



EFFECT OF SEASON ON THE MAJOR PHYSICO-CHEMICAL PARAMETERS OF HONEY PRODUCT

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Abstract

The study was conducted to determine the major physico-chemical parameters and to investigate the effects of season on the quality of honey product collected from Dawuro zone, Southern nationalities of people region of Ethiopia. The contents of physico-chemical parameters within the three honey production seasons ranged as: moisture content, 15-18%; electrical conductivity, 0.27-0.31mS/cm; pH, 4.0 - 4.5; free acidity, 18.7 - 24.7meq/kg; lactone acidity, 5.7 - 8.0meq/kg; HMF content, 16.1-20.3mg/kg; reducing sugars, 70 - 72g/100g and sucrose content from 2.4 - 2.8g/100g. This study showed that there were some significant variations for the contents of the different physicochemical parameters between the different honey production districts and seasons. It was confirmed that the results were below the maximum permissible limit to conform to international standards concerning the good quality of honey. Although, the honey collected in this area should be consumed as fresh to avoid further contamination. Moreover, further studies of other quality parameters of honey are needed.

Keywords: Acidity, Dawuro, electrical conductivity, hydroxymethylfural, moisture, sugars and quality

1. Introduction

Honey is a thick, golden liquid produced by industrious bees and saved inside the beehive for eating during times of scarcity [1, 2]. It is an ingredient of several industrial products and has been used for a long time because of its antioxidant and antibacterial properties [3-5].

The major ingredients of honey include a mixture of carbohydrates, organic acids, amino acids, proteins, minerals, vitamins and lipids [1, 6,7]. The presence of these major food ingredients in honey made human beings to use it as food sweetening and other human health care. As a result, human beings remove and collect it from

the honeycomb and use for different usages without further purification and the quality of honey is unknown. Different factors such as nectar source of the plant, the variety of bee, seasonal variation and improper handling and harvesting may contaminate and reduce honey quality [8-11]. There are different types of honey quality parameters, but in this study moisture content, electrical conductivity, pH and acidity, the content of 5-hydroxymethyl-2-furaldehyde (HMF), and reducing sugars and sucrose contents were considered.

Carbohydrates are the primary components of honey especially for blossom honey glucose and fructose concentrations must

be $\geq 60\text{g}/100\text{g}$ and sucrose concentration $\leq 5\text{g}/100\text{g}$ [2, 6, 12, 13]. Water is the second-highest component of honey and low water content is desirable because honey may begin to ferment and lose its fresh quality if it is above 20% [2, 14]. Electrical conductivity is an important criterion to determine the botanical origin of honey and depends on the mineral content, organic acids and proteins of honey [15-17]. Naturally, all kinds of honey are acidic generally with the pH values between 3.4 and 6.1 [1, 18]. The free acidity of honey was also mostly due to the presence of mineral and organic acids [19, 20].

Honey might also contain 5-hydroxymethyl-2-furaldehyde (HMF) which is a water-soluble heterocyclic organic compound derived from the acid-catalyzed dehydration of hexose sugars [21-23]. HMF is only present in trace amount in fresh honey and its concentration has been reported to increase with storage and the prolonged heating of the honey [10, 11, 24,25]. The amount of HMF present in honey is the reference used as a guide; the higher the HMF value, the lower the quality of the honey is considered.

In Dawuro zone there are different apiculture areas with high potential of honey production in the different season especially during October, December and around June but there was no documented data concerning the quality of honey which makes this zone impossible to be competent in the honey commercial market. Therefore, the main purposes of this study were determining the quality of honey and to generate quality information that can be used as baseline data to create international market linkage.

Abbreviations:

EC: electrical conductivity; FA: free acid; HMF: 5-hydroxymethyl-2-furaldehyde; LA: lactone acidity; SNNPR: Southern

Nation Nationalities and People Region; SD: standard deviation; SPSS: Statistical package for the social sciences

2. Materials and methods

2.1. Description of the study area

The study was conducted in Dawuro zone, SNNPR, Ethiopia with four woredas (districts) namely Loma, Mareka, Tocha and Isara that were selected purposefully depending on the areas where the high honey product is available. Dawuro zone is located in southern nation nationalities and people regional (SNNPR) state of Ethiopia with an approximately 500km from Addis Ababa the capital city of Ethiopia and it is found between 6°36' to 7°21' north latitudes and 36°68' to 37°52' east longitudes with an altitude of 500 to 3000 meters above sea level.

