JIMMA UNIVERSITY
COLLEGE OF AGRICULTURE AND VETERINARY MEDICINE
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EVALUATION OF HONEY QUALITY AND BEEKEEPING SYSTEM IN SELLECTED DISTRICT OF HORO GUDURU WOLLEGA ZONE, OROMIA REGION , WESTERN ETHIOPIA

MSC THESIS

BY
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Evaluation of Honey Quality and BeekeepingSystem In Sellected District of Horo Guduru Wollega Zone, Oromia Region , Western Ethiopia

A Thesis Submitted to the School of Veterinary Medicine, Jimma University College of Agriculture and Veterinary Medicine in Partial Fulfillment of the Requirements for the Degree of Master of Science in Veterinary Public Health

By

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I have completed my thesis research work as per the approved proposal and it has been evaluated and accepted my advisors. Hence, I hereby kindly request the department to allow me to present the findings of my work and submit the thesis.

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DEDICATION

I dedicate this piece of work to my mother, for her committed lives with strong prayers for the piece and health of our family. I dedicate it also to my beloved wife Beshatu Alemayehu for being providing support and inspiration to my academic journey.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my real work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for MSc degree at Jimma University, College of Agriculture and Veterinary Medicine and is deposited at the University library to be made available to borrowers under rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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BIOGRAPHICAL SKETCH

The author was born in 1990 G.C. from his father Dugasa Yadeta and his mother Dinkinesh Dugasa in Amuru Wereda, Horo Guduru Wollega zone, Oromia regional State, Ethiopia. He completed his elementary school in Ejere Goromti elementary school and Amuru primary and secondary school from 1996 to 2003 and high school education in Fiche high school from 2004to 2005 and completed his preparatory school in Amuru Preparatory school from 2006 to 2007.

After passing the Ethiopian School Leaving Certificate Examination in 2008, he joined Haramaya University Faculty of Veterinary Medicine in October, 2008 and graduated with Doctor of Veterinary Medicine on 12th July, 2012. After he received his DVM degree, he was employed by Oromia Special Zone of Amara Regional State Agricultural office on Febreury, 2013 as a junior animal health prevention and quality control expert at Kamissie administrative town, Oromia special Zone and he served there until June, 2015.

Since June 2015 he was transferred to Horo Guduru Wollega Amuru district Livestock Development and Fishery Office served at different position as an expert, team leader, and animal health extension and technology dissemination team coordinator. On October, 2018, he joined Jimma University College of Agriculture and Veterinary Medicine to pursue his postgraduate studies in Veterinary Public Health stream from October, 2018 to date.

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ABBREVIATIONS AND ACRONYMS

AFB	American foulbrood
ANOVA	Analysis of Variance
ARDO	Agricultural and Rural Development Office
BC	Before Christ
CAC	Codex Alimentarius Commission
CAS	Codex Alimentarius Standard
COMESA	Common Market for Eastern and Southern Africa
CSA	Central Statistical Authorit
DAs	Development Agents
Ec	Electrical conductivity
EFB	European foulbrood
EU	European Union
FAO	Food and Agricultural Organization
G	Conductance in ms
ha	hectar
HBRC	Holeta Bee Research Center
HGWZLRDFO	Horo Guduru Wollega Zone Livestock Resource Development and Fishery Office
HHs	House Holds
HMF	Hydroxymethylfurfural
HO	Health Office
IHC	International Honey Commission
JJWANRO	Jardaga Jarte Woreda Agricultural and Natural Resource Office
K	cell constant in cm^{-1}
KAP	Knowledge, Attitude and Practice
LRDFO	Livestock Resource Development and Fishery Office
M1	Weight of dish + ash
M2	weight of dish
Mc	Moisture content
Mo	weight of Honey
MoA	Ministry of Agriculture
$mS .cm^{-1}$	milli Siemens per centimeter
MS excel	Micro Soft excel
NGO	Non-Governmental Organization
QSAE	Quality Standard Authority of Ethiopia
RFI	Radio Frequency Identification
RI	Refractive Index
SH	Electrical Conductivity of honey solution in mS cm-1
SPSS	Statistical package for social science
W	Water content in g/100

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ABSTRACT

Honey is one of the oldest sweetening foods, has medicinal, nutritional and economic importance. The present work has the aim of evaluating honey quality and assessing knowledge, attitude and practices (KAP) of beekeepers by using 24 honey samples and 125 selected beekeepers in Amuru district of Oromia region, west Ethiopia. Of the total of 24 honey samples, of which 18 samples were purposively taken from selected six kebeles from farm gates of beekeepers and 6 samples from honey retailers. The main analyzed parameters were Moisture Content (MC), Hydroxymethylf urfuran (HMF), pH, Free Acidity (FA), Ash, Electrical Conductivity (EC), Reducing Sugar, Sucrose and Color. The analysis was done in Holeta Bee Research Center. A total of 125 respondents were interviewed and field observation was made used as tool of data collection. The laboratory result and the survey were analyzed by SPSS version.23 by using GLM, and using descriptive analysis method respectively. The overall mean ± SD of MC, HMF, pH, FA, ash, EC, RS, Sucrose and Color of honey analyzed were; 20.43 ± 1.32 %, 16.39 ± 2.68 mg/kg, 3.92 ± 0.07, 36.67 ± 2.13 meq/kg, 0.25 ± 0.14, 0.68 ± 0.3 %, 73.08 ± 0.92 %, 1.80 ± 0.35 % and 103.75 ± 2.89 mm respectively. The value of moisture, HMF and FA of honey significantly ($p < 0.05$) influenced by agro-ecologies and sources. pH and Color values of honey from the retailer was significantly higher ($p < 0.05$) than the honey collected from beekeepers. There was no significant difference ($p > 0.05$) revealed among hive types of all parameters analyzed. The moisture value of honey mainly declared significantly correlated with free acidity with r = 0 .63**, ($p < 0.01$) and strong positive correlation between HMF and EC with the highest r= 0.77 **, ($P < .01$) is observed. Beekeeping mainly practiced by males 87.20%. The range of age of respondents were from 25 to 65 years old mostly. 89.60% knew the health benefits of honey and practiced to treat many of the human and animal diseases. Presence of honey bee flora, and bee colony considered to be an essential indicato r for potentialities of the beekeeping of the area. However, pests and predators, indiscriminate agroc hemicals and diseases,were the major identified beekeeping constraints. It can be concluded that most results of honey quality analyzed on honey produced in the area is of good quality and can meet the national and international standards. Beekeepers KAP study indicates honey has a public health benefits which could be a great contribution for the development of modern medicine. Therefore, strong efforts have to be made to promote this indigenous knowledge and practices of beekeepers on honey health benefits and improve beekeeping system through extension

***Key words**: Beekeepers, Honey quality, Indigenous, KAP, Physicochemical, Retailer*

1. INTRODUCTION

Honey is one of nature's wonders, of the oldest sweetening foods, has medicinal, nutritional and economic importance(Abeshu and Geleta, 2016).It is certainly the only sweetening agent that can be used by humans without processing (Yadata, 2014). History has revealed that humans had used bee products such as honey for thousands of years in all societies worldwide (Al-Waili *et al.*, 2012). The method of trapping a colony of bees and then taking them to home yard, allowing them to rear and multiply, prepare honey and beeswax is known as beekeeping (Seeley, 2019).Beekeeping seems as old as time itself and no one knows exactly when and where it was started. However, it is believed that, primitive man, may be even Adam and Eve, harvested honey from bee nests in hollow trees and rock crevices (Fissures) and is portrayed in many rock paintings in Africa and Europe (Wallace, 2007).

Honey has been used as a food and medical product since the earliest times. It is a natural substance produced by honeybees, *Apis mellifera*, from the nectar of blossoms or from exudates of trees and plants giving nectar honeys or honeydews, respectively. As the only available natural sweetener, honey was an important food for Homo sapiens from his very beginnings. Indeed, the relationship between bees and man started as early as the Stone Age (Alvarez *et al.*, 2010).Honey is the sweet and viscid fluid, contains significant amounts of mineral, vitamins, and enzymes (Darko *et al.,* 2017*)*. With respect to carbohydrates, honey is mainly fructose (about 38.5%) and glucose (about 31.0%) (Blasco *et al.*, 2011*)* and other sugars are present as traces, depending on floral origin. Honey composition varies depending on its floral, geographical and entomological sources. In addition, external features such as seasonal and environmental factors honey processing, and storage time and conditions have crucial effects on honey's composition (Gidamis *et al*., 2004).

Amongst the factors that most influence quality is high temperature, length of storage and moisture content greater than 21%. They lead to fermentation, high levels of Hydroxymethylfurfural (HMF), loss of enzymatic activity, changes in flavor, darkening and microbial growth (Kinati *et al.*, 2011).The quality of honey relied to a great extent on the art of the producer in storing and blending the product. In marketing of honey, consumers should have confidence that they are getting good quality for what they are paying so that the country able to earn foreign currency to revamp the national economy (Getachew *et al*., 2014).Agricultural contamination with pesticides and antibiotics is a challenging problem that needs to be fully addressed. Honey, are widely consumed as food and medicine and their contamination may carry serious health hazards. Pesticide residues cause genetic mutations and cellular degradation and presence of antibiotics might increase resistant human or animal's pathogens (Al-Waili *et al.*, 2012). Due to continuous expansion of the world honey market, the importance of apiculture as an industry has also grown. Composition and quality criteria of honey are defined by the Codex Alimentarius standard (Souza *et al.*, 2006) and the EU Honey Directive (Fallico *et al.*, 2006) which state that honey should not have any ingredients added; no particular constituent can be removed from it; it does not have any objectionable matter, flavor, aroma or taint absorbed from foreign matter during processing and storage.; and it should not be heated or processed (Bogdanov *et al.*, 2002).

Honeybees are of critical importance in Africa for both ecological and economic reasons. Their contribution to floral biodiversity and conservation, by virtue of their pollination of indigenous flowering plants is unknown, but certain to be considerable. Economically, honeybees are critical for the pollination of a host of commercial crop plants as well as being the source of energy and livelihood for many thousands of mostly small-scale beekeepers (Allsopp, 2004). In African developing countries agricultural production is expected to become increasingly reliant on pollinator services. However, in response to the increasing challenge of providing food security in sub-Saharan Africa, farmers have been simultaneously encouraged to adopt intensive agricultural practices often characterized by widespread use of pesticides as foliar sprays and seed coatings meaning service provision by bees is contingent upon their ability (Power, 2010). Food security is not only a matterof producing grains but also the financial power to pay for the purchase of grain (Caplan, 2002).

Ethiopia occupies the major part of the Horn of Africa. The country covers approximately 1.11 million square kilometers and it is a country of great geographical and climatic diversity, with varied ecological conditions (Froehlich and Siebrits, 2019).Ethiopia, a potential beekeeping giant. In an Abyssinian grain-market, many honey bees were observed collecting from open sacks of shirro (Cicer arientinum) as a pollen substitute. Usage of honey for making “tej”, and for selling (Hussein, 2001).

In Ethiopia beekeeping sub-sector has been an integral part of agriculture. It has been contributing to the household income and poverty alleviation and national economy through export. Ethiopia has huge apicultural resources that made it the leading honey and beeswax producer in Africa (Fikru, 2015). Beekeeping is a long standing practice in Ethiopia and it accounts 1.3 % of agricultural GDP (Demisew, 2016). According to FAO (2009), report 45,300 metric tons of honey is produced per annum in Ethiopia makes the country to rank first honey producer in Africa and ninth in the world. However, the majority of honey is crude and poorly managed. In Ethiopian, only about 10% of the honey produced in the country is consumed by the beekeeping households. The remaining 90% is sold for income generation and of this amount, it is estimated that 80% is used for tej brewing (Legesse, 2014).

1.1. Statement of the Problems

Beekeeping activities in Ethiopia is mainly one of income generating economic activities to subsistence farmer. However, constrained by inability in the transformation, lack of sufficient awareness on beekeeping practice, promotion, scaling up to rapid growth, lack of commercial beekeeping development and beekeeping technology, limited credit supply, quality issue, lack of market access, research and information transmissions (Winberg, 2011).The reports from different parts of Ethiopia are indicating that extreme use of herbicides, pesticides and insecticide chemicals are increasing. Studies on the physicochemical and quality analysis have been carried out in some parts of Ethiopia by different researchers(Berhe *et al*., 2016). The quantity and quality of Ethiopian honey in generally poor, as 95% of beekeepers follow traditional method of beekeeping practice with no improved techniques or technology (Beyene *et al*., 2014). Honey is of good quality as long as it is in the hive, but faulty handling from the time of its harvest until it reaches to market is responsible for its inferior quality. Several factors have contributed to its low quality among which high moisture content is the major quality problem in Ethiopia. Harvesting unripen honey, unsuitable honey storage container and storage places also attribute to high moisture content (Shunkute *et al*., 2012).

Oromiya, Amahara, Southern National Nationalities and People,Tigray and Benshangul are the major honey producing regions in Ethiopia, with production quantities of 21,403.404tons, 10,834 tons, 9,471.625tons and 3,293.394tons and 2,231.380 tons respectively (CSA, 2017/2018). In

Oromia region beekeeping is also a very long-standing practice in the farming communities and it plays a significant role as a source of additional cash income and nutrition for many subsistence farmer beekeepers. It is an integral part of the smallholder farming system. The natural vegetation coverage is relatively high, as a result in this region the honeybee population is dense and production is relatively high. Besides, the beekeeping potentiality of the region, it is partly attributed to the various cultivated oil crops, pulse and field flowers, which are very important, source of forage (Ambaw and Teklehaimanot, 2018).

Amuru district is found in Horo Guduru Wollega Zone, Oromia region, with suitable agro-ecology that makes the district potential for honey production. The nature of diversified flowering plant species and agro-climatic conditions has enabled the area to sustain a number of honeybee colonies.Despite the potentiality of the areas and large volume of honey production, there is no scientific research which has been done to quantify and document the actual properties and quality of honey, beekeepers Knowledge, Attitudes and Practices (KAP). Therefore, this study has been conducted with the following objectives:

1.2. Objectives

1.2.1. General objective

- The aim of the study was to evaluate honey quality, and assess KAP of beekeepers in the study area.

1.2.2. Specific objectives

- To evaluate honey quality
- To assess beekeepers KAP on health benefits of honey
- To assess beekeeping system and managements

2. LITERATURE REVIEW

2.1. People, Bees and Rural Development

Since ancient times, people have been drawn to the study of bees. Bees are spritely creatures that move about on pleasant bright days and visit pretty flowers (Genise, 2017).Honeybees have the capacity to live together harmoniously in a nest, foraging nectar and pollen grains from flowering plants to satisfy the foodneeds of the colony members in the nest. Honey bees are the most usefuland friendly insects to all living things on earth. Such insects that live and work together for their nest activities in an area selected are called social insects (Seeley, 2010). Bees are sensitive indicators of an intact environment, and as essential and persistent shapers of the environment, have a significance that cannot be estimated highly enough. Honey bees are very important for the maintenance of biodiversity (Judova *et al.*, 2016).

Moreover, bees are important pollinators of both natural vegetation and crops, and certain kinds of bees make useful products, especially honey and wax. We are social animals; some bees are also social. Their interactions and communications, which make their colonial life function, have long been matters of interest; we wonder how a tiny brain can react appropriately to societal problems similar to those faced by other social animals, such as humans. For a biologist or natural historian, bees are also fascinating because of their many adaptations to diverse flowers; their ability to find food and nesting materials and carry them over great distances back to a nest; their ability to remember where resources were found and return to them; their architectural devices, which permit food storage, for example, in warm, moist soil full of bacteria and fungi; and their ability to rob the nests of others, some species having become obligate robbers and others cuckoo like parasites. These are only a few of the interesting things that bees do (Michener, 2000).

Human experience of beekeeping started 5 million years ago from the earliest times in which presumably the honeybees coming to existence. The object of exploitation for their by the ancestors of mankind who appeared 1.8 million years ago, in a manner similar to the one by which chimpanzees today lick honey of tree twigs by inserting them into wild bee beehives (Oldroyd and Wongsiri ,2009).

Beekeeping is of pivotal importance, combining the wide economical aspect of honey production and the important ecological services provided by honeybees (Zoccali *et al*., 2017).

Rural development aims to help people manage their livelihoods better through sustainable use of the available resources. It provides them with greater social and economic power by offering them opportunities to work in line with their capacity, without hampering the eco-services provided by their environment (Ahmad *et al.*, 2007). Beekeeping and honey hunting have been practiced by different societies since ancient times and have always been linked to development. 'Honey hunting' – collecting honey from wild bee colonies – is an ancient practice as shown, for example, in cave paintings dating back to 11,000 BC found in Madhya Pradesh, India (Roué *et al.*, 2015).

Beekeeping is the establishment and tending of colonies of social bees of any species. Most of the world's beekeeping is done with *Api mellifera.* Beekeeping is done mainly to produce honey, but there are also other specialized types of operations. These include the rearing of queens or package bees for other beekeepers that are producing honey. Another type of beekeeping provides colonies of bees to pollinate crops, since in many areas of large-scale agriculture; the native pollinators have been destroyed. Each type of beekeeping requires the management of colonies to stimulate the bees to do what the beekeeper wants-for instance, to rear younger house bees to produce royal jelly or more foragers to pollinate crops. So it is essential that hives and frames have standard dimensions and that an accessory is used to ensure that frames are always exactly the correct distance apart (Crane, 2009).

2.2. Physico-chemical Property and Quality of Honey

The study of the physical and chemical properties of honey has increased in recent years because these parameters are important for the certification process that determines honey quality (Farooq and Maqbool, 2008). Honey features vary depending on the botanical source and geographical origin, as well as climatic, processing and storage conditions. Honey is mainly composed of carbohydrates and water, parameters that influence its shelf life and some of its properties, including color, flavor, density, viscosity, hygroscopicity, and crystallization. Honey also contains small amounts of other components, such as nitrogen compounds, organic acids, minerals, vitamins, Maillard reaction products, volatile compounds, and several bioactive substances that affect sensory and physical characteristics, as well as biological potential (Da silva *et al*., 2016).

