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ADDIS ABABA UNIVERSITY

CVMA

ASSESSING AND EVALUATING HONEY QUALITY AT DIFFERENT MARKET POINTS IN ADAMA DISTRICT AND ADAMA TOWN, OROMIA, ETHIOPIA

By:

Melaku Bekele

A Thesis Submitted to the College of Veterinary Medicine and Agriculture Department of Animal Production

Presented in Partial Fulfillment of the Requirements for the Degree of Master of Science in Animal Production

Advisors: Ashenafi Mengistu (Ph.D., Associate Professor)
Zewdu Ararso (Ph.D., Senior Researcher)

June 2023

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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the thesis prepared by Melaku Bekele Tufa (I.D. No. GSR/8465/13) entitled "Assessing and Evaluating Honey Quality at Different Market Points in Adama District and Adama Town, Oromia, Ethiopia". And recommend that it can be accepted as fulfilling the thesis requirement for the degree of Master of Science in Animal Production Studies.

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BIOGRAPHICAL SKETCH

The author was born on August 8, 1989, to parents Desta Rorisa and Bekele Tufa in Tulu Cabi, Tiyo Woreda, and Arsi Zone. His elementary, junior, and high school educations were completed at Abichu Elementary, Cabi Junior School, Adama Secondary School, and Hawas Preparatory School, respectively. He joined Haramaya University after completing his preparatory schooling in 2007, and on July 8, 2010, he received his B.Sc. in animal science from the College of Agriculture (COA).

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STATEMENT OF AUTHOR

First and foremost, I declare that this thesis's content is my original work and that all informational sources have been appropriately cited. This thesis has been deposited at the university or college library to be made available to borrowers following library policies. It has been submitted in partial fulfillment of the requirements for a MSc degree at Addis Ababa University, College of Veterinary Medicine and Agriculture. I hereby solemnly declare that this thesis is not being submitted to any other institution, anywhere, to receive a diploma, certificate, or academic degree.

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LIST OF ACRONYMS AND ABBREVIATIONS

ATVET	Agricultural Technical, Vocational, and Education Training
C3	Plants that Fix Carbon Dioxide using Calvin Cycle
C4	Plants that Fix Carbon Dioxide using Hatch-slack Cycle
CO_2	Carbon Dioxide
CSA	Central Statistical Agency
CS	Corn Syrup
EHC	European Honey Commission
EU	European Union
FAO	Food and Agricultural Organization
FTC	Farmers Teaching Center
GC	Gas Chromatography
GS	Glucose Syrups
HBRC	Holeta Bee Research Center
HFCS	High Fructose Corn Syrup
HMF	Hydroxy Methyl Furfural
HPLC	High-Performance Liquid Chromatography
IS	Inverted Syrup
$Meqkg^{-1}$	Milli Equivalent Per Kilogram
MC	Moisture Content
$Mgml^{-1}$	Milligram per Milliliter
NIR	Near-Infrared Spectroscopy
PCB	Polychlorinated Biphenyl
TLC	Thin Layer Chromatography
UK	United Kingdom
USA	United States of America
WHO	World Health Organization

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ASSESSING AND EVALUATING HONEY QUALITY AT DIFFERENT MARKET POINTS IN ADAMA DISTRICT AND ADAMA TOWN, OROMIA, ETHIOPIA

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ABSTRACT

The study was conducted to assess and evaluate the quality of honey produced in Adama District and Adama Town at different market points. Factors that affect honey quality and the botanical origins of honey produced and marketed in the areas were assessed at different market points. For this purpose, a total of 105 respondents were interviewed. Moreover, a total of 23 honey samples were collected from beekeepers and different market points and used for quality analyses. The results of the survey showed that honey quality deterioration starts at harvesting time, like the use of too much smoke, harvesting unripe honey, and improper harvesting and storing materials. In addition, the low volume of honey production compared to the demand for honey in the areas had its own contribution to adulterating honey using different adulterants, which affected the quality of the honey in the areas. According to the survey result, the major act of adulterating honey has been performed at the street honey sellers' market point. Most of the quick test results showed that honey sold at most of the market points has quality problems that vary among the market points. The seriousness of the problem is relatively pronounced at the street market and minimarket levels. The ash content, free acidity, and pH value of all honey samples were found to be within the limits of the national standards. Except for the honey samples collected from street (24.62±0.67), minimarkets (23.23±0.58) and retailers (22.60±0.58) the moisture contents of the honey samples were within the national standards. The fructose and glucose contents of the samples were within the ranges of the national standards, *whereas none of the samples met the national sucrose content standard. High sucrose content was observed in the samples obtained from retailers (32.23±1.78%) and the street (31.90±2.06%) market points. In general, the results of this study indicated that there is an overall honey quality problem in the sampled area. However, the level of the problem is more inclined towards the street and minimarket areas. Thus, honey market legislation is needed in the area in particular and in the country in general to protect honey consumers and other stockholders involved in the honey market value chain.*

Keywords: Adulteration, Adulterant, Botanical origin, Detection of adulterations Honey, Market point, Physicochemical, Quality, Quick test.

1. INTRODUCTION

BACKGROUND

Ethiopia is blessed with suitable water resources and numerous honeybee floras, which produce fruitful ground for the growth of beekeeping (Addis, 2016). The majority of beekeepers in Africa are thought to reside in Ethiopia. The apiculture industry has been a part of Ethiopia's economy and continues to support the country's growth. It is evident that beekeeping products, like honey, which supply the required calorie diet, improve the nutritional status of the population. Additionally, apiculture is crucial to Ethiopia's ability to export goods like honey and earn foreign currency. Ethiopia also likely has the longest history of beekeeping among all the African nations. According to Sebeho (2015), Ethiopia produces 21.7% of all honey produced in Africa and 2.5% globally. Ethiopian honeybee colonies present in beehives number 6.99 million, which contributes 1.29 million tons of honey production (CSA, 2020/21).

However, most honey is subpar and badly produced. While in the hive, honey is of high quality, but poor treatment from the time of harvest until it is sold results in honey of lower quality (Crane and Visscher, 2009). High moisture content is also a result of unripe honey being harvested, improper honey storage containers, and inadequate storage locations (Adgaba *et al.*, 2017). Despite having the highest bee population size, a long history of beekeeping, and a significant amount of honey production, but research conducted in the country so far has not covered well enough to know factors affecting honey quality, characterize and document detection mechanisms, as well as the effect and extent of adulteration for locally produced and commercially available honeys (honey available in different markets) in the country (Teferi, 2020).

Water content, ash, pH, electrical conductivity, hydroxymethyl furfuraldehyde (HMF), glucose, fructose, sucrose, and diastase activity were used as physicochemical criteria in the characterization of honey in the Codex Alimentarius Commission (2001) and Ethiopian Standard (2005) specifications. Comparison of the results with naturally occurring values can suggest suspected adulteration (Bogdanov *et al.*, 2003). Poor preharvest handling of honey at producer apiaries and in various markets is a significant operation that is known to have the potential to eliminate spoilage microorganisms, facilitate packaging, and postpone crystallization (Subramanian *et al*., 2007). The flavor, color, and granulation of honey are changed, bioactive components and antioxidants are degraded, and product quality degrades when honey is heated to higher temperatures of more than 70 ^{o}C (Visquert *et al*., 2004). Consumers frequently have no idea what kind of honey they are purchasing. Due to connected occurrences of adulteration, it is vital to control the quality of honey in various markets (Iftikhar *et al*., 2014). It is dishonest and unfair to the consumer when adulterated honey is sold under natural labels at the same price as pure honey (Chen *et al*., 2011). These practices can cause honey's quality to decline at the producer's, during processing, and during marketing. Due to this, it is now crucial for regulatory agencies, retailers, consumers, and processors to identify adulterant substances in honey (Chen *et al*., 2011).

Addis Ababa is the largest and most organized place for honey marketing in Ethiopia, followed by Adama City and its surroundings. In Adama, there are numerous supermarkets, shops, and retail stores that serve as places for honey markets in the area. Most of these honeys are sold without a label or reference to their quality or origins, which may give rise to honey adulteration and/or the marketing of honey with low quality standards. Therefore, a reduction in honey quality could result either from adulteration (deliberate mixing of honey with cheap foreign substances) or from pre- and post-harvest handling, poor storage facilities, or processing of honey with excessive heat treatment. Despite all these facts, information on honey adulteration, adulterants used, and honey quality, starting from beekeepers through various market points in Adama and the surrounding areas, is limited. Hence, assessing factors affecting honey quality, identifying the botanical origin of honey in the area, and characterizing the physicochemical properties of honey produced and sold in the area are highly vital for honey retailers, consumers, and policymakers.

Statement of the Problem

Due to inappropriate post-harvest handling, including transportation, processing, adulteration, excessive heat treatment, and lengthy storage times, the quality of honey was altered. Because reports show that such adulterated honey has no nutritional or therapeutic value, such honey may have poor physical, chemical, organoleptic, and microbiological qualities and, as a result, poor functioning (Bogdanov and Gallman, 2008). Except for a few studies on local conventional tests used by beekeepers and customers, not much research has been reported on the degree of honey adulteration and quality at different market points in Ethiopia (Ambaw and Teklehaimanot, 2018; Gemeda and Negera, 2017). Honey adulteration, detection methods, and degree of adulteration on honey commercially available in Addis Ababa were also studied (Teferi, 2020).

To assess whether the honey meets quality requirements, beekeepers, collectors, cooperatives, honey processors, distributors, supermarkets, minimarkets, retailers, and customers typically rely a great deal on their own experience and observation. For the aforementioned factors as well as numerous others, such as economic loss due to inferior honey quality that does not match international standards, it is impossible to export and earn foreign cash. The detection of honey adulterants by thorough physicochemical parameters and a quick test analysis approach, as well as the factors affecting honey quality for honey available at various market points in Adama city and Adama districts, are therefore crucial to research. Considering that the detection of adulteration requires knowledge of the honey products' physical and chemical properties, this study approach aimed at assessing factors affecting honey quality, evaluating honey quality, and identifying adulterated honey.

Objective of the study

General objective

The general objectives of this study were to evaluate quality and factors affecting honey quality at different market points in Adama city and district.

Specific objective

The specific objectives of this thesis were to:

- ✓ Assess factors affecting honey quality at different market points in Adama city and district
- ✓ Evaluate the quality of honey at different market points in Adama city and district
- ✓ Detect honey adulteration based on their physiochemical, and quick test methods
- ✓ Identify botanical origin of honey found at different market points

2. LITERATURE REVIEW

Bees make honey from nectar, the sugary secretion of flowers. Nectar contains 70-80% water. To make honey, the bees add enzymes and reduce the water content of the nectar to less than 20%. Bees transfer nectar from their stomachs to other bees, who in turn pass it on to other bees. During transfer, the water content decreases and the bees add enzymes from their honey sacs that prevent the nectar from fermenting. The bees then place this in the cells of the comb and further reduce the water content by heating the honey to around 35°C. Therefore, honey is defined as a natural sweet substance that honey bees produce from the nectar of flowers, they collect from living plants, and which they transform themselves through the evaporation of water and through the action of enzymes. The physical and chemical composition of honey can vary depending on the type of flower and the soil the plant is growing on, as well as physical environmental factors.

2.1. Physicochemical Characterization of Ethiopian Honey

Honey is composed primarily of sugars, glucose, and fructose, while its third greatest component is water. Physicochemical parameters of honey include mineral content (%), moisture content (%), acidity ($meqkg^{-1}$), hydroxymethyl furfural ($mgkg^{-1}$), reducing sugar (%), sucrose (%), pH, specific gravity, and color (Gebremedhin *et al*., 2013). These physical chemical properties of honey may vary depending on the botanical origin of the honey and its environmental and ecological conditions.

2.1.1. The Color Analysis of Honey

The Pfund classifier was used to determine the color of honey samples. Just until the cuvette was about halfway full, homogenous honey samples free of air bubbles were put into a cuvette with a 10-mm light path. According to the Codex Alimentarius Commission Standards (2001), the cuvette was placed inside a color photometer Pfund honey color grader (No. 0061, produced in the USA), and the color grades were expressed in millimeter (mm) Pfund grades and compared to an analytical grade glycerol standard. Using the United States Department of Agriculture's (1985) established color standards, measurements were made for each sample.

2.1.2. The Moisture Content of Honey

Honey contains moisture, which has an impact on a number of characteristics, including density, specific gravity, refractive index, viscosity, and optical properties (Ishraga *et al*., 2017). Honey's degree of fermentation is correlated with its moisture content (Rattanathanalerk *et al*., 2005). Honey's moisture content can be affected by the season of the year, the amount of humidity inside the hive, the nectar conditions, the technique of measurement used in the laboratory, and the handling during storage and extraction. Low moisture content is important to protect honey from the attack of microorganisms and to increase shelf life. On the other hand, if the moisture content of honey is high, it is more likely that the honey will ferment upon storage due to high levels of microorganisms (Fredirick *et al*., 2013).

Honey with high moisture would be easily spoiled by microbial fermentation; thus, it would lose its taste and shorten its shelf life. Such a type of honey could be associated with unripe honey harvesting and poor handling management (Fredirick *et al.*, 2013; Masoud, 2014). In Ethiopia, the moisture content of locally produced honey was reported as 20.5% (Nuru, 1999), 14.4 ± 1.5% (Birhanu, 2015) in Guji Zone, and 18.5% (Kinati *et al.*, 2011) in Jimma Zone; 19.7% (Gebru *et al*., 2015) and 18.8% (Kebede *et al.*, 2012) in Tigray Region; and 18.5% (Addis and Malede, 2014) in North Gonder Zone.

2.1.3. The Sugar Content of Honey

In honey, the sugar content is estimated to be 75–85%. Sugar types are mainly fructose, glucose, maltose, raffinose, and sucrose. The main reducing sugars in honey are glucose and fructose, which account for 65-75% of the total sugars (Farh, 2016; Terrab *et al*., 2002). Glucose determines the speed of honey crystallization, while fructose determines the level of hygroscopic features in honey (Kasenburger, 2006). Knowing the main reducing sugars in honey enables us to detect and differentiate between blossom and honeydew honey (Motari, 2010). This is because honeydew honey contains fewer monosaccharides but more di, tri, and oligosaccharides than floral honey (Diez *et al*., 2004).

Additionally, lowering sugar in honey is useful for predicting the phases of honey maturity in various locations from nectar sources (Da Silva *et al.*, 2016), as well as for figuring out the sugar composition of honey (Motari, 2010). Studies were done in Ethiopia by Tadesse and Gebregziabher (2014) (42.4%) on honey samples taken from local markets, pure honey, beekeepers, and adulterants, and by Birhanu (2015) (60.5%) on honey samples collected from farmers' hives and local honey markets.

Adulterations of honey with other products are one of the potential causes of the reduced reduction in sugar content, as the addition of other items reduces the ratio (%) of reducing sugar from the components in the honey. The impact of harvesting honey in hot weather or taking a sample of honey from a hot place may also contribute to the reduced level of sugar in honey. Similarly, the amount of reduced sugar in honey will be smaller if it's hotter when it's being harvested or processed. The invertase enzyme in honey is also destroyed by excessive exposure to heat because of the enzyme's diminished activity, which leads to a shortage of sugars (Lawal *et al.*, 2009); alternatively, the lower reducing sugar content of honey may be caused by the breakdown of sugars into organic acids (Cavia *et al*., 2007; Kirthiga *et al*., 2019).

2.1.4. Hydroxy Methyl Furfural (HMF) Content of Honey

Only honey is taken into consideration for the hydroxymethyl furfural (HMF) concentration, which is a regulatory limit for a food quality criterion (Vorlova *et al*., 2006). HMF is produced when reducing sugars in honey undergo the Maillard reaction, which occurs at higher temperatures and for longer periods of time. It can also happen when processing and storage procedures are deficient. According to Rattanathanalerk *et al*. (2005) and Mahfuza *et al*. (2018), HMF is an appropriate indication of honey standard because it is one of the key parameters used to assess honey freshness and honey degradation.

According to Visquert *et al*. (2014), natural concentrations of HMF withstand increases of up to 10 mg/kg resulting from processing, extraction, manufacturing, or storage, as well as liquefaction and pasteurization, thus improving control and eliminating crystallization. Nevertheless, if HMF concentrations are greater than 40 mg/kg or 80 mg/kg for Tropic, it is thought that the honey is deteriorating as a result of boiling, long-term storage, or adulteration with inverted sugar (Bogdanov & Martin, 2002).

2.1.5. The Enzyme Content of Honey

Honey enzymes are often complex-structured proteins that are essential to certain chemical processes. Bees could get them from plants or from their secretions, then add them to honey. Even though the enzymes in honey have no nutritional value for humans, they play a crucial role in the production of honey. The most important honey enzyme, diastase, is in charge of breaking down starch into dextrin and sugars. Since honey is heat-sensitive, it is crucial to determine diastase activity in order to determine honey age and freshness, storage period, and overheating (Da Silva *et al*., 2016).

Reduced diastase activity in honey may be caused by adulteration, artificial bee feeding, overheating, and prolonged honey storage. The types of bees (associated with age), the physiological stage of the colony, and the floral source of the honey are also thought to be contributing factors to lower enzyme content (diastase activity) (Da Silva *et al.*, 2016; Bogdanov & Martin, 2002). This is because some plants have low diastase content. The inherent variations in pH between different types of honey, nectar flow, and bee foraging habits are additional factors that influence diastase levels. Honey bees fed commercial glucose were unable to consume enough natural glucose to survive. According to Da Silva *et al*. (2016) and Bogdanov and Martin (2002), this encourages an enzyme deficit, specifically diastase, which is needed to convert starch into glucose and fructose.