2.2. Sample Collection

The honey samples were collected in the three different honey production seasons in a year; December (2015), June and October (2016) for each woreda. The collected honey samples were labeled and transported to Wolaita Sodo University and stored at room temperature for 5 - 6 months until sampling preparation and analysis.

2.3. Preparation and analysis of the honey sample

Unwanted materials such as wax, sticks, dead bees and particles of combs were removed by straining the samples by clean cloth before analysis. All measurements during each season were carried out in triplicate to determine the physicochemical properties of honey.

The moisture content of each honey sample was determined by weighing 5g of the sample on a pre-weighed drying dish and the samples were dried at 105°C to

constant weight in an oven (DHG-9055A, Yunboshi, Jiangsu, China)[26].

$$\text{Moisture (\%)} = \frac{W_1 - W_0}{m} \times 100$$

where W_0 is the weight of the empty dish, W_1 is the weight of dried sample + dish and m mass of sample taken

The electrical conductivity of each sample was measured on a solution of 20% honey dray matter at 20 °C with a previously calibrated conductivity meter (HQ40D, Hach Company, Colorado USA).

Free acid, lactones, and pH were determined by equivalence point titration[14, 27]. Five grams of honey [M] were dissolved in a few milliliters of water. The solution was transferred quantitatively to a 50 ml volumetric flask and filled to the mark with water. Twenty-five mL of solution was dropped into a 250 ml beaker in the presence of a bar magnet. The initial pH (pHi) was recorded and titrated first with the sodium hydroxide solution (up to 10 ml), (free acid determination) then (into the same beaker) with the sulphuric acid solution (up to the second equivalence point) for lactone determination. The free acidity (FA) is expressed in milliequivalents of sodium hydroxide required to neutralize 1 kg of honey.

$$\text{F.A.} = V \times T \times (50/25) \times (1000/M)$$

The lactone acidity (LA) is expressed in the same units:

$$\text{L.A.} = [(10-V) \times T - 0.05 \times V] \times (50/25) \times (1000/M)$$

Note from the curve the free acidity neutralization volume in ml [V] and the sodium hydroxide excess neutralization volume (corresponding to pH 7) in ml [V].

The amount of HMF was determined by measuring the UV absorbance of HMF at 284nm by using UV-visible digital spectrophotometer (Model 371, Electronics India, and New Delhi India). To avoid the interference of other components at this wavelength, the difference between the absorbance of a clear aqueous honey

solution and the same solution after the addition of bisulfate was determined.

The HMF content was calculated after the subtraction of the background absorbance at 336nm [14]. The HMF content of the samples was calculated by the following formula:

$$\text{HMF (mg/kg)} = (A_{284} - A_{336}) \times 149.7 \times 5 \times D / w$$

where A_{284} = absorbance at 284nm, A_{336} = absorbance at 336nm, 149.7 = factor

W = weight of sample taken and D = dilution factor, if dilution is necessary

The apparent reducing sugars and content of sucrose were determined by the modification of Lane-Eynon procedure involving the reduction of Soxhlet's modification of Fehling's solution by titration at the boiling point against a solution of reducing sugars in a honey solution using methylene blue as an internal indicator[14, 28].

Two grams and six decigrams of honey were weighed and transferred to 500mL volumetric flask. Five milliliters of standardized Fehling A and B solutions were transferred to a 250mL Erlenmeyer, with 7mL of water and 15mL of honey solution. The Erlenmeyer was heated and 1mL methylene blue (0.2 %) was added. Titration was conducted by adding the diluted honey solution until the indicator was decolorized. Determination of sucrose content was carried out by inversion, adding 10mL of diluted HCl, 50mL diluted honey solution and water to a 100mL volumetric flask, heating in a water bath, then cooling and diluting to mark. Finally, the Lane-Enyon method was applied and sucrose content was obtained by difference.

