JIMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCE

DEPARTMENT OF CHEMISTRY

PHYSICOCHEMICAL AND BIOCHEMICAL PROPERTIES OF MINING AND HONEY BEES PRODUCTS FROM JIMMA RARE DISTRICT, HORO GUDURU WOLLEGA ZONE, ETHIOPIA

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ACRONYMS/ABBREVIATION

ANOVA	Analysis of Variance
BH	Blossom honey
CAC	Codex Alimentarius Commission
CSA	Central Statistical Agency
DMSO	Di-methyl Sulfoxide
EC	Electrical Conductivity
ETB	Ethiopian Birr
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
HBH	Honey Bee Honey
HDH	Honeydew honey
HMF	Hydroxylmethylfurfural
IHC	International Honey Commission
MBH	mining bee honey
meq	milliequivalent
N	Number of trials
QSAE	Quality and Standard Authority of Ethiopia
SNNPRS	Southern Nations, Nationalities and Peoples Regional State of Ethiopia
WIS	water insoluble solids

ABSTRACT

Honey is a natural sweet sticky and viscous solution produced by bees from the nectar of flowers or from living parts of plants. The physicochemical and biochemical parameters of honeys are essential for monitoring of the quality of the products, and for proper handling and storages. In the present study, physicochemical and biochemical characteristics of mining and honey bees' products from the Jimma Rare district, Horo Guduru Wollaga Zone, Ethiopia were investigated. The study was conducted on 2 honey bees and 3 mining bee honeys samples which were harvested in January, April and August of 2019. The average mean ± standard deviation value of moisture content, electrical conductivity, pH value, acidity, total ash, water insoluble matter, color analysis, sugar content and Hydroxylmethylfurfural of homogenized bee hive honey samples were 17 ± 0.003%, 0.54 ± 0.002mS/cm, 3.72 ± 0.118, 25.8 ± 0.144 meq/kg, 0.40 ± 0.004%, 0.13 ± 0.007%, 78.66 ± 0.006 mmpfund. 65.31 ± 0.071% and 30.54 ± 0.016 mg/kg, respectively, and also the overall average for mining bee honeys from three different holes of this parameter were 25.67 ± 0.003%, 1.13 ± 0.007 MS/cm, 3.00 ± 0.088, 229.20 ± 0.327 meq/kg , 0.72 ± 0.001%,1.41 ± 0.049%, 258.16 ± 0.018 mmpfund , 159.22 ± 0.269% and 48.16 ± 0.013mg/kg respectively. The results of the study showed that physicochemical and biochemical characteristics of honey bee products were lie within the ranges of national and international standards for honeys. But mining bee honeys were significantly varied from honey bees honey and were also not satisfy the national and international honey quality standards.

Keywords: *Mining bees, Honey bees, Physiochemical; Biochemical parameter.*

1 INTRODUCTION

Honey is naturally sweet substance, produced by honey bees from the nectar of blossoms or from the secretion of living parts of plants or excretions of plant-sucking insects living in parts of plants, which honey bees collect, transform and combine with specific substances of their own store, and leave in the honeycomb to ripen and mature [1]. Ethiopia has huge potential for beekeeping production due to its notable diversity of Agra-climatic conditions and floral vegetation resources. The country takes the total share of honey production around 23.58% and 2.13% of the African and World's, respectively [1]. Yearly honey production of Ethiopia is estimated to be around 54,000 tones, which make the country leading into honey production in Africa and 9th in the world [3].

Honey contains complex chemical compositions such as plant pigments (carotenes, xanthophylls, and chlorophyll), mineral substances, sugars, and various impurities. Its Physiochemical and biochemical properties can vary based on its botanical origin, geographical and entomological source [4]. They can be also vary based on honey harvest season, botanical origin and environmental factors [5]. Honey contains a mixture of substances; mainly sugars and water as well other compounds such as proteins, minerals, enzymes, vitamins, hydroxymethylfurfural (HMF), volatile compounds, flavonoids and phenolic acids [6]. The chemical compositions of honeys are generally associated with two factors: most importantly on the source of nectar and the extrinsic factors. Extrinsic factors consist of local climatic factors, type of soil, altitude and production methods of the beekeepers [7]. Other factors that affect the compositions and quality of honey production is the lack of skilled manpower and training institutions, low level of technology, lack of conservation of natural vegetation, indiscriminate application of Agrochemicals and inadequate research institutions to address some.

Jima Rare district is found in Horo Guduru Wollaga zone, Oromia regional state, Ethiopia. The district is rich in diversified natural and planted trees, shrub species and crops, which produce flowers throughout the year and thus provide sufficient forage for bees to produce honey. In the district honey bee and mining bee honeys are usually harvested. Mining bee honey, locally named as Damma Daamu, is harvested from underground and it characterized by relatively different taste and aroma from the normal honey bee honeys. Mining bee honey is traditionally used for medicinal purpose for treating of cough, asthma, allergic, respiratory illnesses, treatment of wounds and so on. Traditionally, honey is not only used as a natural sweetener but also as a healing agent [8]. The biological properties that make honey ideal as a medicine are: antibacterial, bacteriostatic, anti-inflammatory, wound and sunburn healing effects, antioxidant activity, radical scavenging activity and antimicrobial activity [8, 9].

Honey is generally evaluated by analysis of its physiochemical and biochemical parameters. Some of the major constituents of physicochemical and biochemical parameters involved in honey quality analysis are moisture content, sucrose, pH, electrical conductivity, ash content, free acidity, diastase activity and Hydroxymethylfurfural (HMF) content [10]. The composition and flavor of honey vary with the floral source used by the honeybees, as well as regional, beekeeping practices, environmental climatic variations and honey harvesting season [11]. The quality and properties of honey are relate to honey maturity, production methods, processing and storage conditions, climatic conditions as well as the nectar source of the honey. Carelessly handling can cause deterioration of honey quality that are caused by heating honey at high temperatures, high moisture content, adulteration, poor packaging and poor storage conditions [12].

Although several studies have been carried out in the investigation of physicochemical and biochemical parameters of honey bees (beehive) product of different origin [8 -12], attention has not been given to study the physicochemical and biochemical parameters of mining bee honeys. Hence, in this study, physicochemical and biochemical properties of mining bee and beehive honeys which were harvested in January, April and August 2019 from the Jimma Rare district, Horo Guduru Wollaga Zone, Oromia Regional State, Ethiopia were investigated. In the study, was also intended to find out whether the mining bees and beehive honeys, which were harvested from the same area were similar or not. The obtained results were also with the honey quality parameters documented in both national and international guidelines.