The quality of honey is normally assessed by physico-chemical test of its ingredients. These ingredients have substantial insinuations (hints) on honey industry as they influence the storage quality, granulation, texture, flavors and nutritional and medicinal value of the product. Internationally, certain constituents are proposed as quality criteria for honey and these include, but not limited to, moisture level, electrical conductivity, reducing sugars, amount of fructose and glucose, concentration of sucrose, free acidity, total acidity, hydroxymethylfurfurale (HMF) and proline content. The magnitude of these physico-chemical properties of honey could be influenced, among others, by the type of storage container used. In this regard, it is assumed that the physio-chemical properties of honey can change with time and kinds of storage containers (Gebremeskel, 2015).Chemical composition of honey varies depending on plant source, season and production methods. Storage conditions may also influence final composition, with the proportion of disaccharides increasing overtime. Amongst the factors that most influence quality is high temperature, length of storage and moisture content greater than 21%. They lead to fermentation, high levels of Hydroxymethylfurfural (HMF), loss of enzymatic activity, changes in flavor, darkening and microbial growth (Kinati *et al*., 2011).

Quality of honey is mainly determined by its sensorial, chemical, physical and microbiological characteristics. Pollen profile, color, moisture content, ash, acidity, electrical conductivity, pH, reducing sugars, apparent sucrose and HMF were the parameters analyzed in each honey sample (Gomes *et al*., 2010).A quality product will go a long way in developing the confidence that encourages return, customers and efficient production of a product to any marketing scheme (Getachow *et al*., 2014).External factors like climate, harvesting conditions and storage can also influence it (Belie, 2009). Inappropriate materials used for honey handling, careless storage conditions of honey leads to reduce its quality (Mitikie, 2017). The quality and properties of honey are also related to honey maturity, the production methods, climatic conditions, processing and storage conditions as well as nectar sources of the honey (Gobessa *et al.*, 2012).

2.2.1. 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 can be difficult to produce good quality honey of sufficiently low water content, which can be measured using a gadget called refracto meter. 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% (Crane,1996). The moisture content of honey should not be more than 20% (Ball, 2007).

2.2.2. Viscosity

Viscosity of nectar- and honey-thick liquids measured at a typical serving temperature. Serving temperature results are contrasted with viscosity measurements collected at room temperature, showing variable thickening patterns especially related to the type of thickening agent (Garcia *et al.,* 2008). Freshly extracted honey is a viscous liquid. 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 reduce honey flow during extraction, pupping, settling, filtration, mixing and bottling (Olaitan *et al*., 2007).

2.2.3. Density

Another physical characteristic of practical importance is density. Honey density, expressed as specific gravity, is greater than water density, but it also depends on the water content of the honey. Because of the variation in density it is sometimes possible to observe distinct stratification of honey in large storage tanks. The high water content (less dense) honey settles above the denser, drier honey. At the temperature of 20 °C, density of honey typically ranges between 1.38 and 1.45 kg/l (Suliman, 2017).

2.2.4. Color

Honey is color graded into light, amber, and dark categories which do not really have any bearing on quality. Some of the most distinctively and strongly flavored honey variety such as basswood is very light, while very mild and pleasant honeys such as tulip poplar can be quite dark. Honey color is measured on the P fund Scale in millimeters. 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. Minerals such as potassium, chlorine, sulfur, iron, manganese, magnesium, and sodium have been found to be much higher in darker honeys (Mahmoud, 2012).

2.2.5. Electrical conductivity (Dry matter of honey)

The electrical conductivity of honey is defined as that of a 20% weight in volume solution in water at 20 ^{0}C, where the 20% refers to honey dry matter. The result is expressed in milliSiemens per centimetre (mS.cm-1).The method is valid for the determination of the electrical conductivity of honey in the range 0.1 - 3 mS.cm-1.This measurement depends on the ash and acid contents of honey: the higher their content, the higher the resulting conductivity (Bogdanov *et al*., 2002). It is a very easy and quick method, needing only inexpensive instrumentation. The conductivity is a good criterion of the botanical origin of honey and thus is very often used in routine honey control. A lower limit has been proposed for blossom than for honeydew honeys. Exceptions have to be made for some blossom honeys (Bogdanov and Peter, 2002).

2.2.6. Moisture

The moisture content is the most essential quality component of honey, because the rate of fermentation, its shelf life span and processing characteristics are greatly determined by the amount of moisture content. The different moisture content of honey depends on harvesting season, the degree of maturity that honey reached in the hive, type of hive used, environmental temperature and moisture content of original plant (Gebremedhin *et al*., 2013). Moisture content of honey can naturally be as low as 13 % or as high as 23 % depending on the source of the honey, climatic conditions and other factors (Meixner, 2010).

2.2.7. pH and free acidity= which indicates degree of fermentation

Honey pH depends on both the ionized acids of this food and mineral elements and influences microorganism's development, enzymatic activity and texture, among other properties. Honey typically has a pH in the range of 3.3–5.6. The natural acidity of honey inhibits growth of many pathogenic bacteria whose minimum tolerated pH is in the range of 4.0–4.5. These two properties of honey can influence honey stability and its storage conditions and also they give some information on honey origin (Derebaşı*et al.*, 2014). The high acidity of honey is an indication of the fermentation of sugars presents in the honey into organic acid particularly the gluconic acid and the inorganic ions such as phosphate and chloride. According to Bogdanov *et al.* (2009) these honey fermentation results are responsible for two important characteristics of honey: flavor and stability against microbial spoilage (El Sohaimy *et al*., 2015). The acidity of honey developed due to the presence of organic acids. The value of honeys acidity, lower than 50 meq/kg of honey, means that honeys will not be fermented (Gebremeskel, 2015).

2.2.8. Reducing sugar

Apparent reducing sugars' are defined as those sugars which reduce a Fehling's reagent under the conditions specified. 'Apparent sucrose' is defined as 0.95 of the difference in 'apparent reducing sugars' before and after the prescribed hydrolysis procedure. This method is a modification of the Lane and Eynon procedure, involving the reduction of Soxhlet's modification of Fehling's solution by titration at boiling point against a solution of reducing sugars in honey using methylene blue as an internal indicator. The difference in concentrations of invert sugar is multiplied by 0.95 to give the apparent sucrose content. This method is based on the original method of Lane and Eynon and is also used in the Codex Alimentarius standard (Bogdanov *et al*., 2002).

2.2.9. Total ash

The ash content is a quality criterion for honey origin, the blossom honeys having lower ash content than the honeydew ones. The amount of minerals present in honey does not significantly contribute to the dietary recommendations. This method will probably be replaced by the faster and easier conductivity measurement (Downey *et al*., 2005).

Mineral content in honey is generally low, ranging between 0.02 and 0.3% in blossom honeys, while in honeydew honeys can reach 1% of the total. It is influenced by soil and climatic conditions, as well as the chemical composition of nectar that varies according to the different botanical sources involved in honey formation. Variations can also be related to harvesting, beekeeping techniques and the material collected by the bees during foraging on flowers. Minerals are absorbed in their salt forms dissolved in water, moving from the roots to the plant sap and then being pumped to the nectar or honeydew and pollen (Ouchemoukh *et al*., 2007). The most important minerals found in honeys are potassium, sodium, calcium and magnesium. Less abundant elements are iron, copper, manganese, chlorine and in minor quantities trace elements such as boron, phosphorus, sulfur, silicon, bare and nickel, among others. Potassium is the main one, standing for 80% of the total, as a result of its quick secretion by nectar sources (Machado *et al*., 2017).

2.2.10.Hydroxymethylfurfural

Hydroxymethylfurfural (HMF) is a furanic compound produced by sugar degradation, from dehydration of hexoses in acidic medium and to a lesser extent, as an intermediate in the Maillard reactions. HMF is a parameter of honey freshness, since it is absent or present in trace amounts in fresh honeys. High values of HMF are naturally present in honeys from warm climate areas, such as tropical and subtropical. HMF concentration increases during honey processing by heat treatment, and also by adulteration with commercial sugars and throughout storage (Shapla *et al*., 2018). HMF content is also affected by the use of metallic containers, pH, bee species and botanical source. In addition to processing, storage conditions affect the formation of HMF, and HMF has become a suitable indicator of honey quality. Its concentration increases when honey is heated and is in its storage (Mehryar *et al.*, 2013).

If you expose samples of honey at the temperature of 60°C for a longer period of time, a significant increase in the concentration of HMF is present. Noticeable increase of HMF concentration is observed by heating the samples at 90°C for 60 minutes where the results show that the average concentration of HMF was 48.8 mg/kg. Therefore, the content of HMF in honey is an important parameter in determining the quality of honey, his age, antioxidant activity, as well as the loss of its nutritional value (Kesic *et al*., 2017).

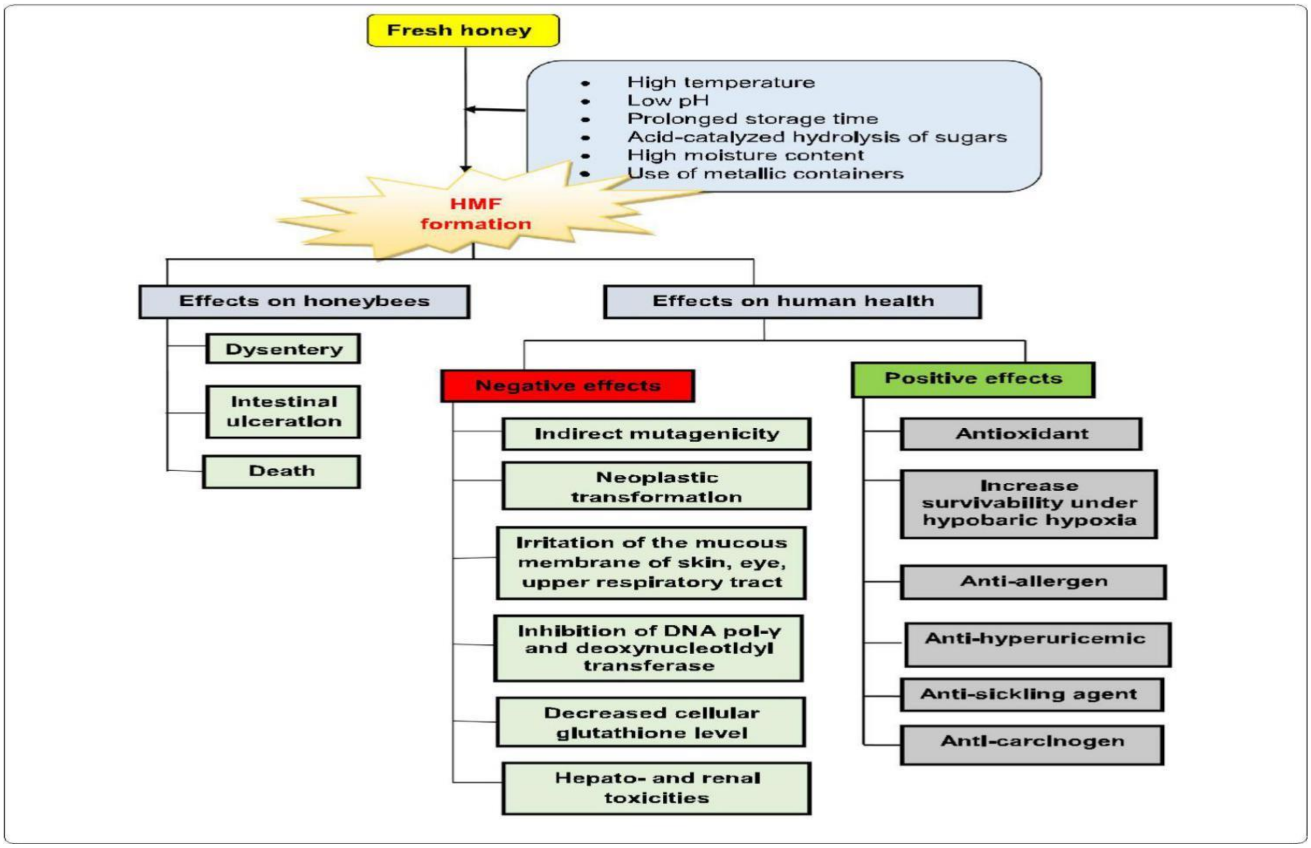

Figure 1. HMF effects on honey bee and human health

Source: Shapla*et al*., 2018.

2.3. Public Health Benefits of Honey

Honey is highly nutritious, it has traces of minerals and vitamins not to mention the antioxidants which destroy free radicals and delay ageing. In short, it is a safe and wholesome food for old, children and adults. Honey is a plant by-product and used medicinally around the world (Pollan, 2008). Honey is also an energizer, helping workers and athletes overcome fatigue and regain energy. Children, young and old can alike take honey, without worrying any side effects. Honey is a multivitamin tonic, has antibacterial properties and has antioxidants. Asthmatic persons can also benefit from taking honey every day. Ayurveda acknowledges honey as a wonder medicine capable of providing longevity. Osteoporosis is another condition, which can be prevented by taking honey regularly. Modern researches have shore up the wonderful effects of honey, proving honey to be effective against advanced cases of stomach and bone cancer.

Taking one tablespoon honey with one teaspoon cinnamon powder three times a day and cancer symptoms receded in one month (Kumar *et al*., 2010).

Honey has been used as medicine in many cultures for a long time. However, it has limited use in medicine due to lack of scientific report. In recent days, honey is becoming acceptable as a reputable and effective therapeutic agent. Its beneficial role has been endorsed to its antimicrobial, anti-inflammatory and anti-oxidant activities as well as boosting of the immune system. Honey has proven antimicrobial activity (Dureja *et al*., 2003). Honey inhibits a broad spectrum of bacterial species. The alcohol extracts of honey exhibit an effect to array of bacterial species including aerobes and anaerobes, Gram positives, and Gram negatives. Honey has powerful antimicrobial effects against pathogenic and nonpathogenic micro-organisms (yeasts and fungi), even against those that developed resistance to many antibiotics. The antimicrobial effects could be bacteriostatic or bactericidal depending on the concentration that is used (Abeshu, 2016).

Furthermore, honey is used as a remedy for diarrhea and gastroenteritis at a concentration of 5% (v/v). Honey reduces the activities of cyclooxygenase-1 and cyclooxygenase-2, thus showing anti-inflammatory effects and demonstrates immune modulatory activities. Ingestion of diluted natural honey showed reduction effect on concentrations of prostaglandins such as prostaglandin E2, prostaglandin F2α, and thromboxane B2 in plasma of normal individuals. Anti-inflammatory activity of honey was as effective as prednisolone, reference drug. Further, honey has an anti-inflammatory action free from adverse side effects such as suppression of immune response and tissue growth, formation of ulcers in stomach (Nweze *et al*., 2019).

Table 1. Traditional and ayurveda recipes with honey

Diseases	Recipes
Indigestion	Ginger (*Zingiber officinale*) juice with honey, Lemon (*Citrus limon*) juice with honey with Navaratna kalka Roasted cumin seeds powder with bee honey, Roasted cloves powder with bee honey.
Peptic ulcers	Paste prepared with Vishnukranti, honey, ghee and sugar
Diarrheawith vomiting	Decoction prepared by Bark of Beli (*Aegle marmelos*) root and internal part of the mango seed with honey.
Cough	Adathoda (*Adatoda vesica*) svarasa with bee honey, Decoction of Adathoda, Elabatu (*Solanum indicum*) roots and Rasakinda (*Tinospora cordifolia*) with honey, Powder of vibitaka (*Terminalia bellirica*) 10 g mixed with bee honey cure Asthma and cough immediately.
Asthma	Most of the rasa preparations “Buddharaja kalka” - prescribed with juice of Ambuldodam (*Citrus aurantium*), ginger, honey and the drug “Svasakuthara rasa” is given with honey.
Hiccup	Curd with bee honey, Ash of peacock feathers with honey, Rasa preparation “Arogyavardhanavati” with honey.
Anorexia	Pomegranate juice and rock salt with honey

Source: Liyanage and Horadugoda, 2017.

2.4. Overview of Beekeeping in Ethiopia

Beekeeping is a long standing practice in Ethiopia and it accounts 1.3 % of agricultural GDP. Currently one out of ten rural households keep honeybees and the activity make a substantial contribution to rural income generation. Ethiopia is the leading honey producer in Africa and one of the top ten worldwide (Demisew, 2016). Although difficult to establish a time reference when beekeeping was started in Ethiopia, it may date 5000 years back and the Hieroglyphs of ancient Egypt refers to Abyssinia (the former name of Ethiopia) as the source of honey and beeswax.

Thus Abyssinia has been known for its beeswax export for centuries during when other items were not exportable (Cannon, 2009). Ethiopia is one of the poorest and most food-insecure countries in the world. It is primarily a net exporter of agricultural products, with 85 percent of its population employed in agriculture. Ethiopian agriculture contributes more than 45 percent to the nation's gross domestic product (GDP) and significantly affects the country's export trade (Dixon *et al*., 2001).

Ethiopia is the first African countries with huge honey and beeswax producer and having rich plant biodiversity for bee forage. The ideal climatic conditions and diversity of floral resources allow the country to sustain around10 million honeybee colonies, of which 7 million are kept in local beehives by farmers and the remaining, exist in the forests as wild colonies (Ambaw and Teklehaimanot, 2018).Ethiopia is famous for its notable variation of agro-climatic conditions and biodiversity which favored the existence of diversified honeybee flora and vast number of honeybee colonies. The country has the largest bee population in Africa with over 10 million bee colonies (Bekele, 2018). Beekeeping is a traditionally well-established household activity in almost all parts of Ethiopia. Ethiopian honey production is characterized by the widespread use of traditional technology resulting in relatively low honey production and poor quality harvested when compared to the potential honey yields and quality gains associated with modern beehives (Arzaga *et al.*, 2017). Considering its importance to the overall growth of the agricultural sector, the government of Ethiopia gives special emphasis to enhance honey production through the promotion and expansion of beekeeping activities (Bewket *et al.*, 2015).