The percentage of reducing sugar before and after inversion was calculated as follows;

$$C = \frac{2}{W_2} \times \frac{1000}{Y_2}$$

where C = g reducing sugar before inversion/100g honey

W_2 = weight (g) of honey sample and Y_2 = volumes (mL) of dilute solution consumed

$C' = \frac{2}{W_2} \times \frac{1000}{Y_2}$, Where C' = g reducing sugar after inversion/100g honey

W_2 = weight (g) of honey sample and Y_2 = volumes (mL) of dilute solution consumed

The percentage of non-reducing sugar (sucrose) was estimated by subtracting reducing sugar content before inversion from reducing sugar after inversion and then multiplying by a common factor:

$$\text{Sucrose content (\%)} = (C' - C) \times 0.95$$

2.4. Data analysis

The data were collected, coded and entered in a Microsoft spreadsheet excel and

analyzed in SPSS (version 16). ANOVA was used to assess the association of each season and location on quality parameters. Further analysis on differences among the means was determined using the Tukey post hoc tests at a 5% significance level.

3. Results

The results of different physic-chemical parameters are shown in tables 1, 2 and 3 below for mentioned woredas; Loma, Mareka, Tocha and Isara during three honey production seasons.

Table 1
Results of the physico-chemical parameters of honey in season one (December 2015)

| Place | Physicochemical results | | | | | | | |
|--------|-------------------------|------------|---------|-------------|-------------|-------------|-----------------------|----------------|
| | Moisture (%) | EC (mS/cm) | pH | FA (meq/kg) | LA (meq/kg) | HMF (mg/kg) | Reducing sugar g/100g | Sucrose g/100g |
| Loma | 15.1±0.2 | 0.31±0.02 | 4.1±0.1 | 20.3±0.6 | 6.2±0.3 | 19.0±0.4 | 73.1±0.5 | 3.9±0.1 |
| Mareka | 20.0±0.7 | 0.22±0.02 | 4.1±0.1 | 28.3±0.6 | 9.9±0.4 | 20.2±0.1 | 69.4±0.4 | 3.1±0.1 |
| Tocha | 15.5±0.6 | 0.26±0.03 | 4.1±0.1 | 20.8±0.8 | 5.7±0.3 | 18.5±0.3 | 71.4±0.6 | 2.2±0.2 |
| Isara | 15.4±0.5 | 0.45±0.03 | 3.9±0.1 | 29.2±0.7 | 10.1±0.6 | 23.4±0.6 | 66.5±0.2 | 2.2±0.2 |

Results represent the average of three measurements ± SD

Table 2
Results of the physico-chemical parameters of honey in season two (June 2016)

| Place | Physicochemical results | | | | | | | |
|--------|-------------------------|------------|---------|-------------|-------------|-------------|-----------------------|----------------|
| | Moisture (%) | EC (mS/cm) | pH | FA (meq/kg) | LA (meq/kg) | HMF (mg/kg) | Reducing sugar g/100g | Sucrose g/100g |
| Loma | 17.0±0.3 | 0.27±0.03 | 4.3±0.1 | 15.0±0.6 | 4.2±0.3 | 17.7±0.2 | 69.3±0.5 | 2.4±0.2 |
| Mareka | 19.1±0.4 | 0.26±0.04 | 4.2±0.2 | 23.7±0.7 | 4.7±0.4 | 20.0±0.5 | 69.7±0.2 | 2.3±0.1 |
| Tocha | 18.1±0.4 | 0.25±0.04 | 4.4±0.2 | 17.2±0.6 | 4.5±0.2 | 16.1±0.3 | 66.9±0.4 | 1.9±0.1 |
| Isara | 16.9±0.4 | 0.30±0.04 | 4.1±0.2 | 30.7±0.5 | 10.2±0.2 | 25.7±0.3 | 73.5±0.2 | 2.9±0.2 |

