UNIVERSITY OF NAIROBI

Physicochemical Characterization of Honey from Selected Counties in Kenya

By

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A Research Thesis submitted to The University of Nairobi in partial fulfillment of the requirements for the award of degree of Master of Science in Nuclear Science.

Declaration

I hereby declare that this research thesis is my own original work and has not been submitted to any other university for purposes of registration or award of any degree.

Evalyne Jepkorir Rotich, S56/78896/2015

Signature ..... Date 19th Nov 2019

This thesis has been submitted with our knowledge as university supervisors:

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DEDICATION

I would like to dedicate this thesis to my late parents; Albert and Mary, who died young but will never be forgotten, my guardians; Rtd Major Bartai and Rose Bartai, who have always supported me and cheered me on, in my studies and in life from a very tender age until now. I love you all so much.

ACKNOWLEDMENT

I would like to acknowledge and thank my two supervisors; Mr Michael J. Mangala and Prof John M. Onyari for their guidance and support.

Many thanks go to the Director and staff of National Beekeeping Institute, for permission to carry out part of my research in their laboratories and for laboratory assistance in the analysis of honey samples.

Lastly, I thank the staff members of Institute of Nuclear Science and Technology, the University of Nairobi and Radiation Protection Board of Kenya and my classmates for their encouragement and assistance to do better, always.

ABSTRACT

Honey is a natural product with unique flavour and is highly nutritious. It is produced by honeybees and consumed by many. It contains sugars, proteins, water and minerals just to mention a few. There is growing interest in the food industry to characterize food samples for mineral content, in particular. The main objective of this study was to characterize local honey based on physicochemical analysis and mineral content. The parameters that were used in characterization of honey, in this study include determining physicochemical parameters like pH, sugars, Hydroxymethylfurfural (HMF) and trace elements. This was achieved by the measurements of pH, free acidity using titration, HMF and sugars using HPLC and trace metals using EDXRF and ICP-OES. A total of sixty (60) samples of refined honey from two regions; Baringo and Kitui were studied. Sixteen (16) elements, namely Fe, Cu, Mn, Zn, Cr, Ba, Mo, Ni, Ti, As, Cd, Co, Hg and Sn were assessed. The most abundant elements were Fe and Zn with mean concentrations of 19.82 ± 2.74 µg/g and 2.89 ± 0.77 µg/g, respectively for Baringo samples and 24.49 ± 3.38µg/g and 1.92 ± 0.41 µg/g for Fe and Zn, respectively for Kitui samples. Cu levels averaged 1.99 ± 0.38µg/g for Baringo samples and 1.04 ± 0.16 µg/g for Kitui samples. Cr and Ba levels were less than 0.25 µg/g in all samples from both regions. The other elements; Mo, Ni, Ti, As, Cd, Co, Hg and Sn were below their detection limits. The pH average values averaged 3.64 ± 0.28 and 3.46 ± 0.31 µg/g for honey samples from Baringo and Kitui, respectively. These levels are within the EU recommended limits and are consistent with most studies. Naturally, honey is acidic and thereby allows for self-preservation. In general, free acidity averaged 29.67 ± 5.38 milliequivalents/Kg for Baringo and 30.85 ± 4.27 milliequivalents/Kg for Kitui, all values below the KEBS recommended limits of a maximum of 50 milliequivalents/Kg. The total sugars were found to be 65.29% and 59.9% for Baringo and Kitui, respectively. The average HMF levels were 30.05 ± 3.55 mg/Kg and 3.91 ± 0.18 mg/Kg for Kitui and Baringo, respectively. A few samples from Kitui had elevated levels of sucrose (> 5%) and HMF (> 100 mg/Kg), an indication of adulteration or prolonged storage periods. After subjecting the results to statistical analysis (student's t-test), all parameters of the samples from Baringo and Kitui had significant difference, except for free acidity, Fe, Mn and Cr levels. Maltose were not present in Kitui samples, and uniquely distinguishes between the honeys from the two regions. In general, these results indicate good quality honey free of toxic heavy metals.

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LIST OF ABBREVIATIONS AND ACRONYMS

FES – Flame Emission Spectroscopy

FAAS – Flame Atomic Absorption Spectrometry

ICP-OES – Inductively Coupled Plasma Optical Emission Spectroscopy

ICP-MS – Inductively Coupled Plasma Mass Spectroscopy

TXRF – Total Reflection X-Ray Fluoroscopy

HMF – Hydroxymethylfurfural

HPLC – High Pressure Liquid Chromatography

HPAEC-PAD- High Performance Anion-exchange Chromatography – pulsed amperometric detection

GCS – Geographic Coordinate System

WGS – World Geodetic System

TDS – Total Dissolved Solids

EDXRF – Energy Dispersive X-ray Fluorescence.

ICIPE – International Centre for Insect Physiology and Ecology

IRMS – Isotope Ratio Mass Spectrometry

RID – Refractive Index Detector

GC/MS – Gas Chromatography/Mass Spectroscopy

KEBS – Kenya Bureau of Standards

FAO – Food and Agriculture Organization of the United Nations

IHC – International Honey Commission

UV – Ultra-Violet

WEEMA – Water Education Economic Empowerment Medical care and Alliance

WHO – World Health Organization

EU – European Union

AOAC – Association of Analytical Chemists

NFIS – National Farmers Information Services

PC – Personal Computer

SCIRA – Stable Carbon Isotope Ratio Analysis

ASAL – Arid and Semi-Arid Land

SRM – Standard Reference Material

GDP – Gross Domestic Product

XRF – X-ray Fluorescence

IPGRI – International Plant Genetic Resources Institute

KHC – Kenya Honey Council

KS – Kenyan Standard

EAS – East African Standard

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CHAPTER ONE

INTRODUCTION

1.1 Introduction

In Kenya, honey production is an economic activity that is practiced by most farmers in the rural. It does not require a lot of investment as other food crop farming except for occasional beehive maintenance. About 80% of Kenya's land has the potential to produce honey (Muli, Munguti, & Raina, 2007) and there is a high demand for honey products worldwide and locally.

In order to create awareness of the economic benefits of bee keeping and their products, the Kenya Honey Council provides a forum for stakeholders in the beekeeping industry, to safeguard and to promote their interests and expand their growth. Specifically, these include; diversity and availability of bee flora, potential for commercial honey production, introduction to new technologies (like the modern hives and equipment), access to financing and marketing.

However, some of the weaknesses that beekeepers experience includes; limited local research studies, awareness of modern bee management practices and lack of enthusiasm for most farmers. Other challenges include, absconding and migration nature of bees from their habitats, which is mainly caused by human activities; deforestation, construction, pest control through spraying using toxic chemicals etc (Le Conte & Navajas, 2008). Differences in climatic conditions greatly affect the apiculture and agricultural sector in general. Honey can be found in both dry and wetlands depending on the types of bees prevalent in the area.

Worldwide, honey has been used as a biological monitor of the environmental air quality for pollution. For example, air quality can be indirectly investigated by physicochemical analysis of honey for mineral content for; toxic heavy metals, pH and conductivity levels. Since these heavy metals cannot be destroyed or be degraded, they can enter into the human body following ingestion (Afzal, et al., 2014). Therefore, it is essential to determine the amount of trace metals that are in honey. Agricultural diversity is an important component for food security in general. In most parts

of Kenya, agricultural diversity is practiced through crop production and livestock keeping, to increase productivity.

Most often, honey is sometimes used instead of sugar as a source for minerals supplement. For diets that are mainly based on carbohydrates and fats, are said to contribute to diseases like obesity, heart diseases, diabetes and other various types of cancers that are on the rise all over the world (Mouillé, Charrondière, & Burlingame, 2006). Other honey parameters, such as sugars, minerals, volatile compounds, flavonoids and organic acids have been used for characterization of honey. Some of the techniques employed include; Flame Atomic Absorption Spectrometry, Flame Emission Spectrometry, Inductively Coupled Plasma-Mass Spectrometry, Inductively Coupled Plasma-Optical Emission Spectrometry, Total Reflection X-ray Fluorescence, Ion Chromatography, amongst others.

The mineral content in honey can be used to identify its botanical and geographical origin as well. The amount of minerals in honey brings out the different colours in honey and they vary from light to very dark colours. Schuette & Remy, 1932, and his colleagues suggested a connection between the amount of minerals and the degree of honey pigmentation which was later confirmed by other scientists. White & Doner, 1978 confirmed that honey with light colour had lower mineral content compared to honey with dark colour which had a higher mineral content. Furthermore, all these parameters depend on the climate and the types of vegetation. The amount of moisture content is linked to the degree of maturity of honey and the climatic conditions; abnormal values may be an indication of honey adulteration. However, all these factors highly depend on the processing techniques, botanical origin of the sample and the storage conditions. Honeys from different regions indicate differences in mineral contents in their compositions.