1.1 Statement of the problem

Honey is naturally sweet sticky and viscous solution produced by bees from the nectar of flowers or from living parts of plants. It is consumed raw and used as an ingredient in food, cosmetics, natural medicine and as a source of sugar for making wine or local beer. The quality of honey varied based on its physicochemical and biochemical parameters, which could vary based on the maturity of honey, harvesting and storage conditions, production procedures, the nectar source (flora) and climatic conditions. In Ethiopia, in addition to beehive honeys, honeys produced by mining bee are also used for its traditional medicinal values. Mining bee honey is widely produced in underground soil. Although, the distribution and its production level have not been well documented, the product is widely used western part of the country such as in eastern and Horo Guduru Wollaga zones. Mining bee honey is characterized its unique taste and aroma. Traditionally it is used for treating coughs, asthma, allergic, respiratory illnesses, wound treatment and so on. Nevertheless, in the literatures, there is no report on the physicochemical and biochemical parameters mining bee honeys. Therefore, in this work, the physicochemical and biochemical properties of mining bee honey and beehive honey harvested from the same region were investigated. Accordingly, Jimma Rare district from Horo Guduru Wollaga, Ethiopia, where both mining bee and beehive honeys are widely harvested and consumed was used as a study site.

1.2 Objectives

1.2.1 General objective

The main objective of this study was to investigate the physicochemical and biochemical characteristics of mining and honey bees' honeys.

1.2.2 Specific objectives

- ❖ To evaluate the physiochemical properties of mining bee and beehive honeys collected from the Jimma Rare District, Horo Guduru Wollega Zone, Oromia regional State, Ethiopia.
- ❖ To evaluate the biochemical properties of mining and beehive honeys of the Jimma Rare District.
- ❖ To compare the physiochemical and biochemical properties of mining bee and beehive honeys of the district.
- ❖ To compare the physiochemical and biochemical properties of the studied honeys with national and international standards.

1.3 Significance of the study

The study findings would have the following significances:

- ❖ It could have a high significance in creating awareness about the physicochemical and biochemical parameters of mining and honey bees' products of the area.
- ❖ It has also great value to know whether mining bee and beehive honeys harvested from the same area have similar physicochemical and biochemical parameters or not.
- ❖ The obtained findings could be used as background information for the concerned body (governmental and nongovernmental organization) to give attention on production and conservation mining bee honeys.
- ❖ The document could be used as reference for other researchers.

2 LITERATURE REVIEW

2. 1 Definition of honey

Honey is a natural sweet substance produced by honey bees using nectar that is collected by the bees from the nectar of plants. For a century, honey has been used for nutrition in different cultures and it has also been used as a traditional medicine due to its healing properties [13]. The nectar gathered is slowly transformed into honey, through a long process involving the addition of enzymes and the gradual reduction of moisture. The chemical composition of honey varies depending on the plant source, season and production methods.

Honey is the oldest and only available unique natural sweetener to mankind and is the last of natural, unprocessed food to be consumed [14]. Honey is used for nutritional, medicinal and industrial purposes and it is an important commodity in the international market. On the nutritional level, honey is a food of first category, high energy value, presenting certain therapeutic properties and industrial purposes and it is an important commodity in the international market [15].

2.2 Uses of honey

Honey is a viscous fluid and consumed raw around the world. It is used as an ingredient in food, cosmetics, and natural medicine; as a source of sugar for making wine or beer; and as a barter commodity of, cash crop, and export crop. Honey has numerous uses and functional applications in traditionally such as in food systems, religious ceremonies as well as for medicinal purpose. Honey has been used by humans since early ancient times as both a dietary source and sweetener, and until recent times it is also highly regarded as a traditional medicinal treatment for many ailments [16]. On the nutritional level, honey is a food of first category, high energy value and presenting certain therapeutic properties. Honey is not only used as a sweetener and flavour enhancer. It is also consumed as food and drinks, and used as ingredients for bakery, beauty and health products.

Honey is the most important primary product of beekeeping in Ethiopia both from a quantitative and economic point of view. About 80% of the total honey produced in the country goes into making, a local beverage, also known honey wine or honey beer, that is made in the homes of farmers or in urban area and only a very small quantity goes for export mainly to Saudi Arabia and Yemen [17]. For those who prefer non-alcoholic drinks, honey is a tasteful sweetener of juices, cocktails and teas to produce a popular soft drink made out of honey in the country. Mining bee honey (Damma Daamu) is one of the types of honey that is used traditionally for nutritional and medicinal purposes like asthma, bronchitis, etc. throughout the society.

2.2.1 Medical uses of honey

Honey is a collection of nectar from many plants processed by honey bees. This natural product is well known for its high nutritional and intended to prevent disease as medicinal value. For a long period of time honey have been used as a medical remedy and was advocated as an excellent source of energy and a panacea for various illnesses. Whilst Hippocrates (3^{rd} and 4^{th} centuries BC) made little use of drugs in treatment he prescribed a simple diet, favoring honey given as oxymel (vinegar and honey) for pain, hydromel (water and honey) for 'thirst', and a mixture of honey, water and various medicinal substances for acute fevers [18]. It has been used not only in foods and beverages as a sweetener and flavoring, but also in medicine since the early human beings. The role of this product in the treatment of burns, gastrointestinal disorders, respiratory illnesses, infected and chronic wounds, skin ulcers and cancer has been studied recently by many researchers [18].

2.2.2 Antimicrobial activity of honey

Honey has been demonstrated in many studies to have antibacterial effects, attributed to different factors. The unique antibacterial initiators of honey are: high sugar content (osmolality), low water activity, hydrogen peroxide, the presence of strong acids, flavonoids and phenolic acids, methylglyoxal and bee defensible [19].The antimicrobial properties of honey are predominantly due to hydrogen peroxide which is used in the treatment of wounds and gastrointestinal diseases such as dyspepsia, bacterial gastroenteritis, gastric and duodenal ulcers [20].

Up-to-date research has highlighted that, because of its antioxidant activity, honey could play an interesting role in the management of oxidative stress-associated chronic diseases [21]. The use of honey in the treatment of chronic wounds and diabetic ulcers, cataracts and other eye ailments and peptic ulcers and other gastric ailments has been documented. This beneficial role of honey is attributed to its antibacterial activity [22]. The presence of hydrogen peroxide, as well as some minerals (particularly copper and iron), in honey, may lead to the generation of highly reactive hydroxyl radicals as part of the antibacterial system [23].

2.2.3 Antioxidant activity of honey

The antioxidant capacity of different honeys depends on the floral sources used by bees to collect nectar, seasonal and environmental factors, as well as processing ways. [24] Unlike other sweeteners, honey has shown antioxidant activity that provides this food with nutritional and technological advantages. Phenolic compounds (flavonoids, phenolic acids), as well as melanoidins (Maillard reaction products), appear to be the most important constituents of honey responsible for its antioxidant activity [25]. Antioxidant substances have different mechanisms of action, among them decrease of the adverse consequences of reactive oxygen and nitrogen species, inhibit the enzymes responsible for producing superoxide anions, metal chelation, radical chain reactions breaking, and eventually, they can play a preventive role inhibiting the reactive oxidants from being formed [26]. Honey’s antioxidant capacity was significantly lower after being stored at room temperature throughout one year [27]. Darker honey is likely to have a higher antioxidant contents than light colored honey. As well, the antioxidant content is higher in honey with higher water content [28].