Inadequate handling during harvesting, processing, and storage also has an impact on the enzyme content of honey (Muthui, 2012). It might also be connected to when honey is ready to be harvested. Due to the diastase enzyme's potential early fulfilment of its task, the honey component exhibits an inferior enzymatic content (Guler *et al.*, 2007).

2.1.6. The Free Acidity Content of Honey

The amount that exists of organic acids like butyric, acetic, formic, lactic, succinic, pyroglutamic, malic, and citric acids in equilibrium alongside their respective lactones, or internal esters, as well as certain inorganic ions, such as phosphate, is referred to as the free acidity level of honey (Codex Alimentarius, 2001; Nanda *et al.*). FAO/WHO set a limit of 50 mg/kg as an appropriate level of free acidity of honey content, and this is a crucial indicator of overall honey quality for blossom honey (Codex Alimentarius, 2001; European Union, 2002). Several investigations carried out by scientists in East African countries for the free acidity of honey content were within the permissible range.

The significant variance in the level of free acids found in honey may represent the length of time nectar takes to fully transform into honey across different environmental circumstances, the colony's vigor, and particularly the nectar's sugar content (Muli *et al.*, 2007). In fact, the acidity may change as a result of the methods and plans utilized for extraction and storage (Terrab *et al.*, 2002), as well as the kinds of tools and materials employed during processing (Nanda *et al.*, 2003). The honey eventually undergoes fermentation when the acidity is high, and the resulting alcohol is transformed into organic acid. Thus, the honey becomes sour to taste, and hence, it is less acceptable since it is of poor quality (Kugonza & Dorothy, 2008; Da Silva *et al.*, 2016).

2.1.7. Minerals, pH, and Insoluble Dry Materials in Honey

According to Terrab *et al*. (2002), the pH level has an impact on the texture, stability, and lifespan of honey, all of which impact its quality while keeping. The presence and multiplication of microorganisms can be inhibited by a low pH (high acidity), which can also increase shelf life. It also produces a tasty product for ingestion. For use in domestic and international markets, this kind of honey would go well with a variety of culinary products (Kinati *et al*., 2011). Since honey is naturally acidic, this is typically possible. Since it contains several organic acids such as gluconic acid, formic acid, oxalic acid, and lactic acid (Nanda *et al*., 2003), it does contain various organic acids. Of the organic acids, gluconic acid is derived from dextrose via the activity of glucose oxidase, added by bees, or from bacteria's action during ripening (Nanda *et al*., 2003).

The average pH in Ethiopia was found to be 3.8 by Kinati *et al.* (2011), 4.5 ± 0.33 by Birhanu (2016) from the Oromia Region, 3.9 by Gebru *et al.* (2015) from the Tigray Region, and 3.8 by Addis and Malede (2014) from the Amhara Region. The pH levels vary just a little bit. On the other hand, Liepelt *et al*. (2010) found variances in the pH levels of various East African honey samples, which they attributed to the various geographic areas' varying vegetation covers and floral nectars' unique properties. Because of this, understanding the pH of various honey kinds allows us to determine and recommend the origins of the honey types, the flavor of the honey, and their potential for preservation (Terrab *et al*., 2002).

The floral origin, kind, and physiology of each and every plant, variations in soil types, atmospheric conditions, and the chemicals acquired by the bees during foraging can all have an impact on the mineral (ash) composition of honey (Nyangoge, 2012). Differences in the mineral composition of nectar-producing plants may also have an impact. According to its botanical origin, honey can be classified as honeydew honey, mixed honey, or floral honey based on its mineral composition (Nyangoge, 2012).

The number of minerals in honey can also have a significant impact on its colour, nutritional value, aroma, flavor, therapeutic value, and storage properties. While exclusively unadulterated blossom honey has a lower ash level than honeydew honey (White, 1962; Ouchemoukh *et al*., 2010), blending floral honey with honeydew honey may result in a greater amount of mineral content; this is in contrast to Ouchemoukh *et al.*'s 2007 study. Ethiopian honey's ash content has been reported to be 0.23% (Kinati *et al*., 2011), 0.28 0.01% (Addis & Malede, 2014), 0.21% (Gebru *et al*., 2015), and 0.15% (Kebede *et al*., 2012). While Ishraga *et al.* (2017) showed that the ash percentage of

Eastern African honey ranged from 0.35 to 0.78%, 0.36–1.5 (Hana'a, 2007), 0.05-0.3% (Motari, 2010), 0.06% (Orina, 2014), 0.08–0.55% (Gidamis *et al*., 2004), and (Masoud, 2014).

Water-insoluble materials in honey are also considered honey quality parameters, and they are indicators of honey cleanliness. The main reason for the high amount of insoluble honey material is related to the farmers' poor management of honey in harvesting, handling, storing, and marketing. Any honey type used in food must, according to Bogdanov and his colleagues' proposal in Bogdanov *et al.* (2000), adhere to the standard allowable limit of insoluble elements in honey as set forth by legislation at the national and international levels. Therefore, before supplying honey for consumers, honey producers, merchants, and processors should evaluate and confirm the quality of the honey's insoluble material composition. Hence, honey quality with insoluble material content should be assessed and checked by honey producers, traders, and processors before they supply the honey to consumers. Customers can be convinced that the honey is visibly untainted, clean, and of high quality because of this (Gichora, 2003; Kinati *et al*., 2011).

2.1.8. Honey's Botanical Origin

The significance of determining the botanical and geographic origin of honey is demonstrated by the pollen spectrum, which reveals the plants that the bees looked at, and by microscopic inspection of pollen grains. It permits the characterization of honey based on its botanical origin. The first method for honey botanical determination is pollen analysis. The technique, which can be both qualitative and quantitative, relies on the microscopic characterization of pollen. Its drawbacks include the necessity for highly specialized employees, extensive training requirements, and a time commitment. The fact that each pollen analyst's expertise differs is another drawback of the procedure (Bogdanov *et al*., 2004).

Table 1. Summary of Physico-chemical Properties of Ethiopian Honey

S. no.	Parameters	Ethiopians Standards	International Standards
1	Moisture content (%)	17.5 – 21	18 – 23
2	Ash (%)	<0.6	0.25 - 1.0
3	Free acidity (meq/kg)	<40	<50
4	pH	-	3.2 - 4.5
5	HMF (mg/kg)	<40	<80
6	Reducing sugar (%)	>65	60 – 70
7	Apparent sugar (%)	<5	<10
8	Enzyme content ($mScm^{-1}$)	-	0.22- 1.52

Source: (Tesfaye *et al*., 2016)

2.2. Factors Affecting Honey Quality

2.2.1. Beehive Technology as a Factor Affecting the Quality of Honey

According to Croft (2007), beekeeping is the conservation of honey bee colonies, usually in hives, by humans. Beekeepers around the world often use several types of hives to produce honey. All of them are classified as modern and traditional beehives (Morad, 2008). Low-technology hives have been established as a means of finding the compensations of movable frame hives (no need to break combs, standardization, manageability, and efficient honey harvest) without the disadvantage of high-cost production (Bett, 2017).

The container for the hive might, like traditional hives, be constructed from whatever materials are nearby and accessible. Low-technology hives can be kept near home and, if built and transported with attention, bee moved between crops as they blossom consecutively (Global Development Solutions, 2009). For modern hives, the combs can be raised from the hive and then replaced, which allows the beekeeper to study the state of the colony without damaging it. Honeycombs can also be detached from the hive for harvesting without troubling combs containing brood. The colony is then not harmed, and the bees can continue gathering honey to substitute for that which has been harvested, which guarantees good-quality honey can be harvested free of contaminating pollen or brood (Bett, 2017).

2.2.2. Effect of Harvesting and Processing Methods on Honey Quality

In Africa, the meeting of the reasons essential for the implementation of beekeeping has permitted this activity to attain a significant place in agricultural growth programmes since the agricultural revolution is pending today (Africa, H.C., 2010). Different management, harvesting, and processing techniques can influence the final quality of honey (Africa, H.C., 2010).

Beekeepers produce honey by cutting the combs, which are then placed in a container. Processing honey would be managed as soon as possible after removal from the hive. Honey processing is an adhesive process in which time and tolerance are mandatory to attain the best outcomes. Careful protection against contamination by ants and flying insects is required at all stages of processing. It is significant to remember that honey is a food and must therefore be handled hygienically, and all equipment must be perfectly fresh. Honey is also hygroscopic and will absorb moisture, so all honey processing equipment must be perfectly dry. Too much water in honey causes it to ferment (Africa, H.C., 2010).

2.2.3. Impact of Agrochemicals on Honey Quality

In Canada, the USA, UK, and Italy, honeybees were used to monitor environmental pollution since accumulations of certain metals and other substances could be measured in hive products, mostly in pollen but also in honey (Chauzat, M.P., and Faucon, J.P., 2007). Each of these factors together makes the ecological effects of contemporary agriculture extremely harmful (Tilman *et al.*, 2001). These pesticides, fungicides, and herbicides are used on crops, but bees are exposed to them via pollen, nectar, air, water, and soil (Oliver, 2012). This occurs when bees are on the blooms when the insecticide is applied, and the bees instantly perish (Evans & Schwarz, 2011). Some other types of pesticides permit the bees to return home, and then they die. Such kinds are easier to detect than the first ones. There are certain pesticides that have no effect on adult honey bees but cause damage to young, immature bees (Evans & Schwarz, 2011).

Historically, insecticide sprays were responsible for several fatal incidents with bees and also for continuous contamination of honey, which leads to the production of low-quality honey (Oliver, 2012). Insecticides and herbicides are now major problems for beekeepers. Worker bees of all ages are sensitive to the effects of pesticide exposure (Rortais *et al.*, 2005), but beeswax contamination primarily affects the brood because it is in direct contact with the brood cell wall. The key cause of poisonousness is grayanotoxins, also recognized as andromedotoxins. Organic pollutants and polychlorinated biphenyls (PCBs), which originate from motor oil, coolants, and lubricants, are still present in the environment and can contaminate bees and their products (Carrié *et al*., 2012).

2.2.4. Effect of Intensive Farm Practices on the Quality of Honey

The decline and division of valued natural to semi-natural perennial bee habitats, such as agroforestry systems, grasslands, old fields, shrublands, woodlands, and hedgerows, are brought on by the expansion of agriculture. According to Brown and Paxton (2009), this is considered to be the main reason for the fall in honey production and its effects on quality.

Sufficient suitable pollen or nectar producing plants may not be available due to grazing/mowing before flowering (Pieper, (2009). Intensification of agriculture has been associated with significant losses of biodiversity on farmland (Aizen *et al.*, 2009). Industrial agriculture monocultures, and more commonly the deficiency of wildflower diversity within and around croplands, limit the quantity of food that bees have contact with both in space and in time. Bees can go starved as farming becomes additionally intensified (Pieper, 2009).

Since bees depend on a proper nutritional balance for growth and reproduction (Vanbergen & The Insect Pollinators Initiative, 2016), this could have detrimental impacts on them. Several natural environments have been destroyed or fragmented as a result of urbanization and growing agricultural intensification (Vanbergen *et al*. 2016). Farms are losing key natural and semi-natural ecosystems as a result of intensified farming practices.

2.2.5. Honey Markets in Ethiopia

Different studies conducted in different parts of Ethiopia on honey market points participants have identified the actors, activities/ functions, and the enabling environment in the honey markets, and their respective study areas; Out of these studies, Gebru (2015), in a study conducted in Kiltie Awlaelo District, Eastern Tigray, identified the honey market's main actors and their functions, as well as support functions and an enabling environment. The main honey market actors identified included the following. Producers (farmer beekeepers and commercial beekeepers), honey collectors, retailers, whole-sellers, beekeeper cooperatives, unions, companies, exporters, and consumers, the main honey market channel functions identified were input supply, production, processing, marketing, and consumption. The honey market supporters identified in the study area were ATVET, the FTC, research organizations, and government and non-government organizations. The enabling environments identified by the author were infrastructure, laws, policies (livestock and agricultural development), investment policies, standards, social and cultural norms, apiculture organizations, and agriculture.

2.2.6. Actors in the Honey Markets

The main actors in the honey markets are explained based on their main roles in the process. Market supporters (ATVET, FTC, research organizations, GOV, and NG. organizations), honey producer (Farmer beekeepers and commercial beekeepers), processors (traditional and semi-processors), market actors (district, regional & national retailers, middle men & honey collectors, whole seller, beekeepers primary cooperative, unions, companies, supermarkets, minimarkets, and roadsides.), consumer (national and international consumers) (Yetimwork, 2015).

2.3. Types of Food and Honey Adulteration

2.3.1. Food Adulteration

Adulterants in food can be categorized into the following categories (Ribeiro *et al.*, 2014):

A). The intentional introduction of inferior ingredients: with properties similar to the products, they are added to is called adulteration. They are therefore difficult to find. The adulterant can be biological or physical in nature. Adding water to liquid milk, sugar syrup to honey, foreign matter to ground spices, removing or substituting milk solids from the natural product, etc. are some instances of intentional adulteration.

b). Accidental adulteration: is the introduction of undesirable ingredients in the manufacture of food due to ignorance, carelessness or poor sanitary conditions. This can take a distinct form, such as B. contamination of food with fungi and bacteria, food decomposition caused by rodents, access to dust and stones, harmful deposits from packaging material, etc., or total adulteration.

c). The intentional or accidental addition of various metals and metal compounds to food is referred to as metallic contamination. Lead, arsenic, mercury and cadmium are considered to be the most dangerous of all pollutants because their use can be extremely chronic.

d). The deterioration of food caused by the introduction of numerous bacteria: from a variety of sources is known as microbial contamination. Microorganisms can contaminate food at any stage of processing, such as during harvest, storage, processing, distribution, handling, or preparation.

2.3.2. Honey Adulteration

Honey adulteration is mainly done in two major ways (Zábrodská and Vorlová, 2014):

a). Indirect adulteration of honey: is performed by feeding synthetic sugars to honeybees at the point when broods are beginning to become naturally available. It might be quite challenging to identify such indirect adulteration.

b). Direct Adulteration of Honey: straight adulteration refers to the straight addition of foreign compounds to honey.

2.3.3. The Adulterants in Honey

According to Ribeiro *et al.* (2014), honey can become adulterated by the direct addition of sucrose syrups made from sugar beetroot, high-fructose corn syrup (HFCS), maltose syrup, industrial sugar (glucose and fructose), syrups obtained from starch through heat, enzyme, or acid treatment, or by feeding bee colonies large amounts of these syrups during the main nectar period. Ethiopian honey is frequently adulterated with cane sugar, according to Woldemariam *et al*. (2014). The severe extra feeding of bee colonies during the major nectar flow season has frequently resulted in indirect adulteration in recent years, which is unfair to both consumers and producers of pure honey (Guler, *et al*., 2014).

Vegetable syrups made by heating vegetable juices or plant juices can also be adulterants. Three different plant syrups have been identified in Spain: palm syrup, sometimes known as honey, must syrup and sugar cane syrup (Guler, *et al*., 2014). The intentional addition of syrup in certain amounts after production to increase the sweetness of the honey, or overfeeding the bees during the primary nectar period to get more honey from the hives are two possible causes of the presence of sugar as an adulterant in the honey. Inexpensive sugar or industrial syrups are often used for this purpose. Popular adulterants are sugar syrups such as corn syrup (CS) and high fructose corn syrup (HFCS), glucose syrup (GS), sucrose syrup, or invert syrup (IS) made from sugar cane or sugar beets (Anklam, 1998; Guler, *et al*., 2014). According to Ajlouni

(2011), sugar-contaminated honey can actually show changes in certain chemical and/or biochemical parameters, including enzymatic activity, electrical conductivity, and concentrations of specific components such as HMF, glucose, fructose, sucrose, maltose, and isomaltose. Bananas, molasses, sugar, shebeb, inverted syrup fed to bees, and low-quality honey mixed with expensive honey are the main adulterants to honey (Teferi., 2020).

Table 2. Commonly Used Adulterant Materials in Ethiopia

Place/area	Name of adulterant materials	Reference
Arsi	sugar, ripened banana, wheat flour, potato, maize flour, pollen, empty combs, melted candy, molasses, and hot water	Ambaw & Teklehaimanot (2018)
Eastern Tigray Region	sugar syrup, maize and/or wheat flour syrup, banana and sweet potato	Gebremariam& Brhane (2014)
Oromia region	sugar, candy, molasses, banana, orange, and cumber	Gemeda & Negera (2017)
Bahirdar	sugar or invert	Ambaw & Teklehaimanot (2018)
Gedo (SNNP)	sugar syrup, maize and/or wheat flour, banana and sweet potato	Sebeho (2015)
Adigrat and surrounding area	Sugar, water, banana	Gebremariam & Brhane (2014)

2.3.4. Reasons for Adulteration

As several authors have shown, food adulteration is encouraged by higher cash receipts and/or increased shelf life. It is difficult to find foods such as flour, legumes, oil, fruits, vegetables, milk, sweets, spices, tea, coffee, honey, pastries, chocolate, fruit juice, etc. which are free from one or the other adulterant. The main reason people are interested in counterfeiting is to increase their cash income by increasing its mass. However, increasing their turnover margins-initiated adulteration done by some selfish producers, processors, and retailers, the main cause for adulteration is dishonesty and lack of accidental quality assessment on products assumed (Asrat *et al*., 2012).

