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INVESTIGATION OF PHYSICOCHEMICAL AND BIOCHEMICAL PROPERTIES OF HONEY, OF GERA WOREDA, JIMMA ZONE, ETHIOPIA

BY: FIKADU TAYE

NOVEMBER, 2018

JIMMA, ETHIOPIA

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I declare that this thesis is my original work and not submitted to any other institution anywhere for the award of any academic degree. And that all sources of materials used for this thesis have been properly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for MSc degree at Jimma University and is deposited at the University Library to be made available to borrowers under rules of the Library.

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ACRONYMS/ABBREVIATION

ANOVA Analysis of Variance

CAC Codex Alimentarius Commission

CSA Central Statistical Agency

EARO Ethiopian Agricultural Research Organization

EC Electrical Conductivity

EU European Union

FAO Food and Agriculture Organization of the United Nations

HMF Hydroxyl-methyl-furfural

IHC International Honey Commission

QSAE Quality and Standard Authority of Ethiopia

SNNPRS Southern Nations, Nationalities and Peoples Regional State of Ethiopia

WHO World Health Organization

IHC International Honey Commission

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ABSTRACT

In this study, the physiochemical and biochemical properties of honey of Gera Woreda, Jimma Zone, Oromia Regional Sate, Ethiopia were investigated. Honey samples were collected from three main honey supplying routes (i.e., Muje, Agaro and Afallo routes) to Gera town in August 2018. The total of 9 honey samples, (three varieties from each sampling route, namely, white, mixed (light amber) and dark (amber) colored honey varieties) were purposively collected in clean plastic containers and were then transported to Jimma University Analytical Chemistry Research Laboratory. Approximately, 200 g of similar colored honey samples were mixed to take a composite sample for each honey variety. Standard analytical methods were used to analyze the physicochemical and biochemical parameters including moisture content, electrical conductivity, ash content, free acidity, pH, water insoluble solid, honey color, hydroxymethylfurfural (HMF) and sugar content of the honey varieties. The obtained results demonstrated that the moisture content, electrical conductivity, ash content, pH, free acidity, water insoluble solid, honey color, total sugar, and HMF, were ranging from 22.6 - 22.8%; 0.18 - 0.46 mS/cm; 0.04 - 0.21%; 3.23 - 4.00; 19.30 - 33.30 meq/kg; 0.15 - 0.35%; 28.52 - 109.85 mm Pfund; 59.49 - 70.36% and 5.09 - 11.43 mg/kg, respectively. One way ANOVA test $P < 0.05$) indicated the existence of significant differences among honey samples, namely white, light amber and amber honeys in terms of their physiochemical and biochemical properties. The observed results also showed that honeys produced in Gera Woreda have physicochemical and biochemical properties within the acceptable range of the national and international standards; except for the water insoluble solids.

***Keyword**: Honey; Physiochemical Properties; Biochemical properties.*

1. INTRODUCTION

1.1 Background of the study

Ethiopia has high honey production potential, resulting from adequate forage availability coupled with favorable and diversified agro-climatic conditions [1]. The country environmental conditions are conducive for the growth of 6000 to 7000 species of flowering plants [2]. This indicates that the existence of large number of bee colonies in the country forests. The country is home of the most diverse flora and fauna, which makes it highly suitable for sustaining a large number of bee colonies [3]. Ethiopia is the largest honey producer in Africa and the 10^{th} largest in the world. Currently, the total amount of honey production of the country is estimated to be more than 45,300 metric tons per year [4]. Honey production in Ethiopia accounts for about 2% of the world production and 22% of African honey production [5].

Ethiopia produces dozens of honey varieties based on pollen sources, seasons, and agro-ecological regions of production [4]. Due to the presence of diverse flora and fauna, the country produces a unique variety of honey; white honey, light amber, amber, brown, and dark honey and etc. Some of Honey producing trees and shrubs include*: Vernonia spp., Triffolium spp. (clover), Eucalyptus spp., Acacia spp., Birbira, Pisum Sativum* and *grass Spp [2]*. The major floras for honey production in Gera district are *Geteme flowers (Schefflera abyssinica), Mekenisa (Croton macrostachyus), Qerero (Croton macrostachyus, Vernonia Spp), Ebicha (Vernonia spp.), and Buna (Coffee arebica)* (The source information was obtained from honey producers in the Woreda).

Honey is the sweet substance produced by honeybees from the nectar of flowers or from secretions on living plants, which bees collects, transforms and stores in honey combs [6]. Honey consists of about 181 different substances [7]. Its composition is variable and primarily depends on the floral source. However, external factors such as seasonal variations and agro-ecological regions could vary the composition of honey [8]. It is a supersaturated mixture, containing wide range of chemicals including organic acids, amino acids, proteins, minerals, vitamins, enzymes and volatile substances which are responsible for its characteristic flavor [7]. Honey and beeswax play a significant role to support national economy through foreign exchange [3].

Ethiopia honey production is increasing from time to time. According to the report of Ministry of Agriculture and the Central Statistics Authority (CSA) of Ethiopia, in 1997 the country produced 13,600 tons and in 2005 the production was grown to 30,000 tons, which indicated about 12% production growth over the 1997 [4].

The quality of honey is usually evaluated by analyzing its physiochemical and biochemical properties. The physicochemical and biochemical properties of honey could be affected by the nectar type that the honey bee used, geographical ecology, type of soil and climate, honey handling (after harvest), processing, and storage practice [9]. Some of honey constituents are of great importance to ensure the quality of the product and also influence its storage, granulation, texture, flavor and the nutritional and medicinal values [9]. Reported literatures, concerning the honey's quality of different botanical and geographical origins illustrates the importance of determining physicochemical & biochemical parameters of honey [9]. Physicochemical and biochemical properties including color, moisture content, reducing sugars, pH, electrical conductivity (EC), sucrose content, minerals, free acidity and hydroxyl-methyl-furfural and microbiological characteristics are used to determine the quality of honey [10]. Since honey is used as food ingredient due to its rich nutritional values, analysis of the physicochemical and biochemical properties of honey is used to authenticate the product to reveal the possible presence of artificial components or adulterant's [11].

