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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

Bishoftu

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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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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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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 pre-

harvest 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.

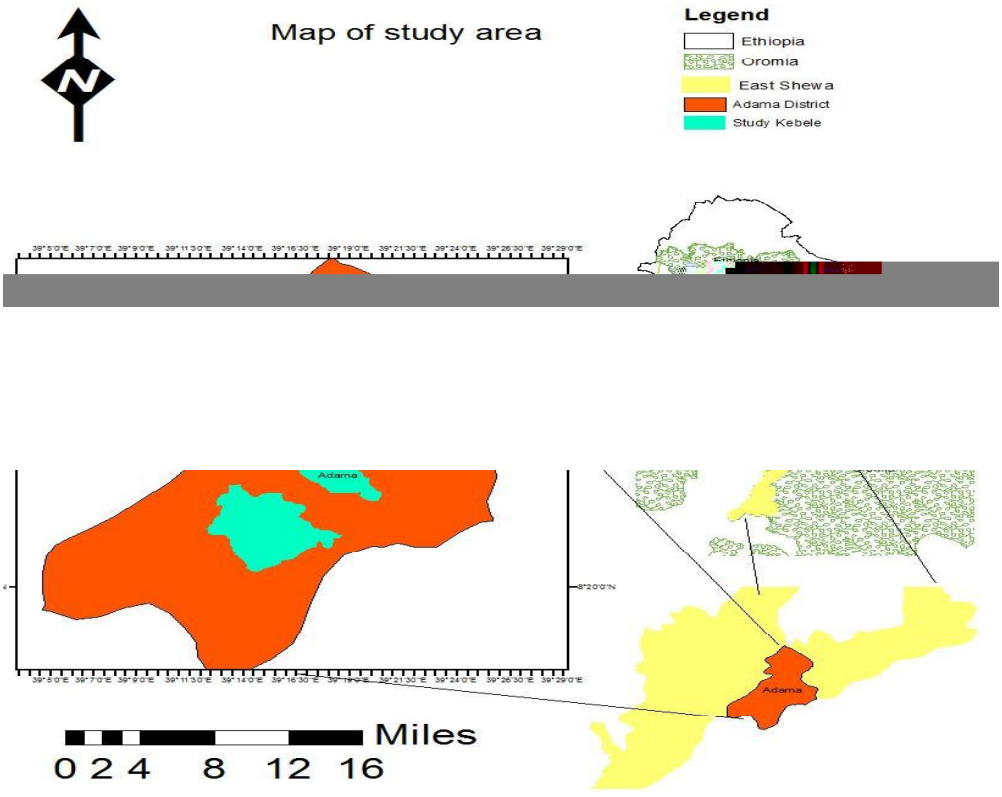


Figure 1. Map of the Study Area (Adama Town and the study kebeles in Adama District)

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

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.

(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±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.

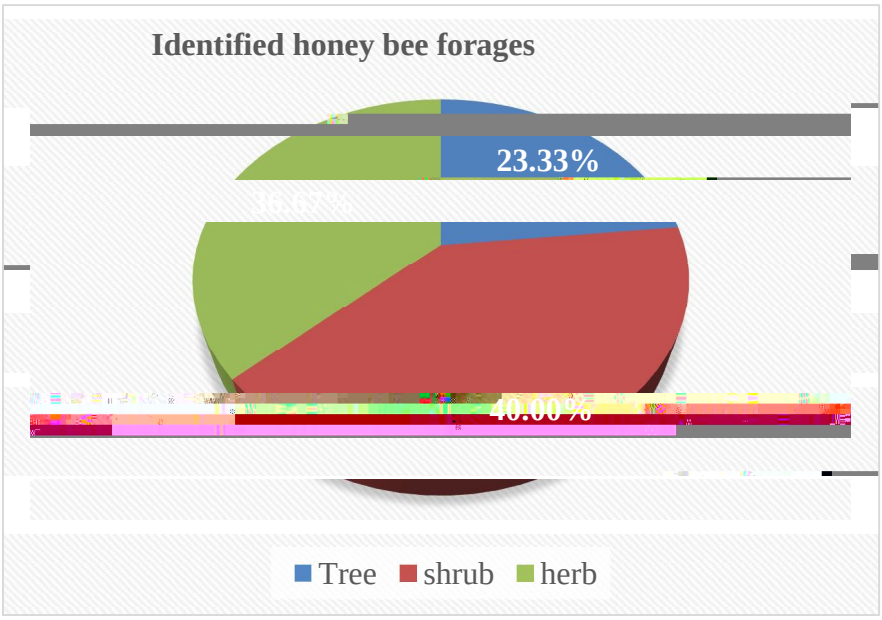
4.5. Sugar Composition Honey from Different Honey Markets

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).