Honey production capacity varies widely from one country to another, for example, China produces 170,000 tonnes, the highest production yields while Argentina produces 45,500 tonnes (Oliveira, et al., 2015). According to the WEEMA, 2016, Ethiopia is the largest producer of honey in Africa; followed by Tanzania, while Kenya is ranked third. Kenya has a high consumption of honey and some honey dealers import it from Tanzania when the demand cannot be met locally. Generally, the highest importers of honey in the world are mainly United States, Germany, Japan, United Kingdom and France (Oliveira, et al., 2015).

Honey residue is a worldwide concern since it affects the quality of honey. Honey residue is the substances that remains after processing and act as a contaminant. Some of these residues include radioactive material, heavy metals and antibiotics. The main sources of these residues are from beekeeping practices and the environment. However, contamination from heavy metals results from traffic and industrial pollution while antibiotics and pesticides result from agricultural practices. Honey therefore is a bio-indicator of environmental pollution (Zane, Maris, Vita, & Arturs, 2013).

Honey is an important nutrition in children; whose daily dietary intake is essential to improve their immune system and to ensure a steady growth. The total mineral content in honey is low but is significant, if consumed frequently. Major elements in honey like sodium plays an important role in maintaining the optimum blood pressure and proper functioning of the kidney, nerves and the muscles. Magnesium is also important since it acts as a cofactor for enzymes in which most of them have antioxidant properties. Lack of magnesium contributes to aging and other disorders related to aging. Trace elements are useful only in certain levels above which, they become toxic to the body since the body cannot get rid of them anymore (Solayman, et al., 2015).

Research shows that some of the bee products contain vitamins, healthy fats and minerals. All these come from the royal jelly, bee venom, propolis and the pollen (Wamwangi, 2012). These bee products can be used for disease treatment and to improve nutritional regulations and health. The pollen is rich in protein and can therefore be a good source for nutritional requirements. Propolis contains essential oils, beeswax, some resins and some pollen and it can also be a very good source of nutritious substances. Propolis also contains minerals, bioflavonoid and amino acids (Shahram, Yarsan, Erici, Tumer, & Demirbas, 2012).

Honey has been characterized based on its physical, biological or chemical properties. The physicochemical parameters that have been used for characterization include; water content, pH, free acidity, ash content or mineral content and electrical conductivity (Kebede, P.A, & Gebrekidan, 2011). For determination of botanical origin of honey, electrical conductivity is used. The electrical conductivity measured, is mostly related to the other contents of honey which include the proteins, mineral salts and organic acids and is, extremely useful when it comes to differentiating the floral origins of the honey (Soares, Soares, Pires, Novaes, & Junio, 2008). The amounts of ash content in honey is highly dependent on the composition of nectar and the main

plants that this nectar comes from. Consequently, the variation in ash content has been related qualitatively to the different geographical and botanical origins of honey. For honey that is generated from plant nectar, the total ash content must be less than 0.06% but for honeys generated from honeydew, it should be less than 1% (Bogdanov & Martin, Harmonised Methods of the European Honey Commission, 1997).

In general, most of the natural honeys are highly saturated with sugars like glucose and fructose and have low pH values which are in the range of 3.2 to 4.5. This pH range is acidic and can be attributed to organic acids like acetic acid, gluconic acid and ascorbic acid which inhibit bacteria growth and allows honey to have a longer shelf life. Fermentation of honey depends on the moisture content and in turn depends on the season when the honey is harvested and the degree of maturity of the honey in the hive. The higher the moisture content, the faster honey will ferment and granulate, and this fermentation process lowers the quality (Equar, Abraha, Lemma, & Amare, 2015). The level of moisture in honey also depends on how it is handled during processing and harvesting.

The minerals in honey are highly stable and are very useful in classifying honey, they also show a relationship to the soil where the vegetation containing the nectar is grown. Approximately 7 km^2 radius has been considered as the distance where most bees forage to look for nectar. In general, the mineral contents of honey ranges between 0.1% to 1.0% and this value varies widely depending on the climatic conditions, extraction techniques used and botanical origin of nectar (Zane, Maris, Vita, & Arturs, 2013).

This study focused on areas in Kitui and Baringo counties where honey is extensively produced. These two counties were chosen for the study, because they are the top honey producing counties in Kenya and contribute substantially to the total amount of honey that is harvested in Kenya. Kitui County is located in the former Eastern Province and it is semi-arid while Baringo County is in the former Rift Valley Province.

In both counties, honey production is a valuable economic commodity and it contributes significantly to creation of employment, food security and poverty reduction.

The government of Kenya, through its strategic development projects for emerging livestock and apiculture, has identified honey production as key for economic growth and is working towards; training of bee farmers and in provision of better beehives among other efforts. Accordingly, Kenya produced an average of 25,000 metric tonnes annually from 2 million beehives. In Kenya, beekeeping is an integral part of the livestock farming and contributes to more than 10% GDP, beekeeping contributes to more than 2% of this GDP (Wambua, Musimba, & Muli, 2016) (Ministry of Agriculture, 2019).

1.2 Problem statement

In Kenya, most honey quality is compromised through adulterations, through addition of food syrup and commercial sweeteners.

This study will enable the characterization of honey from selected parts of two counties of Kenya that produce honey. This includes Baringo county and Kitui county which are some of the counties in Kenya that have high production capacities of honey.

Most research on honey in Kenya has been focusing on physicochemical properties of honey. However, trace elements and mineral content aspect has not been considered for quality evaluation. This study enables for a combination of both aspects to characterize the honey from these two regions.

The main beneficiaries of this study are the consumers of honey and the Apiculture industry and trade.

1.3 Main objective

Characterization of honey based on physicochemical analysis and trace element content

1.4 Specific objectives

a) To determine the variations of physicochemical properties and trace elements in different honey samples from two regions, Baringo and Kitui counties;
b) To evaluate the honey quality and nutritional requirements for compliance with local and international standards;

c) To determine heavy metal residues in the honey samples that are a potential risk to human health.

1.5 Justification, Scope and Limitation

Although research on characterization of honey has been done in many other countries, there is need for getting such information on Kenyan honey especially so, from honey producing counties. Kitui and Baringo counties produce high amounts of honey locally. It is therefore important to know the properties and characteristics of this honey so that its quality can be maintained or even improved. This can greatly increase the market share for honey from both these places, locally and internationally.

There is need for labeling of the amounts of trace elements in Kenyan honey and other physicochemical parameters for purposes of food nutritional labeling and trade requirements.

The legislation on honey production in Kenya needs to be revised to include the levels of the various honey quality indicators, as a basic requirement for the international market requirements and consequently for improvement of trade.

In this study, most of the honey samples used were purchased from the roadside vendors and as such, honey samples could not be categorized in terms of types; unifloral, multifloral or honeydew.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Human beings have consumed honey since time immemorial. Honey is popularly used as food and for medicine, culturally, in most local traditions. Other users of honey and its products include; the textile industry that uses wax, the cosmetic industry and dairy farmers. China, Turkey, Argentina, USA, Ukraine, Russia, India, Mexico, Iran, Brazil and Ethiopia just to mention a few, are some of the countries that produce large quantities of honey. Worldwide, honey has been identified as an economic source for many with an estimated production capacity of about 1.2 million tonnes of honey produced annually (Shahram, et al., 2012).

Current globalization of honey market involves nearly 150 countries and some of the honey dealers have coined a phrase "*the money is in the honey*". This makes honey a very important economic commodity.

However, the consumers are sometimes mainly confronted with challenges presented with the quality of these products, which are subjected to instances of frequent alterations. Some of the factors that lead to adulteration of honey by dealers are economic issues; to satisfy high demands for honey and in some cases, extreme weather conditions. This can happen, for example, when there is not enough nectar at a particular season and the bees are fed on sugar syrup or commercial sweeteners.

Honey has been accepted and is used as medicine and food for a very long time by mankind. Egypt was one of the first nations to practice beekeeping. Both ancient and modern civilizations use honey as a remedy for various ailments; wounds, burns, diabetes, ulcers, cataracts etc. These traditional uses of honey have been validated by many researchers worldwide (Liyanage & Horadugoda, 2017). In some of the Kenyan traditions, honey has been used for wine making and in traditional brew and as payment for bride price.

2.2 The Composition of Honey

Different honeys contain unique combination of elements and properties because of the variety of nectar sources in the geographical location of production, climate of area of production and processing and storage methods (I. Turhan, Karhan, Gurel, & Tavukcuoglu, 2008). The mineral content in honey is affected by the geographical origin (Bogdanov, et al., 2014).

The minerals in honey have nutritional significance for the human body; vitamins like folic acid, vitamin K, vitamin C, thiamine, niacin, riboflavin among others. However, most of these vitamins are lost at extreme heating conditions above 40ºC. It is therefore advisable not to heat honey at high temperatures during processing.

Naturally, honey can be preserved for a long time because it contains acids that contribute to its stability against micro-organisms and its flavour. The volatile components also contribute towards the aroma and flavour. Some of the volatiles in honey are acetaldehyde, formaldehyde, acetone, diacetyl among others (Liyanage & Horadugoda, 2017). Upon storage for a longer period of time, there is increase in alcohol contents such as pentanol, 2-methyl-1-butanol, n-propanol and 3-methyl-1-1-butanol due to fermentation. This may be products of hydrolysis of esters or they may arise from the corresponding amino acids.