Results represent the average of three measurements ± SD

Table 3
Results of the physico-chemical parameters of honey in season three (October 2016)

| Place | Physicochemical results | | | | | | | |
|--------|-------------------------|------------|---------|-------------|-------------|-------------|-----------------------|----------------|
| | Moisture (%) | EC (mS/cm) | pH | FA (meq/kg) | LA (meq/kg) | HMF (mg/kg) | Reducing sugar g/100g | Sucrose g/100g |
| Loma | 15.4±0.5 | 0.29±0.05 | 4.4±0.3 | 18.2±0.6 | 6.7±0.4 | 16.2±0.2 | 71.7±0.30 | 2.9±0.2 |
| Mareka | 13.7±0.4 | 0.28±0.04 | 4.9±0.2 | 13.3±0.4 | 4.8±0.1 | 12.1±0.3 | 72.1±0.26 | 2.5±0.2 |
| Tocha | 17.1±0.1 | 0.28±0.04 | 4.3±0.3 | 25.7±0.6 | 5.1±0.2 | 14.6±0.3 | 68.9±0.48 | 2.1±0.1 |
| Isara | 13.5±0.7 | 0.32±0.04 | 4.5±0.2 | 17.5±0.4 | 6.2±0.3 | 21.6±0.2 | 75.4±0.42 | 3.7±0.2 |

Results represent the average of three measurements ± SD

In season one (December 2015) the moisture contents of the samples varied from 15.1% in Loma to 20.0% in Mareka with the significant difference ($p = 0$) only between Mareka and other three places. For the second season (June 2016), the moisture content varied from 16.9% in Isara to 19.1% in Mareka with significant differences between Loma and Mareka ($p = 0$), between Mareka and Isara ($p = 0$) and between Tocha and Isara ($p = 0.02$). The moisture content of honey during the last production season (October 2016) was ranged from 13.5% in Isara to 17.1% in Tocha with an insignificant difference ($p = 0.95$) only between Mareka and Isara.

In season one the electrical conductivity of the samples ranged between 0.22mS/cm in Mareka to 0.45mS/cm in Isara with insignificant difference between Loma and Tocha ($p = 0.20$) and between Mareka and Tocha ($p = 0.23$), in season two it was between 0.25mS/cm in Tocha and 0.30mS/cm in Isara while in the third season electrical conductivity varied from 0.28mS/cm in Tocha to 0.32mS/cm in Isara in the four studied areas. During season two and three there was no significant difference in electrical conductivity ($p \geq 0.4$) between the four honey production areas.

The pH value of the samples in season one ranged from 3.9 in Isara to 4.1 in Tocha, during the second season it was between 4.1 in Isara and 4.4 in Tocha and in the third season it was in the range of 4.3 in Tocha to 4.9 in Mareka which indicates the honey samples were acidic. The statistical analysis in all seasons showed that there was no significant difference ($p \geq 0.6$) in pH value between different districts. The free acidity of the samples in the first season was between 20.3meq/kg in Loma and 29.2meq/kg in Isara while the lactone content was ranged between 5.7 to 10.1meq/kg. In this season free acidity showed the insignificant difference

between Loma and Tocha ($p = 0.81$) and between Mareka and Isara ($p = 0.4$) while the lactone content showed the insignificant difference between Loma and Tocha ($p = 0.45$) and between Mareka and Isara ($p = 0.88$). In the second season, free acidity was ranged from 15.0meq/kg in Loma to 30.7meq/kg in Isara while lactone content was ranged from 4.2meq/kg in Loma to 10.2meq/kg in Isara. In this season there was a significant difference ($p = 0$) in lactone content only between Isara and the other places. The free acidity of the honey samples in last season was between 13.3meq/kg in Mareka and 25.7meq/kg in Tocha with no significant difference ($p = 0.31$) only between Loma and Isara while the lactone content was between 4.8meq/kg in Mareka to 6.7meq/kg in Loma with no significant difference between Loma and Isara ($p = 0.13$) and between Mareka and Tocha ($p = 0.56$).

The Hydroxymethylfurfural(HMF) content of honey samples during the first season ranged between 18.5mg/kg in Tocha and 23.4mg/kg in Isara, for the second season between 16.1mg/kg in Tocha to 25.7mg/kg in Isara and the third season it was ranged from 12.1mg/kg in Mareka to 21.6mg/kg in Isara. Based on the analysis there was no significant difference ($p = 0.36$) in HMF content only between Loma and Tocha for the first season.