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 Gebremaria havo

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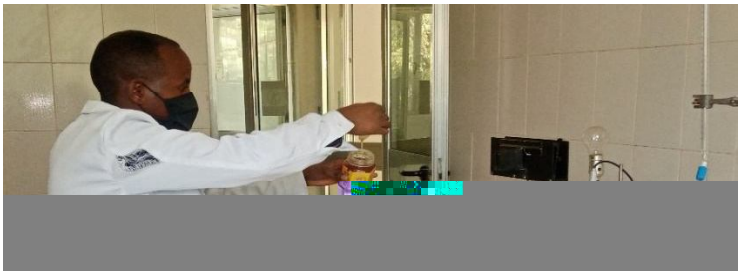
APPENDIX

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

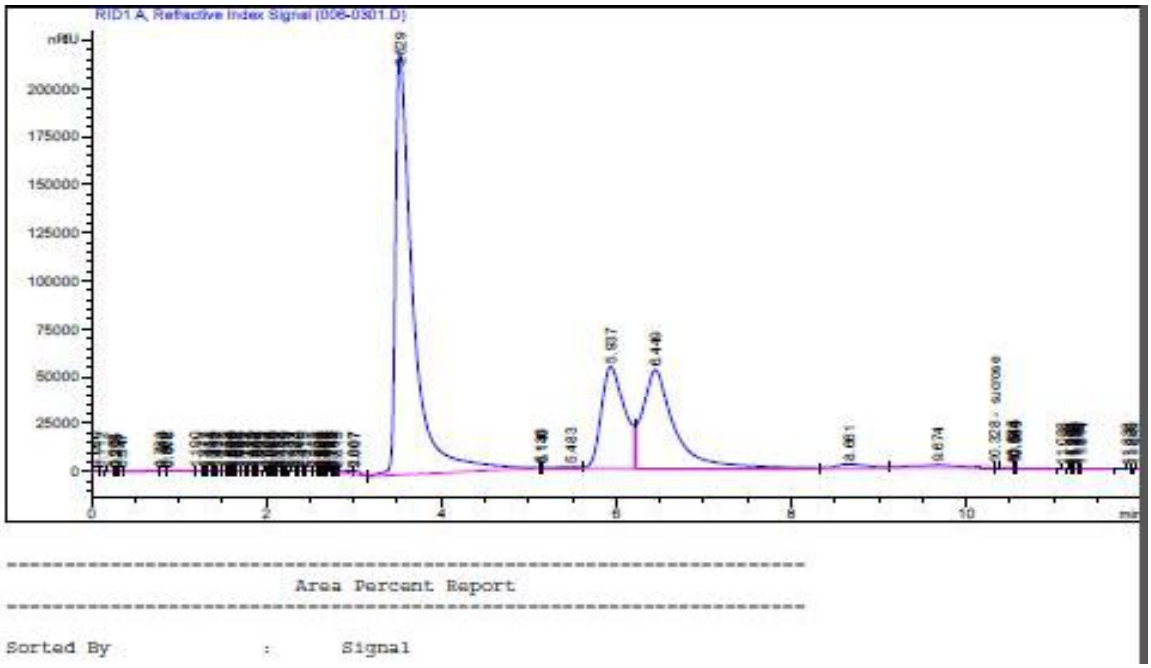
Annex 4: HPLC devices with all its compartments and computerized sets which performs sugar analysis

