey. Additionally, it might be due to the early harvest of honey before sucrose is converted into fructose and glucose (shop traders might purchase unripe honey from their customers).

In line with this result, Eyobel and Miresa (2017) reported the mean sucrose content of 2.60 ± 0.51 g/100g from the Adaberga district of West Shewa zone. Nevertheless, higher than the current finding Alemayehu and Nuru (2011), Chala et al. (2011), Awaris et al. (2014), Addis and Malede (2014) and Abebe (2017) reported the mean of 4.1 ± 1.2 g/100g, 7.55 ± 4.03 g/100g, 4.46 ± 2.59g/100g, 7.55g/100g, and 4.04g/100g sucrose content, respectively, from the different locations of the country. The variation in sucrose content from different parts might be due to harvesting, handling practices and flora sources. The low sucrose content of the studied honey samples indicated that honey produced from the study areas (farm gate) was natural and free of any adulteration. International Regulatory Standards restricted sucrose content not to be greater than 5g/100g of honey. Few nectar source plants are exempted from this restriction.

3.10. Hydroxyl-methyl furfural (HMF)

The mean HMF content of the current finding was 3.42 ± 1.95 mg/kg ranging from 0.00-25.6 mg/kg. The result is within the acceptable range set by QSAE (2005), CAC (2001) and Council (2002), i.e. 40, ≤40 and ≤60, respectively (Tables 2 and 3). Significant difference was not observed between districts, agro-ecologies and district by agro-ecology interaction effect (Table 4). However, there was a significant difference (P = 0.0058) between sources in the accumulation of HMF in honey. The
mean HMF content of the retailer shop honey (10.25 ± 4.99 mg/kg) was higher than the honey samples collected from the farm gate sources 0.00 ± 0.00 mg/kg. This could be due to storage time, heating the honey while crystallized at the time of marketing and adulteration with invert sugar in retailer shop source (Bogdanov et al., 2009).

In general, the current finding revealed that all honey samples of the study area fulfill the national and international standards. Even though HMF is naturally found in honey, in the current study about 83.3% were free of HMF, which might be due to freshness of the honey. In fresh honey, there is practically no HMF, but it increases upon storage, depending on the pH of honey and the storage temperature and condition (Bogdanov et al., 1999).

This result disagrees with Awraris et al. (2014) who reported 19.52 ± 9.41 mg/kg in the Gesha, Masha, and Sheko districts, of Southern Ethiopia. But, the current result is in line with the finding of Chala et al. (2011) who reported 6.32 ± 4.90 mg/kg HMF in a honey sample collected from Gomma district, Southwestern Ethiopia. Lower than the current result Sisay et al. (2012) reported a mean HMF of 1.8 mg/kg from Homessa district of western Ethiopia.

### 3.11. pH of honey

The mean pH value of evaluated honey sample was 4.03 ± 0.21 (Tables 2 and 3), which is within the acceptable standard of CAC (2001). The honey is among the best quality to be stored for longer time. Significant difference (P = 0.0180) was declared between districts concerning the pH of honey. The mean pH of the Doyogena district honey (3.67 ± 0.12) was lower (acidic) than the Kachabira district honey 4.30 ± 0.46. The dissimilarities in pH might predominantly be caused due to different acids found in different floral types (geographical condition). There was no significant difference between agro-ecologies (P = 0.2597) and district*agro-ecology interaction effect (P = 0.0958) in pH of honey and the same was true for honey samples collected from different sources.

pH value is an important variable in the control of spoilage of foods. The low pH content of the honey sample in the current study is very desirable as it discourages the growth of micro-organisms (Ananias et al., 2013).

The current result is comparable with Eyobel and Miresa (2017) who reported the mean pH of 4.01 ± 0.19 from Jeldu districts, of West Shewa zone. However, Chala et al. (2011), Addis and Malede (2014) and Abebe (2017) reported a mean pH of 3.81 ± 0.60, 3.81 and 3.85 ± 0.46, respectively from different locations of the country which are higher than the current result. Bogdanov et al. (2007) stated that honey is naturally acidic with pH content ranging from 3.7 to 4.5 for blossom honey and pH 4.5 to 6.5 for honeydew honey, irrespective of geographical origin. Hence, the study area honey can be categorized as blossom honey and lies within the indicated quality range of 3.7–4.5 (Bogdanov et al., 2007).

### 3.12. Free acidity

The mean honey free acidity of the study area was 13.39 ± 1.43 meq per kg (Tables 2 and 3). The Free acidity values of all honey samples were within the acceptable range of QSAE (2005), 40 meq/kg, CAC (2001), <40 meq/kg and Council (2002), ≤50 meq/kg. Almost all honey samples satisfies the requirement of the national and international standards. There was no statistically significant difference between districts (P = 0.5067) and district by agro-ecology interaction effect (P = 0.6876) in free acidity of the honey. There was significant difference (P = 0.0084) between agro-ecologies. The mean free acidity result of highland agro-ecology honey (13.17 ± 2.53 meq/kg) was statistically higher than the midland agro-ecology honey (7.67 ± 0.42 meq/kg).

The variation in free acidity between agro-ecologies might be due to differences in geographical conditions. Additionally, significant difference (P=0.0001) was observed between sources of honey samples concerning the free acidity of honey. The mean free acidity result of retailer shop sources of honey (19.33 ± 0.71 meq/kg) was statistically higher than the mean free acidity of the honey samples collected from the farm gate sources (10.42 ± 1.48 meq/kg). The higher free acidity level of the retailer shop source sample as compared to the farm-gate source honey might be due to the presence of unwanted fermentation and poor handling practices. The higher moisture content of the honey collected from shop predisposes the honey to undergo fermentation by microbial action. Higher moisture content creates conducive condition for microbial growth that leads to the development of free acidity of the honey from retailer shop sources.

Alemayehu and Nuru (2011) reported 19.32 ± 5.24 meq/kg free acidity level in the silte district which is higher than the current finding. Chala et al. (2011) reported higher free acidity result of 28.24 ± 3.47 meq/kg from Gomma district of Southwestern Ethiopia. Awraris et al. (2014) also reported higher free acidity result of 28.32 ± 14.14 meq/kg from Masha, Gesha and Sheko districts, of Southern Ethiopia. The lower acidity of the study area, in general, might be due to the freshness of honey sample while collected and analyzed, and nonexistence of
unwanted fermentation. In general, the honey of the study area is of good quality that meets the national and international standards set by QSAE (2005), CAC (2001), and Council (2002) concerning acidity.

4. Conclusion and recommendation

The finding of this study indicated that all the physicochemical parameters of honey evaluated lied within the range of national as well as international standards. Some of the parameters were noticeably influenced by both agro-ecologies and honey sources. The influence of agroecology on the physicochemical properties of honey was detected significantly. Honey collected from the highland agro-ecology was characterized by having higher moisture, free acidity, ash, and electrical conductivity as compared to midland agro-ecology. Honey collected directly from the farm gates was found to be superior by many of the parameters evaluated to that of the retailer shop source of honey. The color of honey in the study area varied from extra light amber to amber with the extra-light amber (66.7%) being the dominant one. Appropriate honey handling should be practiced along the value chain to keep the quality of honey. Training should be offered for all actors along the value chain to minimize quality deterioration.

Declarations

Author contribution statement

Teshale Tigistu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Abdo Mohammed, Zemene Worku: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Funding statement

This work was supported by Kembata Tambaro zone Administrative office and Jimma University College of Agriculture and veterinary medicine.

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

No additional information is available for this paper.

Acknowledgements

We would like to acknowledge Holetta bee research center for analyzing honey samples.

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