2.4.1. Role of beekeeping in Ethiopian

Unlike developing countries, in the most developed nations, the primary objective of keeping honeybees is for the pollination of various plants. The secondary use of keeping bees is simply for the production of bee's products namely honey and beeswax. In Ethiopia and other developing countries, the basic purpose of beekeeping is to produce honey and beeswax to get better income and to assure food security (Masehela, 2017). The beekeeping sub-sector has been an integral part of agriculture in Ethiopia. It has been contributing to the household income and poverty alleviation and national economy through export. The country has huge apicultural resources that made it the leading honey and beeswax producer in Africa. Moreover, Ethiopia is a country where apicultural research is being conducted in a coordinated manner under the national agricultural research system. Hence, a lot of information has been gathered on different aspects of the beekeeping. It has been revealed that the country's beekeeping subsector is mainly practiced using traditional basket hives with low productivity. However, attempts by various investigators and development actors showed that both the production and quality can be improved in terms of transforming the beekeeping system, processing and marketing (Gemechu, 2014).

Beekeeping in Ethiopia plays an important role in income generators for beekeepers (farmers). In the country, an average of 420 million ETH. Birr or 35 million $USD obtained annually from the sale of honey. Honey production of the country meets beverage requirement of the urban and rural population. It is also demanded for its nutritional and medicinal values. The others hive products beeswax; royal jelly; propolis and bees venom have high demand globally (Yirga and Ftwi, 2010).

2.5. Factors Affecting Beekeeping and Honey Quality in Ethiopia

Thirty-five percent of global production from crops including at least 800 cultivated plants depends on animal pollination. The transformation of agriculture in the past half-century has triggered a decline in bees and other insect pollinators (Nicholls and Altieri, 2013). Problems and dangers confront the long-time survival of beekeeping as a profitable agricultural enterprise, and changing agricultural and land-use practices threaten the survival of adequate numbers of bees required to pollinate some 90 crops or more. As human population increases, houses, factories, and highways replace open fields of honey and pollen plants. Clean cultivation of farmland and large-scale monoculture reduce the sequence of wild plants needed to provide bee food throughout the season (Kourí, 2004). Honey is produced primarily from floral nectars, and fructose and glucose are the major components. The chemical composition of honey varies depending on plant, season, production methods, and storage conditions (Da Silva *et al*., 2016). Ethiopia has immense natural resources for beekeeping activity. However, like any other livestock sector, this sub Sector has been seriously devastated by complicated constraints.

The prevailing production constraints in the beekeeping sub sector of the country would vary depending on the agro ecology of the areas where the activities is carried out. The major constraints that affect beekeeping sub-sector in Ethiopia are: lack of beekeeping knowledge, shortage of skills man power, shortage of bee equipment's, pests and predators, pesticide threat, poor infrastructure development and lack of research extension (Beyene *et al.*, 2014).

Honey bees are verysensitive organisms, and their vulnerability toward chemical pollution is a priorityenvironmental issue. The "hide model" is able to consider different contamination pathways form inside the hive via pesticide treatments against bee pests or from outsideby means of the eventual contamination present in nectar, pollen, resin, water, air, orvegetation. Radio frequency identification (RFI) systems are noncontact identificationdevices now commonly used in numerous domains, including the study of insectbehaviors, like when faced with pollutants (Judova *et al.,* 2016).The existing production constraints in the beekeeping development of Ethiopia are complex and to a large extent vary between agro-ecological zones and production systems (Ejigu *et al.*, 2009). Most research reports revealed that the pests and predators, shortage of bee forage, lack of skill and knowledge, low level of technology and honey bee disease, agro-chemical, are the top major constraints in most part of the country (Abebe and Puskur, 2011; Godifey, 2015).

2.5.1. Type of flowers

According to the Honey Research Center at the University of Waikato, New Zealand, there is not enough evidence to draw conclusions on the properties of honey, especially the antimicrobial properties, based on the type of flowers used for its production. However, extensive research has been carried out on the honeydew variety obtained from the conifer forests in the central European mountains and the manuka variety obtained from New Zealand. The above-mentioned honeydew kind has been found to have a high microbial activity while the manuka kind has been found to have high non-peroxide activity (Saranraj *et al*., 2016).

2.5.2. Blending

It is also believed that polyfloral honey (which is obtained from more than one flower) provides more benefits than monofloral. Hence, many companies sell blended honey as it offers the benefits from a variety and is, therefore, considered to be healthier than non-blended honey (MUT, 2018).

2.5.3. Storage and heating

When stored for a long duration, it becomes darker in color. It loses some of its properties and may also ferment if the water content is too high. Therefore, prolonged storage should be avoided, while newly harvested honey should be preferred (Ashenafi, 2006). Heating honey leads to drastic changes in its chemical composition. As a result, heating at high temperatures reduces its benefits. No wonder many people prefer raw, organic or raw organic honey. While raw by definition signifies less processing (and no heating), Organic honey is prepared using stringent organic production methods and processing standards, in which heating at high temperatures is not allowed (Coultate, 2009).

2.5.4. Agro-chemicals

A number of factors linked to industrial modes of agriculture affect bee colonies and other pollinators around the world, ranging from habitat degradation due to monocultures with consequent declines in flowering plants and the use of damaging insecticides. Incentives should be offered to farmers to restore pollinator-friendly habitats, including flower provisioning within or around crop fields and elimination of use of insecticides by adopting agro ecological production methods. Conventional farmers should be extremely cautious in the choice, timing, and application of insecticides and other chemicals (Nicholls and Altieri, 2013).

Pesticides are the chemicals that are most widely used to control pests incrop production. When different chemicals are applied to the crops, they are affecting the pests of the crops but also harm the beneficial insects as pollinators, predators and parasites. This harmful effect disturbs the natural balance between the insects and their natural hosts (Wilson and Tisdell, 2001). Older worker bees forage outside the hive for pollen and nectar, and thus are vulnerable to contact exposure to pesticides during foraging as well as dietary exposure during collection or ingestion of pollen and nectar. Workers also serve as a vector for bringing contaminants back to the hive. Young workers clean cells and attend brood, whereas middle aged workers do a variety of tasks mainly within the hive. All the young and middle-aged workers, queen and drone can have secondary exposure to pesticides through contaminated food brought back to the hive (Van der Sluijs *et al*., 2013).

2.5.5. Honey Bee Disease

The biology and health of the honey bee *Apis mellifera* has been of interest to human societies for centuries. The bees and their products are vulnerable to various diseases, parasites and pests. Honey bees diseases, pests and predators are causing a significant economic loss in honey bees and their products. The most commonly known honeybee diseases reported to exist in Ethiopia are Nosema, Amoeba and Chalk brood diseases (Evans and Schwarz, 2011). Nosema is caused by *Nosema apis* and *Nosema ceranae*. It is a microsporidian fungal disease that infects the intestinal tract of adult bees. Nosema cause detrimental effects on honey bees, colony development, queen performance and honey production. In Ethiopia Nosema was reported in low infestation rate in the survey conducted by the initiation of FAO (Fikru, 2015). In Ethiopia Nosema was also reported from different regions with varying prevalence rate such as 58% in Oromia, 60% Benishangul-Gumuz and 47% in Amhara regions (Teferi, 2018). Amoeba is diseases of honey bee caused by a single celled parasite called *malpighamoeba mellificae.* The parasite affects malpigian tubules of honey bees andshortens the life cycle of bees (Evans and Schwarz, 2011).

Diagnosis made on honey bees in field and laboratory at Addis Abeba reported a prevalence rate of 73% of amoeba infestation (Begna and Kebede, 2005). The diseases was also reported with high prevalence rate in different regional state of Ethiopia such as; Oromia region with prevalence rate (88%), Amhara region (95%) and 60 % in Benishangul- Gumuz (Diribe *et al*., 2012). American foulbrood (AFB) is an infectious disease of the larval stage of the honeybee *Apis mellifera. It is caused by a* Gram positive bacterium called *Paenibacillus (*Genersch, 2010). European foulbrood (EFB) is a severe bacterial brood disease caused by the Gram-positive bacterium*Melissocccus plutonius*. The disease has a worldwide distribution and is an increasing problem in some areas. Although the causative agent of EFB was described almost a century ago, many basic aspects of its pathogenesis are still unknown (Forsgren, 2010).

2.5.6. Honey bee predators and pests

Honey bees are active defenders of their colony. Their stinging and biting behavior is very effective at repelling intruders, even intruders as large as bears. Guard bees initiate colony-level responses by identifying threats and recruiting nest mates for collective defense. Recruitment is achieved through chemical communication. Volatile chemicals (pheromones) released by guards 'sound the alarm' and attract recruits to the entrance of the colony. Interestingly, young bees normally stay within the hive and do not participate in colony defense (Breed *et al.,* 2004). However, detection and subsequent avoidance of cryptic predators, including crab spiders that can change color to match their background pose intriguing challenges with respect to the relative investments into decision speed and accuracy, as well as the minimization of false-negative responses, in which over cautiousness might compromise the range of available foraging options (Ings and Chittka, 2008).

Lice are known to infect honey bees in hive. Bee louse are wingless ecto-parasite fly which causes significant damage bee colonies. Bee lice larvae feed on honey and pollen by tunneling under the cell capping (Sarwar, 2016). In Ethiopia infestation of lice in honey bees was reported from the western region of Shoa, Oromia regional state with overall prevalence rate of 42% with highest prevalence rate 70.8% by Gemechis, 50% in Holeta and 17.1% in Jaldu (Gemechu *et al*., 2014). And also in Tigray regional state reported an overall prevalence 4% in brood and 5.5% adult bees (Godifey, 2015). According to Tsegaye, (2015) the small hive beetle was reported in the Oromia regional state; 60% Jimma and 1.1% in Horo Guduru Wollega.

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted between November, 2019 and June, 2020 in Amuru district of Horo Guduru Wollega zone of western Oromia, Ethiopia. Amuru district is located approximately from 09°50'- 10°20'N latitude and 36°50'- 37°20 'E longitude in Horo Guduru Wollega Zone of Oromia regional State of Ethiopia. The district is one of the thirteen districts in Horo Guduru Wollega zone and is located at a distance of 72 km northwest of Shambu town, 405 km north west of Addis Ababa capital city of Oromia regional state and Ethiopia. The district borders with Amara regional state of Abay river in the north, Jardaga Jarte and Abe Dongoro districts in the south, Kiramu distric in the west and again Jardaga Jarte district in the east.

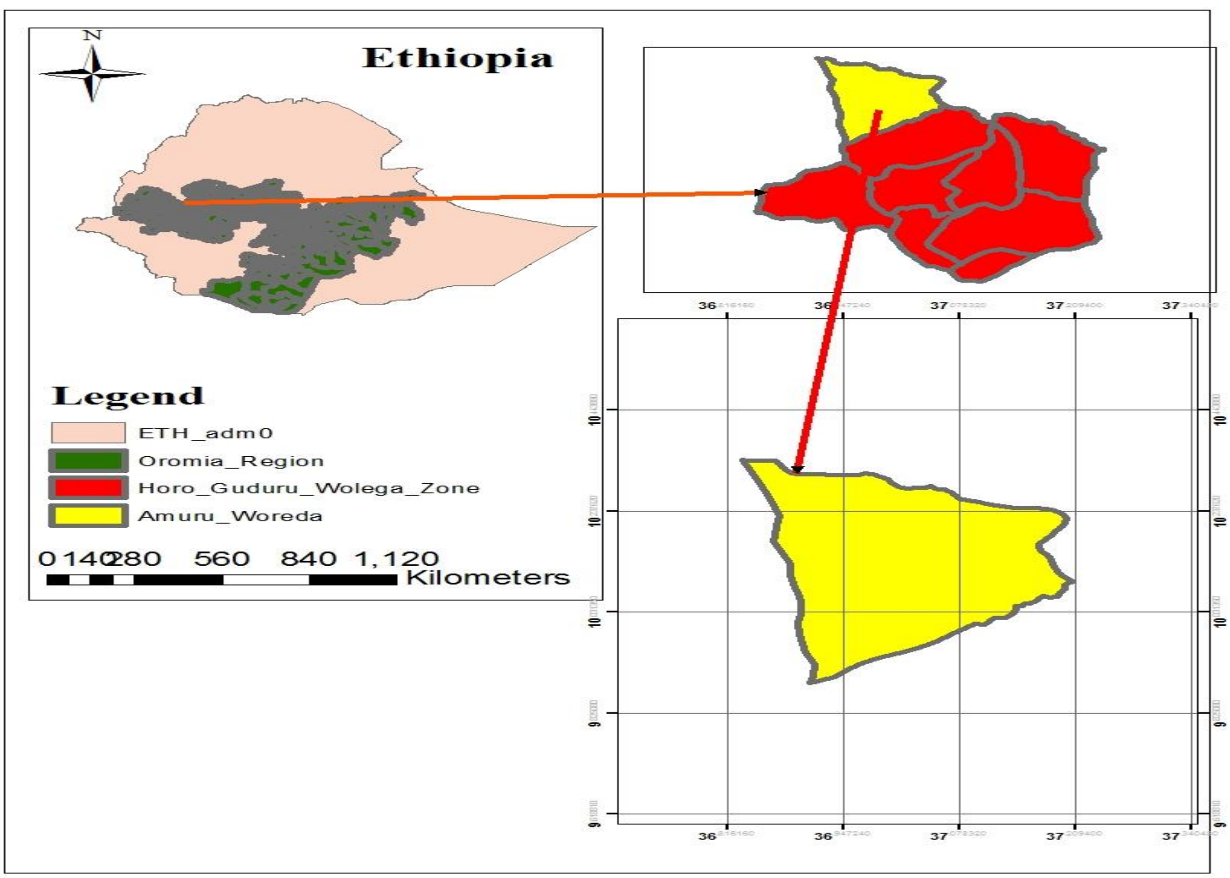

Figure 2. Location map of study area

Source: GIS (2019)

As information obtained from the district Agriculture and Rural Development Office (AWARDO, 2011) there are 18 rural and 3 urban kebele administrations in the district. Obora town is the administrative center of the district. The total population of the district is estimated to be 102,721 with the 52,398 and 50,323 male and female, respectively. From the total population 9548 (9.3 %) were urban dwellers. The majority of the inhabitants are Ethiopian protestant, with 50.81 % ,, while 15.1 % of the population are muslim, 29.86% are orthodox, and 4.23% practiced wakefata in the district (WHO, 2013).According to AWARDO (2011), the altitude of the district ranges from 760 - 2505 m above the sea level and the average annual rainfall and temperature are about and 18 ^{0}C, respectively. It has three agro-ecologies where 14.29 % is highland, 57.14 % midland and 28.57 % are lowland agro-ecology. The district is dominantly mid altitude by agro-climatic condition (AWARDO, 2011).

The dominant and important trees in the district was lafto (*Acacia* specie*s),* bargamo (*Eucalyptus* spp*.), wadessa (Cordia Africana), kombolcha (Dovyalis abyssinica), kello (Bidens* spp.) and other trees, shrubs and climbers that provide nectar and pollen for honey bees (AWARDO, 2011). According to the district agricultural office information, the major soil types, in the wereda include red soil, clay, black soil and brown soil. The rainfall pattern of the Wereda generally is bimodal; the minor raining season starts in January and ends in April, while the main rainy season begins around May and stops in September. There are about four major rivers (*Abay, Hanger, Walage and Kachalu*) and many springs in the midland part of the Wereda(AWARDO, 2011).

The livelihood of the people in the district is very diverse and the main economic activities are mixed farming (crop farming, livestock rearing and beekeeping) is the mainstay for the majority of the population in the area. The main types of crops cultivated by the farmers in the area are maize, wheat, teff, nug, barley, beans, pea, coffee, chat, fruits and vegetables. The livestock population of the district is estimated to be 245,462 in 164,898 cattle, 15,152 sheep, 20,401 goats, 7694 donkeys, 176 horses, 247 mules, 34,*892* poultry and 11,431 managed honeybee colonies. Livestock is considered as an important component in the farming system and beekeeping also practiced mostly as income generating activity of the Wereda (AWLRDFO , 2011).

3.2. Study Design

A cross-sectional studysupported by questionnaire survey and observation was conducted from November to December, 2019 to evaluate honey physicochemical property in different agro ecologies, beehives and beekeepers perception towards honey health benefits and beekeeping system from the beekeepers living in the study area. The sampling units were households keeping honey bee colony and honey retailers. Beekeepers in the three agro-ecology and honey retailers represented the study population of the district.

3.3. Sampling Method, Sample Size Determination and Data Collection

This study had two components: the first part was laboratory analysis which was aimed at determining the quality of honey produced in the study area. The second part was a survey which was conducted to assess the knowledge, attitudes and practices (KAP) of beekeepers towards honey health benefits and beekeeping systems. Prior to sample collection, cooperation letter was sent to Horo Guduru Wollega LRDF Zonal Office from Jimma university post graduate program for selecting beekeeping potential district and Horo Guduru Wollega Zonal LRDFO sent cooperation letter to AWLRDO for sampling in each kebeles. Reconsensus survey and informal data collection from the district Livestock office and key informants discussion were employed before the actual data collection work was started.

The study district (Amuru), consists a total of eighteen rural and three urban kebeles. For this study, the district was stratified into three agro-ecologies (highland, midland and lowland with 3, 12 and 6 kebeles, respectively), such that each stratum was made up of kebeles sharing similar characteristics. Approximately 29 % of the kebeles were sampled from the total 21 kebeles based on the proportion of kebeles in each agro-ecological zone. Therefore, six representative sampled kebeles, 1 from highland, 3 from midland and 2 from lowland were selected from 18 beekeeping practiced kebeles using purposive sampling method proportional to the beekeeping practiced entire kebeles of the district were identified. For honey quality analysis, a total of 24 samples: of which, 18 freshly harvested honey samples from the three agro-ecologies by considering three hive types (modern, transitional and traditional) (1x 3) + (3 x3) + (2 x3) and another 6 samples from the honey retailers in Obora town were collected (Table 2).From list provided beekeepers in kebeles were selected for sample collection. Fresh honey samples from farm gates were collected during night local time (6:00 - 8:00 PM) in order to minimize the biting behavior of the bees. From a total sampling frame 1,325 beekeepers in the district 125 respondents from the three agro-ecologies were selected for questionnaire survey by using the formula (Cochran, 1977). The individual beekeeper selection from selected kebeles was employed by using systematic random sampling technique for interview.