2.3.5. Impacts of Adulteration

The problems of adulteration make the food items used in our daily life unsafe and unhygienic for use due to poor handling (Asrat *et al*., 2012). In the past few decades, honey adulteration has become one of the serious problems and consumption of adulterated honey causes serious diseases like cancer, diarrhea, asthma, ulcers. In general, adulteration of food items has a very serious impact on producers/farmers, processors or manufacturers/enterprises, consumers, and the government. More lately, pollutant practice for example, in the People's Republic of China (Chinese milk scandal case with melamine) in which some children were killed and thousands were injured has inspired much public attention (Asrat *et al*., 2012). So, it is indicative that food adulteration is fast growing worldwide as an industry and the global market of adulteration and fake goods is more than several hundred billion dollars which constitute more than 10 percent of total trade (Ayza and Belete, 2015).

2.3.6. Honey Adulteration Detection Methods

Straightforward adulteration is typically detected using conventional studies of the physical attributes and chemical makeup of honey. Although these methods are frequently employed in the honey business, these analytical techniques take a fair amount of time and necessitate extensive experiment preparation and sophisticated analytical tools (Cozzolino *et al*., 2011). Adulterants in honey can also be identified using a variety of contemporary methods. Some techniques are specifically suited to certain adulterants, such as when the adulterant shares a chemical structure with honey. One of the earliest techniques for identifying adulterants in honey is the C-isotope strategy, which is based on differentiating carbon isotope ratios (13C and 12C) between plant groups (Croft, 2007).

Thin-layer chromatography (TLC), gas chromatography (GC), and high-performance liquid chromatography (HPLC) are the analysis techniques that are often used. Although they are rapid, inexpensive, non-destructive, and user-friendly, cutting-edge methods like near-infrared (NIR) spectroscopy and Raman spectroscopy are frequently used in qualitative as well as quantitative evaluations of food products. It might be difficult to spot adulteration when it is carried out with low-cost sweeteners like corn syrup, corn syrup with a high fructose content, or inverted syrup. However, scientific tests can't tell when the honey has been manipulated by adding concentrated sugars. An effective method for showing the possible existence of adulterants in honey products has always been very difficult to come up with due to the difficulty of tracing heavy metals in honey and the challenge of detecting sugar-contaminated honey (Alemu and Dechasa, 2019).

3. MATERIAL AND METHODS

3.1. The Study Area's Description

The Adama District is situated between 8.14° and 8.44° north latitude and 39.04° and 39.25° east longitude. It shares a border with Lume, Dodota, Boset, the Amhara region, and Bora in the East Shewa zone. This woreda is located between 1500 and 2300 meter above sea level. Sodere, Gergedi, and Boku Femoral hot springs are notable neighboring attractions. Just 30% of the land in this woreda is found to be arable or cultivable; 6.5% is used for pasture; 5.2% is set aside as forest; and the remaining 58.3% is thought to be swampy, degraded, or otherwise unsuitable. According to Kirko *et al.* (2020), fruits, vegetables, and sugarcane are important cash crops.

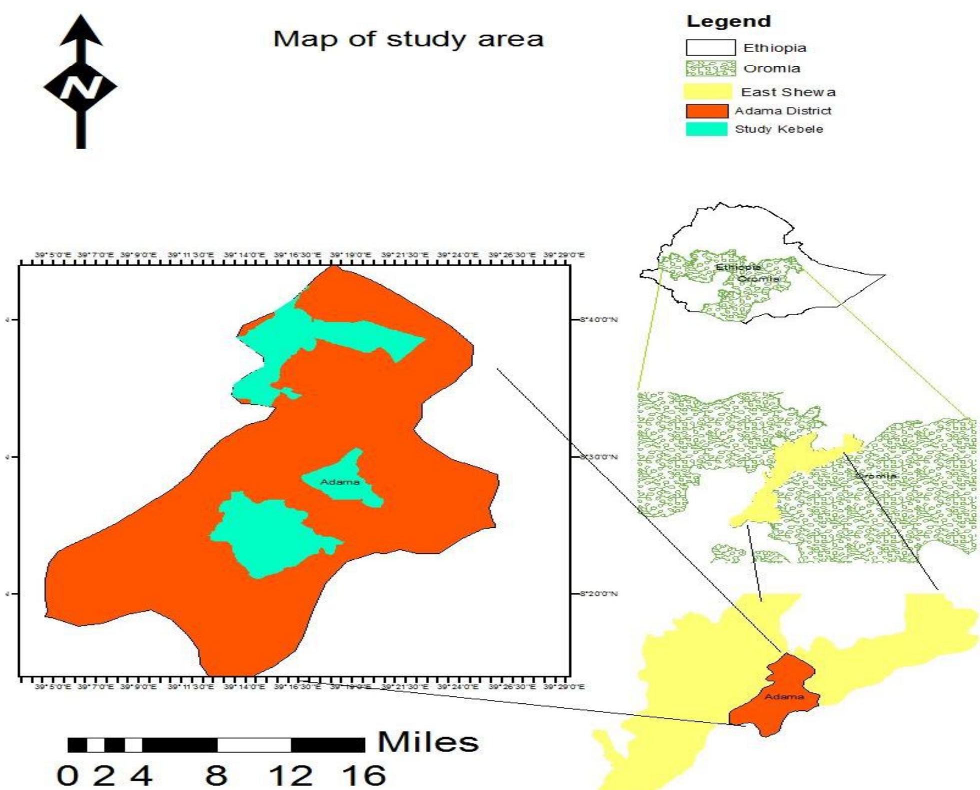


Figure 1. Map of the Study Area (Adama Town and the study kebeles in Adama District)

3.2. Study Area Selection and Sampling Techniques

The survey was conducted in Adama City and Adama District of Oromia Regional State. The study area was selected based on the availability of beekeepers, honey markets (supermarkets, minimarkets, retailers, and on-street honey sellers), the presence of honey processors, exporters, and beekeeping cooperatives, as well as the existence of local farmer beekeepers in the areas. The sample size for the survey was determined based on the total beekeeper households based above mentioned criterions with the assistance of the head of district agriculture and livestock resource, a bee expert, and kebeles development agents, purposeful sampling was used to select kebeles and stakeholders or households based on their experience and long-term interest in honey production and assessment factors affecting honey quality and accessibility of the areas to transportation services. Dick *et al*. (2004) claim that combining techniques like key informant interviews, planned interview schedules, and observation was the only way to comprehend the complexity of the survey study fully.

A questionnaire consisting of structured items was designed to collect primary data at the village level. Primary data was collected through structured and semi-structured questionnaires administered to various respondents. The designed questionnaire and key informants' interview were elaborated and directed to the honey quality, factors affecting the honey quality, potential bee flora or bee forages, and availability to beekeepers, mini markets, supermarkets, retailers, on-street sellers, and knowledgeable elders.

3.2.1. Identifying Relevant Informants

Local community leaders and respected elders played a critical role in finding and choosing competent vital informants in order to gain crucial insights into the complicated field of beekeeping and honey production. These informants were carefully selected, with consideration given to a number of aspects, including their reputation, gender, and beekeeping experience as well as their acquaintance with honeybee flora. The selected informants included a wide range of people with noteworthy knowledge and expertise in the field, including development agents, agricultural and rural development officials, bee experts, seasoned beekeepers, street vendors, supermarket and minimarket retailers, respected elders, and others.

3.2.2. Preparation and Administration of Questionnaire

A checklist of questions was prepared in English and translated into the study area's local language for interviews and discussions. The questionnaire incorporated dichotomous (yes or no questions), multiple choice, and open-ended questions.

3.3. Honey Sample Collection

The samples were collected from market points (available for customers purchase in rural beekeepers, urban beekeepers, supermarkets, mini markets, retailers, and street sellers) located in Adama Town and Adama District. From each market point, four samples were collected. A total of 23 samples were collected. The honey samples were stored in glass jars and kept in the refrigerator at 4^{o}c, until analysis. The sample analysis was conducted at the Holeta Bee Research Center’s laboratory. Honey physicochemical parameters of the sample results were compared with the standard set by the Ethiopian Standard Agency.

Table 3. Sample Collection Areas with their Honey Sample Sources.

Samples codes	**Areas where samples collected**	**Special name**	**Samples source**
1	Adama District	Kecema	Beekeeper
2	Adama District	Mukiye	Beekeeper
3	Adama District	Bubisa Kusaye	Beekeeper
4	Adama District	Wonji	Beekeeper
5	Adama Town	Peacock	Beekeeper
6	Adama Town	Franco	Beekeeper
7	Adama Town	Mebrat-hayil	Beekeeper
8	Adama Town	Kella	Beekeeper

9	Adama Town	Bole	Street
10	Adama Town	Kella	Street
11	Adama Town	Kiden-mihiret	Street
12	Adama Town	Arada	Retailer
13	Adama Town	Mebrat-Hayil	Retailer
14	Adama Town	Posta	Retailer
15	Adama Town	Kella	Retailer
16	Adama Town	Franco	Mini-market
17	Adama Town	Posta	Mini-market
18	Adama Town	Kella	Mini-market
19	Adama Town	Menaharia	Mini-market
20	Adama Town	Franco	Super-market
21	Adama Town	Bole	Super-market
22	Adama Town	Warka	Super-market
23	Adama Town	Mebrat-Hayil	Super-market

3.4. Detection of Adulterations Using Quick Tests

A preliminary assessment was conducted to know the quality of collected honey samples from different market points compared with the properties of pure honey when conducting a quick test according to the method adopted from Teferi (2020).

3.4.1. Flame Test

The honey had been ignited using a match-stick or candle flame. Pure honey produced (gives off) a smokeless flame and lights easily without any difficulty. After being lighted, the flame test, a smoky flame and/or a cracking sound indicated the existence of adulterants.

3.4.2. Heating Effect

The heating procedure was used to see the heating effect on pure and adulterated honey samples. Upon gentle heating of samples to dissolve crystallized substances, pure honey melts into a clear transparent, viscous solution, with wax materials floating on top. A mixture of honey adulterants melts to form a dispersed and non-transparent liquid.

3.4.3. Water Test

The addition of one tablespoon of honey to a glass of water was observed, and the added honey was either dissolved or sank to the bottom of the glass. While tainted (adulterated) honey started dissolving in water as soon as it was added, pure honey settled at the bottom of the glass (Teferi, 2020).

3.4.4. Thumb Test

A drop of honey was placed on the thumb. The honey is not pure if it immediately spread or spilled. It is pure if it stays whole.

3.4.5. Coca-Cola Test

A baker received the honey samples. Coca-cola was then added to the sample in a volume of 4 ml. Whereas pure honey does not produce froth or bubbling sounds, honey mixed with foreign substances does (Teferi, 2020).

3.5. Laboratory Analysis of Honey Quality

In accordance with the international honey commission methodologies (IHC, 2009), the following physicochemical characteristics of honey from various market points were investigated and compared with the Ethiopian Standard Authority honey physicochemical parameters as comparison.

3.5.1. Identification of Botanical Origin

The botanical origin of honey was determined using the pollen analysis method developed by the International Honey Commission for Bee Botany (Louveaux *et al*., 1985). 10 grams of honey were dissolved in 20 ccs of 40°C hot water. The sample, according to Erdman (1960), was centrifuged for 10 minutes at 2500 rpm, the supernatant solution was decanted, and the sediments were gathered into a conical tube for analysis. After being cleansed with distilled water to remove the pollen, the residual particles were centrifuged at 2500 rpm for 5 minutes to separate the pollen from the honey. Under a light microscope, the pollen included in the samples was identified on two slides made from each sample of honey. After deleting the pollen-producing plant species that emerged from the pollen analysis of the honey, the percentage occurrence of pollen taxa in all samples was computed (Louveaux *et al*., 1985).

3.5.2. Honey Color Determination

The color of samples of honey was determined using the Pfund classifier. In a 10-mm light path cuvette, around half of the homogenous honey sample was placed. The cuvette was placed inside a color 27 photometer Pfund honey color grader (No. 0061, made in the USA), in accordance with the Codex Alimentarius Commission Standards (2001), and the color grades were presented in millimeter (mm) Pfund grades in relation to an analytical grade glycerol standard. Readings for each sample were taken using the authorized color standards published by the United States Department of Agriculture (1985).

3.5.4. The Moisture Content of Honey

Applying an Abbe refractometer that was calibrated often with distilled water and thermostated at 20^{o}C, the moisture level in honey samples were assessed. Samples of honey were mixed together before being submerged in water to dissolve all of the sugar crystals. Following homogenization, honey was applied to the prism's surface of the refractometer, and after two minutes, the refractive index for moisture content was calculated. Using a standard table created for this reason, the refractive index value of the honey sample was calculated (Bogdanov, 2009).

3.5.5. The Free Acidity and pH of Honey

Ten grams of honey were taken from every sample and mixed with 75 ml of distilled water in a 250 ml beaker utilizing a magnetic stirrer. The pH of honey was measured while an electrode using a pH meter was submerged into the solution. The solution is then titrated using 0.1 M sodium hydroxide (NaOH) solution to pH 8.30 to determine the free acidity. Using a 10 ml burette, the reading was taken precisely to the nearest 0.2 ml. The measure of free acidity was millimeter equivalents/kg honey. The technique described by Bogdanov (2009) was used to represent the outcome to one decimal place.

Acidity is equal to 10 V, where V is the amount of 0.1N NaOH in 10 g of honey.

3.5.6. Measuring the Overall Ash Content

As a result of burning samples of honey at 600°C in a muffle furnace to a constant weight, the ash concentration was identified (Bogdanov, 2009). The ash in the dish was initially heated to an ashing temperature in an electrical muffle furnace, followed by cooling to room temperature in a desiccator, and weighed 0.001 g (M_2). A platinum dish was used to weigh 5 g (M_o) for every honey sample to the closest 0.001 g, and a pair of drops of olive oil were included to avoid foaming. Applying electrical apparatus, water was extracted and began to ash without loss at a heat of 550–600 ^{o}C.

The ash from the dish was measured after cooling using the desiccators. Until a consistent weight is attained, the ashing process continues (M_1). Finally, the percentage of the mass of ash in g/100 g of honey was determined using the following formula:

$Ash(\%) = \frac{M1-M2}{M0}$ where by,

M_1 as a = ash weight + dish
M_2 is the dish's weight
M_o equals the weight of the honey used

3.5.7. The Sugar Determinations

High performance liquid chromatography (HPLC) was used to determine the sugar profiles of honey. 5g of honey were dissolved in 40ml of distilled water. The resulting solution for all honey samples was passed through a syringe filter (0.45µm) prior to chromatographic analysis using 25ml of acetonitrile pumped into a 100ml volumetric flask. The honey solution was then transferred to the flask and made up to the mark with distilled water. The HPLC isolation system comprised an analytical stainless-steel column with a diameter of 4.6 mm and a length of 250mm containing amine-modified silica gel with a particle size of 5-7µm and a mobile phase of 1.3ml/min, The flow rate, an injection volume of 10 µl of the sample, and an acetonitrile: water ratio of 80:20, v/v. The sugars were identified using a refractive index sensor that was thermostated at 30°C and operated in a column oven at 30°C. By contrasting the retention durations of honey sugars and regular sugar, honey sugars were found (Bogdanov, 2009). A combination of 2g and 1.5g of each of the following sugars: fructose, glucose, sucrose, and maltose were diluted five times in succession. In order to generate a calibration curve, the International Honey Commission (Bogdanov, 2009) states that 2g, 1.5g, 1g, 0.5g, and 0.15g were measured and diluted in 40ml distilled water and 25ml acetonitrile.

3.5.8. Hydroxyl Methyl Furfural (HMF)

HMF was calculated using 6800 UV-Vis spectrophotometers. A 5-gram sample of honey was then weighed into a tiny beaker, 25mL of distilled water was added, and the mixture was then transferred to a 50mL volumetric flask (Bogdanov, 2009). In a separate container, combine 0.5mL of Carrezz solution II (30g Zn Acetate/100mL distilled water) with 0.5mL of Carrezz solution I (15g K4Fe (CN)6.3H2O/100mL distilled water). The solution and the honey solution were combined. Filtrate (10ml) was removed from the solutions filtered through a filter paper. Two test tubes were each filled with 5ml of filtrate, followed by 5ml of distilled water in the first test tube (the sample solution) and 5 ml of sodium bisulfite solution (0.20% of 0.20 g NaHSO3/100 ml of distilled water) in the second testing tube (the reference solution). The ingredients of both test tubes were thoroughly combined using a vortex mixer, and the absorption has been determined spectrophotometrically by comparing the absorbance of reference (the same honey solution treated with sodium bisulfite, 0.2%) at 336 nm to the absorbance determined at

284 nm for HMF in the honey sample solution according to the International Honey Commission (Bogdanov, 2009).

HMF/100 g of honey = (A284 - A336) 14.97 5/g of the sample. Where:

14.97 is a constant,

A284 is the absorbance at 284,

A336 is the absorbance at 336,

g = the mass of the sample of honey, and,

5 = the theoretical nominal sample weight.