The content of reducing sugar in the first season was between 66.5g/100g in Isara to 73.1g/100g in Loma, for the second season it was ranged from 66.9g/100g in Tocha to 73.5g/100g in Isara with no significant difference ($p = 0.54$) between Loma and Mareka and for the last season reducing sugar was ranged from 68.9g/100g in Tocha to 75.4g/100g in Isara with no significant difference ($p = 0.52$) between Loma and Mareka.

The sucrose content during the first season was between 2.2g/100g and 3.9g/100g

with no significant difference ($p = 0.92$) between Tocha and Isara, for the second season it was ranged from 1.9g/100g in Tocha to 2.9g/100g in Isara with significant differences between Loma and Tocha ($p = 0.02$), Mareka and Isara ($p = 0.02$) and between Tocha and Isara ($p = 0$) whilst for the last season it was ranged from 2.1g/100g in Tocha to 3.7g/100g in Isara with no significant difference ($p = 0.08$) between Loma and Mareka.

4. Discussion

4.1. Moisture content

The moisture content of honey samples in the four different honey production areas in Dawuro zone during a year ranged from 15.3% in Isara to 17.6% in Mareka (table 4) with no significant difference ($p \geq 0.45$) between the four honey production areas.

Table 4
Total mean values of physicochemical parameters of honey in four places for Dawuro district in a year

| Place | Physicochemical results | | | | | | | |
|--------|-------------------------|------------|-----|-------------|-------------|-------------|-----------------------|----------------|
| | Moisture (%) | EC (mS/cm) | pH | FA (meq/kg) | LA (meq/kg) | HMF (mg/kg) | Reducing sugar g/100g | Sucrose g/100g |
| Loma | 15.8 | 0.29 | 4.3 | 17.9 | 5.7 | 17.6 | 71.4 | 3.1 |
| Mareka | 17.6 | 0.26 | 4.4 | 21.8 | 6.5 | 17.4 | 70.4 | 2.6 |
| Tocha | 16.9 | 0.27 | 4.3 | 21.2 | 5.1 | 16.4 | 69.1 | 2.1 |
| Isara | 15.3 | 0.36 | 4.2 | 25.8 | 8.8 | 23.6 | 71.8 | 2.9 |

Results represent the average of the sum of values obtained in the three honey production seasons (season 1, 2 and 3) for each place.

Based on the collection of the season the moisture content was ranged from 14.9% in season three to 17.8% in season two in a year (table 5) and the moisture content was significantly different ($p = 0$) only between season two and season three. The study showed that there were some honey production areas and seasons with lower and some with higher moisture contents, which could be due to the difference in the geographical area and climatic conditions.

The higher the moisture content would lead to fermentation upon storage by osmotolerant yeasts and also an indicator of honey adulteration [29, 30]. The values in both honey production area and collection of the season showed that the honey samples in Dawuro zone have a low possibility of fermentation that fairly agreed with different standards and unions which sets a moisture content below 21% [2, 14, 31].

Table 5
Total seasonal mean values of honey samples in Dawuro district

| Parameter | Moisture (%) | EC (mS/cm) | pH | FA (meq/kg) | LA (meq/kg) | HMF (mg/kg) | Reducing sugar g/100g | Sucrose g/100g |
|--------------|--------------|------------|-----|-------------|-------------|-------------|-----------------------|----------------|
| Season one | 16.5 | 0.31 | 4.0 | 24.7 | 8.0 | 20.3 | 70.1 | 2.8 |
| Season two | 17.8 | 0.27 | 4.2 | 21.6 | 5.9 | 19.9 | 69.9 | 2.4 |
| Season three | 14.9 | 0.30 | 4.5 | 18.7 | 5.7 | 16.1 | 72.0 | 2.8 |

Results represent the averages of the sum of values obtained in four honey production areas (Loma, Maraqa, Tocha, and Isara) for each season.