2.3 Physicochemical parameters

Physicochemical analysis of honey enables to form standards for genuine honey samples of any region based on various physicochemical characteristics. Moreover, the physicochemical data of any honey sample is essential for production process, storage purpose and marketing. Physicochemical parameters of natural honeys content have been strictly defined because of quality indicators of individual honey varieties. The quality of honey is generally evaluated in terms of its physicochemical properties which influence its storage, granulation, texture, flavor and the nutritional quality. Physical properties of honey are related to its state, age, presence of water and level of crystallization.

2.3.1 Moisture

The water content of honey is the quality aspect that determines the ability of honey to remain fresh and to avoid spoilage by yeast fermentation. The lower the water content the higher the perceived value of the honey. However, due to honey's high sugar concentration yeasts in honey are less likely to cause fermentation in honey with low water content. In honey with high water content the yeast is more likely to cause fermentation during storage; resulting in higher acidity; directly affecting the honey's quality. It has been reported that higher water content might cause undesirable honey fermentation during storage and formation of acetic acid [29]. In most cases the storage conditions of honey may change its moisture contents after extraction. The moisture is the most important characteristics of honey that influence its quality and granulation property [30]. Properly harvested honey is a viscous liquid with a water content near 18% [31]. The water content of honey depends on humidity levels in the hive during harvest season, the original moisture of the nectar conditions, and the timeliness of extracting the honey from the comb [32]. Moisture and ash contents were determined on the basis of the total weight loss principle of foodstuff [33].

2.3.2 pH and electrical conductivity of honey

The free acidity of honey is the sum of all the free acids expressed in mill equivalents per kilogram of honey. The variation in free acidity among the different honeys could be due to the different floral origins or to the variation in harvest seasons [34]. It has been reported that high free acidity values may indicate the fermentation of honey sugar by yeasts. During fermentation, glucose and fructose are converted into carbon dioxide and alcohol. Alcohol is further hydrolyzed in the presence of oxygen and converted into acetic acid. Thus, it is greatly contributes to the level of free acidity in honey [34]. The acidity of honey is due to a large number of organic acids. The main acid is gluconic acid, which is in equilibrium with its lactones or its esters and inorganic ions such as phosphates and chlorides. The free acidity was expressed in milliequivalent of NaOH required to neutralize 1 kg of honey. Figure 1 shows the reaction of the enzymatic method by glucose oxidase.

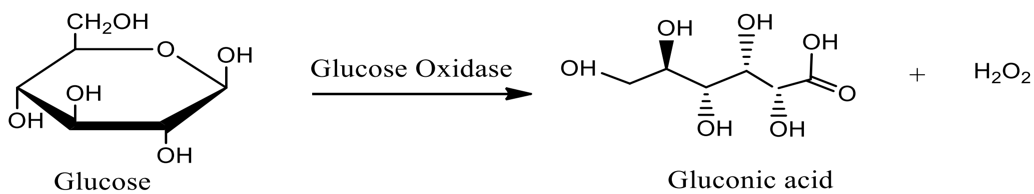

Figure 1: Reaction of the enzymatic method of glucose oxidase.

It influences honey texture, stability and shelf life [36]. The electrical conductivity (EC) of honey is closely related to the concentrations of minerals or total ash, salts, organic acids and proteins is a parameter that shows great variation according to the honey's floral origin [37]. Honeydew contains considerably higher amounts of minerals compared to blossom honeys. Generally honeydew honeys have an electrical conductivity higher than 0.8 mill Siemens per centimeter blends between blossom and honeydew honeys have conductivity values between 0.51 and 0.79 mill Siemens per centimeter [38]

2.3.3 Ash content

The ash represents the mineral residue of the honey after incineration. The mineral content in honey is generally small and depends on the nectar composition of the predominant plants in their formation. The variability in ash contents has been associated in a qualitative way with different botanical and geographical origins of honeys [39]. The determination of this parameter gives an insight of the honeys' quality, as the blossom honeys have a lower ash content than the honeydew honeys [40]. Thus ash content is a quality criterion for honey origin, the blossom honeys having lower ash content than the honeydew ones.

2.3.4 Water insoluble matter

Honey water insoluble matter includes pollen, honeycomb debris, died bee and filth particles and is thus a criterion of honey cleanness. The measurement of water insoluble matter is an important means to detect honey impurities that are higher than the permitted maxima, which is 0.1 g per100 g of honey [41].The water insoluble content is directly dependent upon honey handling and high concentrations are a sign of improper handling during harvest [42].

2.3.5 Color

Color is a physical property immediately perceived by consumers and is an important parameter for evaluating honey quality. Color is related to the botanical origin, climate and soil conditions. Some authors have reported that pollen; sugars related products, carotenoids, xanthophylls, anthocyanins, minerals, amino acids and phenolic compounds, mainly flavonoids, influence the honey color [43]. Honey color standard designations are expressed using the Pfund scale according to the USDA classification shown in Table 1 [44].

Table 1: Color designations of honey

USDA color standard designation	Color ranges Pfund scale (mm)
Water White	≤ 8
Extra white	> 8 and ≤ 17
White	> 17 and ≤ 34
Extra Light Amber	> 34 and ≤ 50
Light Amber	> 50 and ≤ 85
Amber	> 85 and ≤ 114
Dark Amber	> 114

Dark honeys possess a higher mineral content, dextrin and polyphenol contents, and higher acidity than light honeys [45]. The color of dark honeys is strongly correlated with concentrations of Cd, Fe and Pb, while the color of pale honeys with concentrations of Al and Mg [46]. Generally the lightest ones achieve higher prices in the market, but there are some countries such as Germany, Switzerland, Greece and Turkey, where dark honeydew honeys are preferred [47]. Components that could affect darkening are sugars, nitrogen content, free amino acids and moisture [48].

2.4 Biochemical properties

2.4.2. Sugar content

Honey is a supersaturated sugar solution, where carbohydrates are the main constituents accounting for about 95% dry matter [49]. The most important physicochemical and nutritional properties of honey, such as sweetness, viscosity, granulation, hygroscopicity, specific rotation and energy value depend on sugars' composition [50]. Moreover, the osmotic pressure produced by high sugar concentration is an important honeys' antimicrobial factor [51]. As a food commodity, honey has been used for centuries as a sweetener and human energy source. The monosaccharide's (hexoses) fructose and glucose are the main honey sugars. The predominance of these simple sugars, especially fructose gives honeys most of its nutritional and physical characteristics such as crystallization, hygroscopicity and viscosity. Sugars predominate the composition of honey; among them glucose and fructose are the prominent monosaccharaides (60-85% of honey solids) which account for 85-95% of the honey carbohydrates [52].