3.6. Statistical Analysis

The statistical analyses were performed with the SPPSS-statistical software tool, version 20. For the survey data, analysis was done using descriptive statistics and frequency procedures. In order to analyze the variance for honey quality, a completely randomized design (CRD) was used, and the results were given as means and standard deviations. For honey botanical origin analysis, clustering of the honey source's botanical origin was determined from honey pollen analysis using principal component analysis (PCA), and significant differences were assessed for $P < 0.05$.

Model $Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$

where: Y_{ij} = quality of honey

μ = total mean

α_i = effect of market channels

ε_{ij} = random error

4. RESULTS

4.1. Respondents' Characteristics

It was wise to have a thorough understanding of the beekeepers' backgrounds in order to put the study into context. It was necessary to know the respondents' characteristics in terms of the institutional, socioeconomic, and demographic traits of beekeepers in the study area.

4.1.1. Profiles of Respondents by Demographics

According to the findings of the survey, most of the respondents, or (89.5%) of them, were men, and the remaining (10.5%) were women (Table 4). The sample household's average age was 48.94 years, with the oldest members of the sample household averaging 72 years old and the youngest members of the sample household being 32 years old. The result shows that the beekeepers in the study areas getting older and more beekeeping products are in the hands of older farmers. The increase in low quality honey in the area due to the reason that most youngsters are not in beekeeping activities and most young farmers have no enough back yards for beekeeping and are living around the town in most cases.

4.1.2. The Respondent's Level of Education

From the survey result it is clear that educational background of the household head is one of the significant factors that affect the honey quality and issue of honey adulteration. From the survey result, the low background of education mainly the youngsters make honey adulteration. So, enhancing probability of getting education through different means like informal and formal education will enhance the adoption of modern beekeeping technique and moreover, teaching the next generation who are the potential inputs for our modern agriculture will enhance the use of modern technologies that results in ample production of bee products like honey and tackle poor honey quality and avoid adulteration.

Table 4. Demographic Characteristics of the Respondents

	Minimum	Maximum	Mean
Age	32	72	49
Education level	**Frequency**	**Percent**	
Illiterate	4	3.8	
1-8	75	71.4	
9-12	19	18.1	
Higher education	7	6.7	
Sex			
Male	94	89.5	
Female	11	10.5	
Percent of respondents			
Adama district	90	85.7	
Adama town	15	14.3	
Kebeles Respondents			
Kechama	15	14.5	
Mukiye	15	14.5	
Bubisa kusaye	15	14.5	
Jeldiya mukiye	15	14.5	
Wonji	15	14.5	
Adulala	15	14.5	
01	5	4.3	
10	5	4.3	
12	5	4.3	

Source: Own survey data

4.2. Assessment of Honey Quality and Honey Adulteration

The major causes of poor honey quality, as shown in Table 4, are agro-chemicals (68.6%), adulteration (30.8%), and lack of bee product or low production of honey (6.7%). The types of honey produced in the study area also contributed to the low quality of the honey, which was crude honey (86.7%). The major causes of low-quality honey were adulteration (94.3%) and the stage of honey during harvesting (5.7%). The other contributors to low honey quality were the place of honey storage and the honey container, which were the respondent results, shows any dry place (whether clean or not) in a plastic container (whether food standard or not) at 84.8%. The major actors in adulteration are street honey sellers (90.5%), honey processors who pack and distribute honey for different markets (6.7%), and collectors (2.9%). The survey results also showed that sugar, caramel, molasses, and ripe bananas were among the major adulterants used in the study area.

The survey result shows that honey quality deteriorations start at harvesting time, like the use of smoking intensity, stage of honey during harvesting, materials used during harvesting, and storage conditions (materials and place of storage). In addition to these chemicals, low production of honey compared to demand and adulteration were the major causes of low-quality honey; these adulterations were common at regional and national market levels, and on-street honey sellers were the major actors in adulterating honey. Finally, the results show or suggest controlling methods of adulteration were serious law support for punishment development (94.3%), ample honey production (1.9%), and consumers having to buy directly from beekeepers, reducing middle actors.

Table 5. Major Problems Related to Honey Quality and Adulteration issues of Respondents

Variables	**frequency**	**Percentage**
Cause of low quality and adulterated honey		
Lack of bee products	7	6.7
Agro-chemicals	72	68.6

Act of adulteration	26	30.8
Types of honey produced		
Crude honey	91	86.7
Extracted honey	14	13.3
What are major causes of low-quality honey?		
Stage of honey during harvesting	6	5.7
Adulteration	99	94.3
Harvesting materials		
Knife, plastic container, stainless steel plate which were used for other purpose also	91	86.7
Knife, plastic container, stainless steel plate that only uses for harvesting purposes	14	13.3
Stage of honey during harvesting		
½ comb sealed	10	9.5
¾ comb sealed	95	90.5
What types of hives you own?		
Modern hives	8	7.6
Traditional and transitional	18	17.1
All hive types	79	75.2
Who do you think made honey adulterations?		
Collectors	3	2.9
On-street honey sellers	95	90.5
Processors	7	6.7
What materials do they use for adulteration?		
Sugar	85	81
Molasses	4	3.8
Caramella	15	14.3
Ripen banana	1	1
In which season honey adulteration is common?		

During honey flow season	95	90.5
During dry season/ non honey flow season	10	4.8
Where do you suspect adulteration?		
Honey processors who pack and distribute honey for different markets	6	5.7
On-street honey sellers	99	94.3
What is your recommendation to control adulteration?		
Serious law support punishment development	99	94.3
Increase/ ample honey production	2	1.9
Consumer should have to buy directly from beekeepers	4	3.9

4.3. Quick Honey Quality Test for Honey Obtained from Different Markets

Using simple local techniques, a preliminary assessment of honey quality was made at various market points to determine if the honey was adulterated or pure. A quick quality test of honey samples collected from six market locations is presented in Table 5. All (100%) of the honey samples collected on the street were fully or partially dissolved in the water, which was observed by retailers (66.67%), mini-markets (50%), and urban beekeepers (33.33%). (100%) Honey samples collected on the street were dissolved in the water, followed by retailers (66.67% fully and partially dissolved), mini-markets (50%), and urban beekeepers (33.33%). However, 75% of honey collected from rural beekeepers and 66.67% of honey collected from urban beekeepers was undissolved (settled) in the water. This result clarifies that honey collected from the street market contains foreign substances other than honey, while honey from rural beekeepers is pure honey. The partial dissolution of honey from supermarkets and convenience stores indicates that the honeys from these sources contain additives that cannot dissolve in water (Teferi, 2020). In particular, street vendors of honey have become accustomed to selling a product they have made in the name of honey, although the foreign materials used in the process are not precisely known. All of the honey samples collected at various market locations produced a smoky flame with a cracking sound when flame tested. However, the level varied between market points. Honey obtained from the street and convenience store had a maximum (100%) smoky flame with a cracking sound; The lowest percentage was obtained from honey samples collected from beekeepers. The presence of a smoky flame and/or cracking sound during the flame test indicates that honey samples contain some impurities (Teferi, 2020). The thumb methods of quality assessment showed that honey samples collected from rural beekeepers were 100% intact up to the surface of the thumb, while honey samples collected from the road were 100% spread. This result suggests that there is a suspicion that honey sold on the street contains a higher proportion of solid particles and a very low viscosity. In the Coca-Cola test, all samples produced a bubbling sound. Although all samples produced bubbling sounds during the test, the highest percentage (100%) of bubbling sounds was produced in convenience store and street samples, while the lowest percentage was recorded in honey samples from rural and urban beekeeper markets. According to this result, as long as the pure honey sometimes contains solid particles such as wax, pollen, and dead bees, which produce bubbling noise and foam, the bubbling noise is completely suppressed. This level of fermentation in supermarkets could be due to handling issues in prolonged storage conditions (humidity, storage temperature and equipment) (Teferi, 2020). The fermentation of honey leads to spoilage and also reduces its shelf life (Perez-Perez *et al*., 2007). Overall, the findings of most rapid testing methods indicate that the honey sold at most market points has quality issues that vary by market point. The severity of the problem is relatively pronounced at the street market level and less pronounced at the producer level, suggesting that honey quality is degrading at different rates. Therefore, there should be a responsibility not to sell pure honey and the government needs honey purity laws to protect honey consumers and other stakeholders involved in the value chain.

Table 6. Quick Test Results of Honey of Different Market Points

Test type	Supermarket	Minimarket	Street	Retailer	Rural beekeeper	Urban beekeeper
Dissolving method	58.33% (7/12) settled 16.67% (2/12) dissolved 25% (3/12) of 50% dissolved	50% (6/12) settled 50% (6/12) dissolved	100% (9 /9) dissolved	33.3% (4/12) settled 33.3% (4/12) dissolved 33.3% (4/12) of 50 % of the sample dissolved	75% (9/12) settled 25% (3/12) dissolved	66.67% (8 /12) settled 33.33 % (4/12) dissolved
Thumb test	58.33% (7/ 12) intact 25% (3/12) spreads or spills 16.67% (2/12) intact but contain a foreign substance	50% (/6/12) Intact 50% (6/12) spreads	100% (9/9) Spreads	66.67% (8/12) intact 33.33% (4/12) spreads or spills	100% (12/12) intact	83.33% (9/ 12) Intact 16.67% (2/12) spreads or spills
Flame test	75% (9 /12) smokes 25% (3/12) give light	100% (12/12) smokes	100% (9 /9) smokes	66.67% (8/12) smokes 33.33% (4/12) give light	58.33% (7/12) smoke 41.67% (5/12) give light	66.67% (8/12) smokes 33.33% (4/12) give light
Coca-cola test	50% (6/12) no produce bubbling and sound 50% (6/12) produce bubbling and foam	100% (12/12) produce bubbling and foam	100% (9/9) bubbly and foam	66.67% (8/12) production bubbly and frothy 33.33% (4/12) few bubblies and frothy	25% (3/12) produce a bubble and foam 75% (9/12) no produce a bubble and foam	25% (3/12) to produce bubbly and foam 75% (9/12) no bubbly and foam
Fermentation	75% (9/12) not fermented 25% (3/12) fermented	83.33% (10/12) not fermented 16.67% (2/12) fermented	100% (9/9) No fermented	100% (12/12) no fermented	100% (12/12) no fermented	100% (12/12) no fermented

4.4. Physico-chemical Properties of Honey in Different Markets

4.4.1. Honey Color Analysis

The results of the analysis of honey samples collected from different markets are shown in Figure 2. Honey Color Analysis (mm pfund) In this study, 45.83% of the honey samples collected from different markets were water white and 41.67% were extra white, but the remaining samples were 8.33% and 4.17% very pale yellow. Aloisis, (2010) reported a similar result because the color of honey varied according to the fruit area of Argentina.

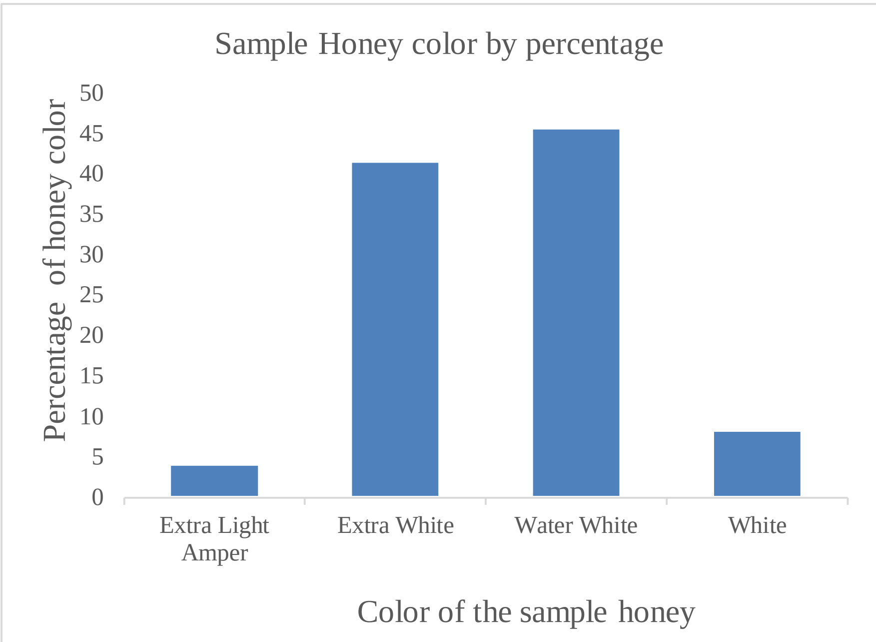


Figure 2. Honey samples from different market points and their colors proportions

4.4.2. The Moisture Content of Honey

Moisture content is the key criterion that determines the ability of honey to remain fresh and free of fermentation (Silva *et al*., 2009; Bogdanov *et al*., 1999). Moisture content, including other main physicochemical characteristics such as ash content, HMF, PH value, and free acidity of the honey from different market points, is presented in Table 6. A statistically significant difference ($P< 0.05$) was observed among rural honey market points. The highest moisture content was recorded for honey collected from a street

(24.62±0.67%) and the lowest was recorded from rural beekeepers (18.90±0.58%). The moisture content of honey collected from rural and urban beekeepers was not significantly different.

Table 7. The Mean ± SE Values for the Physicochemical Properties of Honey from Different Market Points

Treatment	Moisture content (gm/100gm)	Ash content (gm/100gm)	HMF (mg/kg)	pH value	Free acidity (meq/kg)
T1	24.62 ± 0.67^{c}	0.05 ± 0.02^{a}	27.18 ± 3.20^{a}	3.24 ± 0.08^{ab}	23.47 ± 2.33^{ab}
T2	$22.60 \pm 0.58b^{c}$	0.06 ± 0.02^{ab}	29.09 ± 2.78^{ab}	3.13 ± 0.07^{a}	33.88 ± 2.02^{c}
T3	23.23 ± 0.58^{bc}	0.07 ± 0.02^{ab}	35.14 ± 2.78^{ab}	3.41 ± 0.07^{abc}	27.84 ± 2.02^{abc}
T4	21.05 ± 0.58^{ab}	0.06 ± 0.02^{ab}	39.39 ± 2.78^{b}	3.50 ± 0.07^{bc}	28.73 ± 2.02^{bc}
T5	18.90 ± 0.58^{a}	0.13 ± 0.02^{b}	31.92 ± 2.78^{ab}	3.57 ± 0.07^{c}	20.03 ± 202^{a}
T6	19.10 ± 0.58^{a}	0.21 ± 0.02^{c}	28.31 ± 2.78^{ab}	3.67 ± 0.07^{c}	25.88 ± 2.02^{abc}
P-value	<0.000	<0.000	<0.000	<0.028	<0.000

Means with a different superscript letter within a column are significantly different ($P<0.05$), HMF= Hydroxymethyl furfural aldehyde T1= On- street honey market samples, T2= Retailer market honey samples, T3= Minimarket honey samples, T4= Supermarket honey samples, T5= Urban beekeepers honey samples, T6= Rular beekeepers honey samples.

4.4.3. The Ash Content of Honey

The average ash content of honey at different market points is summarized in Table 6. The ash content of samples collected from urban and rural beekeepers was significantly different ($P<0.05$) from samples collected from streets, convenience stores, retailers, and supermarkets. The highest mean ash content was found for honey from rural beekeepers (0.21±0.02 g/100 g), while the lowest ash content was found for honey sold on the street (0.05±0.02 g/100 g).

4.4.4. The pH Value of Honey

Table 6 shows the pH values for different market honey samples. The highest pH was 3.67 and the lowest was 3.13. The pH values in the analyzed honeys differ significantly ($P<0.05$) among market points, with the most acidic values present in honeys from retailers, streets and minimarkets.

4.4.5. The Hydroxymethyl Furfural Aldehyde (HMF) in Honey

The highest HMF mean value was recorded in honey samples collected from supermarkets (39.39±2.78 mg/kg) and minimarkets (35.14±2.78 mg/kg), while the lowest record was for honey samples collected on the street (27.18±3.20 mg/kg).

4.4.6. The Free Acidity of Honey Samples

In this study, there was a significant difference in free acidity ($P<0.05$) between honey samples from different market points, with the higher mean (33.88±2.02 $meq.kg^{-1}$) found in samples from retailers and the lowest in urban beekeepers Samples (20.03±2.02 $meq.kg^{-1}$) (Table 6). However, a statistically significant difference ($P>0.05$) was not observed in the free acidity was not observed among samples from the minimarkets, supermarkets, and rural beekeepers.

4.5. Sugar Composition Honey from Different Honey Markets

4.5.1. Fructose in Honey Samples

The mean fructose content in honey from urban and rural beekeepers (39.01±0.97%) differed significantly ($P<0.05$) from that of convenience store honey (34.48±0.97%) and supermarket samples (37.57±0.97) (Table 7). However, no statistically significant difference ($P > 0.05$) in fructose content was observed between samples collected from the street, retail and supermarkets. The low fructose content indicates heated or adulterated honey. The increase in fructose levels is probably due to the nectar richness of fructose (Louveaux, 1985). The results of most study samples are consistent with previous reports on fructose levels (White *et al.*, 1962; Kucuk *et al.*, 2007; Amir *et al.*, 2010; Amir, 2013; Belay *et al.*, 2017).