3.3.1. Honey sample for honey quality analysis

3.3.1.1. Honey sample size determination and sample types

A total of 24 honey samples (0.5 kg each) which is stated by Bogdanov, 2009 (0.5 -1 kg), were collected from purposively and conveniently selected six (6) potential beekeeping kebeles including Sammo illamu, Mekeno, Ejere Goromti, Tombe Dangab, Chidati and Gulufa considering their agro-ecologies and hive type they owned. Fresh honey samples were collected during the peak honey harvesting season (November to December, 2019 locally known as "***damma tuufoo***") and retailers ***(mana bookaa fi daadhii)*** in Amuru district.Of which eighteen (18) samples of locally produced honey (0.5 kg each) were collected from beekeepers from six purposively and conveniently selected kebeles, were named as HHS-1 through HHS-3, MHS-4 through MHS-12 and LHS-13 through LHS-18, whereas six samples were collected from honey retailers purposively and named RHS-19 through RHS-24 where HHS,MHS, LHS and RHS refers to Highland Honey Sample, Midland Honey Sample, Lowland Honey Sample and Retailers Honey Sample respectively**.** The number shows the sample number (Table 2).

Table 2. Summary of purposively selected kebeles and collected honey samples

No.	Honey from hive types	Agro- ecologies			Sample from beekeepers	Sample from retailers (RHS)	Total
		Highland (HHS)	Midland (MHS)	Lowland (LHS)			
1.	From traditional beehive	1	3	2	6		
2.	From transitional beehive	1	3	2	6	6	
3.	From modern beehive	1	3	2	6		
Subtotal honey sample		**3**	**9**	**6**	**18**	**6**	
Total honey sample collected					**18**	**6**	**24**

N= number of collected honey sample

3.3.1.2. Sample preparation and handling procedure

Starting from sample collection, preparing, straining, storing and handling samples, the following sample handling directions and procedures were followed from farm-gate and retailers house up to laboratory (QSAE, 2005). The required 0.5 kg sample for the laboratory analysis was taken in to securely closed, chemical free, properly cleaned and dried plastic containers without exposing to damp air, dust, dirty, light and smoke. Precautions were taken to protect the samples, the sampling instrument and the containers from contamination by using cool jar. Each container was sealed air-tight and marked with full detail of sampling code number and other important particulars. Sample preparation involves identification and grouping with respect to agro-ecologies, type of beehives, harvested date and source of collection, (Fig.3).

a. During harvest b. Straining and preparing of honey

Figure 3. Prepared and labeled honey samples for laboratory analysis

3.3.1.3. Laboratory methods and procedures

The collected honey sample were prepared according to the “COMESA 002 (2004) Standard for Honey” protocol for the quality analysis.The quality parameters of the interest were: moisture content (%), HMF (mg/kg), pH, free acidity (meq/kg), ash content (%), EC ($mScm^{-}$), reducing sugars, (%), sucrose (%) and color (mm) of the samples were determined according to the IHC, (Bogdanov, 2009). There is quality standards of the honeywhich is stated by national and international norms (see table 4 below). All parameters were measured according to the internationally harmonized commission methods of the honey commission of (2009). The result of the laboratory analysis of honey samples was compared with Quality and Standards Authority of Ethiopia (QSAE, 2005), Codex Alimentarius Commission (CAC) and European Union (EU).The physicochemical properties of the collected honey samples were analyzed at Holeta Bee Research Center (HBRC) analysis laboratory. The procedure, principle, reagents, equipment's used and procedure followed to do both physical and chemical analysis of honey sample according to (IHC, 2009) was elaborated in (Annex 3 to 11).

3.3.2. Questionnaires

Semi- structured questionnaire was prepared both close and open-ended questions were included (Annex 15). Verbal consent was obtained and the objectives of the study were explained for the respondents. Questionnaire was intended to the owners of beehives/ beekeepers to get information related to socio-demographic characteristics, purpose of beekeeping, beekeeping system, honey bee flora, beekeeping constraints and KAP of beekeepers towards health benefits of honey through face to face conversion. Beekeeping system, type of beehives, management and KAP commonly practiced in the study area was collected from district LFRDO, DA`s record and personal communications of selected beekeepers. The questionnaire was pre-tested and adjusted as required translated in to local language (Afaan Oromo) for interviewees. Information collected was ethically respected and beekeepers interviewed from kebeles were proportionally selected from each agro-ecology.

Determining sample size in each agro-ecology and kebele was proportional to entire population of households. Therefore, From a total of six representative kebeles namely; Samo Ilamu from highland, Mekeno, Ejere Goromti and Tombe Dangab from midland, Chidati and Gulufa from lowland were selected purposively based on having large number of beekeepers, beekeeper experience, and potential area for beekeeping, abundance of honey bee colony, availability of common bee flora and agro-ecology of the district.The sampling units were households keeping honey bees. The sample size required for the study questionnaire survey was determined by the formula recommended by Cochran (1977) and Pandey and Verma, (2008) determination formula.

Accordingly, a total of 125 respondents' were selected for the interview from the district as follows. 5% sampling error was used as a standard.

no = Z^2 p q / e^2 ……………………………………………………(Cochran, 1977)

= 138.29 after this by using finite population correction factor

$$n1 = \frac{no}{1 + \frac{no}{N}}$$

n1= 138.29 / 1 + [138.29/1325}

= 125beekeepers were selected from the district for interview

Where;

$n_{o=}$ desired sample size Cochran's (1977) when population greater than 10,000

n_1= finite population correction factors (Cochran's formula, 1977) less than 10, 000

Z = standard normal deviation (1.96 for 95% confidence level)

P = 0.1 (proportion of population to be included in sample i.e. 10%)

q =is 1-P i.e. (0.9)

N = is total number of population

d =is degree of accuracy desired (0.05)

After determining the total sample size from all agro-ecologies of the district to select interviewers from each agro-ecology the following formula was used.

$$n1 = \frac{n * N1}{N} \text{ and } n2 = \frac{n * N2}{N}$$ ………………. (Pandey and Verma, 2008)

Where;

n1, n2 and n3 = is sample size of respondent in each agro ecology

So from this:

n1= 125x134/ 1325= n1= 13 respondents were selected from highland agro-ecology

n2= 125 x 673/1325 = n2= 63 respondents were selected from midland agro-ecology

n3= 125x518/1325 = n3= 49 respondents were selected from lowland agro-ecology

N1 and N2 = is total number of household in each agro ecology

n= total sample size of respondent in three agro ecology i.e. 125

N = is total number of beekeepers in the study area

After determining sample size from each agro-ecologies, we have selecting each beekeepers by usingsystematic random sampling method**.**

Table 3. Total selected kebeles and beekeepers for questionnaire survey

Kebeles / Beekeepers/Beehives	**Amuru district**			
	High land	**Mid land**	**Low land**	**Total**
All kebeles found in the district	3	12	6	21
Kebeles where beekeeping is practiced	3	9	6	18
All beekeepers of the district	134	673	518	1,325
Selected respondents for the study	13	63	49	125

3.4. Data Analysis

Data was managed both in hard and software. The evaluation of honey quality was subjected to statistical analysis using General Linear Model (GLM) of SPSS version.23. In all the analyses, P <0.05 is set for significance and not significant as P > 0.05 level was used to separate the means whenever GLM showed statistically significant difference. Collected data from both primary and secondary sources was documented, organized, analyzed and summarized using MS excel and SPSS version 23 for descriptive statistics. The survey data was coded and tabulated for analysis using SPSS statistical package version. Simple descriptive statistics was employed in order to have a summary description of the data collected from the survey. This involves the use of percentages, means, frequency distributions and standard deviations to describe parameters such as socio-demographic characteristics, KAP of beekeepers, beekeeping costraints and so on.

4. RESULTS

4.1. Results of Honey Quality Parameters

In this finding the results of honey quality analysis of the total of 24 honey samples, of this 18 obtained from beekeepers and 6 from honey retailers of Amuru district in the western part of Ethiopia are presented, in(Table 4, 5, 6, 7 and 8 below). Honey samples acquired purposively from 6 kebeles considering their agro-ecologies and beehives and 6 from honey retailers, were subjected to analyses with regard to moisture, HMF, pH, free acidity, electrical conductivity, ash, reducing sugar, apparent sucrose, color and their results are well summarized. In the present study measurements were performed twice and the average result was taken and most of the overall mean of quality parameters evaluated were indicated in agreement with national and international standards (Table 4 and 5).

Table 4. All mean results of honey samples collected from the study area

Quality Parameters	N	Overall Mean ± SD	Maximum	Minimum	Range	Sources Mean ± SD (N=24) Beekeepers (n=18)	Retailers (n=6)	@ Standards National	International
MC (%)	24	20.43± 1.32	22.8	18	4.8	19.90± 1.04*****	21.90± 0.79 *****	17.5-21	18-23
HMF(mg/kg	24	16.39 ± 2.68	39.2	0	39.2	10.76 ± 9.47*****	33.28 ± 5.78*****	< 40	40-80
pH	24	3.92 ± .07	4.8	3	1.8	4.05 ± 0.24 *	3.54 ± 0.39 *	-	3.2- 4.5
FA (meq/kg)	24	36.67 ± 2.13	55	14	41	33.00 ± 9.23*****	47.67 ± 3.98 *****	< 40	5-54
Ash (%)	24	0.25 ± 0.14	.50	0	.50	0.26 ± 0.15	0.22 ± 0.12	0.6	0.25 – 1
EC (mS/cm)	24	0.68 ± 0.3	1	0.1	0.9	0.6 ± 0.3	0.5 ± 0.2	< 0.6	0.22-1.52
RS (%)	24	73.08 ± 0.92	80	66	14	73.22 ± 4.37	72.67 ± 5.32	65	60-70
AS (%)	24	1.80 ± 0.35	0	4.8	4.8	1.72 ± 1.70	2.07 ± 1.91	10	3- 10
Color (mm)	24	103.75± 2.89	129	75	54	98.94± 12.45*****	118.17± 8.08*****	-	-

[@]Quality and Standards Authority of Ethiopia (2005)

*****= Means within a row and column are significantly different (P < 0.05) both in univariate and multivariate GLM analysis

N= total number of sample, SD= Standard deviation MC =moisture content, HMF = Hydroxyl Methyl Furfural, FA = Free Acidity, Ash = Total ash content, RS = Reducing Sugar, AS = Apparent Sucrose, EC = Electrical conductivity)

Table 5. Mean results of honey samples collected from different Agro-ecology and hive types

	Agro-ecology (Mean ± SD)(n=18)			**Hive types (Mean ± SD)(n=18)**		
Quality Parameters	Highland (n=3)	Midland (n=9)	Lowland (n=6)	Modern (n=6)	Transitional (n=6)	Traditional (n=6)
Moisture (%)	20.50± 1.21*****	20.50±.52*****	18.80±0.53*****	20.30±0.82	20.10±1.01	19.90±1.09
HMF (mg/kg)	1.62±.62*****	6.37±1.08*****	22.15±2.65*****	6.08±2.71	11.85±4.24	14.35±4.28
pH unit	4.02±0.06	3.94±0.03	4.22±0.14	4.20±0.13	3.99±4.11	3.95±0.07
FA(meq/kg)	28.33±0.28*****	38.44±2.02*****	27.17±4.30*****	26.50±3.22	35.50±2.93	37.00±4.05
Ash (%)	.13±0.06	.29±.18	.28±.13	.22±.01	.20±.18	.26±.20
EC (mS/cm)	.40±.1	.6±.3	.7±.2	.5 ±.2	.5 ±.3	.6 ±0.3
RS (%)	72±1.53	73.78±1.29	73±2.48	71.83±1.70	75±1.51	72.83±2.15
AS (%)	0.83±0.38	1.26±0.58	2.85±0.64	2.18±0.84	1.93±0.66	1.03±0.59
Color(mm)	103.67±3.79	99.78±13.82	95.33±13.62	101.5±11.33	100.27±3.79	98.25±13.0

* = Means in the row and column is means which revealed significant difference at (p < 0.05)

4.1.1. Moisture Content

The overall mean value of moisture for the honey samples acquired from the study area was 20.43 ± 1.32 %; with the lowest moisture content of 18 % obtained for the sample acquired from beekeepers and the highest moisture content of 22.80 % obtained for the sample acquired from honey retailer (Table 4, 5 and Annex 12). The average value of moisture content of fresh honey sampled from beekeepers were (19.90 %) which is numerically lower than the overall mean of honey samples obtained from retailers (21.90 %). Therefore, the moisture content of the sampled honey for this study revealed that significant difference (P < 0.05) between honeys sampled from beekeepers and retailers, (Table 6). In this finding the mean value of moisture content of the honey sample collected from highland and midland agro-ecologies were equal (20.50 %); while the moisture content of lowland agro-ecology is 18.80 %. From this result moisture content of honey sampled from different agro-ecologies revealed significant difference ($p < 0.05$) among agro-ecologies. The mean moisture content of the honey sampled from different hive types were numerically 20.30 %, 20.10 % and 19.90 % from modern, transitional and traditional hives, respectively. Even if the numerical mean value of moisture content is varied among hive types; statistically, there is no significant different ($p > 0.05$) among beehives, (Annex 13). Generally, except some value of moisture content of honey samples acquired from retailers, all honey samples collected from the study area has moisture content of honey in the range of national and national standards, (Annex 12).

4.1.2. Hydroxymethyl furfural (HMF)

The HMF overall average for the honeys used in this study was 16.39 ± 2.68 mg/kg; with the lowest HMF amount of 0.0 mg/kg obtained for the honey sample from beekeepers; whereas the highest HMF amount of 39.2 mg/kg was determined for the honey sample obtained from retailers, (Table 4, 5 and Annex 12). The average result of honey obtained from beekeeper (10.76 mg/kg) is much lower than that of retailers (33.28 mg/kg) which indicates the freshness of honey of beekeepers than retailers in the present study. Similarly there is significant difference ($p < 0.05$) between sources of honey collected from beekeepers and retailers of the study area. The HMF mean value of the honey collected from lowland agro-ecology (22.15 mg/kg) is higher than that of midland (6.37 mg/kg) and highland (1.62 mg/kg). Therefore, there is a significant difference ($P < 0.05$) in HMF value among agro-ecologies. The honey sampled from different hives indicated different mean values of HMF numerically 6.08 mg/kg, 11.85 mg/kg and 14.35 mg/kg collected from modern, transitional and traditional hive types respectively. Despite, numerical difference of HMF value in different hive types there is no significant difference ($P > 0.05$) among hive types of this finding, (Annex 12 and 13).

4.1.3. pH

The overall mean of pH for the honeys used in this study was determined as 3.92 ± 0.07 which ranges from 3.00 to 4.80. With the lowest pH determined for the honey sample acquired from retailers with 3.00; whereas the highest pH was obtained for the sample from beekeepers 4.80 (Table 4). In this finding the pH numerical values of honey samples collected from beekeeper is higher than honey samples obtained from retailers, So, significant difference ($P < 0.05$) were observed between the sources (Table 5 and 6). The pH values of honey samples analyzed in this study ranged from 3.00 to 4.80 with the mean value of 4.02 from highland honey, 3.94 from midland and 4.22 from lowland agro-ecologies. The pH value of honey sampled from lowland agro-ecology is higher than that of highland and midland agro-ecology. In other case, the overall mean values of pH honey sampled from modern, transitional and traditional hives were 4.20, 3.99 and 3.95 respectively (Table 5). So, thecurrent finding of pH value of honey sampled was revealed that there is no significant difference among agro-ecologies and hive types analyzed (Table 5 & Annex 13).

4.1.4. Free acidity

The overall mean of the free acidity of the study area was 36.67 meq kg^{-1} with the range of 14 to 55 meq kg^{-1}, with the lowest free acidity value obtained for the fresh honey sample acquired from beekeepers with 14 meq/kg; whereas the highest free acidity value was obtained for the honey sample acquired from the retailer with 55 meq/kg. There is high range (41 meq/kg) between fresh honey sampled from beekeepers and retailers. There was a statistically significant difference between the free acidity values of the honey samples used in this study ($p < 0.05$) between beekeepers and retailers honey (Table 6 and Annex 12). The mean and range of the free acidity of the honey sample obtained from different agro-ecologies were 28.33 meq/kg (22 meq/kg to 30 meq/kg) from highland, 38.44 meq/kg (31 meq/kg to 48 meq/kg) from midland and 27.17 meq/kg (14 meq/kg to 46 meq/kg) from low land agro-ecology. The result of this study was observed that there was significant variation ($P < 0.05$) in free acidity among agro-ecologies. The mean of different hive types of this finding were 26.50 meq/kg from modern, 35.50 meq/kg from transitional and 37.00 meq/kg from traditional hives which is different numerically, however, it showed no significant difference ($p > 0.05$) among hive types.

4.1.5. Ash content

The overall average of Ash amount for the honey samples used in the present study was 0.25 ± 0.03 %; with the lowest and highest ash amount obtained for the honey samples acquired from beekeepers with 0.0 % and 0.50 % respectively. Therefore, there was no statistically significant difference between the ash amounts for the honey samples used in the study ($p > 0.05$) between of sources of honey (Table 6).The mean and range of Ash obtained from different agro-ecologies were 0.13 % (0.1 % to 0.2 %) from highland, 0.29 % (0 % to 0.5 %) from midland and 0.28 % (0.1 % to 0.5 %) honey sampled from lowland agro-ecologies. The ash mean value obtained from modern, transitional and traditional hives were 0.22 % (0.1 % to 0.3 %), 0.20 (0 % to 0.5%) and 0.37 % (0.1 % to 0.5 %), respectively (Table 5). There was no significant difference ($p > 0.05$) both due to agro-ecologies and hive types analyzed, (Annex 13).