Comparable reports to our study area were given on Guji zone of Ethiopian honey [32]; with a moisture content of 14.28 - 15.12%, European Atlantic area honey [4] and Lazio region honey [33], which had an average moisture content of 17.6% and 16.36% respectively. Therefore, the moisture content of our honey samples during the three production seasons and those reports point out good maturity of the samples and were considerably free of fermentation.

4.2. Electrical conductivity

The mean electrical conductivity values of honey samples in the four production areas varied from 0.26mS/cm in Mareka to 0.36mS/cm in Isara (table 4) with a significant difference ($p \leq 0.03$) between Isara and other production areas. These range of electrical conductivity values indicated that the honey samples produced in Isara had high mineral, acid or protein contents and the color of the honey samples collected in this area was slightly black which might contribute to high electrical conductivity values [17]. The difference in electrical conductivity in different honey production places was due to the difference in environmental conditions and botanical origin.

The average electrical conductivity of honey in terms of production seasons was between 0.27mS/cm in the second honey production season and 0.31mS/cm in the first honey production season (table 5). The post hoc multiple comparison tests (Tukey) showed that there was no significant difference ($p \geq 0.28$) in electrical conductivity value between the three honey production seasons in Dawuro zone. It was observed that the honey samples during the first honey production season showed high electrical conductivity values than the second and third honey production seasons. The electrical conductivity value is important for differentiating honey of different botanical

origin and indicated that the honey samples in all production areas and seasons were floral (blossom) types [2, 34]. Comparable result to our study area was given in Chubut, Argentina with an average electrical conductivity value of 0.33mS/cm [35]. In contrary to our study high amounts of electrical conductivity values were reported from Harena forest honey, Bale, Ethiopia; 0.7mS/cm [28], west Shewa zone, Oromia Region, Ethiopia; 0.38- 0.65mS/cm [36] and honey from Algeria; 0.42 - 0.81mS/cm [37].

4.3. pH, Free and lactone acidity

All the honey samples in the study areas were acidic with a pH value generally lying between 4.2 in Isara to 4.4 in Mareka (table 4) and showed no significant differences ($p \geq 0.5$) between all honey production areas. Based on the collection season the pH values ranged from 4.0 in the first honey production season to 4.5 in the third production season (table 5) with no significant difference ($p = 0.07$) between season one and season two.

The average free acidity in different woredas ranged between 17.9meq/kg in Loma to 25.8meq/kg in Isara (table 4) with a significant difference ($p = 0.01$) between Loma and Isara. In the same way, the total average lactone acidity was ranged from 5.1meq/kg in Tocha to 8.8meq/kg in Isara with a significant difference ($p \leq 0.03$) between Isara and other honey production areas. The average values of free acidity in terms of collection season varied from 18.7meq/kg in the third honey production season to 24.7meq/kg in the first honey production season and average values of lactone content varied from 5.7meq/kg in the third season to 8.0meq/kg in the first season (table 5). Based on the Tukey test at 0.05 levels, there was a significant difference ($p = 0.02$) for free acidity and ($p = 0.03$) for lactone acidity between the first and third honey production seasons. These variations in acidity values have been

attributed to the difference in harvest season and are fairly below the maximum acceptable limit [2, 20,31].

Previous studies carried out on honey quality in Tepi areas of Ethiopia varied between 3.36 and 4.26 from pH measurements and 18.3 - 25.3meq/kg from acidity measurements [38] and the study carried out in Guji zone of Ethiopia showed an average pH value of 4.45 and 24.08meq/kg in acidity measurements [32]. Abroad Ethiopia analogous results were reported in Turkish blossom honey with a pH of 3.68 - 4.65, free acidity of 3.86 - 30.42meq/kg and lactone content of 0.99 - 9.50meq/kg [39] and for Australian honey with a pH of 4.02 - 4.69, free acidity of 10.25 - 20.34meq/kg except for the considerably high lactone content of 18.5 - 33.2meq/kg [40]. Our study showed that none of the samples during the three honey production seasons exceeded the limit allowed by international regulations. Consequently, the pH and acidity measurements in Dawuro district indicated that the honey samples were nearly acidic which can inhibit the growth of microorganisms and implies low fermentation of honey.