Table 8. Mean ± SE of the Sugar Profile of Honey from Different Market Points

Sugar Types	Treatment						P value
	T1	T2	T3	T4	T5	T6	
Fructose	37.47±1.12ab	38.69±0.97b	34.48±0.97a	37.57±0.97a	39.01±0.97b	39.01±0.97b	<0.013
Glucose	32.03 ± 0.88^{b}	28.08 ± 0.76^{a}	26.93 ± 0.76^{a}	28.55 ± 0.76^{a}	28.40 ± 0.76^{a}	28.90 ± 0.76^{ab}	<0.003
Sucrose	31.90 ± 2.06^{c}	32.23 ± 1.78^{c}	20.79±0.76b	21.37±1.78b	9.89±1.78a	8.60±1.78a	<0.000

Means within different letters across the row shows significant differences among the treatment (P<0.05). T1= On- street honey market samples, T2= Retailer market honey samples, T3= Minimarket honey samples, T4= Supermarket honey samples, T5= Urban beekeepers honey samples, T6= Rural beekeepers honey samples.

4.5.2. Glucose in Honey Samples

Mean glucose values from street honey samples (32.03±0.88%) differed significantly (P<0.05) from samples from convenience stores (26.93±0.76%) and supermarkets (28.55±0.76). Honey sold on the street had the highest mean glucose value (32.03±0.88 g/l00g).

4.5.3. Sucrose in Honey Samples

From the present analysis (Table 7), a statistically significant difference (P<0.05) in sucrose concentration was found between street vendors, retailers, convenience stores, supermarkets, and honey from urban beekeepers and rural beekeepers. The highest sucrose levels were found in honey samples from retailers (32.23±1.78%) and at street markets (31.90±2.06%). The difference could be due to different levels of maturity of the honey as honey was collected and harvested from the different areas. The study result gave a sucrose honey range from 8.60±1.78% to 32.23±1.78%.

4.6. Pollen analysis of honey

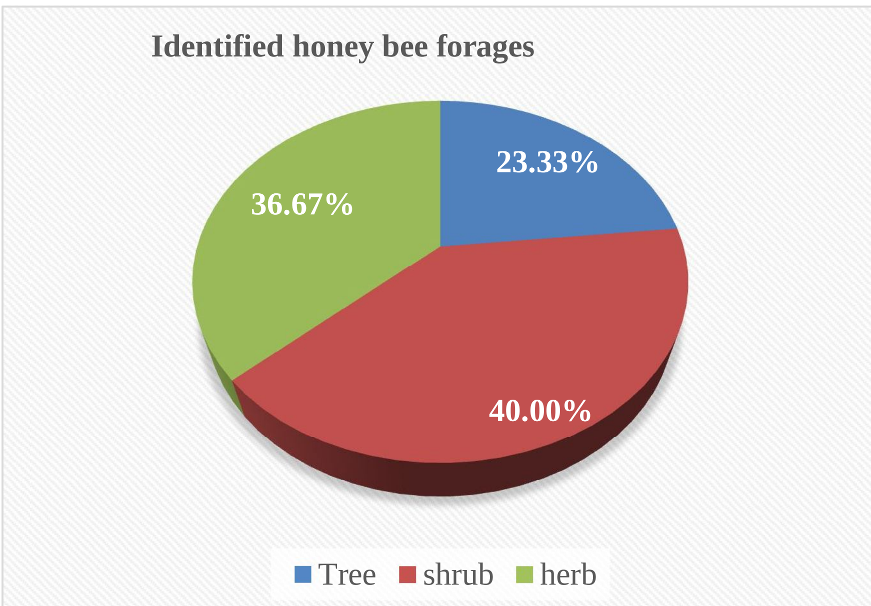
The result of the study revealed that 30 honeybee forages were identified which belong to 20 families. Among the identified plant families, Asteraceae (13.33%) and Fabaceae (13.33%), and Acanthaceae (10%) were the most frequent families, represented by the highest species composition in the area (Table 8). This study result implies there is no dominant monofloral bee forage that results in monofloral honey production in the area and affects the physicochemical properties of the samples in a special way.

Table 9. Bee Forage Plants with their Family, Growth Habit and Flowering Period in the Study Area.

Family	Scientific name	Local name	Habits	Flowering period
Acanthaceae	*Hypoestes forskaoli*	Dargu	herb	Sept. to Nov.
Acanthaceae	*Isoglossa somalensis*	Dargu	herb	Sept. to Oct.
Acanthaceae	*Justicia schimperiana*	Dhumuga	shrub	Nov. to Jaun.
Amaranthaceae	*Achyranthes aspera*	Matane	herb	All year round
Anacardiaceae	*Rhus natalensis Krauss*	Tatessa	shrub	Sept. to Oct.
Anacardiaceae	*Schinus molle*	Tiqur-Berberie (Amh)	Tree	All year round
Apiaceae	*Coriandrum sativum*	Dimbilaala	herb	All year round
Aquifoliaceae	*Ilex mitis*	Wolkite Gaadhii	Shrub	Dec. to Jaun.
Arecaceae	*Phoenix reclinata*	Meexii	Tree	Sept. to Oct.
Asteraceae	*Bidens pilosa*	Matane	herb	Sept. to Oct.
Asteraceae	*Guizotia scabra*	Hadaa, Tuufoo	herb	All year round
Asteraceae	*Vernonia amygdalina*	Ebicha	shrub	Dec. to May
Boraginaceae	*Ehretia cymosa*	Wagi	Shrub	Sept. to Dec.
Brassicaceae	*Brassica carinata*	Raafuu Simbiro	herb	Sept. to Nov.
Brassicaceae	*Crambe hispanica*	Gomena	herb	Sept. to Nov.
Caricaceae	*Carica papaya*	Papaya	Tree	All year round
Euphorbiaceae	*Croton macrostachyus*	Makkanissa	Tree	April to July
Fabaceae	*Acacia abyssinica*	Lafto	Tree	Oct. to Nov.
Fabaceae	*Parkinsonia aculeata*	Jerusalem Thorn (Eng).	shrub	Sept. to Dec.
Fabaceae	*Phaseolus vulgaris*	Adengware	herb	Sept. to Oct.
Fabaceae	*Pterolobium stellatum*	Haragama, Gora	shrub	Oct. to May
Lamiaceae	*Ocimum urticifolium*	Koricha Michi	shrub	Sept. to Oct.
Loranthaceae	*Dracaena spps*	Tieghem (Eng.)	Shrub	Sept. to Nov.
Loranthaceae	*Tapinanthus heteromorphus*	Baleddo	shrub	All year round
Malvaceae	*Hibiscus panduriformis*	Hincinii	herb	Sept. to Oct.

Myrtaceae	*Eucalyptus camaldulensis*	Bargamoo Diimaa	Tree	March to May
Proteaceae	*Grevillea robusta*	Grevillea (Eng.)	Tree	Sept. to Nov.
Rosaceae	*Rubus steudneri*	Gora	shrub	Augst to Jaun.
Simaroubaceae	*Brucea antidysenterica*	Tamichaa	shrub	Sept. to Oct.
Solanaceae	*Datura stramonium*	Asangira	herb	Augst to Feb.

Source: from the study pollen analysis data



Source: from the study pollen analysis data

Figure 3: Life forms of honey bee forages in the study areas

5. DISCUSSIONS

5.1. Physicochemical Properties of honey

5.1.1. Moisture Content

Moisture content is the key criterion that determines the ability of honey to remain fresh and free of fermentation (Silva *et al*., 2009; Bogdanov *et al*., 1999). The highest moisture content was recorded for honey collected from a street (24.62±0.67%) and the lowest was recorded from rural beekeepers (18.90±0.58%). The results are comparable with results obtained for honey samples from different areas of the country (Fikru *et al*., 2015; Tesfaye *et al*., 2016; Gebreegziabher *et al*., 2014; Adgaba, 1999). However, relative to the honey sample directly harvested from the hive (16.0 ± 1.25%), (Tewodros *et al*., 2013). The mean moisture contents of rural beekeepers, urban beekeepers, and supermarkets are in agreement with the national standard (Ethiopian Standard, 2005) and international (Codex, 2001; EU, 2002) ranges recommended, which should be a maximum of 20%. The moisture content of honey could vary depending on different factors, even within the agro-ecological zone, due to differences in harvesting, ways of processing, and improper handling practices among producers, processors, and merchants. The moisture content of some honey samples collected from local markets has been reported to be higher than national and international standards (Gebremariam and Berhane, 2014). The variation in the moisture content of honey might be related to harvesting, handling, processing, and adulteration.

5.1.2. The Ash Content

The variability in ash content of natural honey reflects the abundance of mineral content in honey sources, which is mainly influenced by the nectar's botanical origin, location, processing and handling techniques (Finola *et al*., 2007). In this regard, Biluca *et al*. (2016) pointed out that the mineral content in honey is closely related to its floral origin and the properties of the soil in which the plants are located. The ash content of the current result is similar to that reported in Cuba (Alvarez-Suarez) and Nigeria (Kayode and Oyeyemi, 2014). The maximum level in A. mellifera honey is currently limited to 0.6% for the EU standard (EU Council, 2002) and Ethiopia, showing that honeys with ash levels in this range are of nectar origin (Andrade *et al.*, 1999). In our study, all honey samples were within recommended limits, with ash levels in A. mellifera honey meeting national standards.

5.1.3. The pH Value

The pH values were within the range specified by the codex (2001), except for honey from retailers. Honey pH can provide a good indication of its origin and can also predict honeys degradation during storage (Jeanne, 2005). Thus, the lower pH value of honey from retailers may suggest that may be shelved for a longer time.

5.1.4. The Hydroxymethyl Furfural Aldehyde (HMF)

Hydroxymethylfurfural (HMF) is a compound made from the chemical reaction of some sugars and acids, and it is used as an indicator of honey's freshness and good quality regarding product adulteration or improper storage conditions (Marchini *et al*., 2007). Even though the result of HMF of the honey samples was below the national standards (Ethiopian Standard, 2005) and international quality standards (Codex, 2001, and EU, 2002), the current HMF values are so high, indicating that these honey samples are either not fresh, poorly processed and handled, or may contain foreign materials. The results highly exceeded values reported by different scholars in Ethiopia from different areas, such as Jimma Zone (6.3 mg/kg) (Kinati *et al*., 2011), Tigrai Region (15 mg/kg) (Gebru *et al.*, 2015), and Gonder (6.3 mg/kg) (Getu and Birhan, 2014). However, higher HMF values that reach 85.4 ± 0.15 mg/kg and 103.2 ± 40.5mg/kg have also been reported from supermarkets in Kenya (Fredrick *et al.*, 2013) and Uganda (Kugonza and Dorothy, 2008), respectively. Such high HMF values may be due to the fact that honey samples taken from supermarkets are aged, poorly processed and handled, and/or contain foreign materials, which affect the HMF of the product. Under normal conditions, HMF is absent, while levels may increase during processing or aging, mainly influenced by temperature abuse, pH, storage conditions, and floral origins (Alvarez-Suarez). The high levels of HMF in honey in the study area suggest that the honey at each market point is either aged, poorly processed and handled, and/or contains foreign materials.

5.1.5. The Free Acidity

The acidity of honey is related to organic acids, especially gluconic acid, being in balance with their corresponding lactones or inner esters and inorganic ions, mainly phosphate, sulfate and chloride (Silva *et al.*, 2009; Terrab *et al.*, 2002). This average value of acidity for the stingless bee honey samples is within the acceptable limits of national (<40 meq. kg^{-1}) and international standard values (<50 meq. kg^{-1}) for Apis honey (Codex, 2001). In accordance with those utilized in the present investigation, honey-free acid levels have been reported from Tigray (29.895 meq/kg; Gebreegziabher *et al.*, 2014) and Amhara (27.34 5.06 meq/kg^{-1}; Alemu *et al.*, 2016) that are near to the outcomes attained. According to Nweze *et al*. (2017) and Makarewicz *et al*. (2017), the mean free acid content of the honey from this study was higher than that of Nigerian honey (18.67 0.64 meq/kg) and Polish honey (14.40 0.58 meq/kg^{-1}). According to a study by Gebremariam and Brhane (2014), market samples exhibited free acid values that were greater than those advised for samples of genuine (pure) honey. According to a study report by Muli *et al*. (2007) from Kenya, honey samples obtained from traditional processors, beekeepers, and honey merchants had honey-free acid levels that ranged from 8 to 71.9 meq/kg^{-1}. In contrast to the findings of the current investigation, the report by Fredrick *et al*. (2013) revealed that the free acidity of honey samples obtained from a neighborhood store (supermarket) and honey brands was 56.7 meq/kg^{-1}. The honey eventually ferments when the free acid concentration rises, and the resulting alcohol is transformed into organic acid. This imparts a sour flavor to the honey, making it less palatable (de Silva *et al.*, 2016; Kugonza & Dorothy, 2008). The substantial fluctuation in the number of free acids in honey may be due to colony size, nectar sugar concentration, and the amount of time nectar needs to fully transform into honey under various environmental circumstances. The final quality of honey in terms of the free acid content can also be impacted by different management, harvesting, and processing processes (Krell *et al.*, 1996).

5.2. Sugar Profiles

The amount of carbohydrates in honey is perhaps the most crucial factor in determining its botanical origin, and it also indirectly impacts its proper classification and any indications of adulteration (Muthui, 2012). The majority of the sugars in honey, according to Aljohar *et al*. (2018), are fructose, glucose, and sucrose. In addition to evaluating the specific sugar content of monosaccharides (glucose, fructose) or disaccharides (sucrose), honey samples can also be certified by determining the fructose-glucose ratio (Rybak-Chmielewska, 2007).

5.2.1. Fructose

Fructose is the dominant sugar in honey samples and is responsible for honey's sweetness (Crane, 1990). The values of the current result were not in the EHC range (31.2-42.4%) of the fructose values. The low fructose content indicates heated or adulterated honey. The increase in fructose levels is probably due to the nectar richness of fructose (Louveaux, 1985). The results of most study samples are consistent with previous reports on fructose levels (White *et al*., 1962; Kucuk *et al*., 2007; Amir *et al.*, 2010; Amir, 2013; Belay *et al*., 2017).

5.2.2. Glucose

Honey sold on the street market had the highest mean glucose value (32.03±0.88 g/l00g). This is due to the honey of certain plant species, honey obtained from bee syrups or honey to which artificial substances added had high in glucose have been indicates addition of high glucose adulterants (Abdel-Aal *et al*., 1993). In this study, glucose levels are within the range established by the EHC (23.32%). The current result is relatively lower than that of Belay *et al.* (2017) (37.20±0.4 g/100 g) in Ethiopian mono-flower honey, which may be due to the nature of the nectar source (Muthui *et al.*, 2012).

5.2.3. Sucrose

The difference shown in the result could be due to different levels of maturity of the honey as honey was collected and harvested from the different areas. The highest average sucrose content was found in retail honeys, which could be due to honey of a specific floral origin, the method of honey preparation by beekeepers that overfeeds the bees with sugar, and the addition of sugar and syrup, which is an adulteration of honey with sugar (Kelly *et al.*, 2004). The study result gave a sucrose honey range from 8.60±1.78% to 32.23±1.78% and these results were higher than those previously reported for honey samples from Malaysia (3.02±1.33; Moniruzzaman *et al.*, 2013), Bangladeshi honey reported results.6.1±0.1; Islam *et al.*, 2017) and Nigeria (2.36± 0.05; Nweze *et al.*, 2017), except for a few samples. The result of this study is higher values compared to studies by Kinati *et al.* (2011) (5.68-9.45%), Tadesse and Gebregziabher (2014) (3.8-9.8%) and Getu & Birhan (2015) (7.6%) who performed analyzes on honey samples, collected at the local market, pure honey, adulterated honey or forest areas. Similarly, in Sudan, a study was conducted by Hana'a (2007) (5.5%) on honey samples collected by beekeepers from various flower sources by beekeepers, and by Mohammed et al. (2014) (5.8-8.7%) on honey samples. The results of this study showed that honey samples obtained from urban and rural beekeepers only had sucrose levels which meet the EHC suggested limit of 10 g/l00g (Bogdanov & Martin, 2000; Codex, 2001) and even above the Ethiopian standard (Ethiopian Standard, 2005) which is much more than 5g/100g.

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

In Ethiopia, there has been little research on the commercial quality and adulteration detection of honey samples, despite the many nutritional, medicinal, and expensive uses of honey. Since the authenticity of honey is crucial for several marketing and health aspects, it was very important. In this study, the quality of honey samples available at different market locations was determined by sampling studies, rapid tests, and basic physicochemical characteristics. Evaluation of the quality of honey using a sample survey shows that the quality of the honey is very poor and that the honey was adulterated by street honey sellers. moreover, the adulteration of honey was to increase their performance (income). Research shows that the main ingredients used in the adulteration were sugar, caramel, molasses, and a ripe banana. The survey results indicate a time of year when honey adulteration was common during the high honey flow season. This was due to beekeepers producing or harvesting fake honey with pure honey to simulate or harvest. The evaluation study also reveals that the biggest problems with fake honey were health problems, loss of satisfaction, and reduced honey production. Honey samples obtained from different markets were often not excellent in terms of physical and chemical properties. However, several honey samples from rural and urban beekeepers contained concentrations of components closer to the recommended ranges. This study determined typical honey adulteration in Ethiopia using characteristics useful in identifying pure and adulterated honey samples. Five different rapid test methods were used for the honey samples. Most of the results of rapid tests of honey in different markets show that the quality of the honey is unsatisfactory compared to the standards and that it was adulterated. The physicochemical characteristics of sample honey collected from different marketplaces, apart from individual samples, showed that many of them did not meet the quality criteria of Codex (2001), EU (2002), and Ethiopian Standard (2005). The determination of ash content, free acidity, HMF, pH value, moisture content, fructose, glucose, and sucrose were potentially valuable to differentiate between pure and adulterated honey samples. Overall, this finding showed that the methods used to monitor honey quality were effective in detecting honey adulteration and evaluating honey content.