4.1.6. Electrical conductivity

The electrical conductivity overall mean value of the samples used in this study was determined as 0.68± 0.3 mS/cm (Table 4); with the lowest value obtained for the honey sample acquired from beekeepers with 0.1 mS/cm and the highest value obtained for the honey sample acquired both from beekeepers and retailers with 1 mS/ cm. The overall average electrical conductivity of honey sampled from beekeepers (0.6 mS/cm) where numerically higher than that of retailers honey sample (0.5 mS/cm) but, there was no statistically significant difference ($p > 0.05$) between honey samples of beekeepers and retailers (Table 5 & 6). The mean conductivity of honey sampled for the present study was 0.4 mS/cm (ranges from 0.3 mS/cm to 0.5 mS/cm for highland, 0.6 mS/cm (ranges from 0.1 mS/cm to 1 mS/cm) for midland and 0.7 mS/cm (ranges 0.3 mS/cm to 1.0 mS/cm) honey sampled from lowland agro-ecology. The overall mean value of electrical conductivity from modern, transitional and traditional hive types were 0.5 mS/cm (0.3 mS/cm to 0.7 mS/cm), 0.5 mS/cm (0.1 mS/cm to 1.0 mS/cm) and 0.6 mS/cm (0.3 mS/cm to 1.0 mS/cm), respectively, (Table 6 and 7). The value of electrical conductivity not varied significantly ($p > 0.05$) due to agro-ecologies and type of beehives analyzed (Annex 13).

4.1.7. Reducing sugar

From the total of 24 honey sampled for the study the overall mean of reducing sugar (fructose and glucose) of Amuru district was 73.08 ± 0.92 %; with the lowest and highest reducing sugar ratio obtained for the fresh honey sample acquired from the beekeepers with 66 % and 80 %. Numerically the highest and lowest value was obtained from beekeepers and there was a no statistically significant difference ($p > 0.05$) between the total reducing sugars of the honey samples studied of the honey obtained from beekeepers and retailers (Annex 13). The mean reducing sugar of honey sampled from different agro-ecology and hive types were 72 % (69 % to

74 %) from highland, 73.78 % (71% to 80 %) from midland and 73 % (66 % to 80 %) from lowland agro-ecologies. While 71.83 % (66 % to 78 %) from modern, 75 % (71 % to 80 %) from transitional and 72.83 % (68 % to 80 %) obtained from traditional hive types, (Table 5). The mean reducing sugar of honey sampled from different agro-ecologies and hive types were no significant difference ($p > 0.05$) observed both due to agro-ecology and hive types, (Annex 13).

4.1.8. Apparent Sucrose

In this study the total average of apparent sucrose for the honey samples obtained from the study area was 1.80 ± 0.35%; with the lowest sucrose calculated for the collected sample from beekeepers with 0.00 % value; while the highest apparent sucrose ratio was obtained for the honey sample acquired from the retailers with 4.8 % value (Table 4 and Annex 12). Despite the mean is varied numerically, there was no statistical significant difference ($p > 0.05$) between honey sampled from beekeepers and retailers. The mean sucrose content of honey sampled from the highland, midland and lowland agro-ecologies were 0.83 % (0.1 % to 1.4 %), 1.26 % (0 % to 4.4 %) and 2.85 % (0.1 % to 4.5 %). Whereas the mean sucrose of honey sampled from modern, transitional and traditional hives were 2.18 % (0 % to 4.5 %), 1.93 % (0.1 % to 4.4 %), and 1.03 % (0.1 % to 3.1 5), respectively, (Table 5). There was no significant difference ($p > 0.05$) observed both due to agro-ecology and hive types in apparent sucrose value of the present study (Annex 13).

4.1.9. Color

The overall average value of color of honey samples used in the study area was 103.75± 2.89 mm which indicates amber color, with this the lowest color value obtained from the honey sample acquired from the beekeepers with 75 mm which revealed light amber color; while the highest color value was determined from retailers honey sample with 125 mm with dark amber color. The average color value of honey sampled of this study from beekeepers were 98.94 mm which indicates amber color, while the color mean value of honey sampled from retailers were 117.17 mm reveals dark amber color (Table 4). From the total sample collected 62.5 %, 16.67 % and 20.83 % were amber, light amber and dark amber color respectively. 83.33 % studied honey sample of dark amber color was calculated from retailers honey sample (Annex 12). There was statistically significant difference ($p < 0.05$) between the color values of the honey samples examined from beekeepers and retailers (Table 6). The average color value of honey sampled from different agro-ecologies were 103.67 mm from highland, 99.78mm from midland and 95.33mm from lowland which all indicated amber color, while the color mean value of honey sampled from different hive types were 101.5 mm from modern, 100.27 mm from transitional and 98.25 mm from traditional hive types which all mean values in the range of amber color (Table 5). Therefore there was no significant difference ($p > 0.05$) both due to agro-ecologies and hive types of honey sampled, (Annex 13).

4.2. Results of Univariate General Linear Model Analysis of Honey Quality Parameters

In this finding according to univariate analysis of General linear model (at 95 % CI and $p < 0.05$ significant difference) the sampled honey from different agro-ecology significantly affects the parameters ($P < 0.05$) among the samples for moisture content, HMF and free acidity. However, no significant differences ($P > 0.05$) in pH, Ec, ash content, reducing sugar, apparent sucrose and color were revealed among the honey samples collected from different agro-ecologies. Moisture content, HMF, pH, free acidity, and color were revealed significantly different ($p < 0.05$): whereas, Ash content, Ec, reducing sugar and apparent sucrose revealed not-significant difference ($p > 0.05$) observed between sources of honey (i.e. beekeepers and retailers). Finally, all parameters evaluated in this finding were not showed any significant difference ($p > 0.05$) among samples collected from different hives types (Annex 13).

4.3. Results of Multivarite General Linear Model Analysis of Honey Quality Parameters

In these study variables with a $p \leq 0.25$ in the univariate GLM analysis under different independent variables like source, agro-ecology and type of beehives were included in the final multivariate GLM analysis. Accordingly; moisture, HMF, pH, free acidity and color, were analyzed by multivariate analysis for sources and revealed that all those analyzed parameters have significant difference ($p < 0.05$)between sources (beekeepers and retailers) of honey sample collected like in univariate analysis (See Table 6).

Table 6. Multivariable analysis between sources

Independent variable	*Dependent variable*	*No. of tested*	*F*	*P-value*
Source	MC (%)	24	18.482	.000
	HMF (mg/Kg)	24	29.699	.000
	pH	24	14.882	.001
	FA (meq/Kg)	24	13.943	.001
	Color (mm)	24	12.351	.002

In the same way; moisture, HMF, pH, free acidity and apparent sucrose were analyzed for agro-ecology. However, only moisture, HMF and free acidity have significant difference ($P< 0.05$) among agro-ecologies in multivariate analysis (Table 7). Parameters analyzed by multivariate for hive types having $p \leq 0.25$ like pH, free acidity ash content and EC value, indicated that they couldn't show any significant difference ($p > 0.05$) among beehives. In general, in the present study sources of honey has a significant ($p < 0.05$) effect on the values of honey moisture, HMF, pH, free acidity and color and Agro-ecologies have significant ($p < 0.05$) effect on moisture, HMF and free acidity value of the present analyzed honey.Whereas, type of hive have`t any significant ($p < 0.05$) effect on the parameters of honey analyzed in the study area (See Table 7).

Table 7. Multivarite analysis among agro-ecologies

Independent variable	*Dependent variable*	*No . of tested*	*F*	*P-value*
Agro-ecology	MC (%)	18	13.939	.000
	HMF (mg/Kg)	18	31.116	.000
	FA (meq/Kg)	18	4.412	.031

4.4. Correlation between the Quality Parameters of Honey Sampled

In this finding the physicochemical properties of honey of the study area had different correlation results between each other. Pearson correlation coefficients between all parameters were presented (Table 8). In correlation criterion there is strong negative/ positive and weak negative/ positive ranges from -1 to +1. There were strong positive significant ($p < 0.01$) correlations between moisture content and free acidity and color r = 0.63 and 0.53 respectively. HMF is significantly correlated with free acidity and electrical conductivity with the value of r = 0.45* and 0.77**, ($p < 0.05$) and ($p < 0.01$) respectively. Whereas the overall free acidity of the honey sample was significantly correlated with electrical conductivity with the r = 0.41 value ($p < 0.05$). Other parameters had shown numerical strong and weak correlation between variables. Also in the current evaluation of correlation there is strong relationship between HMF and electrical conductivity with the highest r= 0.77 ** ($P < .01$). The high correlation coefficient (r = 0.77 **) value indicated pure/ fresh honey is characterized by a low HMF and conductance value in , (Table 8 below).

Table 8. Pearson correlation coefficients among the analyzed parameters

Parameters	MC	HMF	pH	FA	Ash	EC	RS	AS	Color
Moisture (%)	**1**	**.19**	**-.58****	**.63****	**-.16**	**.11**	**-.12**	**-.24**	**.53****
HMF(mg/kg		**1**	**-.50***	**.45***	**.09**	**.77****	**.13**	**.14**	**.33**
pH			**1**	**-.79****	**-.05**	**-.32**	**-.29**	**.26**	**-.29**
FA (meq/kg)				**1**	**.00**	**.41***	**.04**	**-.07**	**.19**
Ash (%)					**1**	**.35**	**.11**	**-.14**	**-.09**
EC (mScm-)						**1**	**-.03**	**.27**	**.15**
RS (%)							**1**	**-.54****	**.13**
AS (%)								**1**	**-.34**

**. Correlation is significant at the 0.01 level (2-tailed),

*. Correlation is significant at the 0.05 level (2-tailed). r= correlation

4.5. Questionnaire Survey

4.5.1. Socio-demographic characteristics and beekeeping system of the respondents

In this section, the major socio-demographic characteristics of households interviewed in the survey were described. These characteristics related to the relative frequency distribution of household heads by educational background, gender, age, beekeeping experience and livestock they owned.From the total 125 selected respondents: 10.40 %, 50.40 % and 39.20 % of them were selected from highland, midland and lowland agro ecology respectively, proportional to entire beekeepers. Majority of the respondents are (about 67.20 %) under educated (below high school) (see table 9). Of the selected beekeepers interviewed male to female ratio were of 6.82: 1 (87.20 %) male.In mid and low land there are few female beekeepers than males whereas in highland none of them do not participating in this activity. Females active participation was limited in the study area their support of men in collaboration with the family members in terms of cleaning the apiary, controlling pests and predators, selling hive products, preparation and offering supplementary feed was not ignorable.To maximize women's participation in beekeepin g training should have to be offered to empower them.

The mean age distribution of the respondents were 43.31 ± 19.75 (with the range of 14 to 81 years). In this study people in the range of active and productive age groups are actively participated in beekeeping activities. The beekeepers had an average experience 7.66 (ranges from 3 to 26 years.) of beekeeping in the district. Of 125 respondents the livestock rearing household heads in the study area were presented. Of the total households interviewed, were 97.60 % were cattle owners while 44 % sheep, 48.80 % goats, 72.80 % equines and 72 % rearing poultry (Table 9).

Table 9. Socio-demographic indicators and beekeeping system of respondents (N= 125)

Descriptions	Agro-ecologies			
	Highland (n=13)	Midland (n=63)	Lowland (n=49)	Total (N= 125)
	N (%)	N (%)	N (%)	N (%)
1. Educational Background				
No informal and formal education	2 (15.38)	6 (9.52)	11 (22.45)	19 (15.20)
Elementary school	7 (53.85)	31 (41.27)	27 (46.94)	65 (52)
High school	3 (23.08)	20 (31.75)	9 (18.37)	32 (25.60)
Colleges / University	1 (7.69)	6 (9.52)	2 (4.08)	9 (7.20)
2. Gender				
Male	13 (100)	51 (80.95)	45 (91.84)	109 (87.20)
Female	-	12 (19.05)	4 (8.16)	16 (12.80)
3. Age				
14-24 years	4 (30.77)	18 (28.57)	13 (26.53)	35 (28)
25-65 years	6 (46.15)	32 (50.79)	29 (59.18)	67 (53.60)
> 65 years	3 (23.08)	13 (20.63)	7 (14.29)	23 (18.40)
4. Beekeeping Experience				
< 5 years	4 (30.77)	16 (33.33)	11 (22.45)	31 (24.80)
≥ 5-10 years	7 (53.85)	35 (55.56)	33 (67.35)	75 (60)
≥ 10 years	2 (15.38)	12 (19.05)	5 (10.20)	19 (15.20)
5. Livestock Ownership				
Cattle	13 (100)	60 (95.24)	49 (100)	122 (97.60)
Sheep	5 (38.46)	24 (38.10)	26 (53.06)	55 (44)
Goat	4 (30.77)	20 (31.75)	37 (75.51)	61(48.80)
Equines	9 (69.23)	42 (66.67)	40 (81.63)	91 (72.80)
Poultry	6 (46.15)	51 (80.95)	33 (67.35)	90 (72)

N= Total number of respondents in study area n= number of respondents in each agro ecology, %= percentage

In the sampled population 52 %, 33.60 % and 96.80 % of beekeepers owned modern, transitional and traditional hive types. The average of beekeepers who owned improved beehives was 21.67 households, 14 households owned transitional hive and for beekeepers that had traditional beehives was 40.33 households in each agro-ecology. The user of modern hive was 65.08 %

from midland, 46.15 % from highland and 36.37 % from lowland agro-ecologies this is may be due to proximity of midland agro-ecologies of the interviewed beekeepers to the town of the district and closed to get technology assess and information from the livestock office. All beekeepers lived in the study area in highland and lowland agro-ecologies had traditional type of hives. Transitional hive usage is mostly seen in lowland agro-ecology with 46.94 % than that of highland and midland.

Table 10. Hive types owned by beekeepers of respondents among agro-ecologies

Hive type	Agro-ecologies			Total (N =125)
	Highland (n=13)	Midland (n= 63)	Lowland (n= 49)	N (%)
	N (%)	N (%)	N (%)	
Modern	6 (46.15)	41 (65.08)	18 (36.73)	65 (52)
Transitional	5 (38.46)	14 (22.22)	23 (46.94)	42 (33.60)
Traditional	13 (100)	59 (93.65)	49 (100)	121 (96.80)

N= total no. of respondents n=number of beekeepers in each agro-ecology

4.5.2. Knowledge, attitude and practices of beekeepers on public health benefits of honey

Respondents main purpose of keeping honey bees were 88 % for both household consumption and income generation, 4 % only for income generation and 8 % only for household consumption.The beekeepers have developed indigenous knowledge of keeping bees passing from generation to generation. These indigenous beekeeping knowledge are hive construction from locally available materials, swarm catching, hive fumigation materials, honey and swarming season identification, different medicinal values of honey, identification of important honeybee floras, the medicinal value of honey and identification of adulterated honey.

In these survey beekeepers experiences in purpose of using honey activities the deep indigenous knowledge of beekeepers which may contributes to the development of modern medicine varied from area to area and from agro-ecology to agro-ecology. In this finding most of the selected respondents in the district (89.60 %) had knowledge, attitude and practices of honey health benefits as traditionally using for many diseases, symptoms and values and (10.40 %) of the respondents consume honey as foods without knowing the benefit of honey on the health perspective. About 32.80 % the respondents belief that honey has side effects on infants less than one year old but, they can`t considered as disease rather than the honey side effect.Interviewed beekeepers replied that beekeepers had a long term experience that passed from generation to generation (Table 11).

The knowledge of honey health benefits were mostly adopted 72 (64.29 %) from families, 34 (30.36 %) from neighbors and 6 (5.36) were experienced from other sources (book, radio, television and etc.) Knowledge of honey health benefits has significant correlation with ($p < 0.05$) the beekeepers having long experience of beekeeping and age in the interviewed study area. From the respondents interviewed the aged group has more knowledge of honey health benefits and the highland beekeepers have 100 % knowledge of health benefits while 93.65 % from midland and 81.63 % from lowland agro-ecologies were knew/ heard health benefits of honey. In other hand, this research revealed that educational background; of the respondents have no any significant ($p > 0.05$) relation with the knowledge, attitudes and practices of the honey health benefits. Moreover, knowing the medicinal value of honey, identifying honeybee pests and predators with their time of attack, method of controlling honeybee pests and predators and cleaning hive by smoke provision from predators attack. This makes the beekeepers to know overall things of beekeeping and essential to expand the beekeeping activity. Long term experience as well as indigenous knowledge of beekeepers of the study district is the best opportunity and immense potential to modernize beekeeping industry and widely utilize available ethno-medicinal value.

Table 11. Knowledge, attitude and practices of beekeepers on public health benefits of honey

Descriptions	Highland (n=13)	Midland (n=63)	Lowland (n=49)	Total (N=125)
1. Purpose of beekeeping				
Both for consumption and income	13 (100)	51 (80.95)	46 (93.88)	110 (88)
Only for Consumption	-	9 (14.29)	1 (2.04)	10 (8)
Only for income	-	3 (4.76)	2 (4.08)	5 (4)
2. Know or heard honey public health benefits				
Yes	13 (100)	59 (93.65)	40 (81.63)	112 (89.60)
No	-	4(6.35%)	9(18.37)	13(10.40)
3. From where do you get the Knowledge				
Family	9 (69.23)	38 (64.41)	25 (62.50)	72 (64.29)
Neighbor	2 (15.38)	17 (28.81)	15 (37.50)	34 (30.36)
School	-	-	-	-
Other *	2 (15.38)	4 (6.78)	-	6 (5.36)
3. Know or heard on honey side effects giving for Childs ≤ 1year				
Yes	5 (38.46)	23 (36.51)	13 (26.53)	41 (32.80)
No	8 (61.54)	40 (63.49)	36 (73.47)	84 (67.20)

Others *= book, radio, television, story

Most of the Beekeepers interviewed in the study area were used honey alone or by mixing with other ingredients to treat many diseases, symptoms, disorders and values by different formulation, route of administration, frequency of using, dosage and species they treat as traditional healer for peoples and their livestock (Table 12). Therefore, in this finding almost all (89.60 %) of the respondents knew the health benefits of honey traditionally. The main diseases, symptoms, disorders and values they treat obtained from questionnaires were gastrointestinal tract, respiratory, skin, reproductive and they used as cosmotics specially by females. The scientific and local names of disease and other values were stated in the (Table 12). The formulation of the honey for medicinal value is bolus, syrup, juice, powder and infusion forms. The routes of administrations stated by the respondents were 54.55 % oral and 45.45 topical. The frequency and dosage of drugs given is mostly as needed, from 1, 2 and 3 days as administered by the beekeepers local healers. The species treated by the honey and additives were human being and livestock, (Table 12).