1.2 Statement of the problem

Jimma zone, in particular Gera Woreda (District) is known by producing and supplying quality honey to the surrounding community, Jimma town and other part of the country. Even though honey is widely used in Jimma, there is no significant report which shows the physiochemical and biochemical properties of honey in the study area. Large amount of honey produced in the Gera woreda and stored for long period of time in improper way at farmers' houses. When it is stored for a long time, there is change in its color, aroma, flavor, etc [12] and the quality of honey was deteriorated due to physical and biochemical change in the honey. As a result, large amount of honey products of the woreda could not market access. Many farmers do not get appropriate income due to inefficiency of honey production techniques, lack of awareness the quality of their products, poor handling system and lack of access to market. Farmers (honey producers) in the Woreda produce and store honey in traditional ways that could decrease the quality of their product and also their benefits. Possible adulteration and manipulation affect the physiochemical and biochemical properties of honeys and therefore, it is necessary to test and verify the honey products from different origin.

Honey is used for nutritional, medicinal and industrial purposes and it is an important commodity in the national and international market. Jimma zone, particularly, Gera Woreda, has great potential to supply large quantities of honey for its surrounding markets, particularly Jimma zone. However, there is no report shows information about the qualities of the honey products from the area. Therefore, to increase awareness of the producers as well as to the consumers about the quality of the Gera Woreda honey, the study of physiochemical and biochemical properties of honey quality marker is crucial. The studies have answered about the quality of honey varieties that are produced in the Gera Woreda.

1.3 Objectives

1.3.1 General objective

The main objective of the present study was to investigate the physiochemical and biochemical properties honey of Gera Woreda, Jimma Zone, Oromia regional State, Ethiopia.

1.3.2 Specific objectives

- To determine the physiochemical properties of white, mixed and dark coloured honey varieties of Gera woreda.
- To determine the biochemical properties of white, mixed and dark coloured honey varieties of Gera Woreda.
- To compare the physiochemical and biochemical properties of Gera Woreda honey varieties with national and international standards.

1.4 Significance of the study

Investigation of physiochemical and biochemical analysis of honey is useful for the producers as well as consumers. Generally, the study finding could have the following importances:

- It could be used to control adulteration or poor handling mechanisms by monitoring certain quality indicators honey;
- The finding could motivate the farmer to produce quality honey.
- The study finding could be used as baseline information regarding physiochemical and biochemical properties of Gera Woreda honey.

2. LITERATURES REVIEW

2.1 Honey

Honey is the natural sweet substance produced by the worker of honey bee (apis mellifera) from the nectar of plants or from secretions of living parts of plants, which the bee collect, transform and combine with the specific substances and store in the honey comb to ripen and mature [13]. Honey is the oldest and only available unique natural sweetener to mankind and is the last of natural unprocessed food to be consumed [14].

2.2 Uses of honey

Humans use honey for various purposes. For instance, honey can be a substitute for sugar in many foods. It can be used to make ice cream. In long year, priests used honey and cakes sweetened by honey in many religious ceremonies. In Roman times, Romans used honey as widely as sugar is now. Honey was used for cooking, preserving meats, vegetables, fruits, sauces and dressing [15]. In Ethiopia, honey is commonly used for preparation of honey beer (wine) "tej". Honey beer or "tej "has been a popular drink throughout the country since ancient time. In every town, "tej "production is a big business and it is even served in some big bars and hotels as special cultural drinks. For those who prefer non-alcoholic drinks, honey is a tasteful sweetener of juices, cocktails and teas. Ethiopians´ make a popular soft drink made out of honey which is called 'birz', which is consumed as a delicacy during religious festivities [16].

2.2.1 Medical uses of honey

Carbohydrates are the major constituents (70– 80%), honey contains, in low amounts, various substances such as organic acids, proteins, amino acids, vitamins, enzymes, minerals and different other molecules (pigments, flavonoids, antibacterial factors, etc. Numerous publications pointed out the effectiveness of honey in the treatment of wound ulcers and skin burns. In ancient times honey was used as a medical remedy, and was advocated as an excellent source of energy and a panacea for various illnesses. The ancient Egyptians and Greeks used honey to treat wounds and various gut diseases. Honey is used as traditional medicine in many countries. It has gastro protective properties [15]. Honey is used as an effective dressing of infected wounds,

burns, and ulcers, accelerating their healing. It has also been used successfully with oral rehydration fluid to treat children with gastroenteritis [15].

2.2.2 Antimicrobial activity of honey

Among naturally occurring mixtures containing free fructose in excess of glucose, honey ranks at the top. The antimicrobial activity of honey is probably due to the high osmolarity and to the rather acidic pH**.** It also contains hydrogen peroxide and flavonoids, e.g., phenolic acids that also have antibacterial properties. 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. Honeys contain a number of components to act as preservatives; these include α-tocopherol, ascorbic acid, flavonoids, and other phenolic and enzymes such as glucose oxidase, catalase, and peroxidase. It is suggested that any of these substances owe their preservative properties to their antioxidative activity [15, 17].

2.2.3 Honey as antioxidant

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. 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. Honey contains a number of components known to act as antioxidants; these include vitamin C, vitamin E, enzymes such as catalase, peroxidase and phenolic compounds [15].

2.4 Physiochemical properties

Honey is generally evaluated in terms of its physicochemical properties. Some of its constituents are of great importance to ensure the quality of the product and also influence its storage, granulation, texture, flavor and the nutritional quality. These physicochemical parameters include moisture content, electrical conductivity, reducing sugars, sucrose content, minerals, free acidity and hydroxyl-methyl-furfural [3].

2.4.1. Moisture content

The amount of moisture is important function of the factors of ripening, including weather conditions and original moisture of the nectar. After extraction of the honey, depending on conditions of storage, moisture contents may be changed. The moisture is one of the most important characteristics of honey that influence its quality and granulation property. Quantitatively, water is honey's second most important component. Properly harvested honey is viscous with water content near 18% .Water content much higher than 18% will likely affects storage life and processing characteristics; and is called 'green' or 'unripe' honey .The final water content of honey depends on several environmental factors. Namely 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 [3]. The moisture content of 490 American bee honey samples ranged from 13.4-22.9% and averaged 17.2% [15, 18].The moisture content of Sudanese bee honey samples ranged from 13.1-26.8% and averaged 19.9% [18]. The moisture content of 33 Greek honeys from different botanical and geographical origin was ranged between 13.0 - 18.9 % [18]. In more humid climates even sealed cells can contain honey with more than 24%, even 28% moisture content [3]. International legislation recommends the moisture content of honey to be less than 20-21% [19].