Honey adulteration affects all aspects of honey production and marketing to maintain its quality and safety.

6.2. Recommendations

Based on the results of this study, the following recommendations are made for further consideration:

- ✓ As a result of the study's findings, it is possible to identify honey's quality and adulteration utilizing quick test methods and physicochemical analysis.
- ✓ Training of beekeepers and other stakeholders on rapid test assessment to keep honey quality, identify the adulterated honey and outcome of adulteration, and avoid adulteration should have to be carried out.
- ✓ Ethiopian honey criteria and requirements need to be updated. The moisture and sugar content of some poor-quality honey samples were comparable to pure honey. In addition, the standard for sucrose needs to be updated, and no standard has been established for fructose, glucose, or maltose.
- ✓ Severe laws support punishment development to reduce honey adulteration.
- ✓ Increase ample honey production to reduce low-quality honey in the markets.
- ✓ Consumers ought to be able to deal directly with beekeepers, cutting off brokers and middle actors.
- ✓ Street honey sellers need to be stopped by the law, and they need to be punished.
- ✓ It is advisable to support more investigation into alternative control methods and the impact of honey adulteration on consumers.

REFERENCE

Abdel-Aal, E.M., Ziena, H.M. & Youssef, M.M. (1993). Adulteration of honey with high fructose corn syrup: sucrose syrups: correlations with HPLC-RID results. Food Research International, 64, 634-646.

Addis & Malede (2014). Chemical analysis of honey and major challenges in honey production in and around Gonder, Ethiopia: *Academic Journal of Nutrition* 3(1): 06-14.

Addis, N. (2016). Honey Value Chain Analysis: Amhara in the Semen Shewa Zone of Ethiopia; The case of Basona Woreda. (MBA Thesis) Addis Ababa, Ethiopia.

Adgaba N, Alghamdi A, Sammoud R, Shenkute A, Tadesse Y, Ansari MJ, ... & Hepburn C (2017). Determination of the spatio-temporal distribution of bee forage species in the Al-Baha region based on a soil inventory supported by GIS applications and remote satellite imagery analysis: *Saudi Journal of Biological Sciences*, 24(5): 1038-1044.

Adgaba, N. (1999, June). Quality status of sorting of Ethiopian honey. *Proceedings of the first national conference of the Ethiopian Beekeepers' Association, Addis Ababa, Ethiopia.*

Africa, H.C., (2010). Honey value chain in Kenya.

Aizen, M.A., Garibaldi, L.A., Cunningham, S.A., and Klein, A.M. (2009) How much agriculture depends on pollinators a lesson for long-term crop trends? Annals of Botany, 103 (9): 1579–1588.

Ajlouni, S., and Sujirapinyokul, P. (2011). Hydroxy methyl furfuraldehyde and amylase concentrations in an Australian male. *Food Chemistry*, 119(3): 1000–1005.

Alemu, A.E., Maertens, M., Deckers, J., Bauer, H., and Mathijs, E. (2016). Impact of supply chain coordination on the income of honey farmers in Tigray, northern Ethiopia, *Agricultural and Food Economy* 4(1): 1–21.

Alemu, T., and Dechasa, S. (2019). Overview of honey adulteration and honey adulteration detection, *International Journal of Gastroenterology*, 4(1): 1-6.

Alimentarius, C. (2007). Codex Standard for Honey, Codex Stan 1219.

Alvarez-Suarez, J.M., Tulipani, S., Romandini, S., Bertoli, E. & Battino, M. (2010). Contribution of honey to nutrition and human health: a review. *Mediterranean Journal of Nutrition and Metabolism*, 3(1), 15-23.

Aljohar HI, Maher HM, Albaqami J, Al-Mehaizie M, Orfali R, Orfali R, & Alrubia S. (2018). Physical and chemical analysis of honey samples available on the Saudi market: an important aspect in the authentication process and quality assessment: *Saudi Pharmaceutical Journal*, 26(7), 932-942.

Aloisi, F., Serafini, B., Magliozzi, R., Howell, O. W., & Reynolds, R. (2010). Detection of Epstein–Barr virus and B-cell follicles in the multiple sclerosis brain, 133(12), e157-e157.

Ambaw, M., and Teklehaimanot, T. (2018). "Study on Quality Parameters and Producer Knowledge of Honey Failure in Selected Areas of the Ars Zone, *International Journal of Agriculture and Veterinary Sciences*, 4(1): 1-6.

Amir, R.M., Anjum, F.M., Khan, MI, Khan, MR, Pasha, I. & Nadeem, M. (2013). Application of Fourier transform infrared spectroscopy (FTIR) to identify wheat cultivars. *Journal of Food Science and Technology*, 50(5), 1018-1023.

Amir Y, Yesli A, Bengana M, Sadoudi R & Amrouche T (2010). Physico-chemical and microbiological evaluation of honey from Algeria. *Electronic Journal of Environmental, Agricultural & Food Chemistry*, 9(9).

Andrade, P.B., Amaral, M.T., Isabel, P., Carvalho, J.C., Seabra, R.M., and Da Cunha, A.P. (1999). Physico-chemical properties and pollen spectrum of Portuguese heather honeys. *Food Chemistry*. 66:503-510.

Anklam, E. (1998). A review of analytical methods used to determine the geographic and botanical origin of honey: Food Chemistry, 63(4), 549-562.Asrat, A., Zelalem, Y., and Ajebu, N. (2012). The quality of fresh whole milk produced in and around the town of Wolaita Bodit in southern Ethiopia, *African Journal of Biomedical Animals, Science*, 7(2):95.

Authority, E.Q.S. (2005), Quality and Standard of Ethiopian Honey (ES 1202: 2005). *Addis Ababa, Ethiopia.*

Ayza, A., and Belete, E. (2015). Food adulteration: its challenges and consequences, *Quality Management in Food Science*, 41:50–6.

Belay A, Haki GD, Birringer M, Borck H, Chul Y, Cho CW, (2017). Sugar profile and physicochemical properties of Ethiopian monofloral honey: *International Journal of Food Properties*, 2912 (November), 133.

Bett, C.K., 2017. Factors influencing the quality of honey production: *International Journal of Academic Research in Business and Social Sciences* 7, 281-292.

Biluca, F.C., Braghini, F., Gonzaga, L.V., Costa, A.C.O. & Fett, R. (2016). Physicochemical profiles, minerals and bioactive compounds of stingless bee (Meliponinae) honey: *Journal of Food Composition and Analysis*, 50, 61-69.

Birhanu, T., (2016). Honeybee production and honey quality assessment in Guji zone, Ethiopia. *EC Agri*, 3: 646-652.

Bogdanov S., Gallmann P. (2008), Authenticity of Honey and Other Beekeeping Products and the Latest Technology, *ALP Science*, 2008, No. 520.

Bogdanov, S., and Martin, P. (2003), The authenticity of the honey, *Food Inspection and Hygiene Notices*, 93(3): 232-254.

Bogdanov, S. (2002). Harmonized methods of the International Honey Commission. Swiss Bee Research Center, *FAM, Liebefeld*, CH-3003 Bern, Switzerland.

Bogdanov, S. (2009). composition of honey. Book of Honey, Chapter 5. *Bee Products Science*, accessed 29 August 2012.

Bogdanov, S., Lllmann, C., Martin, P., von der Ohe, W., Russmann, H., Vorwohl, G., Piro, R. (1999). Honey Quality and International Regulatory Standards: *Review by the International Honey Commission: Bee World*, 80(2), 61-69.

Bogdanov, S., Ruoff, K. & Oddo, L.P. (2004). Physico-chemical methods for the characterization of blossom honey: an overview: *Apidology*, 35(Suppl. 1), 4-17.

Brown, M.J., and Paxton, R.J. (2009), Beekeeping: A Global Perspective, *Apidologie*, 40 (3): 410–416.

Carrie, R.J., George, D.R. and Wickers, F.L. (2012). Selection of floral resources to optimize conservation of agricultural insect groups: *Journal of Insect Conservation*, 16(4): 635640.

Cavia, M.M., Fernandez-Muino, M.A., Alonso-Turo, S.R., Huidobro, J.F. and Sancho, M.T. (2007). Development of Acidity in Honey from Continental Climates: Effect of Induced Granulation: *Food Chemistry*, 100(4): 17281733.

Chauzat, M.P., and Faucon, J.P. (2007), Pesticide residues in beeswax samples collected from bee colonies (Apis mellifera L.) in France: *Pest Management Science: Formerly Pesticide Science*, 63(11): 1100-1106.

Chen L, Xue X, Ye Z, Zhou J, Chen F and Zhao J (2011). Determination of Chinese honey adulterated with fructose corn syrup by near-infrared spectroscopy: *Food Chemistry*, 128(4): 1110-1114.

Codex, A. and Intergovernmental, T.F.O. (2001). 24th Meeting of the Codex Alimentarius Commission of the Joint FAO/WHO Program on Food Standards, Geneva, July 27, 2001 Codex.

Cozzolino, D., Corbella, E. and Smyth, S.E. (2011), Quality control of honey by infrared spectroscopy: a review. *Applied Spectroscopy Reviews,* 46(7): 523-538.

Crane, E. and Visscher, P. K. (2009). Hunaya Encyclopedia of Insects (459-461), *Academic Journalism.*

Crane E (1990). A book full of honey. International Bee Research Association, *Oxford University Press,* UK.

Croft, L.R., 2007. Honey and hay fever: a report on the treatment of hay fever with honey. *LR Croft*.

CSA (Centre for Statistics), (2020/21). Agricultural Selection Study of Cattle and Livestock Traits: Vol. (II), No. 581, March 2021, Addis Ababa.

Da Silva P M, Gauche C, Gonzaga L V, Costa AC O, & Fett R (2016). Honey: chemical composition, stability and authenticity: *Food Chemistry*, 196, 309-323.

Dick, C. W., Roubik, D. W., Gruber, K. F., & Bermingham, E. (2004). Comparative mitochondrial DNA phylogeography revealed long-distance gene flow and dispersal in lowland rainforest bees (Apidae: Euglossini) across the Andes. *Molecular Ecology*, 13(12), 3775-3785.

Diez, M.J., Andrs, C. and Terrab, A. (2004). Physico-chemical parameters and pollen analysis of Moroccan honeydew honey: *International Journal of Food Science and Technology*, 39(2):167-176.

Erdman, G. (1960), Method of Acetolysis Correctly Prescribed by Sv. Bot. Tidskr, 54: 561–564.

Ethiopia standard. (2005). Honey Specification: Ethiopian Standard, ES 1202:2005. *In: Addis Ababa, Ethiopia.*

EU Council. (2002). Council Directive 2001/11 O/EC of December 20, 2001 on honey: *Official Journal of the European Communities*.

Evans, J.D., and Schwarz, R.S. (2011). Bees on their knees: microbes that affect bee health, Trends in Microbiology, 19(12): 614-620.

Farh, I.A.E. (2016). Identification of some honey samples from Sudanese bees (PhD dissertation, Sudan University of Science and Technology).

Fikru, S., Gebresilassie, G. & Kassa, A. (2015). Assessment of beekeeping practices (escape, bee forage, and bee diseases and pests) in the Jigjiga Zone, Somali Regional State of Ethiopia: *Poultry, Fisheries and Wildlife Sciences*.

Finola, M.S., Lasagno, M.C. & Marioli, J.M. (2007). Microbiological and chemical characterization of honeys from central Argentina: *Food Chemistry*, 100(4), 1649-1653.

Fredrick, N.; Anam, O.; Antony, G. and Elijah, N. (2013). Physicochemical Analysis of Honey in the Kenyan Retail Market: *Food Science Quality Management*, 12:30-36.

Gebreegziabher Gebremedhin, Gebrehiwot Tadesse and Etsay Kebede. (2014). Physiochemical Properties of Honey Produced from Traditional and Modern Beehive Production Systems in the Tigray Region of Northern Ethiopia: *Momona Ethiopian Journal of Science (MEJS)*, 5(1), 115 128.

Gebremariam, T. & Brhane, G. (2014). Determining the quality and adulterating effects of honey from Adigrat and the surrounding area. *International Journal of Technology Enhancements and Emerging Engineering Research*, 2, 71.

Gebremedhin, G., Tadesse, G. and Kebede, E. (2013). Physicochemical Properties of Honey from Traditional and Modern Bee Production Systems in the Tigray Region of Northern Ethiopia: *Ethiopian Journal of Science*, 5(1): 115-128.

Gebru, Y.G. (2015). Characterization of beekeeping systems and the honey value chain, and effects of storage containers and durations on the physicochemical properties of 120 honeys in Kiltie Awlaelo District, East Tigray, Ethiopia. *PhD Dissertation, Department Animal Production Studies.*

Gemeda, M. and Negera, T. (2017). Assessing the impact of adulterants on the quality of honey and beeswax and drafting an identification method in Oromia, 5 (8): 34-39.

Getu, A. and Birhan, M. (2015). Chemical Analysis of Honey and Major Challenges of Honey Production in and um Gondar: *Ethiopia Academic Journal of Nutrition*, 3 (1): 0614.

Gichora, M. (2003). On the way to realizing Kenya's full beekeeping potential. A case study from Baringo County.

Gidamis, Cuvillier Verlag., A.B., Chove, B.E., Shayo, N.B., Nnko, S.A., & Bangu, N.T. (2004). Quality assessment of honey from selected areas in Tanzania with a special focus on Hydroxymethylfurfural (HMF) content: *Springer Science business Media, Plant Foods for Human Nutrition,* 59(3), 129132.

Guler, A., Bakan, A., Nisbet, C. and Yavuz, O. (2007). Determination of important biochemical properties of honey to differentiate between pure and adulterated honey with sucrose syrup (Saccharum officinarum L.). Food Chemistry, 105(3): 11191125.

Guler, A., Kocaokutgen, H., Garipoglu, A.V., Onder, H., Ekinci, D., and Biyik, S. (2014). Detection of adulterated honey produced by bee colonies (Apis mellifera L.) fed various commercial industrial sugar syrups (C3 and C4 plants) by carbon isotope ratio analysis: *Food Chemistry*, 155:155-160.

Hana'a, Y.A.E.H. (2007). Physico-chemical properties of honey from different floral sources. *A dissertation with the degree "Master of Science in Food Science and Agricultural Technology" University of Khourtum.*

Iftikhar F, Mahmood R, Islam N, Sarwar G, Masood MA & Shafiq, H. (2014). Physico-chemical analysis of honey samples collected from local markets in Rawalpindi and Islamabad, Pakistan: *American Journal of Biochemistry*, 4(2), 3540.

International Honey Commission (2009). Harmonized methods of the International Honey Commission (IHC), Swiss Bee Research Center: *Bundestagebuchstation, Liebefeld.*

Ishraga, G., Thoria, I., Onsa, O., Amna, E., Khalafalla Hajer, I., mIshag, I., & Sayda, O. (2017). Yassin 1 and Safa O Su 2017: Quality Assessment of Bee Honey from Western Sudan Regions. *Sudan Journal of Veterinary Research*, 32, 1–5.

Islam, M. R., Pervin, T., Hossain, H., Saha, B., & Hossain, S. J. (2017). Physicochemical and antioxidant properties of honeys from the Sundarbans mangrove forest of Bangladesh. *Preventive nutrition and Food Science*, 22(4), 335.

Kasenburger, P. (2006). Sugars and free and total acids of various types of Slovenian honey: *University of Ljubljana*: 98.

Kayode, J., & Oyeyemi, S. D. (2014). Physicochemical Investigation of Honey Samples from Bee Farmers in Ekiti State, Southwest Nigeria. *Journal of Plant Sciences*, 2(5), 246–249.

Kebede, N., Subramanian, and M. Gebrekidan. (2012). Physicochemical Analysis of Tigray Honey: An Attempt to Determine Major Quality Markers of Honey". *Bull. Chem. Soc. Ethiopia*, 26(1), 127-133

Kelly, J. D., Downey, G., & Fouratier, V. (2004). An initial study of honey adulteration by sugar solutions using midinfrared (MIR) spectroscopy and chemometrics: *Journal of agricultural and food chemistry*, 52(1), 33-39.

Kinati, C., Tolemariam, T., and Debele, K. (2011). Evaluation of the quality of honey produced in Gomma Woreda, Southwest Ethiopia: *Rural Development Livestock Research*, 23(9): 6–14.

Kirko, V., Kononova, E., Lukyanova, A., & Razumovskaya, V. (2020). Education as an instrument of human capital development. *In Edulearn20 Proceedings* (pp. 8765-8770). Iated.

Kirthiga, S., K., Vikram, P., & Soon, J.M. (2019). Melissopalynological, physicochemical and antioxidant properties of honey from West Coast of Malaysia. *Journal of Food Science Technology* 56, 2508–2521 (2019).

Krell, R. (1996). Value-added Products from Beekeeping. *FAO Agricultural Services Bulletin No*. 124, Rome

Kucuk M, Kolayli S, Karaoglu S, Ulusoy E, Baltaci C & Candan F (2007). Biological activities and chemical composition of three honeys of different species from Anatolia: *Food chemistry*, 100(2), 526-534.