Nectar is the primary source of minor elements in the soil that are transported through the plant and find its way in honey (Hernandez, Fraga, Jimenez, & Arias, 2005) (Equar, et al., 2015). There are external and internal factors that influence the production of nectar; the size of the flower, the age of the flower and its maturity on the plant, the nectary surface and the species and variety or cultivated species to which the plant belongs (Stihi, Chelarescu, Duliu, & Toma, 2015). The external factors include use of fertilizers and the type of soil, soil humidity, the temperature and the direction of wind and the time of day or the time of year.

Other factors that affect the components in honey include, beekeeping techniques, extraction and processing by the beekeeper or the commercial producer and the changes induced by the conditions of storage. Processing of honey at high temperatures destroys some of the significant nutritional components like the vitamins and the volatiles. If heavy metals are present in high amounts in the honey, then it can be harmful to human health. This is because heavy metals cannot be destroyed, and they are non-biodegradable.

2.2.1 Review of Studies on Trace Elements Content in Honey

The minerals in honey vary according to the geographical and botanical origin. The essential trace elements in honey are desirable because of their nutritional value. On the other hand, heavy metals in higher levels are not beneficial because of their toxicity. However, some of these heavy metals are very important when it comes to maintaining metabolism in the human body. Examples of such heavy metals include Zn, Cu and Se (Shahram, et al., 2012).

Kebede et al., 2011 reported that Ethiopian honey samples showed the presence of elements such as Cd, Cu, Cr, Co, Ni, Fe, Mn and Zn. Among the elements investigated, Cd, Cr and Co had the lowest concentration while Fe and Cu had the highest concentration. Table 2.1 shows the typical natural chemical element composition present in honey.

Table 2.1: Natural chemical elements in honey. Adopted from *(Ajibola, Idowu, Oyefuga, & Iquot, 2007)*

Element	**Amount (mg $100g^{-1}$)**	**Element**	**Amount (mg $100g^{-1}$)**
Potassium (K)	40 - 3500	Rubidium (Rb)	0.04 - 3.5
Iodide (I)	10 - 100	Strontium (Sr)	0.04 - 0.35
Calcium (Ca)	3 - 31	Iron (Fe)	0.03 - 4
Phosphorus (P)	2 - 15	Manganese (Mn)	0.02 - 2
Sodium (Na)	1.6 - 17	Copper (Cu)	0.02 - 0.6
Sulphur (S)	0.7 - 26	Arsenic (As)	0.014 - 0.026
Magnesium (Mg)	0.7 - 13	Aluminium (Al)	0.01 - 2.4
Chlorine (Cl)	0.4 - 56	Barium (Ba)	0.01 - 0.08
Fluoride (F)	0.4 - 1.34	Chromium (Cr)	0.01 - 0.03
Bromine (Br)	0.4 - 1.3	Selenium (Se)	0.002 - 0.01
Lithium (Li)	0.225 - 1.56	Lead (Pb)	0.001 - 0.03
Cobalt (Co)	0.1 - 0.35	Vanadium (V)	0 - 0.013
Zinc (Zn)	0.05 - 2	Nickel (Ni)	0 - 0.051
Zirconium (Zr)	0.05-0.8	Molybdenum (Mo)	0-0.004
Boron (B)	0.05-0.3	Cadmium (Cd)	0-0.001
Silicon (Si)	0.05-24		

Cantarelli, Pellerano, Marchevsky, & Camina, 2008, did a study on the chemical composition and trace elements of Argentine honey. They used ICP-OES for the analysis of trace elements and found Fe, Al, Mn, Zn, Cu, Ca, Mg, Na, K among other essential elements. The highest concentration was from K, Ca, Na and Mg, followed by Fe and Zn. Mn and Cu had the lowest concentration.

Boukka, Belouali, & Hakkou, 2008, determined some of the major and minor elements present in honey from Morocco. They found that the most abundant elements were K, Zn, Ca, Mn, Mg and Fe. They also checked the honey for heavy metals; Cd and Pb. They detected these two heavy metals in some of the samples, but they were below the maximum limits according to the European standards.

A study was done to determine the trace elements in raw honey and processed honey, in Kitui and Nyeri, by Wamwangi, 2012 and he found that raw honey had a lower level of Fe than processed honey.

2.3 Spectrometric Methods used to characterize honey for quality

Determination of metals in foodstuffs that are rich in sugar presents a challenge in analytical procedures because of interference that arise from the matrix (Ioannidu, Zachariadis, Anthemidis, & Stratis, 2004).

Chemical elements in honey can be determined using several techniques. The techniques that are commonly used are; flame atomic absorption spectrometry, flame emission spectrometry; and inductively coupled plasma mass spectrometry and inductively coupled plasma optical emission spectrometry. In some situations, precipitation titrations and acid titrations are used to determine Ag and Ca, respectively (Skoog, West, & Holler, Fundamentals of Analytical Chemistry 7th Edition, 1996).

Some other methods include; Total Reflection X-ray Fluorescence Spectrometry (Enrich, Boeykens, Caracciolo, Custo, & Vazquez, 2006), Spectrophotometric Analysis (Afzal, et al., 2014) and Infrared Spectroscopy Analysis (Lidija, Nikola, Dragan, & Domagoj, 2014). In most of these techniques, preparation of honey samples requires acid digestion before analysis.

High Performance Anion-exchange Chromatography – pulsed amperometric detection (HPAEC-PAD) has been used to detect adulterated honey products as well as floral origin characterization (Christophe, Julio, Clement, & Daniel, 2003). The use of this method has embraced pattern recognition procedures to characterize honey samples. The pattern recognition uses full sugar profiles of honey and mathematical and statistical techniques in order to identify possible adulteration by non-reducing sugars. Most of these methods incorporate other techniques so as to get chemically relevant information. Chemometric tools like Principal Component Analysis have been employed in some of the cases.

The principle of the TXRF method is based on the measurement of absorbed radiation at specific wavelengths which corresponds to the mineral of interest. Calibration of measurement instruments and the use of relevant working standards is very important in order to get accurate and reliable data.

There is a big challenge when it comes to analysis of minerals in honey. This is mainly because of the complex organic matter of the matrix in honey (Ajibola, Idowu, Oyefuga, & Iquot, 2007).

Other quality indicators of honey that are based on physicochemical analysis include: Total acidity, the higher the acidity the earlier the fermentation of honey. Acidity is mainly caused by factors such as nectar sources, organic acid variation, and enzyme activity of sugars, the bacterial activity and the mineral content present in honey. To ensure a longer shelf life of honey, there is need to keep moisture content low to avoid early fermentation. Therefore, honey should be capped when harvested and moisture content kept below 20% (Kebede, P.A, & Gebrekidan, 2011). Some common physical methods used to determine moisture content include; tilting the honey container, dip a stick in the honey container and observe how it flows and pour honey in the water in a glass to observe whether it flows in a straight or wavy line. An Abbey refractometer is used to precisely determine the moisture content (Kebede, et al., 2011).

The most common tool for investigating HMF and sugars is HPLC. Content of HMF (Hydroxymethylfurfural) is the main quality indicator especially on honey freshness and excess heating. Fresh honey may not contain hydroxymethylfurfural but the amount increases with storage time, storage temperature and the pH of honey. Sugar content includes reducing sugars like fructose and glucose as the main sugars in honey. Some reducing sugars (sucrose and maltose)

are also present in small amounts, usually 5% or below. A high amount of non-reducing sugars indicates adulteration or bees feeding on sugar syrup (Zappala, Fallico, Arena, & Verzera, 2004).

Honey parameters like flavour contributes to the uniqueness of honey. The flavour and aroma of honey can be affected by poor processing procedures, over smoking at harvest time, poor storage conditions and packaging materials. Ripeness of honey is associated by proline. The amount of proline present in honey is directly related to the ripeness of honey. As proline content reduces, the quality of honey also reduces. On the other hand, Fiehe reaction is the test mainly done to detect the presence of commercial sugar and the elimination of nutritional properties due to heating above 40ºC (Zappala et al., 2004). Diastase activity utilizes the enzymes present in honey. The enzyme amylase is important in detecting heating in processed honey. This enzyme is thermally unstable and therefore it indicates freshness and overheating. Furthermore, Lund reaction is mainly done to determine substances that naturally precipitate. Natural honey should be able to precipitate after some time of storage. Adulterated ones may not precipitate or if they do it is not as much as natural one.

Mineral content or ash present in honey is a criterion for the determination of botanical origin. However, it is much replaced by electrical conductivity which is now accepted as a worldwide standard. Water insoluble solids content generally measures the cleanliness of honey. Some of the substances that are not soluble in water include wax and any other foreign material that is insoluble in water (Bogdanov et al; 2014, Cantarelli et al; 2008).