2.4.2 Electrical conductivity

Electrical conductivity (EC) depends predominantly on the mineral content of honey. EC can be determined with conductometer and it is very widely used for discrimination between honeydew and blossom honeys and also for the characterization of unifloral honeys. Conductivity is a good criterion of botanical origin of honey and today it is determined in routine honey control instead of the ash content. This measurement depends on the ash and acid content of honey; the higher their content, the higher the resulting conductivity. There is a linear relationship between the ash content and electrical conductivity [15]. The range of EC in honey lies from 0.06 - 2.17 milli Siemens per centimeter. Honeydew contains considerably higher amounts of minerals compared to blossom honeys. Generally honeydew honeys have an EC higher than 0.8 milli Siemens per centimeter .Blends between blossom and honeydew honeys have conductivity values ranging from 0.51 -0.79 milli Siemens per centimeter [20].

2.4.3 Ash content

The ash content in honey is generally low and influenced by the chemical composition of nectar that varies according to the different botanical sources involved in honey formation. It can vary between 0.02 and 1.0 % and the maximum limit allowed for honey from floral sources is 0.6%. Normally, ash contents between 0.1 & 0.3 % are found for floral honeys. Very high mineral contents about 1.0% are actually encountered only in honeydew [20]. The ash content is a quality criterion for honey origin. The ash content of honey depends on the material gathered by the bee during foraging; nectar normally has low ash content ranged from 0.02 - 1.03% and an average of 0.169% [18]. The ash content ranged from 0.16 - 0.60 % with an average of 0.32 %. [18].The ash content of American bee honey samples ranged from 0.02 - 1.028% and averaged 0.17% [15]. The average ash content of Egyptian honeys was 0.17% [15].

2.4.4 pH value

All honeys are acidic with a pH-value generally lying between 3.5 and 5.5, due to the presence of organic acids that contribute to honey flavour and stability against microbial spoilage. The pH is indeed a useful index of possible microbial contamination and adulteration. The pH of adulterated honey samples is higher than that of pure samples and has high relevance during the extraction and storage of honey because it is related to the stability and the shelf life of the product [20]. The pH value of American bee honey samples ranged from 3.42 - 6.10 and average 3.91 [15]. An average pH value of 3.9 for Egyptian honey samples analyzed [15]. The pH value of Sudanese bee honey samples ranged from 3.3 - 4.3 and an average of 3.8 [15].

2.4.5 Acidity

Acidity is an important quality criterion. Honey fermentation causes an increase of acidity and because of this a maximum acidity value has proven useful, although there is a considerable natural variation. The acidity of honey is due to the presence of organic acids, particularly the gluconic acid, in equilibrium with their lactones or esters and inorganic ions such as phosphate and chloride. The variation in acidity among different honey types may be attributed to variation in these constituents due to extraction season [15]. Honey fermentation results are responsible for two important characteristics of honey: flavor and stability against microbial spoilage. The

acidity of honey developed due to the presence of organic acids. The value of honeys acidity, lower than 50 milli equivalent per kg of honey, means that honeys will not be fermented [19]. The great variation in the acidity honey samples were observed Sudan Honeys, which were varied from 6 – 171 milli equivalent per kg [18]. The acidity ranged between 17.59 – 39.81 milli equivalents per kg with an average of 27.2 milli equivalent per kg [18]. The acidity ranged between 9.2 – 41.51 milli equivalents per kg and the mean 22.49 milli equivalent per kg [18] and ranged from 17.1 – 50.9 milli equivalents per kg with a mean of 32.7 milli equivalent per kg [18].

2.4.6. Viscosity

Freshly extracted honey is viscous. Its viscosity depends on a large variety of substances and therefore varies with its composition and particularly with its water content. Viscosity is an important technical parameter during honey processing, because it reduces honey flow during extraction, pumping, settling, filtration, mixing and bottling. Raising the temperature of honey lowers its viscosity a phenomenon widely exploited during industrial honey processing [16].The rheological behaviour of honey has been investigated for in shelf-life, proper handling, packing and processing issues. The honey viscosity depends on the water content, floral source, amount and size of crystals and, finally, the temperature. Honeys with higher water contents flow faster than those with lower ones [17]. Viscosity is the measure of the internal friction of a fluid. The viscosity measurements were performed at ambient temperature, using a Brookfield disc-type viscometer [21]

2.4.7. Hygroscopicity

The strong hygroscopic character of honey is important both in processing, storage and for final use. Because of this character, it easily absorbs moisture from the air. Thus, in areas with a very high humidity it is difficult to produce good quality honey, which can be measured using a gadget called refractometer. Different researches show that normal honey with a water content of less than 18.3 % or less will absorb moisture from the air if a relative humidity is above 60% [16].

2.4.8 Water insoluble solids

Honey water insoluble matter includes pollen, honey-comb debris, 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 .The water insoluble content is directly dependant up on honey handling and high concentrations are a sign of improper handling during harvest [3].

2.4.9. Color

One of the most physical characteristics of honey is color. Colors of honey form a continuous range from very pale yellow through amber to a darkish red to black and this can be helpful in the identification of floral source of the original nectar of differing honeys. The variations are entirely due to the plant source of the honey, although heat may modify the color of honey by darkening action. The color and consistency of honey is not only affected by the source of flower from which the nectar was collected but is also affected by factors such as weather and climatic change [3]. The color of honey is varies from clear and colorless (like water) to dark amber or black categories which do not really have any bearing on quality. While it is not an indicator of honey quality and there are exceptions to the rule, generally speaking, the darker color the honey, the higher its mineral contents, the pH readings, and the aroma/flavor levels [16].

2.5. Biochemical properties

2.5.1 Protein content

It has been known for many years that honey contains protein materials. Interest in protein content was used to distinguish natural honey from artificial mixture and blend. Studied the United States honey and found that the protein content ranged between 0.25 and 0.76 % [18].Proteins account for 0.5-1% of the honey composition with proline constituting 50-80% of the total amino acids [22].A range of 0.077-1.378% and an average of 0.53% protein in Sudanese bee honey [18].

2.5.2. Sugar content

Honey is above all a carbohydrate material, with 85 – 90.0 percent of the solids being sugars, and the identity of these sugars has been studied for many years. Glucose and fructose are the main

sugars in honey. These are the building blocks for the more complex honey sugars and account for about 85 percent of the solids present in honey [3]. 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 [3].