Table 12. Summary of medicinal value of honey in the study area

Indication	Disease local names	Form of preparation	Route	Frequency	Dosage	Spp. it treats
Diarrhea	Garaa kaasaa	Bolus: by pounding 3-4 tea spoon of honey with coffee powder	Oral	For 2-3 days morning and evening before meal	1-2tea spoon	Human
Abdominal pain	Garaa dhukkubbii	Juice: by mixing 1-2 tea spoon of honey with garlic	Oral	As needed	1-2tea spoon	Human & Cattle
Common cold	Utaalloo	Infusion: by mixing 2 tea spoon of honey with 1 raw egg	Oral	As needed	1cup of coffee	Human
Asthma	Asmii	Infusion: by mixing 3-4 tea spoon of honey with ½ lit. warm water and garlic at morning	Oral	For 2-3 weeks	½ liter	Human
Hangover	Machii	Infusion: by mixing 3-4 tea spoon of honey with 1/2lit. of water	Oral	Stat	½ lit.	Human
Wound	Madaa	Juice/ Sap: mixing 1 tea spoon of honey with salt	Topical	Morning& evening for 3 days	1tea spoon	Human & Animals
Cuts and Burns	Muraa fi Gubaa	Juice: mixing 2-3 tea spoon of honey with yoghurt	Topical	Morning evening for 1-2 weeks	1-2 tea spoon	Human & Animals
Dermatophytosis	Baarollee	Poultice: mixing honey with lemon, endode and salt	Topical	For 1-2 weeks	As needed	Human
As cosmetics	Miidhaginaaf	Paste/ Poultice: mixing 1 coffee cup of honey with papaya, yoghurt and lemon	Topical (face)	Evening before sleep as needed	1coffee cup	Humans mostly females
Increase male sexual Potency	Fedhii saala dhiiraa dabaluu	By mixing honey with red teff as beverage as drinking	Oral	As needed	From 2-3 glass as needed	Humans
Wart	Kintaarotii	Mixing honey with salt powder and dissolve with cold water	Topical	After washing with soap morning and evening	1 tea spoon of honey	Human

4.5.3. Honey bee flora and their flowering season

Honeybee flora and trees of the current study area are considered to be an essential indicator for potentialities for beekeeping activity in the district of the study area. According to the results of this survey, the honey bee plants of the study area were trees, shrubs, herbs and cultivated crops which are a source of nectar and pollen. Some important honey bee plants of the areas were recorded in local and scientific names with flowering seasons from the beekeepers and observations on the field (Table 13). The honey bee flora of Amuru district was perennial trees and annual shrubs, cultivated crops and some herbs having significant contribution of the honey production. This wide range of plants and vegetation variety of the area could be potentially suitable for effective quantity and quality production of honey in various seasons. Experienced beekeepers familiar with the plants that produce nectar or/and pollen, when it blooms and how long they remain in blooming.

Most beekeepers of the district knew the plant flora type and medicinal value of honey depending on the color and test of honey. The annual production of honey mostly comes from trees like Makanisa *(Croton macrostachys*), Bargamo *(Eucalyptus spp),* Waddessa *(Cordia Africana) and* Baddessa *(MavlaVerticillate)* usually flowers from March to June. Many beekeepers in the study area explained that annual shrubs, crops and herbs like Hadaa (Bidens spp), Siddisa *(Trifolium Spp) and Nugi* had yellow color and thick viscous honey. Respondents stated that the flowering season of this herbs, crops and shrubs were totally from September –November, (Table 13) and the medicinal value of this honey was very higher than autumn harvested honey from April to May.

Figure 4. Some flowering plants in the study area

Beekeepers in the study area replied that they depended on many kinds of flowering plants including trees, to be indicator shrubs, undergrowth and different kinds of flowering crops and weeds. According to the beekeepers view, most important bee flowering shrubs, crops and weeds flowered between September and November and most important plants flowered in March to May and some in January. In Amuru district, the major honey harvesting season is between October and December from *Gizotia scabira, Bidens pachyloma,Cordia Africana, crops (maize. wheat and barley)* and other weedy species. Local names, and flowering seasons are given based on field observations and local knowledge of beekeepers (Table 13).

Table 13. Major honey bee floras and blooming season in the study area

No.	Scientific Names	Local Names	Blooming Season
1.	*Vernoia amygdalina*	Eebicha	December-February
2.	*Cordia Africana*	Waddeessa	January- July
3.	*Dovyalis caffra*	Kombolcha	May –June
4.	*Coffea arrabica*	Buna	April- May
5.	*Acacia abyssinica*	Laaftoo	March- September
6.	*Ekebergia capensis*	Somboo	September –November
7.	*Ficus vasta*	Qilxuu	October- December
8.	*Bidens spp*	Hadaa/ Keelloo	September –December
9.	*Trifolium spp*	Siddisa	July – October
10.	*Zea mays*	Boqqolloo	September- October
11.	*Mavla Verticillate*	Baddessaa	April- June
12	*Guizotia abyssinica*	Nuugii	October –December
13	*Schefflera abyssinica*	Gatamaa	May – June
14	*Syzygium guineese*	Waaleensuu	October- November

4.5.4. Beekeeping challenges in the study area

Based on the results obtained from this study, beekeepers of Amuru district have faced with a number of challenges and difficulties that affect their desired production. Major challenges in beekeeping a rises from environmental factors that are beyond the control of the beekeepers, while others have to do with poor marketing, infrastructure and management system. Beekeepers were interviewed to list the constraints in order of their importance. According to the beekeepers response and information the major challenges of the beekeeping are detailed. The major constraints of beekeeping in the study area were pests and predators (38.40 %) (Table 14).

Table 14. Major constraints of beekeeping identified in the study area (N= 125)

Major constraints	N (%) of respondent who experienced constraints
Pests and predators	48 (38.4)
Agro-chemicals	26 (20.8)
Equipment and price of hive	17 (13.6)
Disease	15(12)
Deforestation	11 (8.8)
Market problem	5 (4)
Lack of beekeeping knowledge	4 (3.2)

5. DISCUSSION

This study is conducted in Amuru district of Horo Guduru Wollega to evaluate honey quality and assess the knowledge, attitude and practices of beekeepers from November, 2019 to June, 2020.The moisture content is the most essential quality component of honey, because the rate of fermentation, its shelf life span and processing characteristics are greatly determined by the amount of moisture content (Gebremedhin *et al*,. 2013).As indicated in this finding themean value of moisture is 20.43 %. The present study nearly agrees with findings of Belay *et al.* (2013) who reported 20.50 % as mean test result for Ethiopian honey. However, the finding islower than the average honey sample (22.86 %) reported by Getachew *et al*, (2014).This finding much higher than the average moisture content of (18.80 %), (17.89 %) and (14.41 %), of honey moisture reported by Belay *et al*, (2013) ; Getu and Birhan (2014) and Tesfaye *et al*, (2017) in Harenna and Bale forest southeastern Ethiopia and in and around Gondar town, respectively.

Honey moisture determines the capability of honey to remain stable and resist spoilage by yeast fermentation: the higher the moisture, the higher the probability that honey will ferment uponstorage (Getu and Birhan, 2014).Lower moisture limits (e.g. 19%), ensuring a better shelf-life of honey which would be met by a large majority of the commercial honeys, have been for the revision of the Codex Alimentations (Oddo and Bogdanov, 2004).The mean value of the moisture content of honey collected from beekeepers (19.90 %) was lower than the mean of honey samples obtained from retailers (21.90 %).This variation might be freshness of honey from beekeepers and hygroscopicity, poor handling and storage of honey samples obtained from retailers (Bogdanov, 2004). The moisture content of honey depends on various factors such as the harvesting season, the degree of maturity that honey reached in the hive, type of hive used, and environmental temperature (Nigussie *et al.*, 2012).

HMF: HMF is defined as a breakdown product of fructose that is formed slowly and naturally during the storage of honey and much more quickly when honey is heated (Getachew *et al.,* 2014). One of the most commonly monitored parameters for determining honey freshness and good practices by beekeepers are HMF(Pasias *et al.,* 2018). As indicated in the HMF value of the honey samples in this study ranges from 0 mg/kg to 39.20 mg/Kg with the mean value of 16.39 mg/kg which is within acceptable range set by QSAE, CAC and EU, i.e. 40, $\leq$ 40 and $\leq$ 60 respectively. Similarly, Tesfaye *et al*, (2016) have also reported that mean HMF content of different honey samples ranged from 27.10 to 40.80 mg kg^{-1}. In fresh honey, HMF is present only in small amounts and its concentration increases with storage time and prolonged heating of honey (Da Silva *et al.*, 2016). The average result of honey obtained from beekeeper (10.76

mg/Kg) is much lower than that of retailers (33.28 mg/Kg) which indicates the freshness of honey of beekeepers than retailers in the present study. Similarly, it showed significant difference ($p < 0.05$) among the sources of honey. The average HMF (16.39 mg/Kg) value of the present study was much lower than that of Adaba (38.81 mg/kg) and Dinsho (33.86 mg/kg) districts which is evaluated by Tesfaye *et al,* (2016). The HMF mean value of the honey collected from lowland agro-ecology (22.15 mg/Kg) is higher than that of midland (6.37 mg/Kg) and highland (1.62 mg/Kg). Therefore, the finding revealed statistical significant difference ($p < 0.05$) among agro-ecologies. The honey sampled from different hives indicated different mean values of HMF numerically 6.08 mg/Kg, 11.85 mg/Kg and 14.35 mg/Kg collected from modern, transitional and traditional hive types. Despite, numerical difference of HMF value in different hive types there is no significant difference ($P > 0.05$) between hive types of this finding.

pH: Most honeys are acidic and have low pH values. The overall mean of pH for the honeys used in the study was determined as 3.92 ± 0.07 which ranges from 3.00 to 4.80. With the lowest pH determined for the honey sample acquired from retailers with 3.00; whereas the highest pH was obtained for the sample from beekeepers 4.80. All honeys are acidic with a pH-value generally lying between 3.50 and 5.50, due to the presence of organic acids that contribute to honey flavor and stability against microbial spoilage (Bogdanov *et al*., 2004). All pH value of this study is in line with pH values reported earlier. This parameter has of great significance during the extraction and storage of honey as it influences the texture, stability and shelf life of honey (Gomes *et al*., 2010).

According to Tesfaye *et al,* (2017), the average pH of honey samples in Dellomenna (3.92) district of Bale Natural Forest, Southeastern Ethiopia is similar to this study. A published report indicates that the pH of honey should be between 3.20 to 4.50 (Kinate *et al*., 2010). In this finding the pH numerical values of honey samples collected from beekeeper is higher than honey samples obtained from retailers, similarly, there is significant difference ($P < 0.05$) were observed between the sources.

In the current study, the mean value of pH honey sampled from modern, transitional and traditional hives were 4.20, 3.99 and 3.95 respectively (Table 5). So, a current finding revealed that there is no significant difference between hive types and agro-ecologies. Similarly, the result investigated by Gobessa*et al, (*2012) in Homesha district of Benishangul Gumuz was showed that there is no significant difference ($p > 0.05$) in pH were observed between honey samples obtained from different hives and also between honey samples obtained from the different locations. In general the pH value of the present study was agreed with that of Belay *et al,*(2013), Kinate *et a,l* (2013) and Alvarez *et al,* (2009) who were reported the pH values of 3.53 to 5.01, 3.45 to 4.18 and 3.47 to 4.24 respectively.Therefore, in the current result the low pH of honey confirms that it well inhibits the presence and growth of micro-organisms and makes honey compatible with many food products in terms of pH. The variations in pH might mainly be resulted due to different acids found in different floral types. The low pH of the honey samples showed that they are acidic and this indicated their ability to inhibit the presence and growth of microorganisms (Ward, 2014).

Free acidity: Free acidity has been used as a quality criterion for assessing whether fermentation has taken place; honey fermentation causes an increase in acidity. The overall mean of the free acidity of the study area was 36.67 meq kg−1 with the range of 14 to 55 meq kg−1, with the lowest free acidity value obtained for the fresh honey sample acquired from beekeepers with 14 meq/kg; whereas the highest free acidity value was obtained for the honey sample acquired from the retailer with 55 meq/kg. There is high range (41 meq/kg) between fresh honey sampled from beekeepers and retailers, which is in line with the range of national (< 40) and international (< 50) standards (Alemu *et a*l., 2013)except honey sample obtained from retailer which is 55 meq kg−1. There was a statistically significant difference between the free acidity values of the honey samples used in this study ($p < 0.05$) between beekeepers and retailers honey.The result of this study was in accordance with the observation made by Belay *et al*, (2013) ofthe mean free acid content of the Harenna forest honey samples was 34.57 meq/kg (ranges from 25.49 to 48.81 meq/kg).

Ash content: as indicated in the above result the ash content of the honey samples obtained ranged from 0.00-0.50g with the mean value of 0.25g. The result is consistent with (Tesfaye *et al.*, 2016), who reported the overall mean 0.21 % ash content of honey samples of Bale natural forest, Southeastern Ethiopia. The ash percentage found in honey expresses its richness in mineral content and constitutes a quality parameter.The mineral content of blossom honey ranges from 0.1 to 0.3 % (Bogdanov, 2009). The present study revealed average ash content of honey collected from beekeepers was numerically higher than the average ash content of retailers. In other hand, the average ash content with agro-ecologies were 0.13% highland, 0.29 % midland and 0.28 % lowland and also there was numerical difference between hive types as 0.22 from modern, 0.20 from transitional and 0.37 from traditional. But, there was no significant difference ($P > 0.05$) in ash content of honey samples from all agro-ecologies, type of hives and beekeepers and retailers. This is because ash content of honey depends on the material contained in the pollen collected by the bees during foraging on the flora.

In highland there were higher plant coverage than the midland and lowland. Due to this there will be higher organic matter content in the soil. This makes the nutrient holding capacity of highland soil to be high and higher accumulation of minerals which can be easily available to plants. This result is also agrees with findings of Getu and Birhan (2014) in and around Gondar town and in contrast with Gobessa *et al*, (2012) in Benishangul gumuz which is 0.23 % and 0.17 % respectively. Therefore, the average result of current study revealed that honey produced in Amuru district was in the international limit range which is 0.25-1% and which is good for consumption and industrial purposes.

Electrical conductivity: electrical conductivity depends on ash, organic acids, proteins and some complex sugars, and varies with botanical origin. Electrical conductivity is widely used for discrimination between honeydew and blossom honeys and also for the characterization of unifloral honeys (Chefrour *etal*., 2009).The overall mean of this study of conductivity was 0.68 mS/cm and ranges 0.2 to 1mS/cm which agrees with the international standards (0.22 to 1.52 mS/cm). The current finding of the mean electrical conductivity of the study area of 0.68± 0.018 was in line with the finding of (Getu and Birhan, 2014) who reported the mean electrical conductivity of 0.62 Amahara region in and around Gondar. The mean electrical conductivity reported by (Belay *et. al*, 2013) from Bale Harenna forest honey was slightly higher than the current finding. From this finding is numerical difference between agro-ecologies in such that 0.33 mS/cm from highland, 0.54 mS/cm from midland and 0.78 mS/cm from lowland. And also from this finding there is variation between beekeepers and retailers honey of the study area, however, there is no significant difference ($p > 0.05$) among agro-ecologies and source of collected honey.

The conductivity is a good criterion of the botanical origin of honey and thus is very used in routine honey control (Oddo andBogdanov, 2004).A lower limit has been proposed for blossom than for honey dew honeys (Pita-Calvo and V`azquez, 2017). The conductivity measurement collected honey samples from analysis of different location with respect to Amuru district; is in the range of 0.3 - 0.4 mS/cm, 0.2 - 1 mS/cm, and 0.7 - 0.9 mS/cm in case of highland, midland and lowland respectively was in line with Yadata (2014) from different areas of Tepi. The conductivity value from lowland agro-ecology is greater than in two cases of hives. According to the current study conducted the conductivity was 95.83 % found to be less than 1mS/cm.

Reducing sugar: Sugars comprise about 95- 99% of honey dry weight and are the main constituents of honey. Glucose and fructose are the main sugar constituents of honey and they are produced by sucrose hydrolysis and represent 85-95% of the total sugars (Rebiai and Lanez, 2014). From the total of 24 honey sampled for the study the overall mean of reducing sugar (fructose and glucose) of Amuru district was 73.08 ± 0.92 %; with the lowest and highest reducing sugar ratio obtained for the fresh honey sample acquired from the beekeepers with 66 % and 80 %. The general standard for a minimum content of the sum of fructose and glucose is 60 g/100 g for all blossom honeys and 45 g/100 g for all honeydew honey (Bogdanov.*et al*, 1999). Accordingly, about 100 % honey samples qualify national and international standard for content of reducing sugars in honey.