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

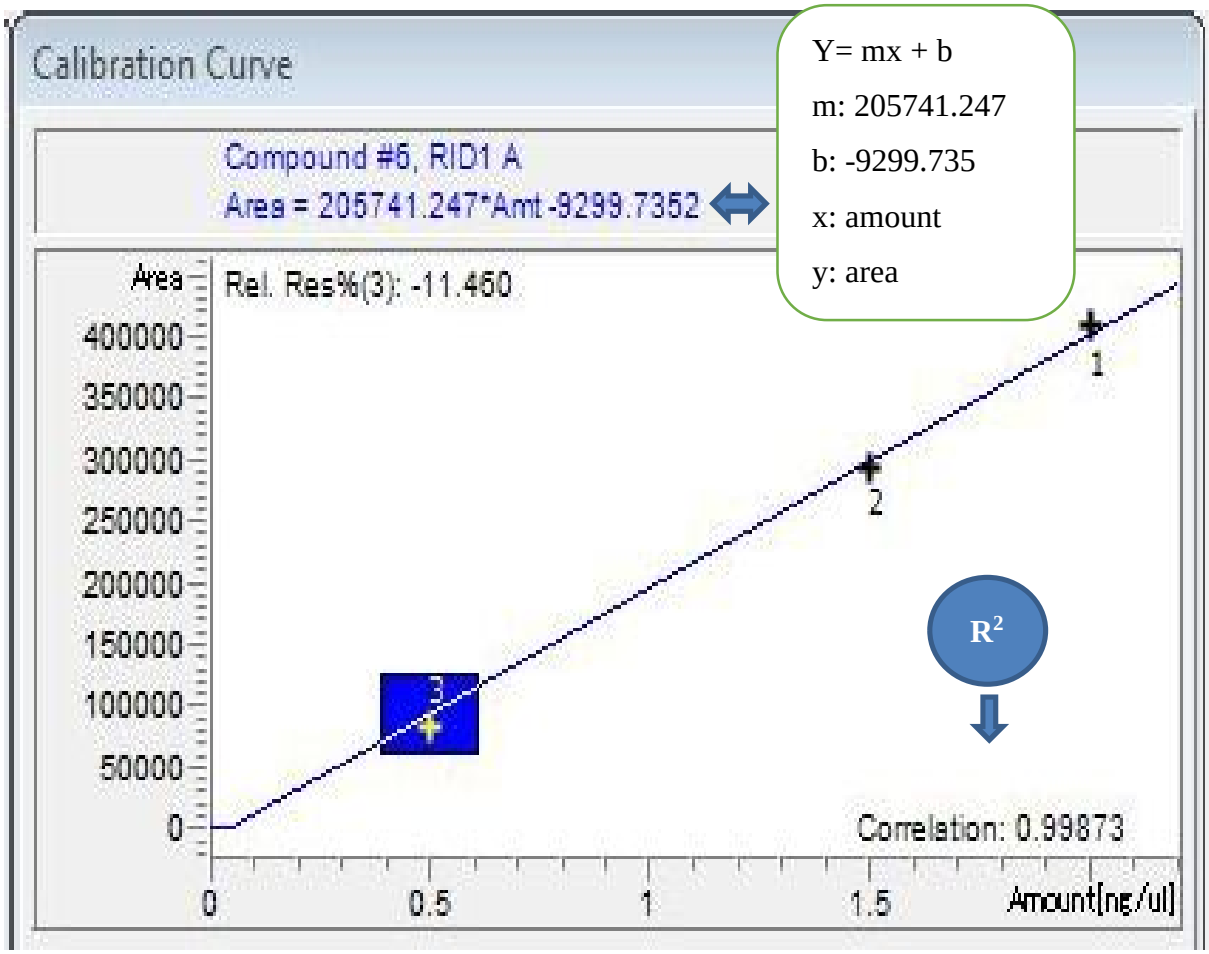
Annex 6: HPLC devices with all its sampler unit (injection unit), pumping unit, column unit, pressure unit and temperature unit and sample analyzed result