2.5.3. Hydroxyl-methyl-furfural

Hydroxyl-methyl-furfural is a break-down product of fructose (one of the main sugars in honey) formed slowly during storage and very quickly when honey is heated. The amount of HMF present in honey is therefore used as a guide to storage guide to storage length and the amount of heating which has taken place. Nearly HMF absent in newly produced honey; it is a byproduct of fructose decay, formed during storage or during heating. Thus, its presence is considered the main indicator of honey deterioration [16]. The heat treatment during processing of honey should be limited as excessive amounts of HMF are indicative of the loss of freshness and overheating [23].Several factors influence the formation of HMF in honey: temperature and time heating, storage conditions, use of metallic containers and it is connected to the chemical properties of honey, which are related to the floral source from which the honey has been extracted, like pH, total acidity and mineral content [17].

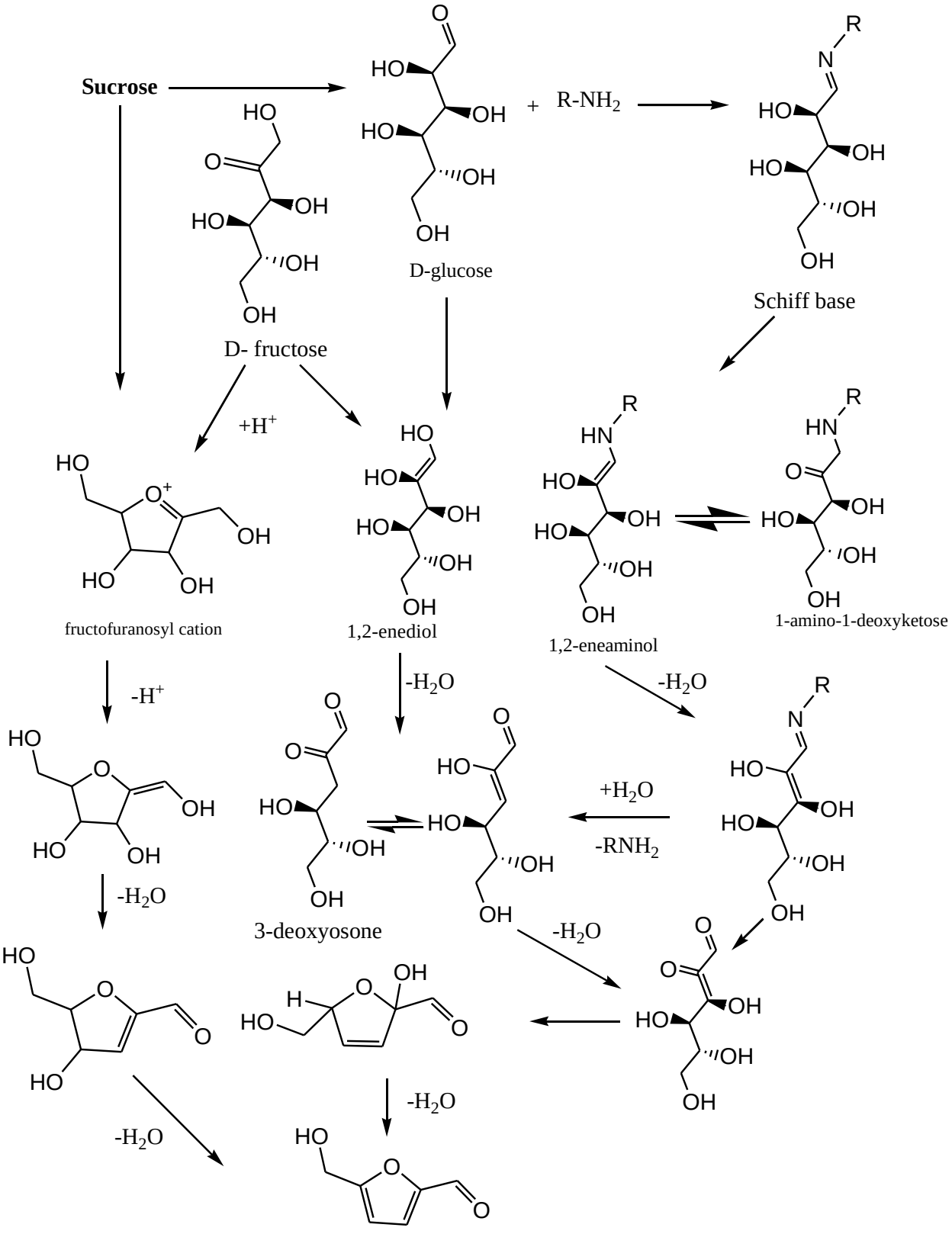

Figure 1.Scheme for the formation of hydroxyl methyl furfural [16].

3. MATERIALS AND METHOD

3.1 Study area

This study was carried out in Gera Woreda, Jimma zone, Oromia Regional State, Ethiopia. Gera Woreda is located of at 95 km in southwest of Jimma town, the zone capital. It has rugged topography with an altitude ranging from about 1500 m - 2900 m. Its average annual minimum and maximum temperatures are 10 ºC, and 26 ºC, respectively. Gera has an average annual precipitation of 1700 mm and has also 113,514 ha dense forest [24].

3.2. Chemicals and reagents

Chemicals and reagents that were used in this study are analytical grades. Meta bisulphite, ($Na_2S_2O_5$, 98%) was obtained from Riedei-deHaen (Rupert-Mayer-Str. Munich, Germany); sodium hydroxide (NaOH), sulfuric acid (H_2SO_4, 98 %), ethanol (96%), and hydrochloric acid (HCl, 37%) were from Blulux Laboratories PLtd. (Faridabad, Haryana, India). Potassium ferro cyanide ($K_4Fe(CN)_{6.}3H_2O$) and Zinc acetate, Zn $(CH_3COO)_{2.}2H_20$, 98%) were obtained from Merck Laboratory Chemicals (Nagpur, Maharashtra, India). Distilled water was use for cleaning of apparatus and dilution purposes.