2.4.3 Determination of hydroxymethylfurfural

Hydroxyemthylfurfiral (HMF) is a breakdown product of fructose that is formed slowly and naturally during the storage of honey and much more quickly when honey is heated [53]. HMF occurs naturally over time in most honeys from the decomposition of fructose in acidic conditions [54]. Figure 2 shows how fructose is converted to HMF.

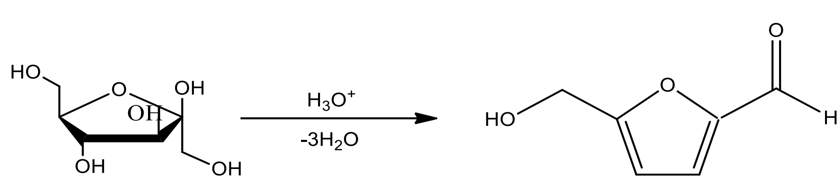

Figure 2: Formation of hydroxymethylfurfural from fructose.

The low level of HMF parameter of honey indicates the freshness of honeys, since it is absent or present in trace amounts in fresh honeys. International regulations set a maximum HMF content of 40 mg/Kg [55]. The HMF content is indicative of honey freshness [56]. Several factors influence the levels of HMF, such as temperature and time of heating, storage conditions, pH and floral source, thus it provides an indication of overheating and stored in poor conditions [57].

3. MATERIALS AND METHOD

3.1 Study area

The study was conducted on mining bee and beehive honey that were harvested from Jimma Rare District, Horo Guduru Wollega Zone, Oromia Regional State, Ethiopia. Wayu is the administrative town of the district which is found from Adds Ababa at 160.275 Km is located at 2348 m above sealeval. The district has diversified Agro-climate with natural floras that are suitable for beekeeping honeys. Mining and honey bees' honey samples were collected from framers at harvesting sites.

3.2 Apparatus and instruments

The equipment's and materials such as pH meter (Bante Portable multipara meter 900P Wag-Wt 3020), refractometer, Muffle Furnace, crucible, vortex mixer, UV Spectroscopy (6705 Jen Way, UK), conductivity meter other classical laboratory apparatus were used during the experiment.

3.3. Chemicals and reagents

Analytical and reagent grades, chemicals and reagents were used. Sodium bisulphite ($NaHSO_3$) UNI-Chem (AR), sodium hydroxide (NaOH) (AR/ACS), conc. Sulfuric acid (H_2SO_4), methanol (CH_3OH), 5% Phenol, ethanol, hydrochloric acid (HCl), potassium Ferrocyanide ($K_4Fe(CN)_6.3H_2O$), Zinc acetate (Zn $(CH_3COO)_2.2H_2O$) and hydrogen peroxide (30%) were used during the experiment. Distilled water was used for cleaning and dilution purposes throughout the study.

3.4 Sample collection, preservation and pretreatment

Availability sampling was used to collect mining and honey bees' honey samples from the study area. Two beehive honey samples (each about 1 kg) were collected from beekeeper in Wayu Town. Totally, 3 mining bee honey samples each about 0.5 kg) were taken from Keku Kallo, Sochosa, and Wayu Town in January, April and August in 2019, respectively. The mining bee samples were collected the holes of mining bee and beehive honey samples were collected from hive of beekeeping in Wayu Town.

After straining out unwanted matters such as wax, dead bees, particles of comb, etc., the samples were packed and sealed in a clean plastic container and transported to Jimma University Analytical Chemistry research laboratory and kept in refrigerator until analysis.

3.5 Sample preparation

To make ready the sample for analysis, samples free from granulation was thoroughly mixed by stirring with spatula and the granulated samples were placed in awater bath and heated at 60 ºC – 65 ºC for 30 min to liquefy. During, heating the samples were occasionally shacked to thoroughly mix them. After cooling the liquefied samples, foreign matters such as wax, bees, and particles of comb, etc. were strained out.

3.5.1 Moisture content

The moisture content was determined using refractometer at 20 °C using refractive index of distilled water (1.33) as a reference. During the measurement the refractometer was regularly calibrated with distilled water. Then, the surface of the prism was covered with homogenized honey samples and the refractive index was recorded after 2 min, and then, the corresponding moisture content values were matched from the refractive index for honey [58 - 61]. Replicate measurements were used to take the average values.

3.5.2 Electrical conductivity

Electrical conductivities (EC) of the honey samples were measured using a digital Portable multipara meter. Accordingly, 10% aqueous solution of the honey samples was prepared by dissolving 10 g honey sample in distilled water to make a 100 mL solution. Then 40 mL of the resulting solution was poured into a beaker and placed in thermostated water bath at 20 °C. EC was measured in µS/cm by immersing conductivity cell into the sample solution [62].

3.5.3 pH value

The pH values of honey samples were determined at 28 ± 2 °C using pH meter [63]. To measure pH of the sample, 10% aqueous solution of the honey was prepared by dissolving 10 g of honey in 75 mL distilled water in a 250 mL beaker. The mixture was stirred thoroughly using magnetic stirrer to form a homogeneous solution. After calibrating the pH meter at pH 4 and pH 7 using standard buffer solutions, the electrode was immersed in the honey solution and the pH value was recorded [64].

3.5.4 Acidity

Acidity indicates the contents of all free acids in honey and it is expressed in milliequivalents/kg honey. Free acid was determined by dissolving 10 g honey sample in 75 mL distilled water and then titrating with standardized 0.1 M NaOH. The end point of the titration was observed at pH 8.3, which was controlled by pH glass electrode [66].

3.6.5 Ash content

The ash content of honey was measured after incineration in Muffle Furnace. To determine, the ash content, firstly, the dish was cleaned and heated in the electrical furnace at 550 ^{o}C and subsequently cooled in a desecrator to room temperature and weighed (M_2). Then, 10 g of honey sample was weighed (M_0) and two drops of olive oil were added to prevent frothing. Then, water was removed and commences ashing without loss (by foaming and overflowing) at a low heat rising to 350 - 400 ^{o}C on a hot plate. Bunsen burner was used to char the sample before inserting into the furnace. After the preliminary ashing with a Bunsen burner, the dish was placed in the preheated muffle furnace at 550 $^{\circ}$C for 1 h. The ash dish was cooled in the desiccator and weighted. The ashing procedure was continued until constant weight was reached (M_1). Percent ash in g/100g honey was calculated using the following formula [65].

$$\text{Ash (\%)} = \frac{M_1 - M_2}{M_0} \times 100$$

Where: Mo, M_1 and M_2 were the weight of honey taken, weight of dish + ash and weight of dish respectively.