The highest and lowest value was obtained from beekeepers and there was a no statistically significant difference ($p > 0.05$) between the total sugar amounts of the honey samples studied of the honey obtained from beekeepers and retailers. The mean reducing sugar of honey sampled from different agro-ecology and hive types were 72 % (69 % to 74 %) from highland, 73.78 % (71% to 80 %) from midland and 73 % (66 % to 80 %) from lowland agro-ecologies, while 71.83 % (66 % to 78 %) from modern, 75 % (71 % to 80 %) from transitional and 72.83 % (68 % to 80 %) obtained from traditional hive types. In this study numerically the mean RS content of traditional hive is higher than that of the frame hive. This is b/c all traditional hives in the area were hanging on selected tallest tree, while the frame hives were on the ground. There is a general truth that stated by Belay *et al*, (2013) during his finding in Bale as height increase the possibility of evaporation increase. So, the traditional hive helps to concentrate the solid component of honey which increases reducing sugar.

The mean reducing sugar of honey sampled from different sources, agro-ecology and hive types were no significant difference ($p > 0.05$) observed both due to sources, agro-ecology and hive types, (Table 6 and 7). The current finding of 73.08 ± 0.92 %; higher than the finding of (Getachew *et. al*, 2014), who reported 66.79 ± 6.96 %, (Behonegn, 2017) who reported 64.3 % in South Wollo zone and (Getu and Birhan, 2014) who reported 67.83 % in and around Gonder.

Apparent Sucrose: Analysis of sucrose content is used to detect the adulteration of honey with table sugar or to determine the amount of sucrose naturally found in a given honey sample (Guler *et al.*, 2007).In this study the total average of apparent sucrose for the honey samples obtained from the study area was 1.80 ± 0.35%; with the lowest sucrose calculated for the collected sample from beekeepers with 0.00 % value; while the highest apparent sucrose ratio was obtained for the honey sample acquired from the retailers with 4.8 % value. The result showed that 100 % of the samples were in the acceptable range set by QSAE i.e. 10g/100g, the range of CAC and EU i.e. < 5 g/100g. Despite the mean is varied numerically, there was no statistical significant difference ($p > 0.05$) between honey sampled from beekeepers and retailers.

Higher sucrose contents could be the result of an early harvest of honey, i.e., the sucrose has not been converted to fructose and glucose (Gomes *et al*., 2010). The mean sucrose content of honey sampled from the highland, midland and lowland agro-ecologies were 0.83 % (0.1 % to 1.4 %), 1.26 % (0 % to 4.4 %) and 2.85 % (0.1 % to 4.5 %). Whereas the mean sucrose of honey sampled from modern, transitional and traditional hives were 2.18 % (0 % to 4.5 %), 1.93 % (0.1 % to 4.4 %), and 1.03 % (0.1 % to 3.1 5), respectively. There was no significant difference observed both due to agro-ecology and hive types of the present study.

Color: The overall average value of color of the samples used in the study area was 103.75 ± 2.89 mm which indicates amber color, with this the lowest color value obtained from the honey sample acquired from the beekeepers with 75 mm which revealed light amber color; while the highest color value was determined from retailers honey sample with 125 mm with dark amber color. Higher finding reported by Tesfaye *et al.* (2016) greatest color of honey sample studied on Pfund value was observed at Dinsho (140 mm Pfund) or dark amber and the lowest Pfund value was observed at Adaba (20 mm pfund) or white color. The color of honey varies depending up on the mineral content of honey, the darker the color the higher the mineral percentage of honey.

Quality parameters of honey of the study area had different correlation results between each other. The moisture content of honey is positively and significantly correlated with acidity and color content ($P < 0.01$). Moisture content has no correlation with the remaining parameters. The HMF content is strong positive and significantly correlated electrical conductivity ($P < 0.01$) and significant correlation ($P < 0.05$) with the free acidity. And also have weakly correlated with ash, reducing sugar, sucrose and color and no correlation with pH. If honey is adulterated with saturated sugar solutions, it will display greater conductance than pure honey as well for HMF value. In fresh honeys there is practically no Hydroxy-methylfurfural (HMF), but it increases upon storage, depending on the pH of honey and on the storage temperature (Bogdanov *et al*. 1999). Free acidity has significant correlation ($p < 0.05$) with the electrical conductivity. The higher free acidity content, the higher the resulting conductivity (Bogdanov *et al*., 2002).Other parameters had shown numerical strong and weak correlation between variables.

The questionnaire survey of this study was assessed the socio-demographic characteristics, KAP of beekeepers on honey health benefits, major honey bee flora and challenges of beekeeping in the study area. These characteristics related to the relative frequency distribution of household heads by educational level, gender, age, beekeeping experience and livestock they owned. Interestingly, the present study was further revealed that 15.20 % of the respondents have no formal education which is in line with the reports of Ambaw and Teklehaimanot (2018), who noted that 16 % of the interviewed beekeepers didn`t received either formal or informal education. In the current finding the literacy level is 84.80%, which is higher than the national average, i.e., 35.5% (Bogale *et al.*, 2010), (Jenberie *et. al,* 2008) and (Behonegn, 2017) who have reported that more than 60% and 62.5% of the sampled respondents of Amahara Region and Sekota district, respectively were literate.Similarly, this finding is in agreement with Kinati *et al.*(2011), who reported the majority of the interviewed beekeepers were educated in Gomma district, south western Ethiopia.

The male to female ratio of the current study was 6.82: 1 (87.20 %) male. The aggressive behavior of honeybee, time of harvest, burden of home job and overall house responsibility limited the active participation of women's in beekeeping practices in the study area. Similarly, Hartmann (2004), reported that traditionally beekeeping is mainly men's job in Ethiopia. The traditional idea of the study area underscores beekeeping to be men`s job due to physical reasons. This very limited number of female participation in beekeeping agrees with the findings of (Gela and Negara, 2017) and (Abebe, 2011), who reported low level of women participation in beekeeping. The very small number of female participation in beekeeping activities in the study area is in line with the result of (Bihonegn, 2017) who reported 92.5% of total sampled households (120) were male headed households while the rest were female headed in Tehulederie district south wollo zone Amahara region, but incompatible with the report of (Fikru *et.al*, 2015) who reported almost all of the respondents were males.

Average ages of the respondents were 43.31 ± 19.75 (with the range of 14 to 81 years). The average age of the respondents of this study was nearly similar with the finding of Ambaw and Teklehaimanot (2018), in Arsi zone, which is 42 years. Somewhat highest mean age result also indicated by Getu and Birhan, (2014), reported mean age of respondents were 47.63 years in and around Gondar. This proves that beekeeping is an important economic activity that can be performed by all age groups, *i.e.* by younger and old people (Alemayehu, 2011).Differences in beekeeping experience might be responsible to influence the attitude and adoption of new beekeeping technologies (Wolters and Hussain, 2015). The beekeepers had an average experience 7.66 (ranges from 3 to 26 years.) of beekeeping in the current study area. Higher average experience and ranges of beekeepers experience was reported 12.47 years (ranges from 3 to 37 years) by Getu and Birhan (2014) in and around Gondar. The current finding of 96.80 % of honeybee colony kept by traditional beehive is in line with the finding of (Tadesse and Kebede, 2014). According to Banchiamlak (2019), only about 10% of the honey produced in the Ethiopia is consumed by the beekeeping households. The remaining 90% is sold for income generation and of this amount, it is estimated that 80% is used for tej brewing. The same thing in this study the respondents main purpose of keeping honey bees were for both household consumption and income generation (88%), only for income generation (4%), and only for household consumption (8 %) according to their need in this study. The main areas of Indigenous beekeeping knowledge are hive construction from locally available materials, swarm catching, hive fumigation materials, honey and swarming season identification, different medicinal values of honey, identification of important honeybee floras, the medicinal value of honey and identification of adulterated honey (Alemayehu, 2011). Beekeepers experiences in purpose of using honey activities the deep indigenous knowledge of beekeepers which contributes to the development of modern medicine varied from area to area and from agro-ecology to agro-ecology in the area.

Honey used as medicine has been limited due to lack of scientific report. In recent days, however, there is resurgence. Its greatest medicinal potential is its application as topical agent to wounds and skin infections. Honey has anti-inflammatory, immune boosting property, and exhibits broad spectrum antibacterial activities (Motuma and Bekesho, 2016).Similarly, beekeepers of the present finding used honey for the treatment of many diseases, symptoms and values but, some of the respondents consume honey as foods without knowing the benefit of honey on the health perspective. Infant botulism occurs in infants less than 1 year of age following ingestion of spores in honey and syrup.The spores germinate in the gastrointestinal tract with toxin production (Jackson, 2017). Of interviewed beekeepers 32.80 % aware on the honey side effects on infants less than one year old while, 84 (67.20 %) hadn`t knew or heard before about the risk associated with consumption of honey for infants less than one year old. In contrast to this, children, young and old can alike take honey, without worrying any side effects (Kumar *et al*, 2010).

Dureja *et al*, (2003), stated that honey has been used as medicine in many cultures for a long period of time. However, it has limited use in medicine due to lack of scientific report. In recent days, honey is becoming acceptable as a reputable and effective therapeutic agent. Its beneficial role has been endorsed to its antimicrobial, anti-inflammatory and anti-oxidant activities as well as boosting of the immune system. In the present survey the knowledge of beekeepers honey health benefits where mostly adopted 72 (64.29 %) from families, 34 (30.36 %) from neighbors and 6 (5.36) were from other sources (book, radio, television and story). Knowledge of honey health benefits have significant correlation with the beekeepers having long experience of keeping honey colonies and age in the sampled study area. From the respondents interview the aged group has more knowledge of honey health benefits and the highland beekeepers have 100 % knowledge of health benefits while 93.65 % from midland and 81.63 % from lowland agro-ecologies were knew/ heard health benefits of honey from the total 125 beekeepers interviewed. In other hand, this research revealed that educational background; of the respondents have almost no relation with the knowledge, attitudes and practices of the honey health benefits.

In Ethiopia, people used traditional methods to treat both human and livestock diseases for generations (Sori *et al*., 2004). Similarly, in the study area beekeepers used honey alone or by mixing with other ingredients to treat many diseases, symptoms, disorders and values of peoples and their livestock. Similarly, the main diseases, symptoms, disorders and values they treat obtained from questionnaires were gastrointestinal tract, respiratory, skin, reproductive and they used as cosmotics specially females. According to Dugassa *et al*,(2012) oral administration of infusions, bolus, and other preparations used as route of administration to treat livestock and humans by ethno-medicines. Likewise, in this finding the formulation of the honey for medicinal value is bolus, syrup, juice, powder and infusion forms. The frequency and dosage of drugs given is mostly as needed, from 1, 2 and 3 days as administered by the beekeepers local healers. The species treated by the honey and additives were human being and livestock.

According to Mesfin *et al*,(2009), in Ethiopia 6500 melliferrous plant species of which more 1500 identified as bee forage. There are 58 National Forest Priority Areas in the Country that are suitable for beekeeping .Of the total land mass of the country about 71% is suitable for fruit and other crops growth which serve as source forage for the bees. Similarly, honeybee flora and trees of the current study area are considered to be an essential indicator for potentialities for beekeeping activity. A good beekeeping area is one in which honey and pollen plants grow abundantly and with a relatively long blooming season. It requires the presence of appropriate crops and plants to favor foraging. Many agro-forestry tree species were found in gardens of farmers and were good sources of forage for the beekeepers (Gebru *et al*., 2015).As the results of this survey indicated, the honey bee plants of the study area were trees, shrubs, herbs and cultivated crops which are a source of nectar and pollen. Experienced beekeepers familiar with the plants that produce nectar or/and pollen, when it blooms and how long they remain in blooming.

Most beekeepers of the district knew the plant flora type and medicinal value of honey depending on the color and test of honey. As indicated by Ambaw and Teklehaimanot (2018) in selected districts of east and west arsi zone of Oromia region, different bee forage species were identified by the respondents in local name as trees, shrubs and herbs. Accordingly the beekeepers of the study area ststed that production of honey mostly comes from trees like Makanisa *(Croton* *macrostachys*), Bargamo *(Eucalyptus spp),* Waddessa *(Cordia Africana) and* Baddessa *(MavlaVerticillate)* usually flowers from march-July. Many beekeepers in the study area explained that annual shrubs, crops and herbs like Hadaa (Bidens spp), Siddisa *(Trifolium Spp) and Nugi* had yellow color and thick viscous honey. Similar report is presented in selected districts of Arsi zone by Ambaw and Teklehaimanot (2018). Respondents of the stuy area stated that the flowering season of this herbs, crops and shrubs were totally from September to November, and the medicinal value of this honey was very higher than autumn harvested honey from April to May.In this finding most important bee flowering shrubs, crops and weeds flowered between September and November and most important plants flowered in March to May and some in January the same finding is reported by Kumsa and Takele (2014) in Jimma Zone. In Amuru district, the major honey harvesting season is between October and December.

The major constraints of beekeeping is the environmental condition which includes: honeybees' enemies, bee poisoning due to agrochemicals, lack of knowledge to manage bees and bee products, lack of bee colonies and bees poisoning from plants (Tesfaye *et al*., 2017). In the same manner beekeepers of the study district have faced with a number of challenges and difficulties that affect their desired production. The challenges a rises from environmental factors that are beyond the control of the beekeepers, while others have to do with poor marketing infrastructure and management system. Beekeepers were interviewed to list the constraints in order of their importance. The beekeepers of the study area experienced constraints of beekeeping 38.40 % pests and predators, 20.80 % agrochemicals, 13.60 % Equipment and price of hive, 12 % disease, 8.80 % deforestation, 4 % market problem and 3.20 % lack of beekeeping knowledge. Approximately similar report is presented by Bihonegn (2017) that, 60.74%, 46.67%, 45.93%, 27.41% and 22.22% of the respondents agree that the main reasons for the colony decline are chemical application, lack of management, predators, pests and drought respectively.

6. CONCLUSION AND RECOMMENDATIONS

This study was conducted to evaluate honey quality, knowledge, attitude and practices of beekeepers in Amuru district, Horo Guduru Wollega zone, Western Ethiopia. The main evaluated parameters of honey samples were moisture content, HMF, pH, free acidity, ash, electrical conductivity, reducing sugar, sucrose and color of honey analyzed and their evaluated mean values were; 20.43 ± 1.32 %, 16.39 ± 2.68 mg/kg, 3.92 ± 0.07, 36.67 ± 2.13 meq/kg, 0.25 ± 0.14 %, 0.68 ± 0.3 mS/cm, 73.08 ± 0.92 %, 1.80 ± 0.35 % and 103.75 ± 2.89 mm respectively. The value of moisture content, HMF, pH, free acidity and color significantly ($p < 0.05$) influenced by sources of the sample (i.e. beekeepers and retailers). Whereas, the value of moisture content, HMF and free acidityis significantly ($p < 0.05$) affected among agro-ecologies. Statistically there was no significance difference ($p > 0.05$) among hive types in terms of all analyzed parameters.The moisture content value of honey sampled declared significant positively correlated with free acidity and color r = 0 .63 and r = 0.53 respectively, ($p < 0.01$).While, HMF value has significant ($p < 0.01$) positive correlation with Electrical conductivity value r = 0.77 and significantly ($p < 0.05$) correlated with free acidity r = 0.45. Free acidity has significant ($p < 0.05$) correlation with electrical conductivity value r = 0.41.

The beekeepers have developed indigenous KAP of keeping bees passing from generation to generation in the study area. These indigenous beekeeping knowledge are hive construction from locally available materials, honey health benefits, major constraints of beekeeping, swarming season identification, identification of important honeybee floras and flowering season of bee flora.Most of the respondents were keeping honey colony for the purpose of both consumption and income. Majority of the respondents (89.60% knew or heard the health benefits of honey and practiced to treat many of the human and animal disease and symptoms. The major constraints identified for beekeeping in the study area are pests and predators, indiscriminate agro-chemicals disease, lack and expensiveness of equipment, deforestation and other factors. In conclusion results revealed that honey produced in Amuru district is of good quality and can meet the national and international market demands. This result provides baseline data on pattern of honey quality and production and identifies perception gaps that can be used as reference for future in the study area.

Based on the research work conducted in the study area, the following recommendations were forwarded:

- To improve the honey quality defects associated with poor management, harvesting and handling in the study district specially by honey retailers, there is a need to provide a practical training to local beekeepers and retailers about proper ways of handling, processing, packaging and sale of honey, moreover, facilitating supply of quality apicultural equipment is crucial.
- Address the skill gap on bee colony management and post harvest handling of hive products, further consistent practical training on bee and bee products management for community is recommended.
- Now a days, there is alarming time to search 3rd generation medicine due to newly emerging and re-emerging disease, so government and non-governmental sectors should have to promote KAP of beekeepers and other social practices.
- Female beekeepers should be empowered to participate effectively in beekeeping activities by offering practical training, organizing in different cooperatives and providing credit service by governmental and non-governmental institutions.
- The government should integrate multidisciplinary experts with the traditional healers to develop modern pharmaceutical products which may solve world's current straining. e.g. the indigenous knowledge on honey health benefits
- Farmers of the study district were using numerous chemicals for the protection of crop during day time when honeybees foraging, the toxicity effect of each chemical on honeybee is too high, so, the farmer should spray the chemical during night time.
- Further study should be recommended to assess honey production and physicochemical properties of honey in the study area.

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8. ANNEXES

Annex 1. Sample collecting sheet

No.	Type of Sample	Amount (kg)	Sample code (HS)	District	Type of bee hive	Agro-ecology (High, Mid and Lowland	Source		Remarks
							Beekeepers	Retailer	
1	Honey	0.5 kg							
2	>>	>>							
3	>>	>>							
4	>>	>>							
5	>>	>>							
6	>>	>>							
7	>>	>>							
8	>>	>>							
9	>>	>>							
10	>>	>>							

Annex 2. Questionnaire recording sheet

No.	Name of respondents	Zone	District	Kebele	Age	Sex	Educational background	Agro ecology			Type of hive		
								Hl	Ml	Ll	Trd	Trns	Mod
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
12													
13													
14													
15													

Annex 3. Procedure for honey moisture determination

1. Preparation of the sample
2. Determine the refractive index of the test sample using a refractometer at a constant temperature near 20 ^{0}C.
3. Convert the reading to moisture content (percent by mass) using the table.
4. If the determination is made at a temperature other than 20 ^{0}C, convert the reading to standard temperature of 20 ^{0}C, according to the temperature corrections quoted.