3.3 Apparatus and instruments

Different apparatus and instruments were used during the research work. pH meter from Hanan instruments (Póvoa de Varzim, Portugal) Atago Abbe Refractometer supplied by Bellinghant Stanley Ltd (, England), Karl Kolb Furnace from Scientific technical supplies (Dreiech,.West Germany), JENWAY single beam UV Spectroscopy, model 6705, obtained from Bibby Scientific Ltd (Beacon Road, Stone, OSA, UK) , vortex mixer model FB15024 obtained from Fisher scientific (Merelbeke, *Belgium*)and Conductivity meter purchased from HACH company (Lal Bagh Road, Bengaluru, India)

3.4 Sampling and sample pretreatment

Honey samples were collected from the three main routes (Muje, Agaro and Afello routs) of honey supply to the Gera town, the capital of Gera Woreda. Totally, 9 honey samples (three varieties from each sampling route, namely, white, mixed and dark honey varieties) were purposively collected in clean plastic container in August 2018. From each route 1 kg was separately taken for different variety of heney. Before sampling the containers were washed thoroughly with distilled water and dried in open air. All the collected honey samples were transported to Jimma University Analytical Chemistry Research laboratory. Then, soon after arriving the laboratory, approximately 200 g of each variety were mixed in round plastic wash basin dish pan to make composite sample.

Subsequently, following the international guideline [25] foreign matters such as wax, bees, particles of comb, were separated manually.Then, the resulting sample was mixed thoroughly by stirring before heating to 60 – 65 ºC in water bath until it was liquefied. The liquefied honey sample was then carefully filtered through 0.5 mm sieve mesh before cooling down to room temperature. The resulting honey samples were then kept under ambient conditions in the laboratory until the time of analysis.

3.5 Method of analysis

Physicochemical and biochemical properties of honey samples were determined by the harmonized methods of the international honey commission [26]. Detailed procedure for each parameter was presented as follows.

3.5.1 Moisture content

Moisture content was determined using refractometer at 20 °C using refractive index of distilled water as a reference. The method correlates the refractive index of honey measured to its moisture content. The refractive index readings were converted to moisture content using table presented in the standard method [26].

3.65.2 Electrical conductivity

The measure the EC 20 g of honey was dissolved in 100 mL of distilled water. A portion of the resulting, approximately, 40 mL was transferred to a beaker and placed in thermostated water bath at 20 °C and then. EC of the solution was measured [27].

3.5.3 pH value

pH value was determined using pH meter [28]. To measure pH of the samples, 10 g of honey was accurately weighed and dissolved in 75 mL distilled water in a beaker. Then, after thoroughly stirring with magnetic stirrer the pH of the resulting solution was measured. The pH meter was calibrated using pH 4, pH 7, and pH 10, prior to using for measurement.

3.5.4 Acidity

The acidity of honey samples was determined according to the method of Quality and Standard Authority of Ethiopia (QSAE) [3].Accordingly; 10 g honey sample was dissolved in 75 mL distilled water in a beaker and the resulting mixture was stirred with a magnetic stirrer. Subsequently, the solution was titrated against 0.1 M NaOH until the pH 8.50, which indicated the end point of the titration. Eventually, the acidity (meq/kg) of the sample was calculated.

$$\text{Acidity} = 10V$$

Where V is the volume of 0.1M NaOH consumed for titration and 10 is the dilution factor of honey sample.

3.5.5. Ash content

The ash content of honey samples was determined using the standard method used by the Quality and Standard Authority of Ethiopia (QSAE) [3]. Accordingly, a crucible dish (quartz dish) was first heated in a furnace at 600 °C and then, after cooling in a desiccator to room temperature its weight (M_2) was measured. Following this, 5 g of honey sample was weighed (M_0) into the crucible dish. After adding, 2 drops of olive oil, to prevent frothing, the dish was placed in preheated furnace and heated for about 3 h at 600 °C, until constant weight (M_1) was obtained. Finally, the ash content (% by mass) was calculated:

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

Where, Mo, M_1 and M_2 are mass of the honey sample taken, weight of the ash and crucible, weight of empty crucible, respectively.

3.5.6 Water insoluble solids

The water insoluble solids content of the honey samples was determined according to the method of Quality and Standard Authority of Ethiopia (QSAE) [3]. Accordingly, 20 g of honey was weighed and dissolved in 200 mL of distilled water. The mixture was boiled at 80 ^{o}C and then mixed well. A crucible (sintered glass, pore size 30 microns), was used to filter the sample. Before measuring the weight of the crucible, it was first dried in an oven and then cooled to ambient temperature in a desiccator containing an efficient desiccant (silica gel). Then, the resulting sample solution was filtered through the crucible and the sample was washed repeatedly with warm distilled water until it was free from sugars. Then, to check for the complete removal of sugars, the filtrate of the washing was taken in the test tube and tested by adding few drops of 1% phloroglucinol in ethanol solution and conc. H_2SO_4. When the sugar was completely removed, the crucible was dried at 135 ^{o}C for about 1 h, and then, weighed after cooling in desiccators, the water insoluble honey content was then determined as follows:

$$\%\, Water\ insouble\ mater\ = \frac{M_1 - M_2}{M_0} \text{ x } 100$$

Where, Mo, M_1 and M_2 are mass of the honey sample taken, weight of the residue and crucible, weight of empty crucible, respectively.

3.5.7 Colour analysis

To determine colours, honey samples were heated to 50 ºC to dissolve sugar crystals, and then, honey colours were determined by measuring the absorbance of a 50% honey solution (*w/v*) at 635 nm. The honey colours were classified according to the Pfund scale after conversion of the absorbance values [7].

mm Pfund (intensity of honey colour) = -38.70 + 371.39 x A (absorption of honey solution).

3.5.8 Sugar content

To determine the sugar content, 5 g of honey samples were taken into a beaker and 100 mL warm water was added to it. The solution was stirred until all the soluble matters were dissolved and then, it was filtered through Whatman filter paper into a 250 mL volumetric flask. After adjusting the volume to the mark using distilled water, 100 mL of the solution was pipetted into a conical flask. Then, following the addition of 10 mL of diluted HCl the solution was boiled on water bath for 5 min. On cooling, the solution was neutralized to phenolphthalein with 10% NaOH and it was titrated against Fehling's solution. Finally the total sugar (%) was calculated as follows [29]:

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

3.5.9 Hydroxyl-methyl-furfural

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 of 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 to 100 ml.5 g of honey sample was taken in a beaker and dissolved in 25 mL distilled water. After transferring the resulting mixture in to 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 [30].

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

A_{284} is absorbance at 284 nm.

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

3.6 Data analysis

Analysis were performed in triplicate, and the results were expressed as mean $\pm SD$.Statistical tests such one way ANOVA and Person correlation were used to compare the physicochemical and biochemical properties of the honey varieties.