2.3.1 Principles of ED-XRF for Trace Elements Analysis

ED-XRF spectrometry use X-ray fluorescence emission to identify and quantify the elements that are present in the sample. The principle of the energy-dispersive-X-ray-fluorescence is that, the atoms present in the sample (which can be in the form of powder, solid or liquid) are excited by interacting with incident X-rays emitted from the X-ray tube. In some cases, if increased sensitivity is required, polarization of the radiation of excitation can be done by the use of particular targets placed between the sample and the X-ray tube or other geometries may be considered such as the TXRF (Van Grieken & Markowicz, 2001). The intensity of radiation for each signal in the spectrum is unique for each element constituent and is also proportional to amount of the analyte

in the sample. X-ray fluorescence is suitable for determining metal elements in their various composition levels.

As shown in figure 2.1, the x-ray tube irradiates the sample directly, then the x-ray fluorescence coming out of the sample is measured with the energy dispersive detector. The detector directly measures the energies of the fluorescence radiation. Apart from the x-ray fluorescence from the sample, some scattered radiation might also reach the detector and form the background noise which makes it difficult to measure low concentrations of analytes (Brouwer, 2010). For such situations, lower concentration analytes are given a longer counting times or other sampling preparation techniques are used prior to the analysis.

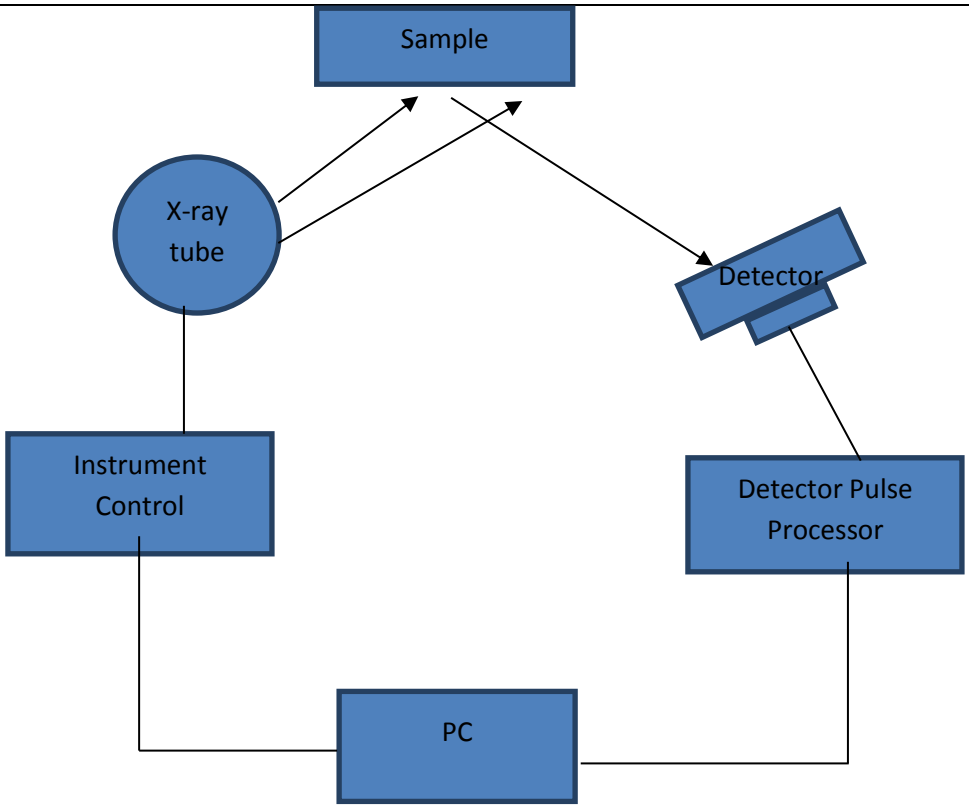

Figure 2.1: The basic components of the EDXF spectrometer and its geometry. (Adapted from Van Grieken et al., 2001).

2.3.2 Principles of HPLC for Analysis of Organic Compounds

High-Pressure Liquid Chromatography (HPLC) is basically a form of column chromatography with a pressure pump that pushes the solvent through the column. The HPLC is mainly composed

of the solvent reservoir, stationary and mobile phases, the high-pressure pump (of up to 400atm), the column, the injector system and the detector system. The main aim of HPLC is to separate, identify and to quantify compounds that are dissolved in a liquid solvent (Skoog, Holler, & Crouch, Principles of Instrumental Analysis 6th Edition, 2007). There are five main components of a HPLC and they are shown in figure 2.2.

The use of small amount of sample during analysis using HPLC enables the interaction of the small particles and the stationary phase. This leads to a better separation of the mixture and therefore improved sensitivity. The separation technique of HPLC is also very efficient and relatively fast.

Analysis of sugars and HMF using HPLC is done using different absorption wavelengths of UV absorption. HMF absorbs UV rays well between 266nm and 330nm but mostly 285nm is used because of the quality of the spectrum formed at this wavelength. The spectrum will show many other flavonoids and that is where the retention time comes in for the identification of the HMF peak (Zappala et al., 2004). As for sugars, the peaks are seen well at wavelengths between 245nm and 254nm. Depending on the detector on the instrument, the retention times will vary slightly but fructose will be first, then glucose, then sucrose and finally maltose. This is the reason why it is important to give time to the analyte to elute and in the case of HPLC such time can be up to 20 minutes (Skoog et al., 2007).

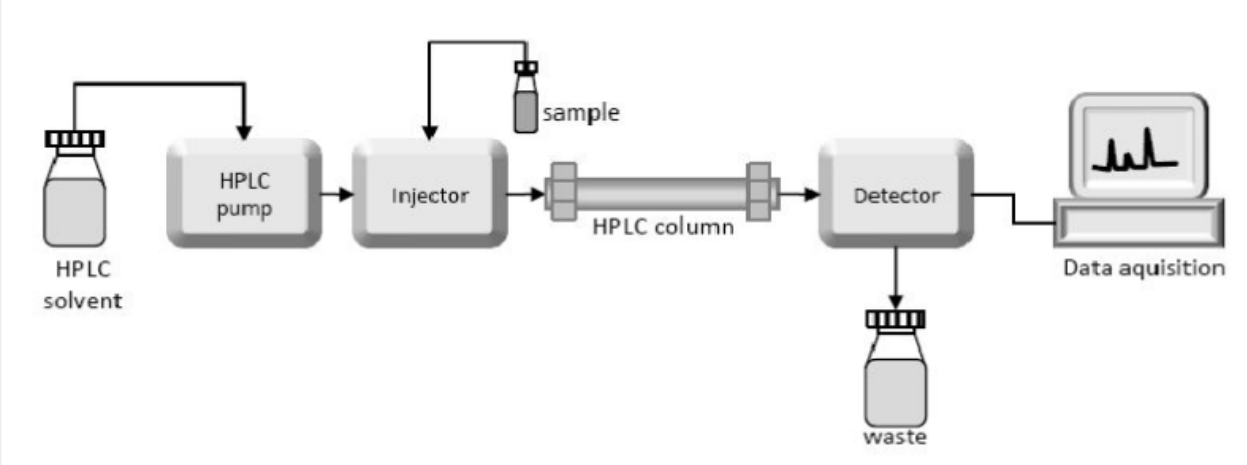

Figure 2.2: Components of HPLC

2.3.3 Principles of ICP-OES

This analytical method relies on atomic emission spectrometry and the principle of ICP-OES is that the sample is put under high temperatures which causes dissociation of the sample into atoms. The atoms are in turn excited and ionized and hence moving from the ground state to excited state. The atoms must eventually go back to lower state and in the process of decay through radiative or thermal release of energy.

In optical emission spectrometry, the intensity of the emitted light is measured at specific wavelength and this is used to determine the concentration of specific element being analyzed (Xiandeng & Jones, 2000). The Ultraviolet/Visible region of the electromagnetic spectrum is mainly used by analytical methods that employ atomic spectrometry. This is basically a range of 160 – 800 nm wavelength. This region is precise, accurate, relatively inexpensive and flexible. It is therefore suitable to use for analysis of trace elements. The main advantage of OES is the high temperature sources that it subjects to the sample (Boss & Fredeen, 2004). This creates a large number of energy levels that can be chosen from several emission wavelengths for a particular element. This means many elements can be analyzed, concurrently. However, this has a disadvantage of increasing the probability of interference because of the many emission wavelengths which might be too close to be distinguished from one another. This is illustrated by figure 2.3.