4.4. Hydroxymethylfurfural (HMF)

The mean HMF value in the four honey production areas was ranged from 16.4mg/kg in Tocha to 23.6mg/kg in Isara (table 4) with a statistically significant difference ($p = 0$) between Isara and the other honey production districts. The average HMF value of honey in terms of production seasons in a year varied from 16.1mg/kg in season three to 20.3mg/kg in season one (table 5) with no significant difference ($p = 0.95$) between season one and season two. These range of HMF values indicated that the content of HMF in season one was high compared to others. The values both in studied areas and collection season indicated that the HMF content did not exceed the international

regulations which set a maximum HMF value of 80mg/kg for tropical countries [2, 31, 34].

The HMF content is an indicator of freshness and in fresh honey its value was very low; 1.89 - 8.24mg/kg in Libyan honey [41]; and 0.84mg/kg in Hareenna forest honey, Bale, Ethiopia[28]. This study showed that the HMF value of honey in Dawuro zone was considerably high after six months of storage at room temperature ($< 25^{\circ}\text{C}$) which might be due to environmental, harvesting and storage conditions. Previous studies carried out on Malaysian honey which were stored for 3 - 6 months showed HMF content of 2.80 - 24.87mg/kg, but after 12 - 24 months storage, its value was increased from 128.19 - 1131.76mg/kg [24]. These HMF values indicate that honey must be handled under appropriate temperature and storage conditions. Although Dawuro district of Ethiopia is under the tropical country, the HMF values were within the international standard limit and encouraged the quality criteria for honey.

4.5. Reducing sugars and sucrose

The average reducing sugar content for the different honey production areas varied between 69.1g/100g in Tocha to 71.8g/100g in Isara (table 4) and the values showed no significant difference between all the study districts ($p \geq 0.2$). The average content of reducing sugars in terms of honey collection season varied from 69.9g/100g in season two to 72.0g/100g in season three (table 5) with no significant difference ($p \geq 0.1$) between all collection seasons.

Determination of sucrose content is used to detect adulteration of honey with table sugar or to determine the amount of sucrose naturally found in honey[28]. The content of non-reducing sucrose in the different production areas was varied between 2.1 g/100g in Tocha and 3.1g/100g in Loma (table 4) and Tukey test

indicated that there were significant differences ($p = 0$) in sucrose content between Loma and Tocha and between Tocha and Isara. Sucrose content based on collection season was ranged from 2.4g/100g in season two to 2.8g/100g in season one (table 5). Based on the Tukey tests, there was no significant difference ($p \geq 0.1$) in sucrose content between the three honey production seasons. The low amounts of sucrose content in our study ($< 5\%$) indicated a good maturity of honey and good conversion of sucrose to fructose and glucose. All these sugar measurements met the requirements of blossom kinds of honey and showed the absence of adulteration. The variation in the collection season and region caused changes in the composition of honey [42] however, in our study the variations were not that much necessary.

The sugar values of this study area were analogous with other reports such as Ethiopian Sekota honey; 63.4 - 71.7 g/100g of reducing sugars and 1.0 - 5.2g/100 of sucrose content[43] Algerian honey; 62.8 - 70.0g/100g content of reducing sugars and 1.80 - 2.54g/100g in sucrose content[37]. Turkish blossom honey showed 65.63 - 80.47g/100g of the sum of fructose and glucose but the high amount of sucrose; 2.85 - 8.44g/100g which could be because of inadequate conversion of sucrose to glucose.[39]. European Atlantic honey reported a slightly lower sum of fructose and glucose (65.6g/100g) and low sucrose content (0.37g/100g) than our study [4].

5. Conclusion

The study showed that the variation in the honey production season and area had some effect on the major physicochemical parameters. However, the physicochemical parameters considered indicated that all the values were within

the standard limits given by different organizations. Therefore, the honey samples produced from Dawuro district, SNNPR Ethiopia, could conform to international standards concerning a good level of quality which smooth the progress of its national and international commercialization. Based on the aforementioned conclusion, the honey produced in this area should be consumed as fresh to avoid further contamination and further studies of other quality parameters of honey are needed.

6. Acknowledgments

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7. Competing Interests

The authors declared that they have no competing interests.

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