3.5.6 Water insoluble matter

The water insoluble solids content of the honey samples were determined by procedure reported in the literature [66]. So, 20 g honey was weighed (M_1) and dissolved in 200 mL of water at 80

^{o}C. A crucible (sintered glass, pore size 30 microns) was dried in an oven and cooled to ambient temperature in desiccators. Then, after weighing the crucible (M_2), the sample solution was filtered through the crucible. The prepared honey sample was carefully washed with warm distilled water until it was free from sugars. Then, 1% phloroglucinol in ethanol was added into some filtrate in a test tube and a few drops of concentrated H_2SO_4 was added and then, the content was mixed to check presence of sugars. Finally, the crucible was placed in oven 135 ^{o}C to dry for 1 h, cooled in a desiccator and weighed. It was returned into oven at 30 min interval until constant weight was obtained (M_3). The result was calculated and expressed.

$$\text{\% Insoluble matter g/100g} = \frac{M}{M_1} \times 100$$

Where, M and M_1 were mass of dried insoluble matter and mass of honey taken respectively.

3.5.7 Colour analysis

To determine colour, honey sample was heated to 50 ^{o}C to dissolve sugar crystals, and the colour was determined by measureming the absorbance of 50% honey solution (*w/v*) at 635 nm. The honeys were classifying according to the Pfund scale after conversion of the absorbance value [67].

Intensity of honey colour in the Pfund scale = -38.70 + 371.39 × Abs

3.5.8 Sugar content

To determine the sugar content of honey, 5 g honey sample was taken into a beaker and 100 mL of warm water was added. The solution was stirred until all the soluble matters were dissolved. The prepared solution was filtered through Whatman number one filter paper into a 250 mL volumetric flask and filled to the mark with distilled water. Then, 100 mL of the solution was taken into a conical flask and 10 ml of diluted hydrogen chloride (HCl) was added and boiled for 5 min. On cooling, the solution was neutralized with 10% NaOH using phenolphthalein as indicator. The resulting solution was then titrated against Fehling's solution and the reading was calculated as follows [68].

$$\text{Total sugar (\%)} = \frac{\text{Factor (4.95) x dilution (250) x 2.5}}{\text{Titre x wt of sample x 10}}$$

3.5.9 Hydroxylmethylfurfural

The HMF value was determined using the procedure presented in the methods of the International Honey Commission [26]. Accordingly, Carrez Solution I was prepared by dissolving 15 g $K_4Fe(CN)_6$, in 100 mL distilled water. Carrez Solution II was prepared by dissolving 30 g $Zn(CH_3COO)_2.2H_2O$ in 100 mL distilled water. 0.2 % g metabisulphite, ($Na_2S_2O_5$) was dissolved in distilled water and volume made with 100 mL. 5 g of honey sample was taken in a beaker and dissolved in 25 mL distilled water. After transferring the resulting mixture into 50 mL volumetric flask, a solution containing the mixture of Carrez solution I and Carrez solution II (in 1:1 ratio) was added and mark with distilled water. The solution was then filtered through the filter paper and the first 10 mL of filtrate was rejected. Afterwards, 5 mL of sample was pipetted in two test tubes. 5 mL water was added to the first test tube and mixed well. To the second test tube 5 mL of 0.2 % metabisulphite ($Na2S_2O_5$) was added and mixed well as reference solution. The absorbance of the sample was determined against the reference solution at 284 and 336 nm wavelengths using 1 cm quartz cuvettes within 1 h [62].

$$\text{HMF expressed as mg/kg} = (A_{284} - A_{336}) \text{ x } 149.7 \text{ x } 5 \times / W$$

A_{284} is absorbance at 284 nm, A_{336} is absorbance at 336,149.7 is constant, and W is weight in g of honey sample

4. RESULTS AND DISCUSSION

4.1. Analysis of Physicochemical properties

The observed results of physicochemical of beehive honey and mining bee honeys are presented in Table 2. It was observed that the moisture content of beehive honey sample was 17.00 ± 0.00% which is inacceptable range of national and international standards. Similarly, the moisture contents of mining bee honeys were 26.00 ±0.00%, 25.00 ± 0.00% and 26 ± 0.00% from Keku Kallo, Sochosa and Wayu Town, respectively, and they were all above the standard moisture contents for mining bee honey. It is internationally recognized that good quality honey contains < 20% water, higher water may accelerate fermentation and lose of freshness of the product. Thus, moisture content of honey is essential to control the quality of honey. The rate of fermentation, the shelf life and processing characteristics of beehive honey is greatly influenced by its moisture content [70].

In addition, in honey containing high water content, the yeast in honey may more likely cause fermentation during storage, resulting in higher acidity. High moisture content can change the quality of honey in many aspects including flavor, preservation, crystallization, specific weight, viscosity, color and also accelerates the growth of microorganisms. The lower the water content the higher the perceived value of the honey. Furthermore, moisture content has positive correlation with acidity since the activity of glucose oxidase to produce gluconic acid increases in higher moisture containing honey [71]. Honey easily absorbs water from the air, since it is hygroscopic in nature. Thus, it is important to avoid environmental moisture uptake during honey processing and packaging [72].

The observed higher moisture content in mining bee honey samples could be due to various factors like the botanical and geographical origin of nectar, soil and climatic conditions, season of harvesting, degree of maturation that honey reached in the soil, as well as extraction, processing and storage conditions.

Table 2: The mean result of physicochemical properties of honey samples collected from different areas of Jimma Rare district, Oromia, Ethiopia (n = 4) and with national and international data.

Parameter	Sample site name					EU		
	Wayu Town HBH	Keku Kallo MBH	Sochosa MBH	Wayu Town MBH	National	HDH	BH	CAC
Moisture (%)	17.00 ± 0.00	26.00 ± 0.00	25.00 ± 0.01	26.00 ± 0.00	17.5-21	< 20	< 20	< 20
EC (mS/cm)	0.54 ± 0.00	0.93 ± 0.00	1.40 ± 0.02	1.05 ± 0.01	< 0.8	< 0.8	> 0.8	< 0.8
pH	3.72 ± 0.12	3.07 ± 0.04	3.05 ± 0.087	2.87 ± 0.14	-	3.2 - 4.5	-	-
Acidity (meq/kg)	25.8 ± 0.14	143.80 ± 0.44	253.80 ± 0.44	290.00 ± 0.50	< 40	< 50	< 80	< 50
Ash (%)	0.40 ± 0.00	0.50 ± 0.00	0.63 ± 0.00	1.04 ± 0.00	< 0.6	< 0.6	< 1.0	< 0.6
WIS (%)	0.13 ± 0.01	1.75 ± 0.07	0.23± 0.01	2.25 ± 0.07	< 0.1	< 0.1	< 0.1	<0.1

WIS is water insoluble solids; BH is Blossom honey; HDH is Honeydew honey; EC is electrical conductivity; CAC is Codex Alimentarius Commission; EU is European Union; HBH is honey bee honey from Wayu Town; Quality and Standards Authority of Ethiopia (2005).