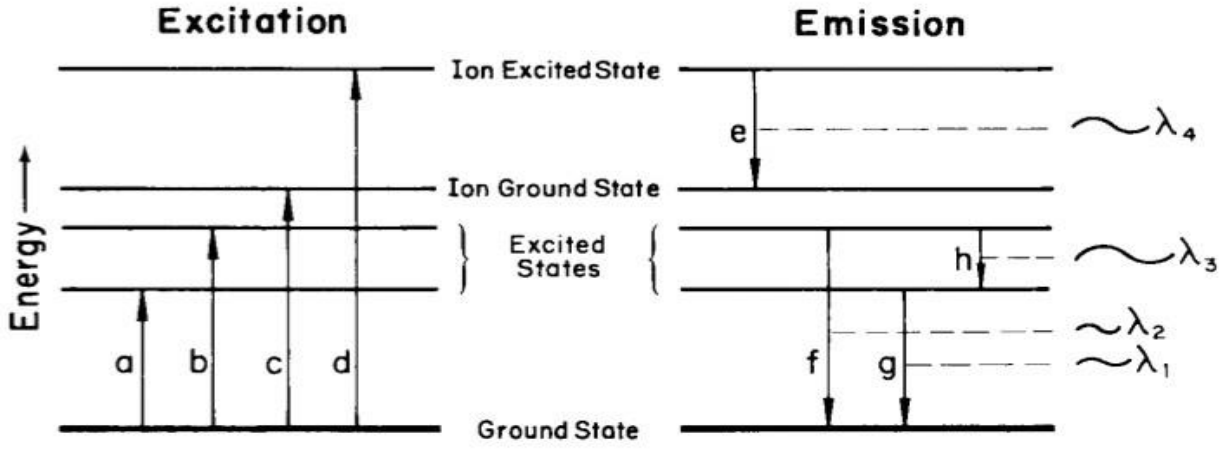

Figure 2.3: Energy Level Diagram showing energy transitions of atoms

2.3.4 Other Complimentary Methods used in Honey Quality test: Carbon Isotope Ratio Analysis for Honey

A very powerful analytical tool that uses stable carbon isotope ratio analysis for detecting adulterated honey is Isotope Ratio Mass Spectroscopy (IRMS). It can detect honey that has been adulterated using syrups of low cost which usually show sugar profiles that are very identical to natural honey (Gilberto, et al., 2008). These sugars may only be detected by the common physicochemical techniques when they are above 13%. However, other techniques like the Gas Chromatography/Mass Spectroscopy (GC/MS) can detect as low adulteration as 0.01%.

IRMS is a special technique whose measurement of changes in natural abundance of stable isotopes of Carbon (the stable isotopes mainly ^{13}C and ^{12}C) are accurate and precise. The ratios of the carbon isotopes are determined in relation to a reference gas that is calibrated using accepted international standards. The carbon isotope ratio analysis was used by Gilberto et al., 2008 to do physicochemical evaluation of Brazilian honey. The study showed that five out of twenty-one samples of honey were adulterated when physicochemical analyses alone were done. However, by analysing $^{12}C/^{13}C$ ratios, it was determined that up-to half of the samples were adulterated with C4 sugars which gave a range of 12.45% to 25.15% which is above the 7% international limit (Gilberto, et al., 2008).

2.4 Regulations on Honey Production in Kenya

In the 53rd report of the Joint FAO/WHO expert committee on food additives. The Expert committee established a maximum level of 2 mg kg^{-1} for Pb and 1 mg kg^{-1} for Cd and Hg, except where there is a good reason for establishing a higher or a lower maximum level. The committee also confirmed that it would include limits for arsenic (As) only when the source from which the additive is prepared, or the nature of the manufacturing method showed that such a limit was necessary.

Labeling food systems and packages of honey for example should include information on the product's floral or vegetable origin and regional or topographical origin (Enrique, et al., 2014). The European Union has described the general and specific definitions of the contents of honey and its characteristics and they include acidity, humidity, sugar content, hydroxymethylfurfural,

diastase activity and electrical conductivity. It is therefore important that the characterization of honey is done so as to provide this information to the consumers and for trade.

Legislation plays a very big role in regulation of foods for public consumption and minimize on potential contaminants or substances that can harm the human health. In Kenya, legislation on honey is found in Cap 254 Food, Drugs and Chemical Substances (General) Regulations, 1978), Part VII Section 88. It states that honey shall be the food derived solely from the nectar of flowers and other sweet exudation of plants by bees; it shall contain not less than 60% invert sugar and shall contain no more than 20% moisture, 8% sucrose and 1% ash.

These requirements might not necessarily cover all specifics for adulterations, but the limits imposed to moisture and sugar contents are adequate to discriminate the adulterated honey products, nevertheless. Furthermore, sugars can also be categorized further to differentiate between pure honey and adulterated honey. However, these regulations need to be more specific to include these categories and their limits.

Honey can be adulterated using cheaper sweeteners like sugar syrups and molasses which are inverted by enzymes or acids from sugar beet, corn, sugar cane and other natural origin syrups such as maple (Blanka & Lenka, 2015). From an economic point of view, honey adulteration brings unfair competition to the market. This might destabilize the market which is not good for business.

Adulteration of honey was first encountered in the world market in the 1970s. This was also the time when the industry introduced high-fructose corn syrup into the market. The main components of honey are sugars which make up to the range of 60 – 77.8%. Fructose and glucose are the most dominant and account for 85–95% of the total sugars. Glucose to fructose ratio in the honey largely depends on the nectar sources. Generally, the average fructose to glucose ratio is normally 1.2:1. At normal temperatures, honey contains glucose at highly saturated levels. However, when the temperature and water content is reduced, crystallization of the glucose can take place. In its dry weight, honey contains approximately 1% sucrose. The moisture content of honey is normally between 12.4% and 24.5%. Fermentation will start when the moisture content of honey drops below 17% (Blanka & Lenka, 2015).

The National Beekeeping Institute, Lenana is a government institution that offers many services to the public and the farmers. These services include quality analysis of honey and other hive

products, production of bee equipment, marketing and utilization of the products, training of the beekeepers and bee management and investigation (World, 2018).The Institute plays a very big role in encouraging the farmers to practice beekeeping and supporting them on the modern equipment to use so as to improve the yield and also to train them on the good practices of beekeeping.

2.5 Honey Health and Nutrition

A large part of honey is made up of carbohydrates, but it also contains enzymes, amino acids, proteins, minerals, vitamins, taste building compounds, polyphenols and aroma compounds. The sugars in honey are the main building compounds because of their sweet nature, the more the amount of fructose, and the sweeter the honey. Polyphenols have antioxidant properties due to the presence of flavonoids such as luteolin, quercetin, galangin, apigenin, kaempferol, chrysin etc. The aroma compounds are responsible for the honey flavour which is very important when it comes to industries that manufacture foods with honey as an ingredient (Bogdanov, Jurendic, Sieber, & Gallman, 2008). The functional properties of honey and its high amount of carbohydrates makes it an ideal source of strength and vitality for athletes and other sport groups. Most of the honey properties that promote health are only achieved by using high amounts of honey like 50 to 80 g per intake.

Human health is a very important aspect of life and recently a branch of medicine called apitherapy has been developed and it uses honey and other bee products as ingredients in the medicine against a number of diseases (Bogdanov, et al., 2008).

The minerals in honey have nutritional significance for the human body; vitamins like folic acid, vitamin K, vitamin C, thiamine, niacin, riboflavin among others. However, most of these vitamins are lost at extreme heating conditions above 40ºC.

The minerals in honey vary according to the geographical and botanical origin. The essential trace elements in honey are desirable because of their nutritional value. On the other hand, heavy metals in higher levels are toxic (Shahram, et al., 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of the Sampling Area and Sampling.

Baringo County borders Turkana County and West Pokot County to the North, Samburu County and Laikipia County to the East, Nakuru County and Kericho County to the South, Uasin-Gishu County to the South West and Elgeyo-Marakwet County to the west. It has six sub-counties. The County has an area of 11,015 square kilometers. This study focused on Marigat and Koibatek sub-counties.

Kitui County borders Machakos County and Makueni County to the west, Tana River County to the east, Taita Taveta County to the south, Embu and Tharaka Nithi counties to the North. It covers an area of 20,501.6 square kilometers. The county has 16 sub-counties. This study focused on Kitui central, Matinyani, Mwingi Central and Mwingi East.

Thirty honey samples each weighing approximately 100 g, were collected, specifically from, Marigat and Koibatek sub-counties and Kitui central, Matinyani, Mwingi central and Mwingi east sub-counties. The sampling was done in May 2018 for Baringo samples and in June 2018 for Kitui samples. The sampling was only limited to roadside vendors.

Specifically, Baringo samples were obtained from; Esageri, Molo River, Muserechi and Ravine Junction and those from Kitui were collected at; at Kalundu market, Kitui animal market and Kitui town.

Figure 3.1 and 3.2 show the sampling points for Baringo and Kitui.