Kugonza, D.R., and Nabakabya, D. (2008). Processing and distribution channels affect the quality of honey in Uganda: *Tropicultura*, 26 (2): 113–118.

Oliver, R. (2012). Neonicotinoids: trying to understand the science: *American Bee Journal*, 152 (8):805.

Ouchemoukh, S., Louaileche, H., & Schweitzer, P. (2007). Physicochemical characteristics and pollen spectrum of some Algerian honeys: *Food Control*, 18(1), 52-58.

Ouchemoukh, S., Schweitzer, P., Bey, M.B., Djoudad-Kadji, H., & Louaileche, H. (2010). HPLC sugar profiles of Algerian honeys: *Food Chemistry*, 121, 561–568.

Lawal, R.A., Lawal, A.K., and Adekalu, J.B. (2009). Physicochemical studies on honey adulteration in Nigeria: *Pakistan Journal of Biological Sciences*: PJBS, 12(15): 1080–1084.

Liepelt, S., Mayland-Quellhorst, E., Lahme, M., et al. Contrasting geographic patterns of ancient and modern genetic lineages of Mediterranean Abies species: *Plant Syst Evol* 284, 141151 (2010).

Louveaux J, Maurizio A and Vorwohl G (1985) Methods of Melissopalynology, *Bee World* 59, 139157.

Mahfuza, S., Tanvir, E. M., Sakib Hossen, M., U., Mondal, M., Afroz, R., Mandal, M., ... & Hua Gan, S. (2018). Antioxidant, brine shrimp lethality and analgesic properties of propolis from Bangladesh. *Journal of Food Biochemistry*, 42(5), e12596.

Makarewicz, M., Kowalski, S., Lukasiewicz, M. & Maysa-Pako, M. (2017). Antimicrobial and antioxidant properties of some commercial honeys available on the Polish market. *Czech Journal of Food Science*,35(5), 401-406.

Marchini, J., Howie, B., Myers, S., McVean, G., & Donnelly, P. (2007). A adulteration and improper storage association. *Nature genetics*, 39(7), 906-913.

Masoud, H. M. (2014). Assessment of quality of Tanzanian honey based on physicochemical properties. *Journal of Food Science and Quality Management* 33: 61–72.

Mohammed, SA (2014). Commercial Sudanese Honey: National Research Center. Faculty of Agricultural Sciences, University of Khartoum, Khartoum, Sudan.

Moniruzzaman, M., Sulaiman, S.A., Khalil, MI. & Gan, S.H. (2013). Evaluation of the physicochemical and antioxidant properties of sourwood and other Malaysian honeys: a comparison with manuka honey: *Chemistry Central Journal,* 7(1), 138.

Morad, M.O., 2008. Quality state of grading Ethiopian honey. Addis Ababa, *Ethiopia: Government of Ethiopia*.

Motari, EM (2010). Structural studies of oligosaccharides bound to proteins expressed in different organisms and PE Gylation of a non-glycosylated protein (PhD thesis, Ohio State University).

Muli, E., Munguti, A. and Raina, S.K. (2007) Quality of honey collected and processed using traditional methods in rural areas of Kenya*: Acta Veterinaria Brno,* 76(2): 315320.

Muthui, B., N. (2012). Physiochemical properties of honey from Mwingi and selected urban areas in Kenya, effects of adulteration and some levels of population awareness of honey adulteration. *Master's thesis, Kenyatta University*.

Nanda, V., Sarkar, B.C., Sharma, H.K. and Bawa, A.S. (2003) Evaluation of the physicochemical properties and mineral content of honey from various plants in North India: *Journal of Food Composition and Analysis,* 16 (5): 613619.

Nweze, J.A., Okafor, J.I., Nweze, EI. & Nweze, J.E. (2017). Evaluation of the physicochemical and antioxidant properties of two stingless bee honeys: a comparison with Apis mellifera honey from Nsukka, Nigeria. *BMC Research Notes,* 10(1), 566.

Nyangoge, Orina., I. (2012). Quality and Safety Characteristics of Honey Produced in Different Regions of Kenya: *Repository of Jomo Kenyatta University of Agriculture and Technology, Thesis.*

Orina, I. N. (2014). Quality and safety characteristics of honey produced in different regions of kenya *[MSc. thesis]*. *Jomo Kenyatta University of Agriculture and Technology*.

Perez-Perez, E., Rodriguez-Malaver, A. J., & Vit, P. (2007, October). Effect of fermentation after harvest on the total antioxidant activity of Tetragonisca angustula honey. *In Free Radical Research* (Vol. 41, Pp. S47-S47). *4 Park Square, Milton Park, Abingdon Ox14 4rn, Oxon, England: Taylor & Francis Ltd*.

Pieper, Barbara (2009). Honey-Based Dressings and Wound Care. *Journal of Wound, Ostomy and Continence Nursing*, 36(6), 588–589.

Rattanathanalerk, M., Chiewchan, N., and Srichumpoung, W. (2005) Effect of heat treatment on quality deterioration of pineapple juice, *Journal of Food Engineering*, 66(2): 259–265.

Ribeiro, R.D.O.R., Mársico, E.T., and da Silva Carneiro, C., Monteiro, M.L.G., Junior, C.C., and Jesus, E.F.O. (2014) Detection of high-fructose corn syrup honey adulteration using low-field nuclear magnetic resonance (LF 1H NMR): *Journal of Food Engineering*, 135: 39–43.

Rortais, Agns; Arnold, Grard; Halm, Marie-Pierre; Touffet-Briens, Fredrique (2005). Modes of honey bees' exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees: *Apidologie*, 36(1), 71–83.

Rybak-Chmielewska, H. (2007). Changes in the carbohydrate composition of honey undergoing during storage. *Journal of Apicultural Science*, 51(1), 39-47.

Sebeho, H.K. (2015). Honey production and quality characteristics in Ethiopia, review: Academic Journal of Entomology: *Academic Journal of Entomology*, 8(4), 168–173.

Silva, L.R., Videira, R., Monteiro, A.P., Valento, P. & Andrade, P.B. (2009). Honey from the Luso region (Portugal): Physico-chemical properties and mineral content: Microchemical Journal, 93(1), 73-77.

Subramanian, R., Umesh Hebbar, H. Kaj Rastogi, and N.K. (2007). Mielprilaborado: superrigardo: *International Journal of Foods*, 10(1): 127–143.

Teferi Damto (2020). Quality assessment and failure detection using multiple analytical approaches using commercially available honey samples from Addis Ababa*(thesis), Ethiopia: Addis Ababa University.*

Terrab, A., Vega-Pérez, J.M., Díez, M.J., and Heredia, F.J., (2002). Characterization of Northwest Moroccan honey by gas chromatographic mass spectrometric analysis of its sugar components: *Journal of the Science of Food and Agriculture*, 82 (2): 179-185.

Tesfaye, B., Begna, D., and Eshetu, M. (2016). Evaluation of the physico-chemical characteristics of honey produced in Bale Natural Forest, Southeast Ethiopia: *International Journal of Agricultural Science and Food Technology*, 2(1), 21–27.

Tilman, D., Fargione, J., Wolff, B., Dantonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W.H., Simberloff, D., and Swackhamer, D. (2001). Predicting global environmental change from agriculture: Science, 292(5515): 281-284.

Tosun, M. (2013). Detection of adulteration in honey samples spiked with different sugar syrups using the 13C/12C isotope ratio analysis method: *Food Chemistry*, 138(2-3): 1629–1632.

Vanbergen, A.J., and Initiative, T.I.P. (2016). Threats to ecosystem services: pressures on pollinators: Frontiers in Ecology and the Environment, 11 (5): 251-259.

Visquert, M., Escriche, I., Andres, A., and Fito, P. (2004). Changes in honey quality parameters caused by thermal processes: *Alimentacion-Equipos-y-Tecnologia*, 23(188), 87-92.

Visquert, M., Vargas, M., and Escriche, I. (2014). Effect of post-harvest storage conditions on the color and freshness parameters of raw honey: *International Journal of Food Science and Technology*, 49(1): 181-187.

Vorlova, K. V. I. B. K., & Večerek, V. (2006). Hydroxymethylfurfural contents in foodstuffs determined by HPLC method. *Journal of Food and Nutrition Research*, 45(1), 34-38.

White, J. W. (1962). Composition of American honeys: US Dept. of Agriculture.

Woldemariam, H.W., and Abera, B.D. (2014). Extent of Adulteration of Selected Foods in Bahir Dar, Ethiopia: *International Journal of Interdisciplinary Research*, 1 (6): 1-6.

Yetimwork, G., T. Berhan and B. Desalegn, 2015. Honeybee production trend, potential and constraints in Eastern Zone of Tigray, Ethiopia: *Agriculture and Biology Journal of North America*, ScienceHuβ

Zábrodská, B., & Vorlova, L. (2014). Adulteration of honey and available methods for detection–a review: *Acta Veterinaria Brno*, 83(10), 85-102.

APPENDIX

Annex 1: Sample collected from different markets in Adama district and Adama Town

Annex 2: Analyzing free acidity and pH content of the honey samples by using a pH meter

Annex 3: Honey samples preparation in vials for sugar content analysis by HPLC

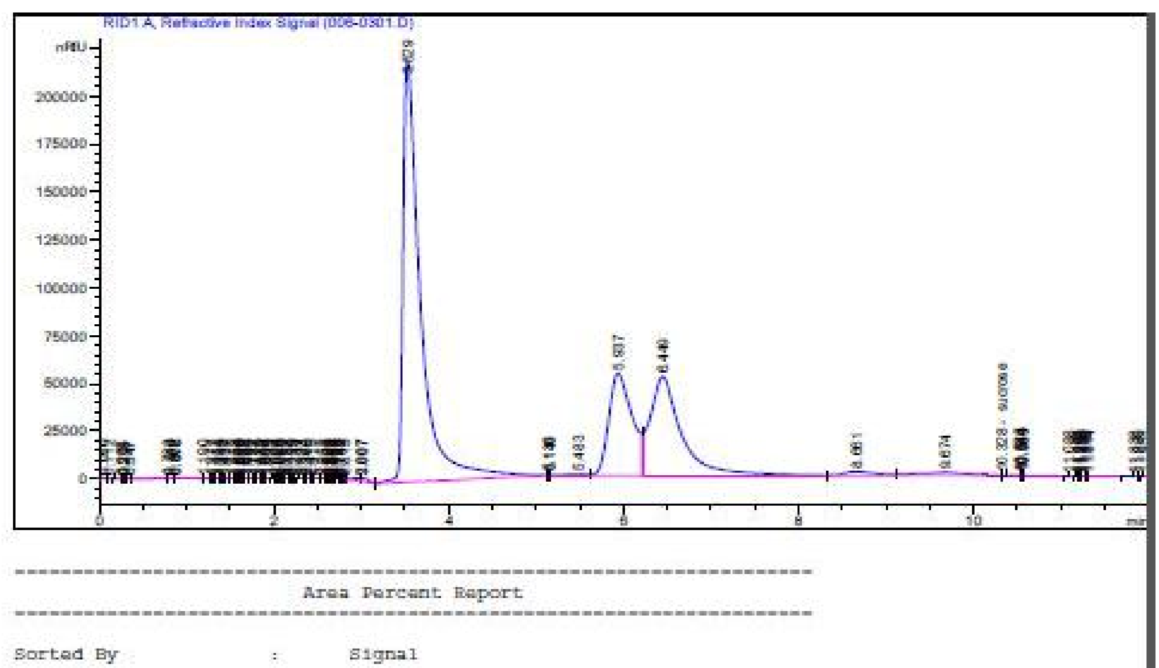
Annex 4: HPLC devices with all its compartments and computerized sets which performs sugar analysis

Annex 5: Sample coding, entering and introducing to HPLC devices

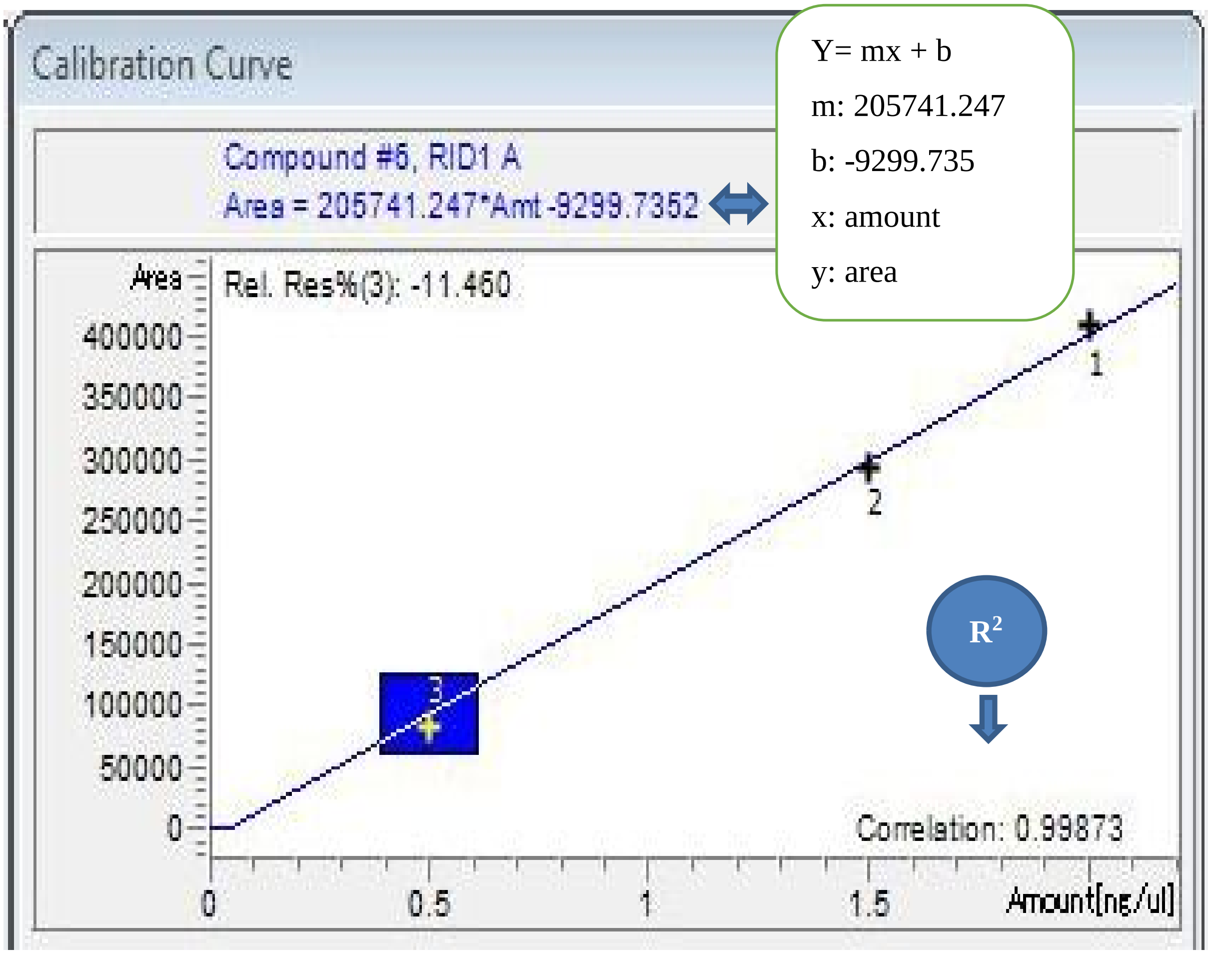
Annex 6: HPLC devices with all its sampler unit (injection unit), pumping unit, column unit, pressure unit and temperature unit and sample analyzed result