Honey contains organic acids and mineral salts which chemically ionize and conduct electricity in a solution. EC of honey is closely related to the concentrations of minerals or total ash, salts, organic acids and proteins. It also shows variation according to the honey's floral origin [73 – 77]. In this study, EC of beehive honey was 0.54 ± 0.002 mS/cm which was within the national and international standard limits. The EC of the mining bee honeys were 0.93 ± 0.001 mS/cm for Keku Kallo; 1.4 ± 0.016 mS/cm for Sochosa and 1.05 ± 0.005 mS/cm for Wayu Town samples, which were much higher than the EC of studied beehive honey from the same area. The EC of mining bee honeys were also exhibited significant differences at $p < 0.05$. The values of EC indicated that the studied beehive honey was typical of blossom type, whereas mining bee honeys were honeydew honey.

EC is correlated to honey ash content and alkalinity of ash and both ash and the EC are related to the honeys mineral content [78]. Mineral content in honey is strictly related to its nutritional power. High EC values reflect high mineral content, which is a positive nutritional property of mining bee honeys that leads to their prescription as a source of minerals for diseases associated with mineral deficiency.

Honey is generally acidic in nature, with pH values lying between 3.5 and 5.5, due to the presence of organic acids that contribute to its flavor and stability against microbial spoilage [79]. This parameter has of great importance during the extraction and storage of honey as it influences the texture, stability and shelf life of honey [80]. In the current study, beehive honey demonstrated pH 3.72 ± 0.12, which was similar to reported pH values for beehive honeys. Similarly, for the studied mining bee honeys the observed pH were 3.07 ± 0.04 for Keku Kallo; 3.05 ± 0.09 for Sochosa and 2.87 ± 0.14 for Wayu samples. The pH of honeys can provide good indication of its botanical origin. Different authors reported that honeys originated from nectar have pH ranging from 3.5 – 4.5 and those from honeydew have pH varying between 4.5 and 6.5 [79]. In present study, beehive honey showed significantly different pH value from the studied mining bee honeys (Table 2).

Although there is no fixed limit of national and international standards, honeys that have low pH are preferred to prevent growth microorganisms. The main acid content in honey is gluconic acid which is in equilibrium with its lactones or its esters and inorganic ions such as phosphates and chlorides. High acidity of honey correlates with the fermentation of sugars present in the honey to organic acid, which is responsible for two important characteristics of honey: flavor and stability against microbial spoilage [81]. Furthermore, it might also indicate that honey samples have high content of minerals [82]. Therefore, the low pH values of mining bee honeys were evidently due to their higher moisture and ash content as compared to beehive honey sample of the area.

The free acidity of honey may express the presence of organic acids in equilibrium with their corresponding lactones, or internal esters, and some inorganic ions, such as phosphate and chloride [83]. This parameter has of great significance during the extraction and storage of honey as it influences the texture, stability and shelf life of honey [84]. High acidity can be indicative of fermentation of sugars into organic acids. Differences in honey acidity could be caused by differences in geographical condition, harvesting procedure and storage conditions [85]. As has been presented in Table 2, the observed free acidity values for beehive honey was 25.80 ± 0.14 meq/kg, which was acceptable according to CA, EU and Ethiopian standards [86]. But, the free acidity for mining bee honeys were 143.8 ± 0.437 meq/kg, 253.8 ± 0.437 meq/kg and 290 ± 0.500 meq/kg for Keku Kallo, Sochosa and Wayu Town, respectively, which were far higher the national and international standards. The obtained free acidity values of the studied mining bee and beehive honeys exhibited significant differences ($p < 0.05$). Acid measurement is useful for evaluation of honey fermentation, authentication of unifloral honeys and differentiating nectar from honeydew honeys [87]. Low acidity content indicates absence of undesirable fermentations [88].

The ash content is used to verify botanical origin of honey. For instance; blossom honeys have lower ash content than honeydew honeys [89]. The ash content represents the mineral residues of honey after incineration and thus determination of the ash content offers the possibility of knowing the overall mineral content of the honey [90, 91]. The permissible limit of ash content of honeys nectar is 0.6% [92] and 1.2% for honeydew honey [93]. There is a linear relationship between the ash content and the EC [94]. In this study, the observed ash content of beehive honey was 0.40 ± 0.00 %, whereas, for mining bee honeys 0.5 ± 0.00%, 0.63 ± 0.00% & 1.04 ± 0.00% were recorded from Keku Kallo, Sochosa, and Wayu Town, respectively (Table 2). Except, the mining bee honey sample from Wayu Town, the ash contents of all honey samples were acceptable according to the national, CA and EU standards. The variability of the ash content observed for different honey samples could be due to the number of pollinated plants, soil type and processes and beekeeping techniques used [95].

The measurement of insoluble matter is used to detect honey impurities that are higher than the permitted maxima. The water insoluble component of honey includes wax, pollen, honey-comb debris, bees and filth particles. The water insoluble contents of the studied beehive and mining bee honeys were 0.13 ± 0.007% for beehive honey and 1.75 ± 0.007%, 0.23 ± 0.01% & 2.25 ± 0.07% for Keku Kallo, Sochosa and Wayu Town mining bee honeys, respectively. The water insoluble solids contents were exhibited significant differences ($p < 0.005$). The water insoluble content of beehive honey was almost at the maximum limit of CA, EU and Ethiopian standards guideline limit. But, all mining bee hones had higher water insoluble solids, above the maximum limits in the national and international guideline.

4.2 Analysis of Biochemical properties

The results of biochemical of beehive honey and mining bee honeys are presented in Table 3.

Table 3: The mean result of biochemical properties of honey samples collected from different areas of the Jimma Rare district, Oromia, Ethiopia (n = 4) and with national and international data

Parameter	**Sample site name**				**National**	**EU BH**	**BDH**	**CAC**
	Wayu Town HBH	**Keku Kallo MBH**	**Sochosa MBH**	**Wayu Town MBH**				
Colour (mm pfund)	78.66 ± 0.01	156.56 ± 0.04	272.15 ± 0.00	336.78 ± 0.01	-	-	-	-
%Sugar	65.31 ± 07	49.58 ± 07	59.06 ± 0.70	50.58 ± 0.04	> 65	< 45	< 65	< 65
HMF (mg/kg)	30.54 ± 0.02	36.23 ± 0.02	56.29 ± 0.01	51.95 ± 0.01	< 40	< 40	< 40	< 80

The color of honey is characteristic of its floral source, which is related to its minerals and other minor components. It is one of the factors used for determining its industrial use, price in the world markets, and also its acceptability by the consumer. Honey color is generally related to its sensory properties such as flavor and odor and can give information on its floral source, mineral contents and storage conditions [96]. Study report indicated that honey color is an indicator of its mineral content [97]. Honeys that contain higher minerals have darker color. Dark honeys are preferred by consumers because their higher mineral contents and antioxidant capacities [98]. The color of honey varies from clear to dark amber or black, depending on its origin (floral source) and constituents (mineral content). It also depends on its chemical composition especially pigments like chlorophlls, carotenoids, flavonoids, tannin derivatives and polyphenols [99].