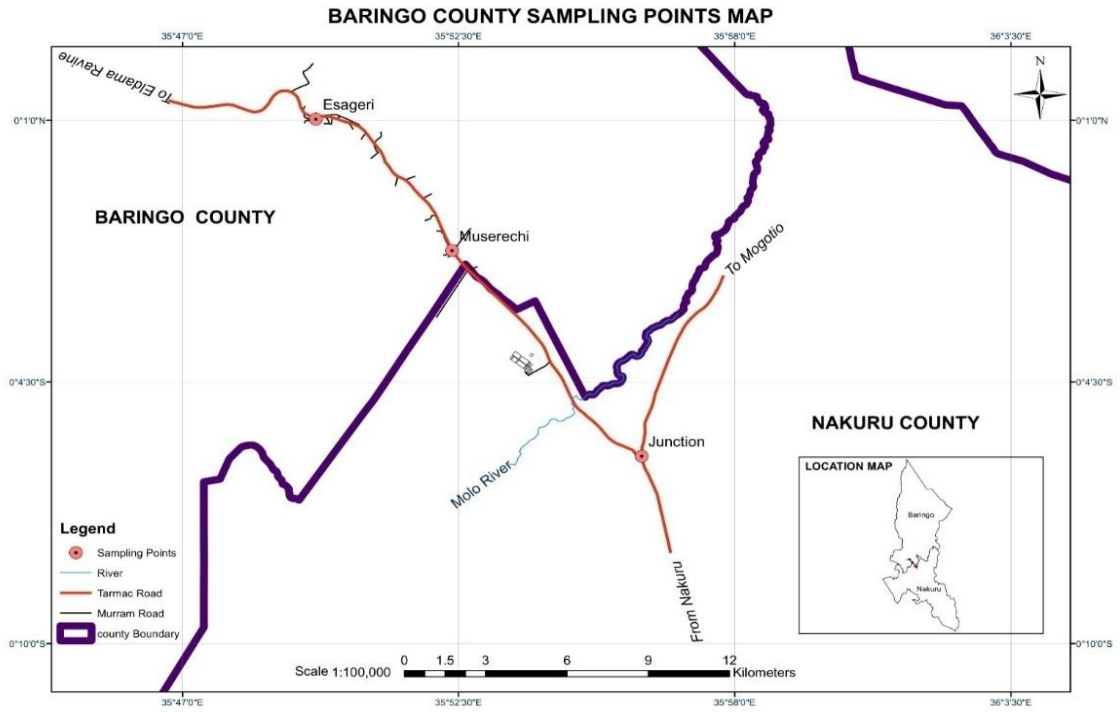

Figure 3.1: Sampling points: Baringo county

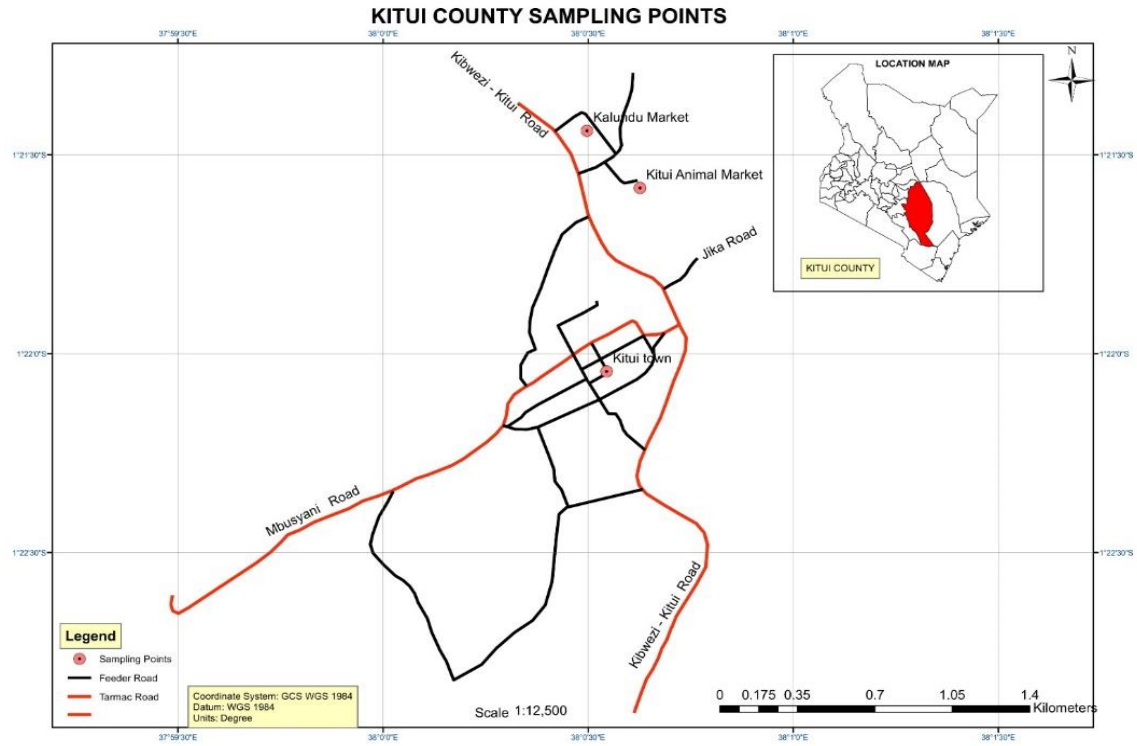

Figure 3.2: Sampling points: Kitui County

The table 3.1 and 3.2 indicates the sampling points in this study and how they were labelled for identification.

Table 3.1: Baringo samples n=30

Esageri	Muserechi	Molo River	Junction a	Junction b	Junction c
Esageri 1	Muserechi 1	Molo River 1	Junction 401	Junction 411	Junction 421
Esageri 2	Muserechi 2	Molo River 2	Junction 402	Junction 412	Junction 422
Esageri 3	Muserechi 3	Molo River 3	Junction 403	Junction 413	Junction 423
Esageri 4	Muserechi 4		Junction 404	Junction 414	Junction 424
Esageri 5	Muserechi 5			Junction 415	Junction 425
	Muserechi 6			Junction 416	
				Junction 417	

Table 3.2: Kitui samples n=30

Mbusyani Road	Open Market	Kibwezi-Kitui Road	Kitui Town
Kitui 101	Kitui 201	Kitui 301	Kitui 401
Kitui 102	Kitui 202	Kitui 302	Kitui 402
Kitui 103	Kitui 203	Kitui 303	Kitui 403
Kitui 104	Kitui 204	Kitui 304	Kitui 404
Kitui 105	Kitui 205	Kitui 305	Kitui 405
	Kitui 206		Kitui 406
	Kitui 207		Kitui 407
	Kitui 208		Kitui 408
	Kitui 209		Kitui 409
	Kitui 210		Kitui 410

3.2 Sample Preparation

All the glassware and containers that were used for sample preparation were soaked in detergent and 10% nitric acid solution for 24 hours. The glassware was then rinsed in pure water and dried. All the honey was shaken for homogeneity before sample preparation and analysis.

For environmental control, reagents and standards were prepared using concentrated acids and they were done in a fume chamber. During digestion, the microwave digester's exhaust unit was

directed at the fume chamber at all times and the unit of extraction was on throughout the period of analysis.

3.3 Sample analysis

3.3.1 Determination of Trace Element Content with EDXRF

Approximately 5 g of honey of each honey sample was accurately weighed into an XRF cup, the top was covered using a mylar foil. Then the sample was placed on the sample holder for EDXRF irradiation for 1000 seconds. Prior to measurements, calibration curve was prepared for use from pure elements of interest of standards in the liquid form to quantify the trace metals in the honey samples.

The Amptek EDXRF Kit used for analysis of heavy metals in this study, contains the X-ray tube, X-ray analysis software, the spectrometer with signal processor and detector and a sample chamber with safety features such as safety interlocks and radiation shielding.

For this study, the spectrometer consisted of a lithium-silicon drift detector, an x-ray tube made of silver and it was operated at 30 keV maximum energy and 80 μA current. Each sample was analyzed in triplicate (figure 3.3).

Figure 3.3: The EDXRF spectrometer at the Institute of Nuclear Science and Technology

3.3.2 Determination of Trace Element Content with ICP – OES

An Agilent 5100 ICP-OES spectrometer, which is available at Kenya Bureau of Standards was used to determine trace elements in this study (figure 3.4). Prior to measurements, the spectrometer was optimized for geometry of measurements using the Agilent ICP Expert software.

Approximately 0.5g of homogenized honey sample was accurately weighed into a Teflon tube to be digested in the microwave. 6mL of Conc. HNO_3 and 3mL of H_2O_2 were added to the sample and then left for 30 minutes in the fume hood. The vessel was well capped and digested in the microwave oven for 30 minutes at various temperatures according to the procedures. This was then transferred to the sample holder for analysis (Boukka, Belouali, & Hakkou, 2008).

The peaks corresponding to each of the element of interest were determined at different wavelengths, from which the concentration of the element in the sample was determined as shown in table 3.3. All the samples were analysed in duplicates.

Table 3.3: Operating conditions of the Agilent 5100 ICP-OES

Analyte	**Wavelength (nm)**	**Read time (s)**	**View**	**Nebulizer**
Ba	455.403	5	Axial	Concentric
Cr	267.716	5	Axial	Concentric
Al	396.152	5	Axial	Concentric
Ca	396.847	5	Axial	Concentric
Pb	220.353	5	Axial	Concentric
Cu	327.395	5	Axial	Concentric
Mg	279.553	5	Axial	Concentric
Fe	238.204	5	Axial	Concentric
Ni	231.604	5	Axial	Concentric
K	766.491	5	Axial	Concentric
Mo	202.032	5	Axial	Concentric
P	213.618	5	Axial	Concentric
Zn	213.611	5	Axial	Concentric

Na	589.593	5	Axial	Concentric
Ag	328.068	5	Axial	Concentric
Ti	190.794	5	Axial	Concentric
Si	251.611	5	Axial	Concentric
Sn	189.925	5	Axial	Concentric

Figure 3.4: ICP-OES Spectrometer at Kenya Bureau of Standards

3.3.3 Determination of pH and Free Acidity

The bench top model HI-2210, Hanna Instruments Digital pH meter was used for pH measurements. It was well calibrated using the buffer 4, buffer 7 and buffer 9 solutions. 0.1M NaOH was prepared prior to measurements (Chemists, 1999).