Annex 7: Sample honey ash content identification performing

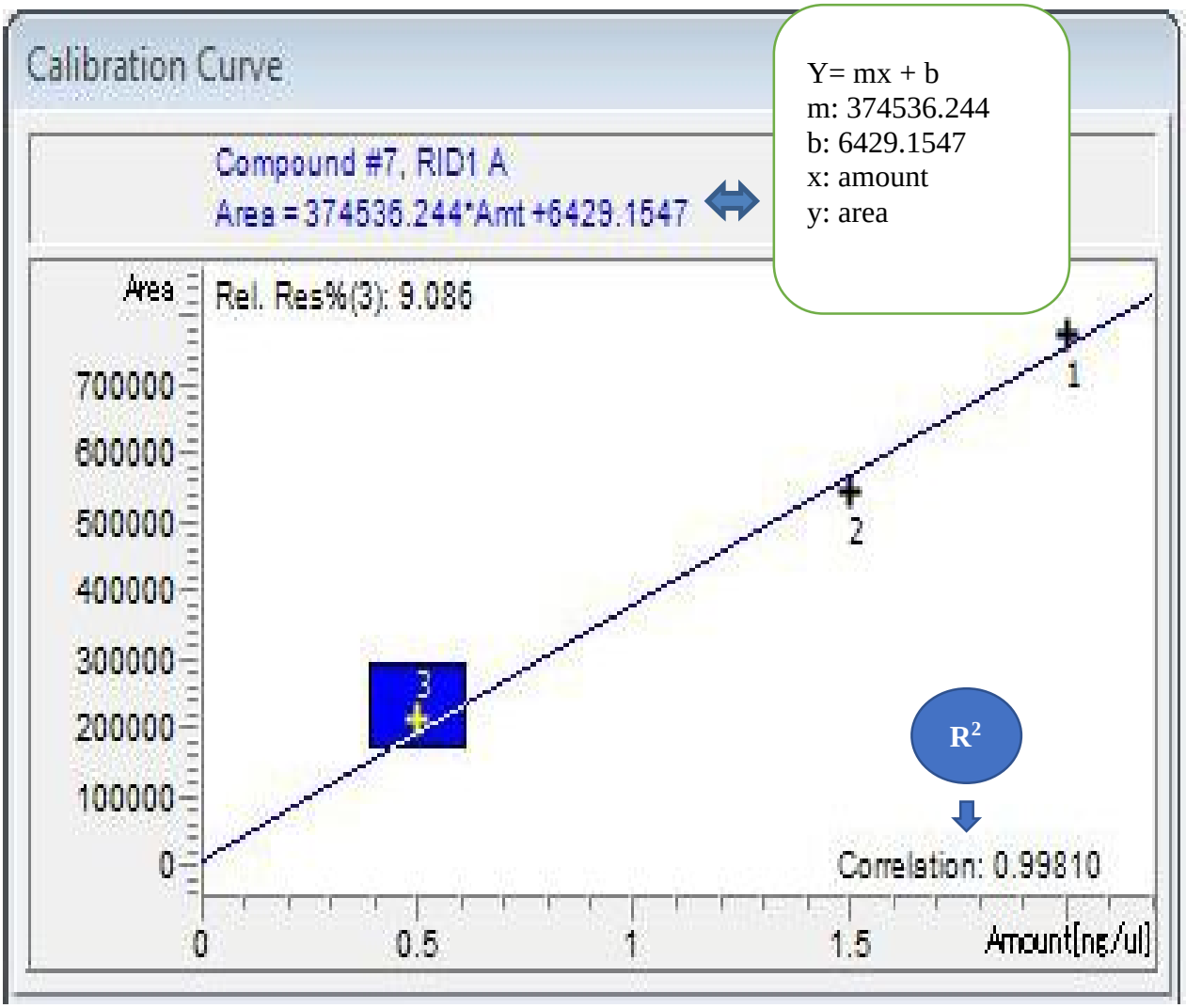
Annex 8: Chromatogram by HPLC, of fructose, glucose, and sucrose of honey samples different market point