4. RESULT AND DISCUSSION

4.1 Physiochemical analysis

The observed physicochemical properties of the honey samples are presented in Table 1

Table 1: Physiochemical properties of Gera honey samples.

	Honey type			QSAE	CAC	EU	WHO
Parameter	**White**	**Mixed**	**Dark**	[1,3]	[33]	[33]	[1]
MC (%)	22.67 ± 0.11	22.33 ± 0.11	20.60 ± 0.00	≤ 21	≤ 21	≤ 21	21-23
EC (mS/cm)	0.18 ± 0.00	0.39 ± 0.00	0.46 ± 0.00				
Ash (%)	0.05 ± 0.01	0.17 ± 0.04	0.19 ± 0.00	≤ 0.6	≤ 0.6	≤ 0.6	0.6-1
pH	3.24 ± 0.00	3.66 ± 0.01	3.99 ± 0.01				
FA (meq/kg	19.4 ± 0.10	23.4 ± 0.10	33.2 ± 0.10	≤ 40	≤ 50	≤ 40	40
WIS (%)	0.20 ± 0.00	0.37 ± 0.03	0.27 ± 0.115	≤ 0.1	≤ 0.1	≤ 0.1	
Color (mm Pfund)	28.64 ± 0.21	61.81 ± 0.21	109.23 ± 0.77				

MC = moisture content, EC= electrical conductivity,
FA=free acidity), WIS= Water Insoluble Solid,

4.1.1 Moisture content

Moisture content is one of the most important parameter to be considered in the quality of honey. It determines the capability of honey to remain stable and to resist spoilage by yeast fermentation [31]. The moisture content in the investigated honey samples ranged from 20.6 - 22.8% with a mean value of 21.86%. The obtained values were relatively higher than the moisture contents of other areas of the country such as Burie district, Amhara region- (18.83%) [1] and Sekota district, northern Ethiopia (15.98%) [32].The moisture contents of the white and mixed Gera honey varieties were above the maximum limit (21%) established by QSAE, CAC and EU [1, 33]. But they were within the range (21-23) set by FAO/WHO guidelines of honey moisture content [1]. The moisture content of honeys of different origins shows variations and it may range from 13% to 29% [34]. High moisture content could lead to undesirable honey fermentation during storage caused by the action of osmotolerant yeasts resulting in the formation of ethyl alcohol and carbon dioxide. The alcohol can be further oxidized to acetic acid and water resulting in a sour taste [34]. This variation in water contents could be due to various factors such as the harvesting season, the degree of maturity that honey reached in the hive, type of hive used and environmental temperature [31]. The low moisture content of the honey samples indicates good storage ability of honey, since high moisture content could lead to fermentation during storage [31].

4.1.2 Electrical conductivity

Electrical conductivity is usually closely related to mineral salts, organic acids, and proteins contents of honey [35]. In present study, EC Gera honey varieties were varying from 0.18 - 0.46 mS/cm with a mean value of 0.34 mS/cm. Botanical origin of honey can be determined by its EC [15]. Honey could be classified as blossoms or honeydew origin depending its EC values. Honeys that have EC higher than 0.8 mS/cm are considered as honeydew honeys, whereas having lower EC values than 0.8 mS/cm are blossom honeys or blends of blossom and honeydew honeys [11]. Accordingly, the Gera Woreda honey varieties are categorized as blossom honeys. The EC of Gera honey samples was relatively lower than the Harenna forest honey, Bale, Ethiopia (0.70), [11]. The variability of the results could be due to fluctuation of mineral salts, organic acids, and protein concentrations [35].The EC of the studied honey varieties with is the range of the European Directives ($\leq$ 0.8 ms/cm) for the nectar honey [11].

4.1.3 Ash content

The ash content in the three honey samples is presented in Table 1.The % ash content indicates the mineral content of honey's and used quality criterion indicating the botanical origin of honey [34]. The % ash content of the studied honey samples ranged from 0.04 - 0.21% with a mean value of 0.14%.The mean % ash content of Gera Woreda honey varieties is lower than that of Bale natural forest honey (0.21%) [27], but it is the same as the mean % ash content of Sekota district, northern Ethiopia [32]. The difference in ash content of honey could be due to the variability of soil type, concentration of minerals found in the nectar and the botanical origin of honey samples [11]. The mean % ash content of the studied Gera honey varieties is lower than the maximum limits (0.6%) set for ash content of the honey by EU, CAC and QSAE [1, 33].

4.1.4 pH

Honey is acidic in nature irrespective of its variable geographical origin [11]. The pH values of the analysed honey samples were ranged from 3.23 - 4.00, with a mean value of 3.66.The observed mean pH value is lower than the mean pH values of Bale natural forest honey (3.75) [27] and, Gonder honey (4.02) [31]. But it is relatively higher than the pH of Burie district honey (3.53) [1]. Low pH value inhibits the presence and growth of microorganisms and makes honey compatible with many food products in terms of pH and acidity. This parameter has great importance during extraction and storage of honey, as they influence the texture, stability and shelf life of honey. Legal documents from national and international institutions have no reports about pH of bee honeys. A published report indicates that acceptable pH for honey is between 3.2 - 4.5 [3].

4.1.5 Free acid

The free acidity level of Gera honey samples were ranged from 19.30 - 33.30 meq/kg with a mean value of 25.33meq/kg. The obtained free acidity is higher than the free acidity of Gonder honey (23.9 meq/kg), [31], but, it is lower Bale natural forest (32.43 meq/kg) [27]. Variation of free acidity among different honeys can be attributed to floral origin or variation of the harvest season [36]. None of the Gera honey samples exceeded the acidity limit suggested by national (40 meq/kg) [1] and international standards (50 meq/kg) [33]. The low free acidity may indicate that the freshness of the honey samples and thus, the absence of fermentation due not stored for

long period of time storage. Although, honey contains several organic acids such as formic acid, acetic, citric, lactic, malic, oxalic, pyroglutamic and succinic acids, it is acidity is mainly attributed to its gluconic acid content [37]. Honey acidity is needed since it contributes to the flavor and antimicrobial property of honey, but when acidity is exceeding certain limits the honey becomes sour [1].