10 g of honey was measured using a well calibrated digital weighing scale. It was then diluted in 75 ml of distilled water, stirred well and the pH readings recorded.

The solution was titrated with 0.1M NaOH to a pH of 8.3. The volume for NaOH used was recorded for determining the free acidity (Chemists, 1999).

3.3.4 Determination of HMF and sugars

5 g of honey was measured using a digital balance. It was then diluted into 100 ml of double distilled water, the solution was filtered through 0.45 µm filters and approximately 10 µl of the filtrate was immediately injected into the HPLC. It was run for 10 minutes then the process repeated for duplicate samples.

Shimadzu Prominence HPLC system, which is available at Beekeeping Institute was used in this study for the determination of HMF is shown in figure 3.5.

Figure 3.5: The HPLC equipment at Beekeeping Institute for analysis of sugars and HMF

For determining HMF, the HPLC spectrometer was reconfigured with SPD-20A detector and the operating conditions optimized for use. Prior to measurements, the system was calibrated using analytical grade standard HMF. The amount of HMF was determined from the peak areas determined using LC Solution software. For determination of sugars; fructose, sucrose, glucose and maltose, the Shimadzu Prominence HPLC system with detector RID-10A was used. The operational conditions of the HPLC were optimized for measurement of sugars as shown in table 3.4.

Prior to sugar measurements, various sugar standards were prepared in the following proportions; fructose; 2%, glucose; 1.5%, sucrose; 0.25% and maltose; 0.15%. The mixture was dissolved with distilled water to 100 ml or in a beaker and left overnight in the refrigerator. The standard solution was run every morning prior to the measurements.

5g of honey was measured using a digital balance. It was then diluted into 100 ml of double distilled water, the solution was filtered through 0.45 µm filters and approximately 10 µl of the filtrate was immediately injected into the HPLC. It was run for 10 minutes then the process repeated for duplicate samples.

Peaks corresponding to fructose, glucose, sucrose and maltose were identified and quantified for the analyte composition using the standards (Bogdanov, et al., 2014)

Table 3.4: Operational conditions of Shimadzu Prominence HPLC

Flow rate	0.6ml/min
Cell temperature	40ºC
Column Temperature	80ºC
Mobile phase	Water
Analysis time per sample	20 min
Column	Shim-pack, SPR-Ca (250 mm L ×7.8 mm I.D, 8 µm)
Detection	RID-10A

The Sugar content was calculated using the formula:

$$C_i = \left(\frac{A_{sample}}{A_{standard}}\right) X \left(\frac{C_{standard}}{W_{sample}}\right) X100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\text{Eqn 3.1}$$

Where,

C_i = percentage concentration of sugars

A_{sample}= Area of the analyte in the sample

$A_{standard}$= Area of the analyte in the standard

$C_{standard}$= Concentration of the analyte in the standard

W_{sample} = Weight of the sample

3.4 Quality Compliance of Measurements and Statistical Analysis

It is important that the obtained results are compared to the standards established locally and internationally for compliance.

Statistical analysis for the parameters analyzed in this study were done using the Student's t-test for comparison of the means (Montgomery & Runger, 2011). Student's t-test was used to compare the two-independent means of the honey sample distribution to sought information of whether there was a significant difference between the two data sets of Baringo and Kitui. A two-sample t test was suitable for this study because it compared the means or averages. By using this method of data analysis, a value was generated as t_{cal} and a table is used to get t_{tab} (Bluman, 2009). When the absolute t-value was greater than the tabulated value ($t_{cal} > t_{tab}$), it indicated that there was a significant difference between the two data sets (Pirk, et al., 2013).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

This section summarizes the results of measurements of physicochemical properties of honey samples from the areas of Baringo and Kitui.

4.2 Physicochemical Parameters of Honey Samples

Table 4.1 and 4.2 show the results of physicochemical parameters of honey samples from Baringo and Kitui counties respectively. Summarized tables were generated from the data in the appendix 1 – 12.

Table 4.1: Physicochemical parameters of honey samples from Baringo County n=30.

Parameter	Mean ± Standard Deviation	Range	KS – EAS 36 Standards	EU Standards
pH	3.65 ± 0.28	2.99 - 4.20		
Free Acidity (meq/Kg)	29.67 ± 5.38	23 - 38.5	50 meq/Kg	40 meq/Kg
Fructose (%)	36.86 ± 2.59	30.6 - 43.25		
Glucose (%)	26.17 ± 1.69	21.89 - 30.2		
Sucrose (%)	1.56 ± 0.98	0.28 - 4.6	5%	5%
Maltose (%)	0.7 ± 0.37	0 - 3.35		
Total sugars (%)	65.29 ± 4.07	61.35 - 74.90	min 60g/100g	min 60g/100g
HMF (mg/kg)	3.91 ± 0.18	1.2 - 8.66	40 mg/kg	40 mg/kg

**EU Standards: Council Directive 2001/110/EC*

Table 4.2: Physicochemical parameters of honey samples from Kitui County n=30

Parameter	Mean ± Standard Deviation	Range	KS EAS 36 Standards	EU Standards
pH	3.46 ± 0.31	2.95 - 4.21		
Free Acidity (meq/Kg)	30.85 ± 4.27	24 - 39.5	50meq/Kg	40meq/Kg
Fructose (%)	29.8 ± 2.07	25.66 - 33.88		
Glucose (%)	26.33 ± 2.48	23 - 30.68		
Sucrose (%)	3.77 ± 1.02	1.92 - 5.86	5%	5%
Maltose (%)	-	-		
Total sugars (%)	59.9 ± 3.82	54.72 - 67.30	min 60g/100g	min 60g/100g
HMF (mg/Kg)	30.05 ± 3.55	6.52 - 114.06	40mg/kg	40mg/kg

In general, free acidity averaged 29.67 ± 5.38 milliequivalents/Kg for Baringo and 30.85 ± 4.27 milliequivalents/Kg for Kitui, all values are within the KS EAS recommended values of a maximum of 50 milliequivalents/Kg. The total sugars were found to be 65.29% and 59.9% for Baringo and Kitui, respectively. The average HMF levels were 30.05 ± 3.55 mg/Kg and 3.91 ± 0.18 mg/Kg for Kitui and Baringo respectively. A few samples from Kitui had elevated levels of sucrose (> 5%) and HMF (> 100 mg/Kg), an indication of adulteration or prolonged storage periods.

4.3 pH Content in Honey Samples

The figure 4.1 and 4.2 show the variations of the pH values for honey samples from Baringo and Kitui respectively. The summarized graphs were generated from the pH values recorded in appendix 1 and 2.