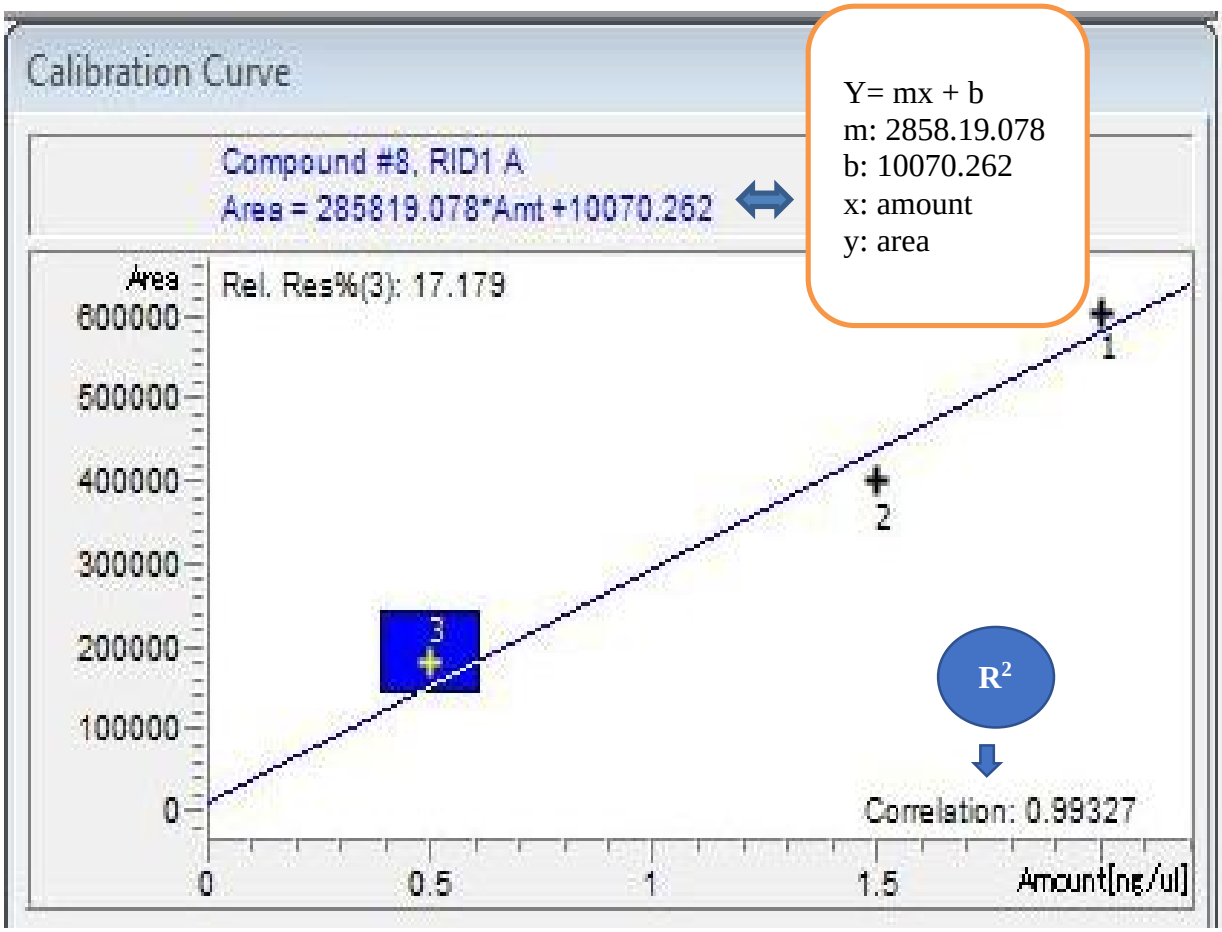
Annex 9: Standard fructose calibration curve



Annex 10: Standard glucose calibration curve



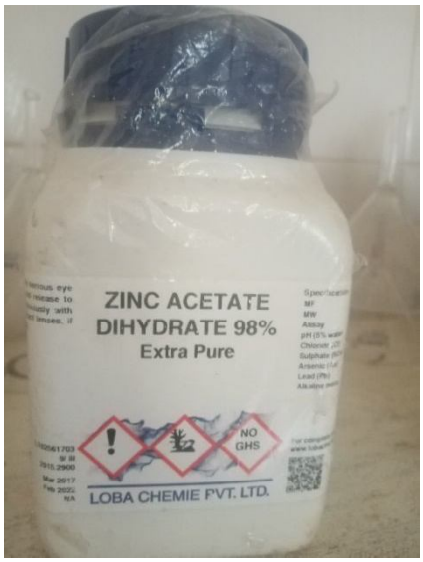
Annex 11: Standard sucrose calibration curve