Annex 7: Sample honey ash content identification performing

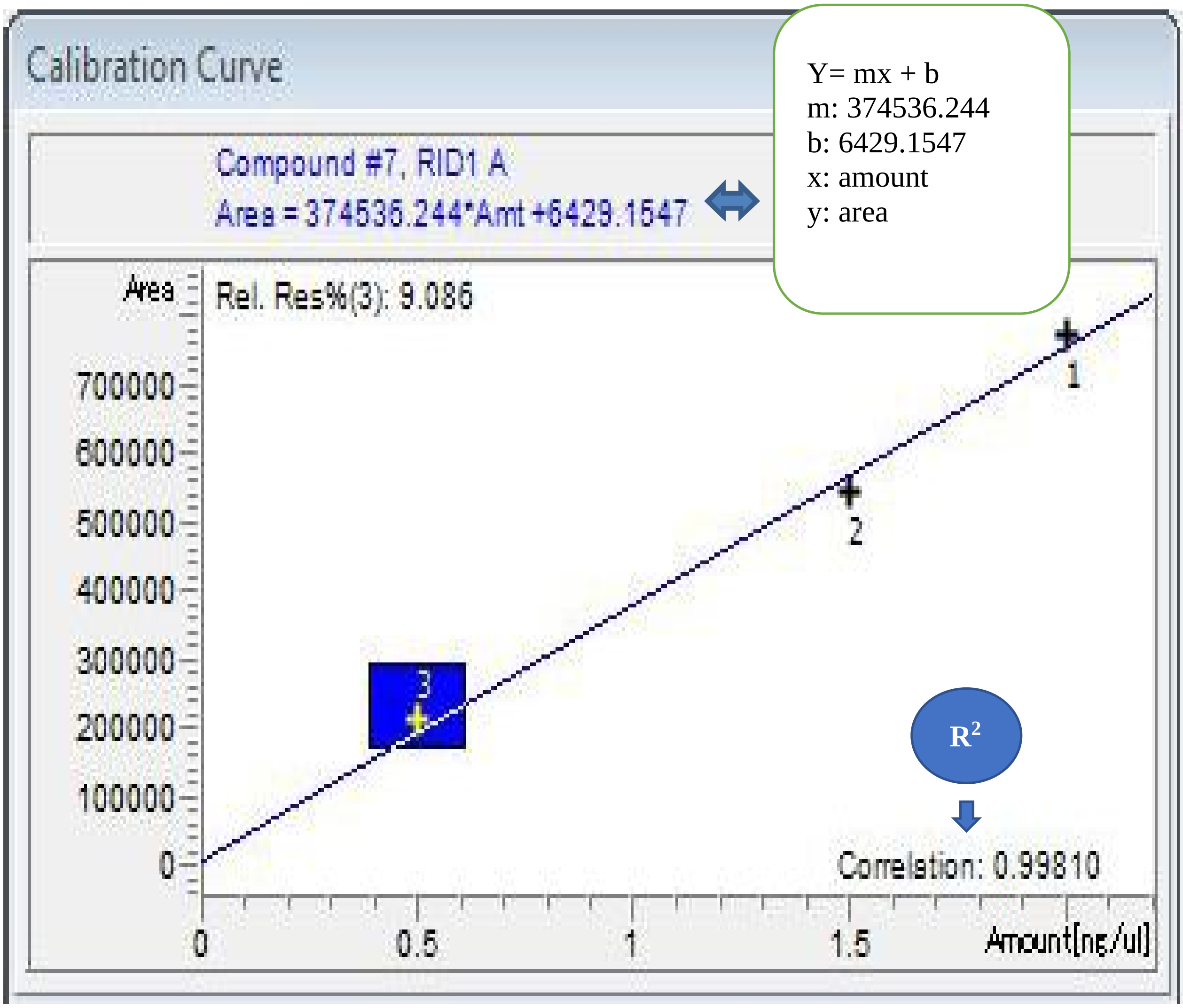
Annex 8: Chromatogram by HPLC, of fructose, glucose, and sucrose of honey samples different market point



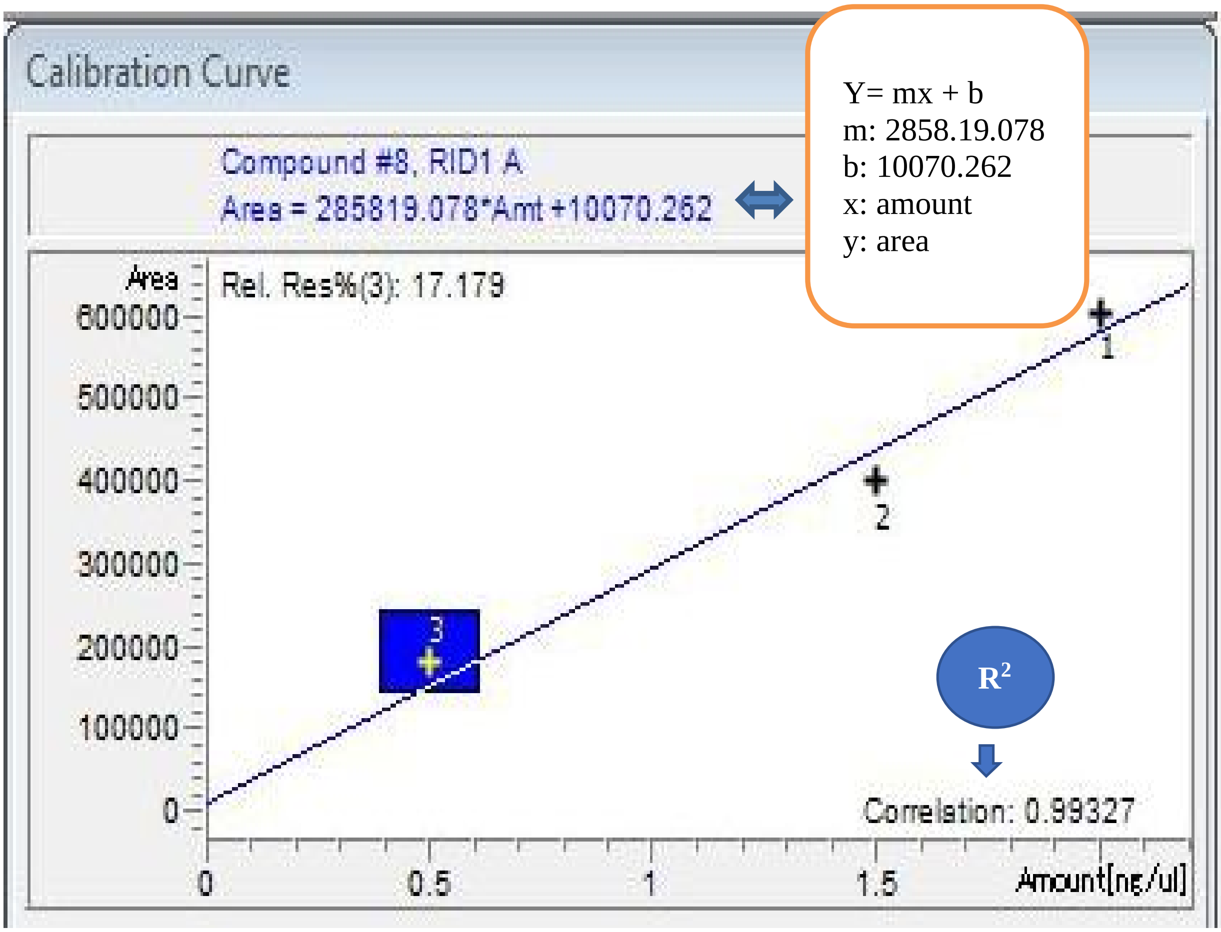
Annex 9: Standard fructose calibration curve



Annex 10: Standard glucose calibration curve



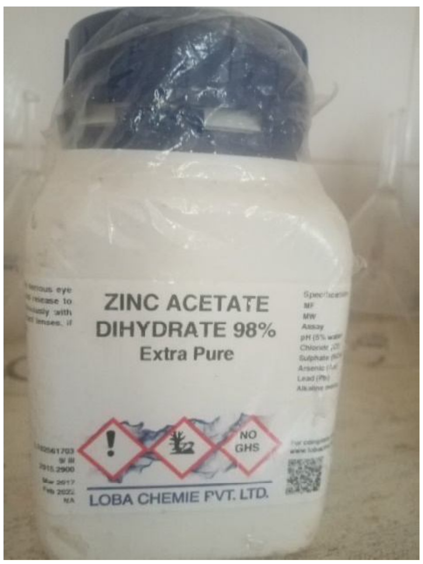
Annex 11: Standard sucrose calibration curve

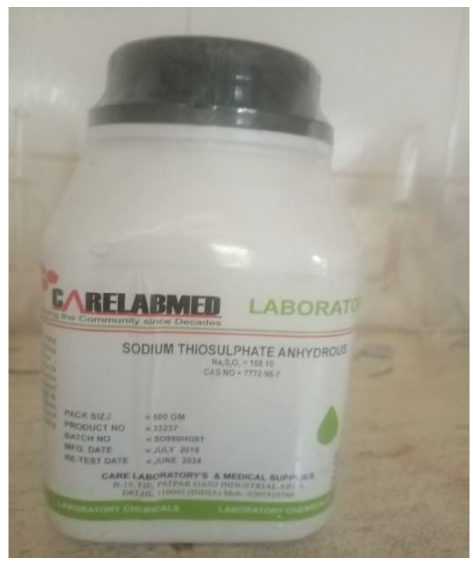


Annex 12: Standard fructose, glucose and sucrose sugar used for HPLC calibration

Annex 13: Buffer solutions for calibration the pH meter and reagents used for determination of pH and free acidity

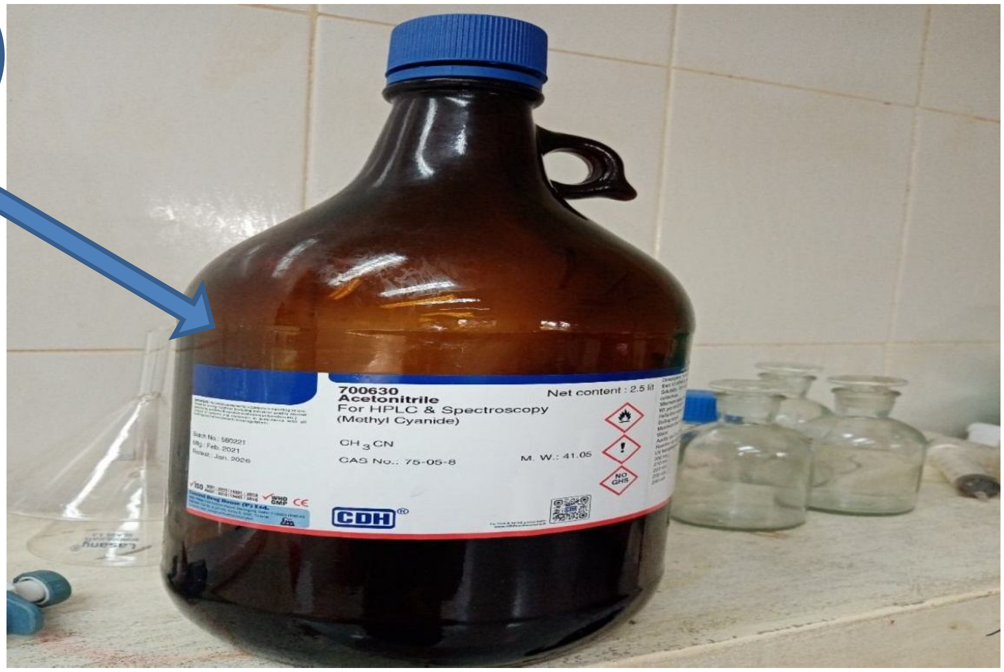
Annex 14: Reagents used for HMF determination by UV-VIS Spectroscopy





Annex 15: Reagents used for determination of sugars by HPLC

Acetonitrile for HPLC and Spectroscopy



Standard water for HPLC

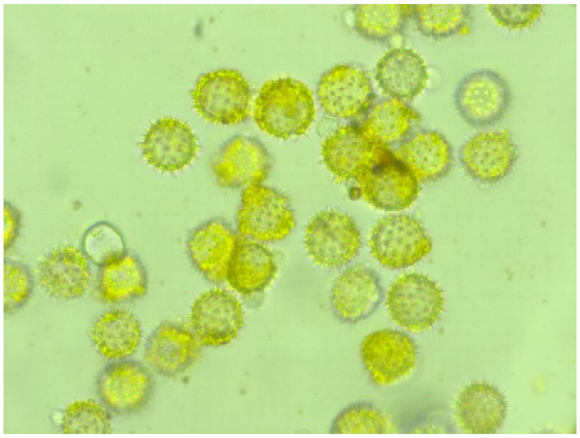
Pure Methanol

Annex 16: Summary of ANOVA

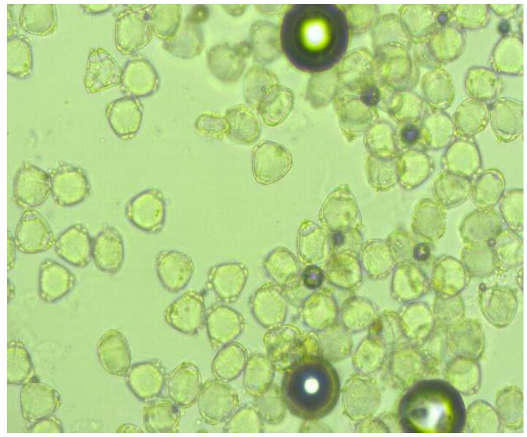
ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Moisture content	Between Groups	290.464	5	58.093	14.485	.000
	Within Groups	252.668	63	4.011		
	Total	543.132	68			
Ash content	Between Groups	.217	5	.043	14.967	.000
	Within Groups	.183	63	.003		
	Total	.400	68			
HMF	Between Groups	1248.299	5	249.660	2.702	.028
	Within Groups	5821.733	63	92.408		
	Total	7070.033	68			
pH value	Between Groups	2.385	5	.477	8.186	.000
	Within Groups	3.671	63	.058		
	Total	6.057	68			
Free acidity	Between Groups	1319.431	5	263.886	5.381	.000
	Within Groups	3089.572	63	49.041		
	Total	4409.003	68			
Fructose	Between Groups	177.982	5	35.596	3.168	.013
	Within Groups	707.865	63	11.236		
	Total	885.847	68			
Glucose	Between Groups	143.546	5	28.709	4.131	.003
	Within Groups	437.787	63	6.949		
	Total	581.334	68			
Sucrose	Between Groups	5879.493	5	1175.899	30.900	.000
	Within Groups	2397.435	63	38.055		
	Total	8276.928	68			

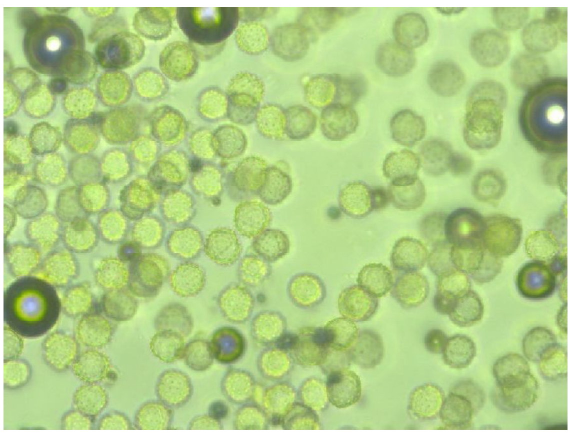
Annex 17: Pollen pictures of some major bee flora that provide honey in the study area



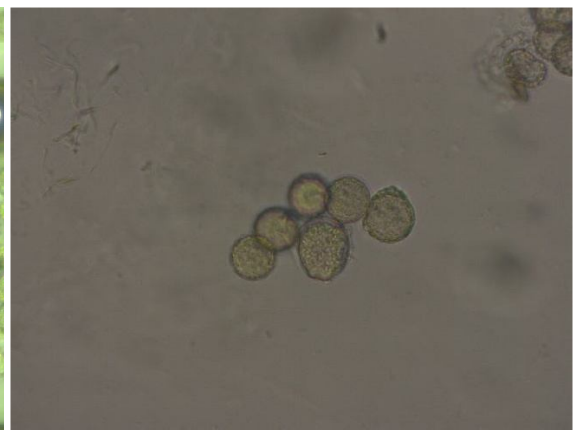
Pollen of Guzotia spps



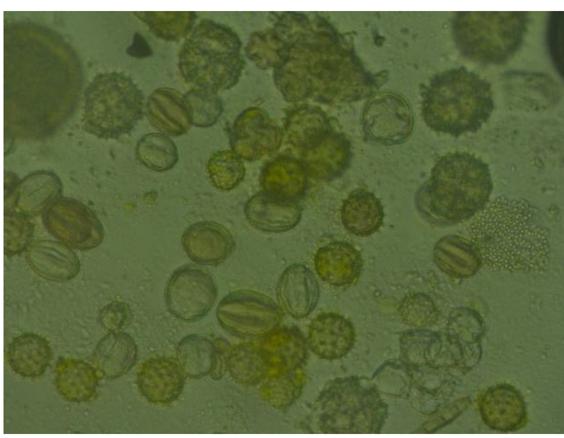
Pollen of Eucalyptus



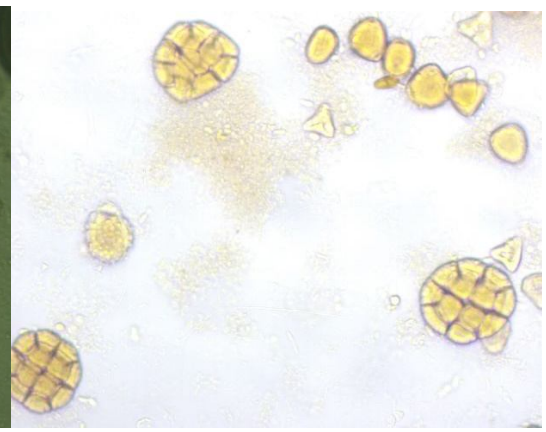
Pollen of Biden spps



Pollen of *Hypoestes forskaoli*



Pollen of Vernonia Spps



Acacia spps

Annex 18: Ethical clearance certificate

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/31/02/15/2023

Name and affiliation of applicant: **Melaku Bekele Tufa (BSc, MSc student)**
Department of Animal Production Studies, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Assessing and evaluating honey quality at different market points in Adama District and Adama towm*

Date of application:	**November, 2021**
Nature of the project:	**Field investigation**
Target animal species:	**None**
Number of animals involved:	**None**
Study area:	**Adama, Ethiopia**

Minutes No. and date of review: **VM/ERC/02/14/022, 01/03/2022**

The Animal Research Ethical Review Committee of the College of Veterinary Medicine and Agriculture of Addis Ababa University has reviewed the above research project and unanimously approved the application of Melaku Bekele.

Professor Getachew Terefe (DVM, PhD) — Signature
Chairman

The Federal Democratic Republic of Ethiopia — Addis Ababa University College of Veterinary Medicine and Agriculture

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Annex 19: Whole document plagiarism test report result

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