4.1.6 Water insoluble solids

The water-insoluble solids content is directly dependant up on honey handling and high concentrations are indications of improper handling during harvest. The water-insoluble solids of honey include wax, pollen, honey comb debris, bees and filth particles. This indicates that honey's water insoluble matter and used as a criterion for honey cleanliness [3]. The water-insoluble solids of the studied honey samples were ranging from 0.15 - 0.35 with a mean value of 0.25. The observed mean value is higher than the national and international maximum limits [3, 33], indicating that the studied honey's contain wax, pollen, honey comb debris, bees and filth particles [3]. The mean water-insoluble solids obserbed in this study is lower than the mean value of Sekota district (0.62%) [32] and Silti Woreda (0.26%) [3].

4.1.7 Colour analysis

Honey is classified by the US Department of Agriculture into seven colour categories (Table 2) according to the Pfund scale [27].

Table 2: Colour classifications of honey, according to the Pfund scale [27].

Categories colour	Pfund scale (mm)
Water White	< 8
Extra white	9 - 17
White	18 - 34
Extra light amber	35 - 50
Light amber	51 - 85
Amber	86 - 114
Dark amber	114

The color of honey varies depending on its origin (floral source) and constituents (mineral content). It also depends on its chemical composition especially chlorophylls, carotenoids, flavonoids and polyphenols [38]. Honey consumers' preferences are usually determined by the color. Color is the single most important factor determining import and whole sale prices [39].

The greatest pfund value in the analysed honey samples was observed in the dark honey (109.23 mm pfund and the lowest pfund value was in white honey 28.64 mm pfund (Figure 2). According to the U.S Department of Agriculture Standard, the three Gera honey varieties were classified as the white sample as white color (28.64 mm Pfund), the mixed sample as light amber color (61.81 mm Pfund) and dark samples as amber color (109.23 mm Pfund). The variations are entirely due to the plant source of the honey. The color and consistency of honey is not only affected by the flower from which the nectar was collected but it's also affected by factors such as weather and climatic change [3].

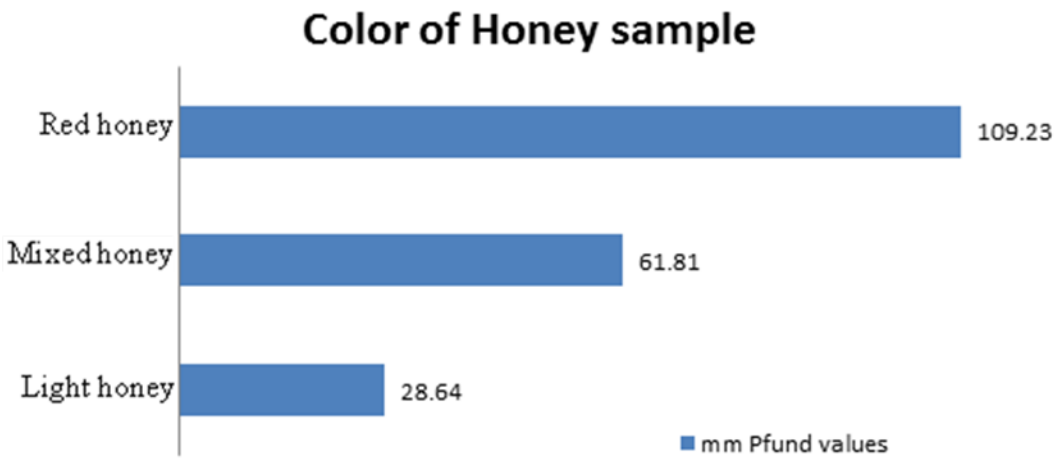

Figure 2.**Color characteristics of honey samples**

4.2 Biochemical analysis

The total sugar content and HMF of the three honey types were determined and their results were shown in Table 3.

Table 3: Biochemical parameters of analyzed honey samples produced in Gera Woreda.

Parameter	**Honey Type**			QSAE	CAC	EU	WHO
	White	**Mixed**	**Dark**	[1]	[33]	[33]	[1]
Sugar content (%)	69.32 ± .79	65.38 ± 1.61	61.93 ± 2.48				
HMF(mg/kg)	5.09 ± 0.59	7.63 ± 0.79	11.43 ± 0.31	≤40	≤60	≤40	≤80

HMF = Hydroxyl methyl furfural

4.2.1 Sugar content

All honey samples tested contained total sugars at different concentrations (Table 3). The total sugar content the samples were ranged from 59.49 - 70.36 with a mean value of 65.54. These results confirmed that sugars are the major constituents of honey. The obtained total sugars are comparable to honey from Pakistan (61.7 - 72.4%), and Brazil (67.6 - 72.4%) [35], but lower than honey from Iran (74.9 - 81. %), Burkina Faso (73.9 - 85.5 % and Cameron (77.9 - 83.1%) [35].The analysed sugar content values are higher than those of honey collected in western India (42.8 - 60.6%) [35].

4.2.2 Hydroxyl-methyl-furfural

The amount of HMF in the honey is one of the most important indicators of honey quality and widely recognized as a parameter in evaluating the freshness of honey. In fresh honey, HMF is present only in small amounts [9]. HMF is a by-product of fructose decay and formed during heating. Its excessive value indicates overheating during processing, or prolonged storage and thus considered as the main indicator of honey deterioration [16]. HMF content of the studied honey samples ranged from 5.09 - 11.43 mg/kg with a mean value of 8.05mg/kg. The mean HMF value of the studied honey samples is higher than the mean HMF of Honey from Gonder (1.8 mg/kg) [31], but it was lower than honey of Bale natural forest (36.35 mg/kg) [27] and Burie district (38.55 mg/kg) [1]. Generally, the mean HMF content Gera honey is much lower than the national and international limits [1, 33], indicating that the collected honey samples were fresh or not stored for long period of time. HMF content is less in fresh honey and its content increases during conditioning and storage, depending on storage temperature [40].

4.3. Analysis of variance

One way ANOVA results (Appendix 2) indicated the presence of significant differences in the studied physiochemical and biochemical parameters among honey samples, namely white honey, mixed honey and dark honey at $P < 0.05$. All of the parameters studied, moisture content, EC, pH, free acidity, ash content, differences in colour, HMF content, total sugar content and water-insoluble solid of honey samples were demonstrated significant differences.