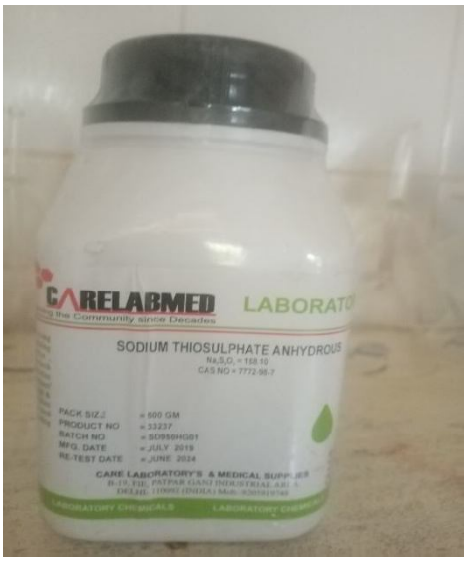


Annex 12: Standard fructose, glucose and sucrose sugar used for HPLC calibration

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





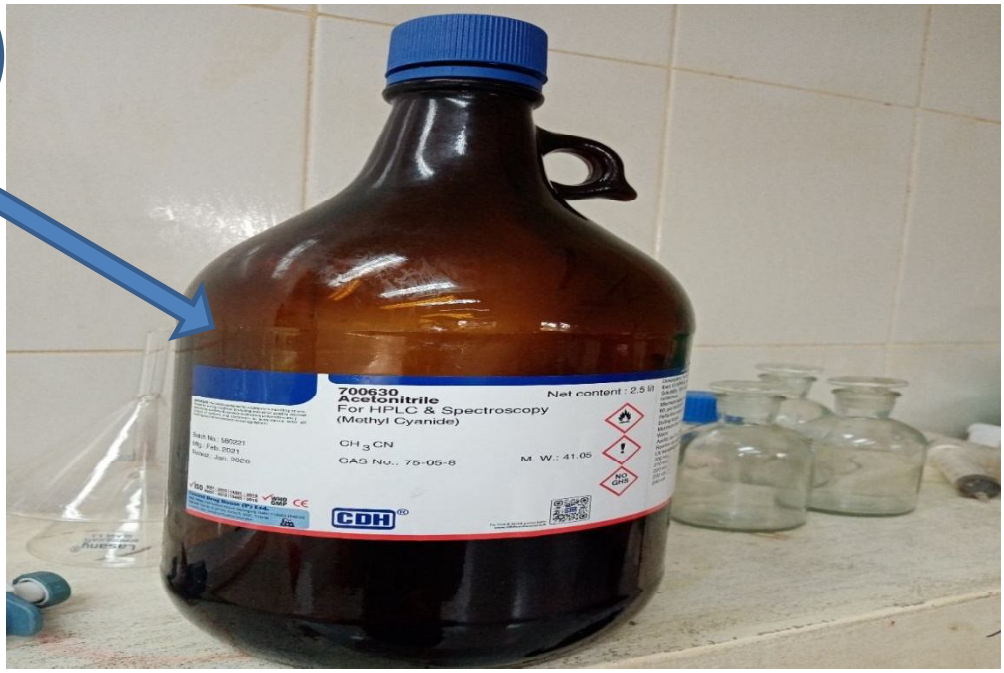
Sodium bisulphite

Potassium hexacyanoferrate

Zinc acetate

Annex 15: Reagents used for determination of sugars by HPLC

Acetonitrile for HPLC and Spectroscopy



Standard water for HPLC

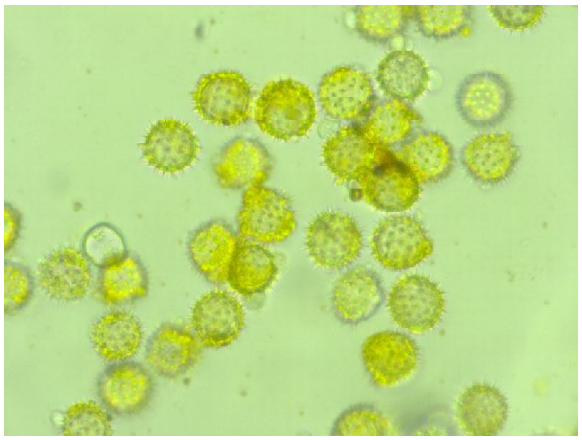
Pure Methanol

Annex 16: Summary of ANOVA

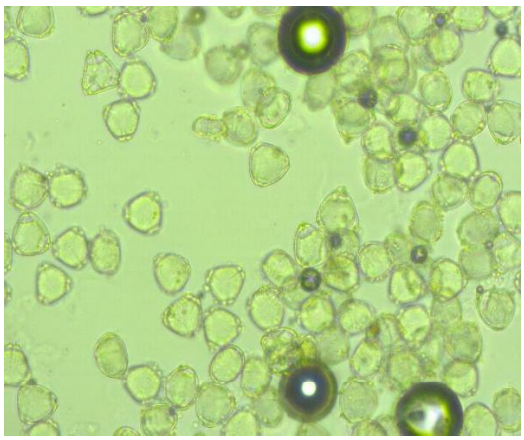
ANOVA

		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			