Figure 1: Abbe refractometer

Annex 4. Conversion factor for the estimation of moisture content from refractive index data

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

Source: (Wedmore 1955)

Annex 5. Procedures for hydroxyl-methyl furfural

1. Accurately weigh 5 g honey in small beaker and transfer with total of 25 ml distilled water to 50 ml volumetric flask
2. Add 0.50 Carrez solution I, mix, add 0.50 ml Carrez solution II, mix, and dilute to volume with distilled water drop of alcohol may be added to suppress foam.
3. Filter through filter paper, discarding the first 10 ml filtrate.
4. Pipette 5 ml filtrate into each of two 18 X 150 mm test tubes.
5. Add 5.0 ml distilled water to one tube (sample) and 5.0 ml $NaHS0_3$ solution to other.
6. Mix well by using Vortex mixer and determine.
7. Absorbance of sample against reference at 284 and 336 nm in 1 cm cells.
8. If absorbance is greater than 0.6, dilute sample solution with water and reference solution with 0.1% $NaHS0_3$ solution to same extent and correct absorbance for dilution.

Annex 6. Dilution of sample and reference solutions carried for estimation of HMF

Addition to test-tubes	Sample solution (in ml)	Reference solution (in ml)
Initial solution	5.0	5.0
Water solution	5.0	-
Sodium bisulphate (0.2 %)	-	5.0

Source: IHC, 2009

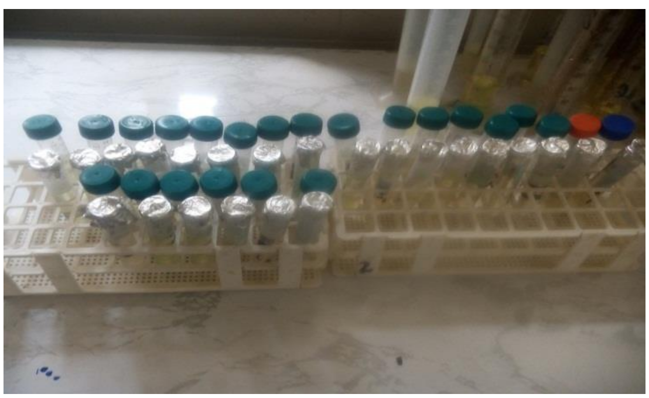

Figure 2. Sample solution and reference solution

Dilution (D) = $\frac{\text{Final volume of solution}}{10}$

Calculation and expression of result:

HMF expressed as mg/kg = **(A284-A336) x 149.7 x 5 x D/W**.

A284= Absorbance at 284 nm.

A336 = Absorbance at 336 nm.

149.7 = 126 x 1000 x 1000/16830 x 10 x 5.

126 = Molecular weight of HMF.

16830 = Molar absorptive and HMF at 284 nm.

10 = Conversion of g into mg.

1000 = Conversion of g into kg.

5 = Theoretical nominal sample weight.

D = Dilution factor (in case dilution is required)

W= Weight in g of honey sample

Annex 7. Procedure for Acidity and PH

1. Preparation of test sample; weigh 10 gram of honey accurately and dissolve it in 75ml in distilled water
2. The test sample is titrated against carbonate free 0.1N sodium hydroxide solution using 4-5 drops of phenolphalein indicator
3. The end point colour should persist for 10 seconds
4. For dark cooled sample a smaller weight shall be taken
5. As an alternative a PH meter may be used and the sample titrated to PH 8.3
6. Expression of the result

Acidity=10V

Where V = the volume of 0.1M NaOH used and 10 is the amount of honey sample

Figure 3. pH meter and Magnetic strirer

Annex 8. Procedures for determining ash content

1. Weigh 5 to 10 grams Honey accurately into ignited and pre-weighed platinum, silica or porcelain crucible (shiinii).
2. Gently heat in muffle furnace until the sample is black and dry.
3. An infra-red lamp or Bunsen burner can also be used to char the sample before inserting in to the muffle furnace.
4. If necessary; a few drop of olive oil may be added to prevent frothing.
5. The sample is then ignited at 600^{o}c to constant weight.
6. The sample is cooled in desiccator before weighing.
7. Finally weigh and calculate the percentage**.**

- Percent ash in g/100g honey was calculated using the following formula, following the procedure of (Bogdov, 2009)

$$\mathbf{WA} = (\boldsymbol{m1} - \boldsymbol{m2})/\boldsymbol{mo}\ \ \boldsymbol{x}\mathbf{100}$$

Where; mo = weight of honey take m1 = weight of dish + ash, m2 = weight of dish. Proposed ash content not more than 0.6 % for normal honey.

Figure 4. Hot plate and muffle furnace

Annex 9: Procedure of determining reducing sugar and sucrose by HPLC Method

A. Reagent

- ❖ Chemicals of analytical purity grade were used.

1. Distilled water HPLC (purchased from the Finfine Kirkos Heparin Trading private limiting Company) **4500 ETB (2.5 Litre).**
2. Methanol for HPLC (from lab.)
3. Acetonitrile for HPLC. Purchased from Finfine, Kirkos, Heparin Trading private limiting Company **5200 ETB (2.5 Litre).** Warning: Acetonitrile is a dangerous substance laboratory safety guidelines on dangerous substances at work were used
4. Eluent solution for the HPLC. Mix 80 volumes of acetonitrile with 20 volumes of water and degassed prior to use.
5. The standard substances: fructose, glucose and sucrose obtained from lab.

- fructose: 2.000 g
- glucose: 1.500 g
- sucrose: 0.250 g

B. Equipments

Sample vials, Ultrasonic bath, Calibrated flasks, volume 100 ml, 25-ml-pipette, Membrane filter for aqueous solutions, pore size 0.45 µm, Filter holder for membrane filters with suitable syringe, High Performance Liquid Chromatograph consisting of pump, sample applicator, temperature regulated RI-detector thermostated at 30o C*, temperature regulated column oven at 300C, integrator, analytical stainless-steel column, silica gel with 5-7 µm particle size. Before use, a system suitability test to ensure all the sugars can be separated was carried out.

* Note: the chromatography can be carried out at room temperature without influence on the results of the sugars, determined by the present method.

C. Procedures

Preparation of the sample solution

1. Weigh 5g of honey into a beaker and dissolve in 40 ml water.
2. Pipette 25ml of methanol into a 100ml volumetric flask and transfer the honey solution quantitatively to the flask.
3. Fill to the mark with water.
4. Dissolve the amounts detailed of the standards in approximately 40ml water and transfer quantitatively to the flask and fill to the mark with water
5. Pour through a membrane filter and collect in sample vials.
6. Store as for the standard solution.

❖ High Performance Liquid Chromatography (HPLC)

-After a column of the type described above is used, the following conditions have been found to give satisfactory separation.

- Flow rate: 1.3 ml/min
- mobile phase: Acetonitrile:water (80:20, v/v)
- column and detector temperature : 30^0C
- sample volume: 10 µl

Note: Identical volumes of sample and standard solution should be injected.

D. Calculation and Result Expression

The honey sugars are identified and quantified by comparison of the retention times and the peak area of the honey sugars with those of the standard sugars. The mass percentage of the sugars, W, to be determined of fructose, glucose, etc..and maltose in g/100g is calculated according to the following formula (external standard procedure):

W = A1 x V1 x m1 x 100/ A2 x V2 x m0

Where,

A1 = Peak areas or peak heights of the given sugar compound in the sample solution, expressed as units of area, length or integration.

A2 = Peak heights of the given sugar compound in the standard solution, expressed as units of area, length or integration.

V1 = Total volume of the sample solution in ml

V2 = Total volume of the standard solution in ml

m1 = Mass amount of the sugar in grams in the total volume of the standard (V2)

m0 = sample weight in g. The final result is rounded to one decimal place.

Figure 5. Honey sample processing in HPLC machine to calculate reducing sugar and sucrose

Annex 10. Procedures for color determination

1. Homogeneous honey samples free of air bubbles was transfer into a cuvette (transparent tubularLaboratory vessel) with a 10-mm light path until the cuvette is approximately half full.
2. The cuvette was inserted into a color photometer Pfund honey color grader (No. 0061, made in USA)
3. The color grades were expres sed in millimeter (mm) Pfund grades compared to an analytical gradeglycerol standard following the procedure of IHC (2009).

Annex 11. USDA color standard

Standard	P-fund Color Grader (mm)
Water white	0-8
Extra white	≥ 8-17
White	≥17-34
Extra light amber	≥ 34-50
Light amber	≥ 50-85
Amber	≥ 85-114
Dark amber	≥ 114

Source: (IHC, 2009)

Figure 6. Pfund color grader

Annex 12. The results of all the honey quality parameters analyzed in the study area (N = 24)

Source of honey samples		Quality Parameters								
Agro-ecology	Hive types	MC (%)	HMF (mg/kg)	pH	FA (meq/cm)	Ash (%)	EC (mS/cm)	Rs (%)	AP (%)	Color (mm)
Highland	Modern	19.4	0	4.1	22	0.2	0.4	73	1	101
	Transitional	20.4	1.4	3.9	33	0.1	0.3	74	1.4	108
	Traditional	21.8	2.1	4.1	30	0.1	0.3	69	0.1	102
Midland	Modern	20.2	3.2	4	33	0.3	0.6	78	0.1	96
	Modern	20.4	1.8	4	34	0.2	0.5	74	3.4	108
	Modern	21.0	2.8	4.1	32	0.1	0.3	71	0	109
	Transitional	20.4	6.6	3.9	40	0	0.2	78	0.1	109
	Transitional	19.6	6	3.9	43	0.2	0.4	71	4.4	75
	Transitional	20.0	7.3	3.9	42	0.5	1	71	0.4	108
	Traditional	20.6	9.8	3.7	48	0.4	0.9	70	2.7	78
	Traditional	21.0	8.8	4	31	0.5	0.3	71	0.1	107
	Traditional	21.2	11	3.9	43	0.4	0.7	80	0.1	108
Lowland	Modern	19.0	16.4	4.2	14	0.3	0.7	69	4.5	102
	Modern	19.2	12.3	4.8	24	0.2	0.7	66	4.1	110
	Transitional	18.0	26.7	4.3	26	0.3	0.8	76	2.9	105
	Transitional	19.0	23.1	4	29	0.1	0.8	80	2.4	79
	Traditional	19.2	29.1	3.8	46	0.3	0.8	68	3.1	78
	Traditional	18.2	25.3	4.2	24	0.5	0.9	79	0.1	98
Honey retailers	Retailer	22.4	39.2	3.1	49	0.4	0.9	79	0.1	125
	Retailer	21.8	38.6	3	55	0.2	0.9	78	0	129
	Retailer	21.0	36	3.8	45	0.2	1	75	2.5	118
	Retailer	21.0	30.5	3.6	47	0.1	1	67	3.5	112
	Retailer	22.6	24.1	3.8	46	0.3	1	68	4.8	107
	Retailer	22.8	31.3	3.9	44	0.1	1	69	1.5	118

Annex 12. Results of Univariable GLM analysis of honey samples parameters

Independent variable	Dependent variable	No. of tested	*p-value*
Agro-ecology	MC (%)	18	*.000*
	HMF (mg/Kg)	18	*.000*
	pH	18	*.073*
	FA (meq/Kg)	18	*.031*
	Ash content (%)	18	*.306*
	EC (mS/cm)	18	*.295*
	RS (%)	18	*.838*
	As (%)	18	*.124*
	Color (mm)	18	*.642*
Source	MC (%)	24	*.000*
	HMF (mg/Kg)	24	*.000*
	pH	24	*.001*
	FA (meq/Kg)	24	*.001*
	Ash content (%)	24	*.526*
	EC (mS/cm)	24	*.528*
	RS (%)	24	*.800*
	As (%)	24	*.675*
	Color (mm)	24	*.002*
Type of hive	MC (%)	18	*.449*
	HMF (mg/Kg)	18	*.318*
	pH	18	*.148*
	FA (meq/Kg)	18	*.097*
	Ash content (%)	18	*.114*
	EC (mS/cm)	18	*.156*
	RS (%)	18	*.466*
	As (%)	18	*.496*
	Color (mm)	18	*.437*

Annex 13. Quality of honey sample of the study area as compared to QSAE, EU and CAC**:**

Parame ters	N	The current result of the study area			National and International institutions and their standards				
		Mean ± SD	Max.	Min.	QSAE	EU	CAC	World	FAO/WHO
Moistur e %	24	20.4± 1.32	22.8	18	17.5-21	< 21	< 21	18-23	21-23
HMF (mg/kg)	24	16.39 ± 2.68	39.2	0	40	< 40	< 60	40-80	≤ 80
PH	24	3.92 ± .07	4.8	3		-	-	3.2-4.5	-
FA(me q/kg)	24	36.67 ± 2.13	55	14	40	< 40	< 50	5-54	40
Ash (%)	24	0.25 ± 0.14	.50	0	0.6	<0.6	< 0.6	0.25 – 1	0.6 – 1
EC mS/cm	24	0.6 ± 0.3	1	0.1	<0.6	<0.8	<0.8	.22-1.52	-
RS (%)	24	73.08± 0.92	80	66	65	< 65	< 65	60 – 70	≥ 65
Sucrose (%)	24	1.80 ± 0.35	0	4.8	10	< 5	< 5	3-10	5-10
Color (mm)	24	103.75±2.89	129	75	-	-	-	-	-

Source : CAC (2001); QSAE (2009); EU Council (2002) and QSAE (2000)

QSAE, quality and Standards Authority of Ethiopia; CAC ; CAC; EU; European Union; meq; milli equivalent

Annex 14. Questionnaires used in the study

- Jimma University College of Agriculture And School of Veterinary Medicine
- Department of Veterinary Public Health

Evaluation of Honey Quality and the KAP of beekeepers In Amuru district Of Horo Guduru Wollega Zone, Oromia, West Ethiopia.

- This questionnaire is prepared for an academic purpose for the fulfillment of MSc degree in **Veterinary Public Health.**
- Dear respondent: This questionnaire is designed to collect necessary information to assess beekeepers practices on health benefits of honey and the beekeeping system in *Amuru* district; Western Ethiopia.
- So your response to the question has great contributions. Therefore you are politely requested to give the accurate and necessary information.

I. Direction for Enumerator

- Please greet them and introduce yourself patiently to the beekeepers before starting the interview.
- Tell the respondents politely about the aim and purpose of the study
- Try to start with easy questions
- Use pencil to tick on **Yes** or **No** and to write broad idea on provided space
- Create sufficient awareness for beekeepers regarding with questions to get accurate data
- Write down properly the idea provided by the beekeepers
- Ask the questions by using the language that beekeepers can understand and know easily

A. Interview guideline for district Office of Livestock Resource Development and Fishery

1. What is the agro-ecology of the district?

   A. High land B. Mid land C. Low land D. All E. Other______________________________

2. How many kebeles are high, mid and low land agro-ecology?

   Highland__________ Midland__________Lowland__________________________________

3. Number of beekeepers in the district in 2011 E.C. ________________________________

4. Total number of beehives in the district in 2011 E.C.___________________________________

A.Traditional_______________ B.Transitional_____________ c. Modern____________________

5. What is the average productivity of the different hives (Kg/hive/harvest) in the the district?

Traditional _____________, Transitional___________Modern______________________________

6. Which kebeles have high beekeeping practices? ____

Why?____

8. At what season honey mostly harvested in the district? __________________________________

9. What is the general perspective of the beekeeping activities in the district?
____

10. How is the potential and what is the good opportunities for beekeeping in the district ?_____________________________

B. Questionnaires for Beekeepers

1. Socio-demographic history of beekeepers

- Name of beekeeper________________________________Zone_________District_________ Kebele_________Village________________Agro-ecology___________
- Sex: a. Male b. Female
- Age: a. 14-25 b. ≥25-65 c. ≥ 65
- Educational background A. Illiterate B. Elementary school C. High school D. College/University

2. Hive type and honey production potential

2.1. What type of bee hive do you have?

Hive type	✓ or x	Amount
Modern		
Transitional		
Traditional		

2.2.When did you start beekeeping? A. < 5 year B. ≥ 5-10 years C. > 10 year

2.3. At what time do you harvest honey? A. day B. night C. specify if any other_____________

2.4. If your answer in question number 2.3. Is A or B why at that time? _____________________

2.5. The amount of honey produced

No.	Type of hive	Frequency of harvesting/year	Average of honey harvested (season/hive/ kg)	Total amount of honey produced hive/year in Kg
1	Modern			
2	Transitional			
3	Traditional			

2.6. What are peak honey producing months____?

3. Honey health benefits KAP

3.1. For what purpose you keep honey bee colony?

A. both for income and consumption B. only for household consumption C. only for income

3.2. Do you know or heard the health benefits of honey? A. yes B. no

3.3. If your answer on question no.3.2. Is yes;

A. For what disease, disorders, symptoms / values you use? ____________________________

B. Formulation: ____

C. Route of administration: ____

D. Frequency; ____

E. Dosage: ____

F. Species it treats; ____

3.4. From where do you get the knowledge and practices of honey health benefits?

A. From family B. from neighbors C. from School D. Others _______________

3.5. Have you heard before the honey side effect on child < 1 year old? A. yes B. no

3.5. If your answer on question no. 3.4. Is yes, what is the side effect?

____

4. Honey Bee Plants and Flora

4.1. What are the major honey bee plants or floras in your area and their flowering season?

No.	Local name of honey bee plants/floras	Flowering season
1		
2		
3		
4		
5		

4.2. What are the peak flowering seasons of the year? ___________________________________

5. Factors Influencing Beekeeping system in the study area

5.1. What are the factors that influence beekeeping in your area?

A. Predators and Pests B. Agro-chemicals C. Deforestation D. Lack of beekeeping knowledge

E. Lack Market F. lack of equipment and prices of hive

5.2.Is there a trend of using agro-chemicals in your locality for crop production? A. yes B. no

- **Name of Enumerator:______________________________**
- **Signature: _______________________________________**
- **Date of interview:_________________________________**