4.4. Correlation of honey parameter

Table 4 shows the Pearson correlation (at $p \leq 0.05$) between the analyzed physiochemical and biochemical parameters of studied honey samples. Strong positive correlations (were observed between moisture content and total sugar content and very strong positive correlation between EC and pH value, EC and free acidity, EC and ash content, EC and honey color, pH value and ash content, pH value and honey color, pH value and HMF. There are also strong positive correlations between free acidity and ash content and very strong positive correlation between free acidity and honey colors, ash content and honey color, ash content and HMF, honey color and HMF. There was also a good negative correlation between moisture content with all parameters except sugar content, EC and sugar content, pH value and sugar content, free acidity and sugar content, ash content and sugar content, water insoluble and sugar content, honey color and sugar content, HMF and sugar content.

Table 4: Pearson Correlation between physiochemical and biochemical parameters of studied honey.

Parameter	MC	EC	pH	FA	AC	WIS	CA	TSC	HMF
MC	1	-0.793	-0.897	-0.987	-0.702	-0.699	-0.960	0.797	- 0.949
EC		1	0.979	0.870	0.952	0.848	0.929	-0.855	0.909
pH			1	0.950	0.926	0.826	0.984	-0.886	0.963
FA				1	0.796	0.760	0.991	-0.841	0.975
AC					1	0.761	0.861	-0.841	0.821
WIS						1	0.800	-0.555	0.794
CA							1	-0.875	0.982
TSC								1	-0.826
HMF									1

A linear relationship known to exist between the EC and ash content. Ash and EC values depend on the mineral content of the honey: ash gives a direct measure of inorganic residue after carbonization, while EC measures all ionisable organic and inorganic substances. The coefficient correlation between EC and ash was found to be (r = 0.95), which indicated a strong positive correlation between the parameters. The positive correlation between honey colour and HMF content is due to the fact that white coloured honeys turn darker when heated or stored in hot conditions for long periods. Thus, dark coloured honeys may partly be a result of heating or long storage, the processes that generate high level of HMF.

The negative correlation between colour and sugar content suggest that darker honeys may have lower amount of sugars than white honeys. The sugar content derived from the moisture content showed a negative correlation with HMF content, indicating that the HMF build up depends on the degeneracy of sugars through the Maillard reaction. It is however difficult to explain the positive correlation between electrical conductivity and water insoluble solid, electrical conductivity and HMF, pH value and water insoluble solid, ash content and HMF levels observed in this study.

5. CONCLUSIONS AND RECOMMENDATIONS

5. 1 Conclusions

The analytical laboratory result shown that the honey produced in Gera Woreda natural forest is from nectars of blossoms of flowering plants and found excellent quality characters according to the national and international honey quality standards. There is a significant variation between different honey types according to floral origin. This study demonstrated that honey collected from different floral origin significantly varied in physiochemical and biochemical properties. The Gera Woreda forest honey moisture, EC, pH, free acid, ash content, total sugar, and HMF content values satisfied the CA, EU and Ethiopian standards; except for the water insoluble solids.

5.2 Recommendations

Based on the obtained results the researcher would like to forward the following recommendations:

- Further studies are needed in order to know the direct effect of pollen and floral origin on the physiochemical and biochemical properties of honey.
- Further studies are suggested to find out the effect of storage on the major components of bee honey and hydroxyl-methyl furfural (HMF).
- More studies are needed to evaluate the quality of Gera honeys based on medicinal, nutritional and antioxidant properties.
- To improve the honey quality defects associated with water-insoluble matter in the study area, there is a need to provide a practical training to local beekeepers and traders about the way how to harvest, handle, process, package and market honey; moreover, facilitating supply of quality apicultural equipment is crucial.

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7. APPENDEX

Appendix 1: Relationship of water content of honey to refractive index.

Water Content,	Refractive Index	Water Content	Refractive Index
g/100 g	20°C	g/100 g	20°C
13.0	1.5044	19.0	1.4890
13.2	1.5038	19.2	1.4885
13.4	1.5033	19.4	1.4880
13.6	1.5028	19.6	1.4875
13.8	1.5023	19.8	1.4870
14.0	1.5018	20.0	1.4865
14.2	1.5012	20.2	1.4860
14.4	1.5007	20.4	1.4855
14.6	1.5002	20.6	1.4850
14.8	1.4997	20.8	1.4845
15.0	1.4992	21.0	1.4840
15.2	1.4987	21.2	1.4835
15.4	1.4982	21.4	1.4830
15.6	1.4976	21.6	1.4825
15.8	1.4971	21.8	1.4820
16.0	1.4966	22.0	1.4815
16.2	1.4961	22.2	1.4810
16.4	1.4956	22.4	1.4805
16.6	1.4951	22.6	1.4800
16.8	1.4946	22.8	1.4795
17.0	1.4940	23.0	1.4790
17.2	1.4935	23.2	1.4785
17.4	1.4930	23.4	1.4780
17.6	1.4925	23.6	1.4775
17.8	1.4920	23.8	1.4770
18.0	1.4915	24.0	1.4765
18.2	1.4910	24.2	1.4760
18.4	1.4905	24.4	1.4755
18.6	1.4900	24.6	1.4750
18.8	1.4895	24.8	1.4745
		25.0	1.4740

Appendix 2: ANOVA analysis of honey samples on the physiochemical and biochemical parameters

		ANOVA				
		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
MC	Between Groups	7.387	2	3.693	415.500	0.000
	Within Groups	0 .053	6	0.009		
EC	Between Groups	0 .130	2	0.065	105201.975	0.000
	Within Groups	0 .000	6	0.000		
pH	Between Groups	0 .856	2	0.428	-	0.000
	Within Groups	0 .001	6	0.000		
FA	Between Groups	302.480	2	151.240	15124.000	.000
	Within Groups	0 .060	6	0.010		
AC	Between Groups	0 .035	2	0.018	30.207	0.000
	Within Groups	0 .003	6	0.001		
WIS	Between Groups	0 .022	2	0.011	7.800	0.021
	Within Groups	0 .008	6	0.001		
CA	Between Groups	9843.558	2	4921.779	21570.983	0.000
	Within Groups	1.369	6	0.228		
TSC	Between Groups	81.966	2	40.983	10.253	0.012
	Within Groups	23.982	6	3.997		

MC = Moisture Content (%), EC = Electrical Conductivity (mS/c), FA = Free Acidity (milli equivalent /kg), AC = Ash Content (% by mass), WIS = Water Insoluble Solid (%), CA = Color Analysis, TSC = Total Sugar Content (%), HMF = Hydroxyl- methyl-furfural (mg/kg).