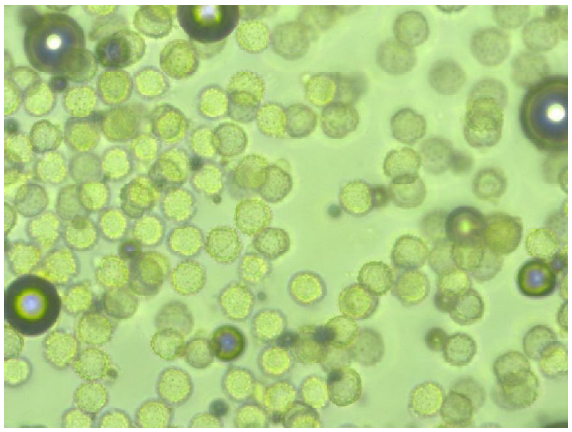
Annex 17: Pollen pictures of some major bee flora that provide honey in the study area



Pollen of Guzotia spps

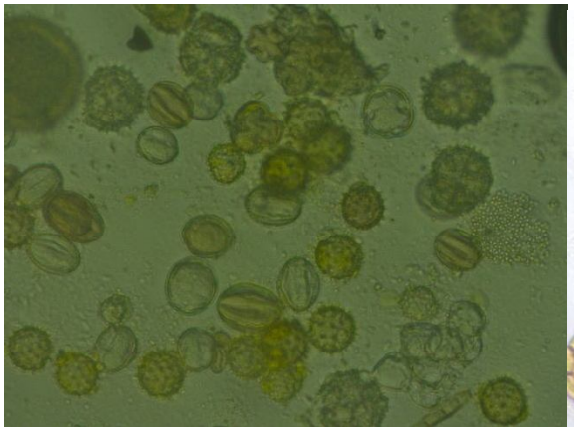


Pollen of Eucalyptus

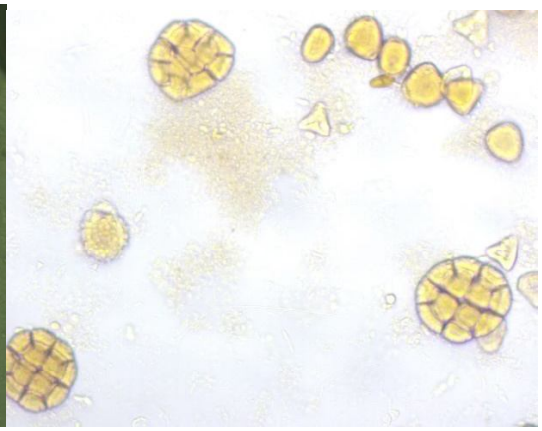


Pollen of Biden spps

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
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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)
Chairman

Signature

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

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