In the present study, the observed colours were 78.66 ± 0.006mm pfund for beehive honey, which is the Light Amber and 156.56 ± 0.041, 272 ± 0.003 & 336.78 ± 0.011 mm Pfund for mining bee honeys from Keku Kallo, Sochosa and Wayu Town, respectively and all were dark. The variation of mining bee honeys from that of the beehive honey is not surprising as they also exhibited varied mineral contents [100]. Mineral content of honey is highly dependent on the soil type where the nectar producing plant is located [101]. Mineral contents of honey influence the various characteristic such as colour, taste, flavor, medicinal value, keeping quality and other physical characteristics [102].

Honey is a supersaturated sugar solution; where carbohydrates are its main constituents accounting about 95% dry matters [103]. The most important physicochemical and nutritional properties of honey such as sweetness, viscosity, granulation, hygroscopicity, specific rotation and energy values depend on its sugar composition [104]. Moreover, the osmotic pressure produced by high sugars concentration is an important honeys' antimicrobial factor [105]. As a food commodity, honey has been used as a sweetener and human energy source. Monosaccharides dextrose (glucose) and laevulose (fructose) are the main sugars in honey. They are produced by honey bees during the ripening process by the transformation of nectar sucrose through the enzyme invertase from the bee's salivary glands. The amount and type of carbohydrates in honeys varied based on vegetal sources, being useful for the classification of unifloral honeys. Thus, the fructose/glucose ratio and the sucrose concentrations are good criteria for differentiating between different unifloral honeys. Carbohydrate concentrations were used to distinguish blossom and honeydew honeys. Honeydew honeys contain lower levels of monosaccharides, higher levels of trissacharides (mainly melezitose, erlose, raffinose and maltotriose), as well as higher levels of other oligosaccharides than blossom honeys [106].

In the current study, the sugar content of beehive honey was 65.31 ± 0.071% and the mining bee honeys had 49.58 ± 0.07%, 59.06 ± 0.70% & 50.58 ± 0.04% in Keku Kallo, Sochosa and Wayu Town samples, respectively. The studied honeys were exhibited significantly different of sugar contents ($p < 0.05$). The content of reducing sugars might be varied due to the storage factor, enzyme activity and acid reversion in honey. Besides, time of samples collection could also affect the total amount of reducing sugars in honeys. For honey collected in flowering season, the total amount of reducing sugars is expected to be higher. During honey storage, the content of monosaccharides decreases, and the content of oligosaccharides increases due to enzymatic activity and acid reversion [107, 108]. Sugars' degradation, the acid-catalyzed dehydration of hexoses or Maillard reactions, where sugars react with amino acids, darkens honey [109]. Indeed, according to Codex guideline the minimum sugar contents, which is the sum of glucose and fructose, is 45 g and 60 g/100 g for honeydew and blossom honeys, respectively, is almost identical to the standard values for apparent reducing sugars, which are 45 g and 65 g/100 g.

HMF is a decomposition product of fructose in acidic conditions. It is slowly formed in nature, during the storage of honey and much more quickly when honey is heated. HMF concentration is widely recognized as parameter affecting honey freshness. It is usually absent or present in only in trace amounts in fresh honeys. Rise of its concentrations during processing and ageing of the product, is used as an indicator of poor quality of the honey. Several factors influence the formation of HMF including temperature, time of heating, storage conditions [109, 110] and some chemical properties of honey such as pH, total acidity, mineral content, quantity and type of reducing sugars [111]. For instance, high values of HMF are naturally present in honeys from warm climate areas, such as tropical and subtropical countries [109]. Codex Alimentaries and EU commission has set the maximum limit for HMF in honey to be 40 mg/kg (with a higher limit of 80 mg/kg for honeys originating from tropical regions) to ensure that the product has not undergone extensive heating during processing for safe consumption [112].

In this study, the observed HMF contents were 30.54 ± 0.02 mg/kg for beehive honey; 36.23 ± 0.02 mg/kg, 56.29 ± 0.01 mg/kg & 51.95 ± 0.01 mg/kg for Keku Kallo, Sochosa kebele and Wayu Town mining bee honey samples, respectively. High acidity, moisture content, sugars (mainly fructose), amino acids (such as alanine) and minerals (such as Mg, Mn, Fe and Zn) in honeys speed up HMF production [113, 114]. The HMF results of this study indicated that both beehive and mining bee honeys studied were fresh according to the national and international standards.

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

In this study, different physicochemical and biochemical parameters affecting the quality of mining and honey bees' honeys were investigated. Mining and honey bees' honey samples were collected from the same area, from Jimma Rare district, Horo Guduru Wollega, Oromia Regional State, Ethiopia. The two varieties of honeys exhibited different physicochemical properties. Mining honey samples contained higher moisture, EC, free acidity, Ash and WIS contents than beehive honey sample collected from the same area. But, mining honeys showed lower pH values than the beehive honey sample. The physicochemical parameters of mining bee honeys samples were also above the maximum limit set in national, EU and CAC guidelines to control of the quality honeys.

The biochemical studies also demonstrated as beehive and mining bee's honeys are varied in terms of colour, sugar and HMF contents. Beehive honey sample has light amber colour, but all the mining bee samples have dark colour. Beehive sample has relatively higher sugars' content than mining bee honey samples. In contrast, mining bee honey samples have relatively higher HMF content than beehive honey samples. Generally, both honey studied honey types have good %sugar and HMF contents.

5.2. Recommendation

The author would like to forward the following recommendations:

- Our community, traditionally, use especially mining bee honeys for medication of gastritis, asthma, cough, and so on. Thus, thorough studies should be conducted on it antimicrobial and anti-oxidant activities to come up with scientific justifications.
- Additional studies are recommended on physicochemical and microbial properties of mining bee honeys based on seasonal variation, maturity levels and origins.
- Further meticulous study is needed to identify whether beehive and mining bees' honeys are different in physiochemical and biochemical properties.

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Appendix I

Physicochemical and Biochemical Parameters Tables for the present study at Jimma Rare district Horo Guduru Wollaga Zone, Oromia regional state, Ethiopia.

Descriptives

Moisture

Site name	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
WHBH	3	1.4947	.00306	.00176	1.4871	1.5023	1.49	1.50
kekukallo	3	1.4660	.00000	.00000	1.4660	1.4660	1.47	1.47
Sochosa	3	1.4727	.00058	.00033	1.4712	1.4741	1.47	1.47
WMBH	3	1.4663	.00208	.00120	1.4612	1.4715	1.46	1.47
Total	12	1.4749	.01233	.00356	1.4671	1.4828	1.46	1.50

WHBH = Wayu Town honey bee honey; WMBH = Wayu Town mining bee honey

ANOVA

Moisture

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.002	3	.001	156.659	.000
Within Groups	.000	8	.000		
Total	.002	11			

Descriptives

EC

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
HBH	3	.5203	.00153	.00088	.5165	.5241	.52	.52
kekukallo	3	.9040	.00100	.00058	.9015	.9065	.90	.91
Sochosa	3	1.3647	.01550	.00895	1.3262	1.4032	1.35	1.38
WMBH	3	1.0247	.00513	.00296	1.0119	1.0374	1.02	1.03
Total	12	.9534	.31524	.09100	.7531	1.1537	.52	1.38

ANOVA

EC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.093	3	.364	5395.649	.000
Within Groups	.001	8	.000		
Total	1.093	11			

Descriptives

pH

Site name	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
WHBH	3	3.7200	.11790	.06807	3.4271	4.0129	3.59	3.82
kekukallo	3	3.0733	.04041	.02333	2.9729	3.1737	3.05	3.12
Sochosa	3	3.0500	.08660	.05000	2.8349	3.2651	2.95	3.10
WMBH	3	2.8700	.13856	.08000	2.5258	3.2142	2.71	2.95
Total	12	3.1783	.34803	.10047	2.9572	3.3995	2.71	3.82

WHBH = Wayu Town honey bee honey; WMBH = Wayu Town mining bee honey

ANOVA

pH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.248	3	.416	39.397	.000
Within Groups	.084	8	.011		
Total	1.332	11			

Descriptives

Acidity

Site name	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
WHBH	3	2.5833	.14434	.08333	2.2248	2.9419	2.50	2.75
kekukallo	3	14.3833	.43684	.25221	13.2982	15.4685	13.90	14.75
Sochosa	3	25.3833	.43684	.25221	24.2982	26.4685	24.90	25.75
WMBH	3	29.0000	.50000	.28868	27.7579	30.2421	28.50	29.50
Total	12	17.8375	10.7863	3.1137	10.9842	24.6908	2.50	29.50

WHBH = Wayu Town honey bee honey; WMBH = Wayu Town mining bee honey

ANOVA

Acidity

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1278.486	3	426.162	2612.487	.000
Within Groups	1.305	8	.163		
Total	1279.791	11			

Descriptives

Ash

Site name	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
WHBH	2	.0400	.00424	.00300	.0019	.0781	.04	.04
kekukallo	2	.0495	.00212	.00150	.0304	.0686	.05	.05
Sochosa	2	.0630	.00141	.00100	.0503	.0757	.06	.06
WMBH	2	.1040	.00141	.00100	.0913	.1167	.10	.11
Total	8	.0641	.02619	.00926	.0422	.0860	.04	.11

WHBH = Wayu Town honey bee honey; WMBH = Wayu Town mining bee honey

ANOVA

Ash

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.005	3	.002	240.220	.000
Within Groups	.000	4	.000		
Total	.005	7			

Descriptives

WIs

Site name	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
WHBH	2	.0150	.00707	.00500	-.0485	.0785	.01	.02
Kekukallo	2	.3250	.03536	.02500	.0073	.6427	.30	.35
Sochosa	2	.0550	.00707	.00500	-.0085	.1185	.05	.06
WMBH	2	.3750	.03536	.02500	.0573	.6927	.35	.40
Total	8	.1925	.17119	.06053	.0494	.3356	.01	.40

ANOVA

WIs

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.203	3	.068	103.872	.000
Within Groups	.003	4	.001		
Total	.205	7			

Descriptives
Color

Site name	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
WHBH	3	.3163	.00586	.00338	.3018	.3309	.31	.32
kekukallo	3	.5500	.04078	.02354	.4487	.6513	.50	.58
Sochosa	3	.8370	.00300	.00173	.8295	.8445	.83	.84
WMBH	3	1.0107	.01102	.00636	.9833	1.0380	1.00	1.02
Total	12	.6785	.27848	.08039	.5016	.8554	.31	1.02

WHBH = Wayu Town honey bee honey; WMBH = Wayu Town mining bee honey

ANOVA

Color

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.849	3	.283	619.662	.000
Within Groups	.004	8	.000		
Total	.853	11			

Descriptives
sugar

Site name	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
WHBH	2	.9500	.07071	.05000	.3147	1.5853	.90	1.00
kekukallo	2	1.2500	.07071	.05000	.6147	1.8853	1.20	1.30
Sochosa	2	1.0500	.07071	.05000	.4147	1.6853	1.00	1.10
WMBH	2	1.2250	.03536	.02500	.9073	1.5427	1.20	1.25
Total	8	1.1188	.14126	.04994	1.0007	1.2368	.90	1.30

WHBH = Wayu Town honey bee honey; WMBH = Wayu Town mining bee honey

ANOVA

Sugar

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.123	3	.041	10.128	.024
Within Groups	.016	4	.004		
Total	.140	7			

Appendix II

The estimation of Moisture content Table corresponding Refractive Indices and Percent Moisture in Extracted Honey

Refractive Index 20 °C	Moisture Content%	Refractive Index 20 °C	Moisture Content%	Refractive Index 20 °C	Moisture Content%
1.5044	13.0	1.4935	17.2	1.4830	21.4
1.5038	13.2	1.4930	17.4	1.4825	21.6
1.5033	13.4	1.4925	17.6	1.4820	21.8
1.5028	13.6	1.4920	17.8	1.4815	22.0
1.5023	13.8	1.4915	18.0	1.4810	22.2
1.5018	14.0	1.4910	18.2	1.4805	22.4
1.5012	14.2	1.4905	18.4	1.4800	22.6
1.5007	14.4	1.4900	18.6	1.4795	22.8
1.5002	14.6	1.4895	18.8	1.4790	23.0
1.4997	14.8	1.4890	19.0	1.4785	23.2
1.4992	15.0	1.4885	19.2	1.4780	23.4
1.4987	15.2	1.4880	19.4	1.4775	23.6
1.4982	15.4	1.4875	19.6	1.4770	23.8
1.4976	15.6	1.4870	19.8	1.4765	24.0
1.4971	15.8	1.4865	20.0	1.4760	24.2
1.4966	16.0	1.4860	20.2	1.4755	24.4
1.4961	16.2	1.4855	20.4	1.4750	24.6
1.4956	16.4	1.4850	20.6	1.4745	24.8
1.4951	16.6	1.4845	20.8	1.4740	25.0
1.4946	16.8	1.4840	21.0		
1.4940	17.0	1.4835	21.2		