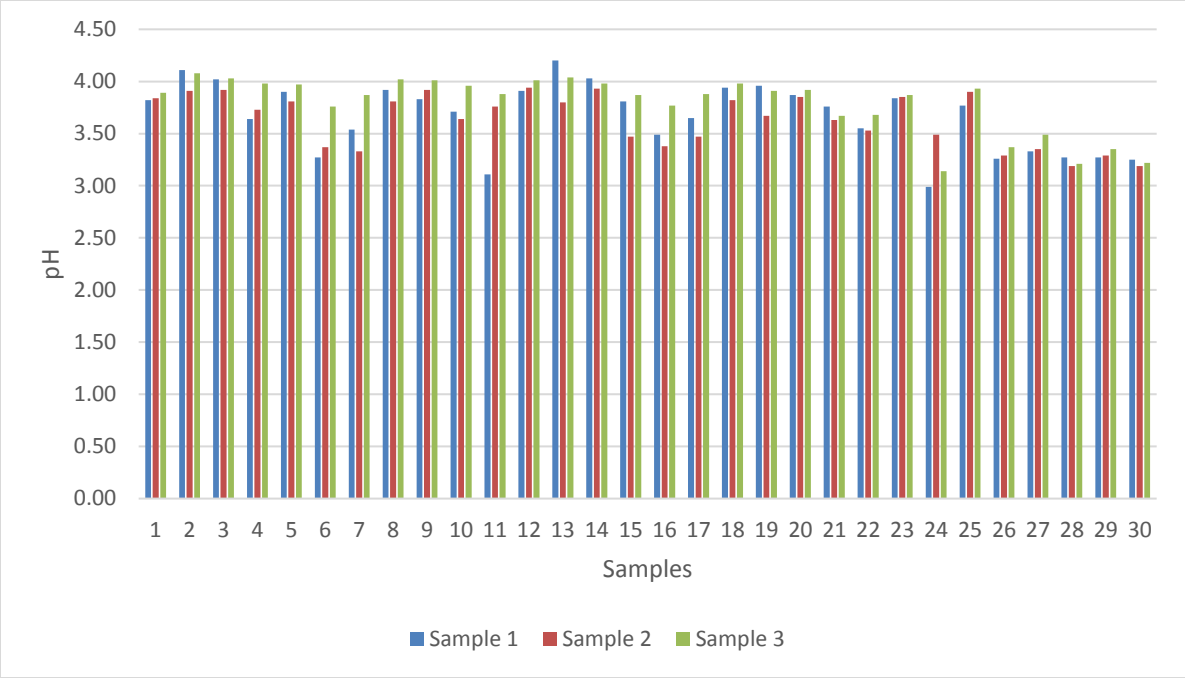

Figure 4.1: pH for Baringo honey samples**.**

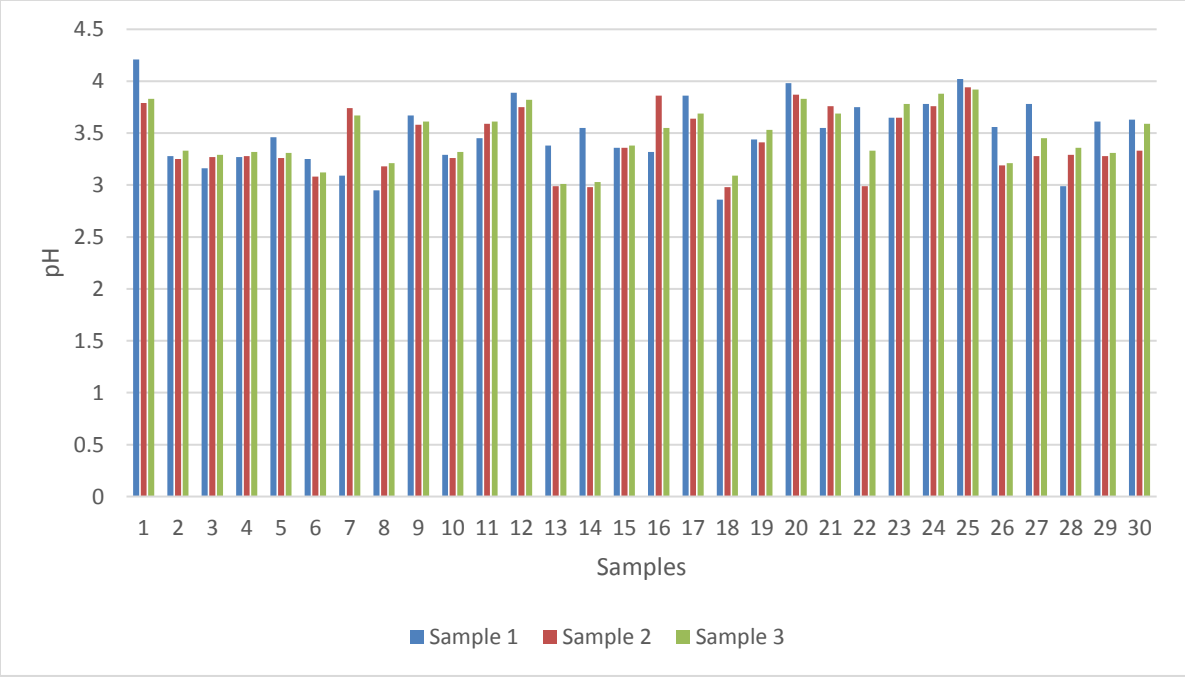

Figure 4.2: pH for Kitui honey samples.

The pH from Baringo samples had a range of 2.99 to 4.20. Those from Kitui had a range of 2.95 to 4.21. The mean pH for Baringo and Kitui are 3.64 ± 0.28 and 3.46 ± 0.31 respectively. The highest readings for Baringo were from Molo River1, Molo River 2, Molo River 3 and Junction

402 which had a pH of 4.02, 4.2, 4.03 and 4.11, respectively. The lowest levels of pH were from Muserechi 4 which had a pH of 2.99.

For Kitui samples, the highest pH measurements were recorded in Kitui 401 with a pH value of 4.21 and Kitui 105 with a pH value of 4.02. The lowest values correspond to Kitui 208, Kitui 408 and Kitui 303 samples with pH 2.86, 2.95 and 2.99, respectively.

Enrique, et al., 2014, characterized Argentine honey based on their quality parameters and mineral content and they found the mean pH of unifloral honey to be 4.12 ± 0.21 and for multifloral honey to be 3.81 ± 0.27. The honey from Kitui and Baringo are more likely to be multifloral according to these classifications. Nganga, Onditi, Gachanja, & Ngumba, 2013, also did some studies on physicochemical parameters of honey and they found pH in the range of 3.82 to 4.43. In Ethiopia, Kebede, et al., 2011, analyzed honey and they found a pH range of 3.82 to 4.45.

4.4 Free Acidity in Honey Samples

In honey, acidity occurs because of the different organic acids present in natural honey. With the different nectar sources used by bees and the activity of the enzymes like glucose oxidase, there is formation of gluconic acid which contributes to the formation of acids in honey. Furthermore, during ripening of the honey, there is the action of bacteria and the minerals that are present in the honey composition (Stihi, et al., 2015). Free acidity of honey is a very important quality indicator. Fermentation of honey leads to increased acidity. Natural honey has varying amounts of free acidity but the maximum value set by the Codex Alimentarius is 40 milliequivalents/Kg. This value was revised to 50 milliequivalents/Kg to cater for the honeys which have an elevated amount of natural acidity. Figure 4.3 and 4.4 show the content of free acidity in honey samples for Baringo and Kitui. The graphs were generated from the data in appendix 3 and 4.

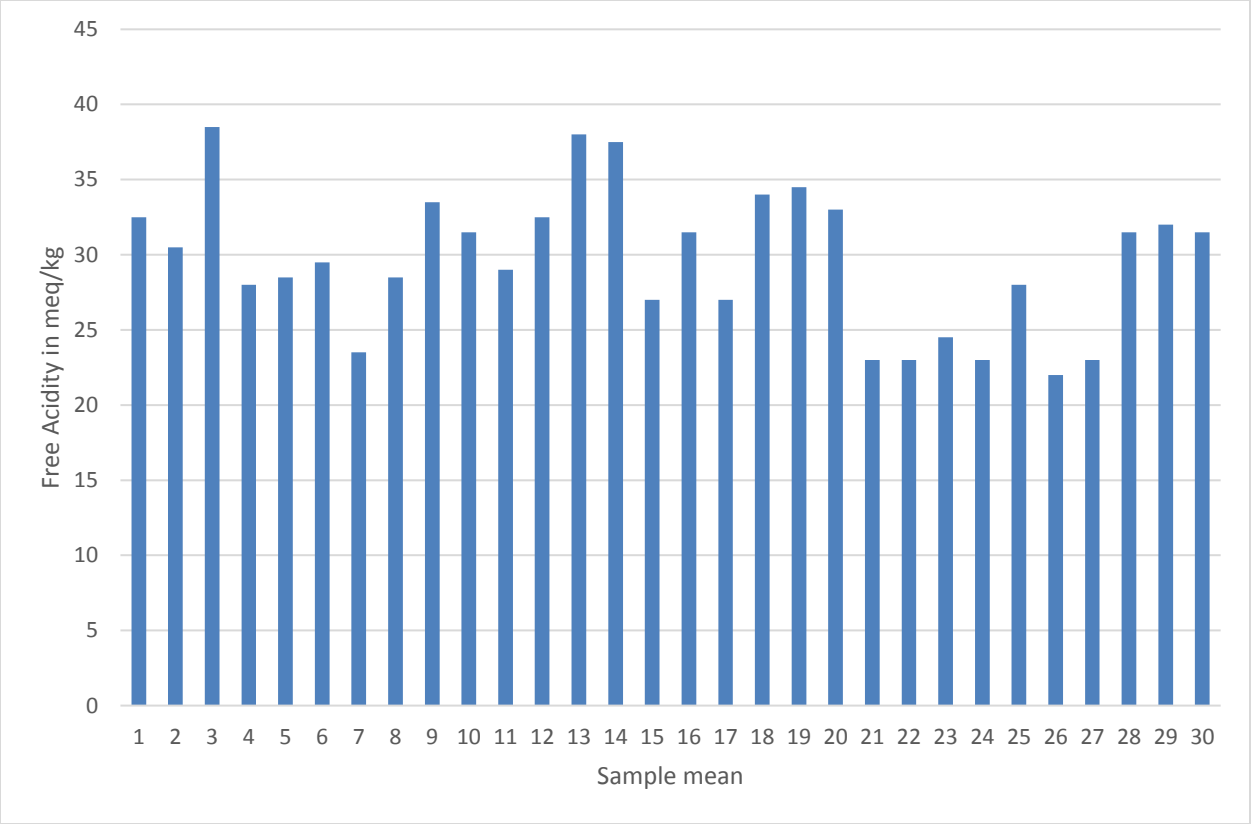

Figure 4.3: Free acidity in Baringo samples.

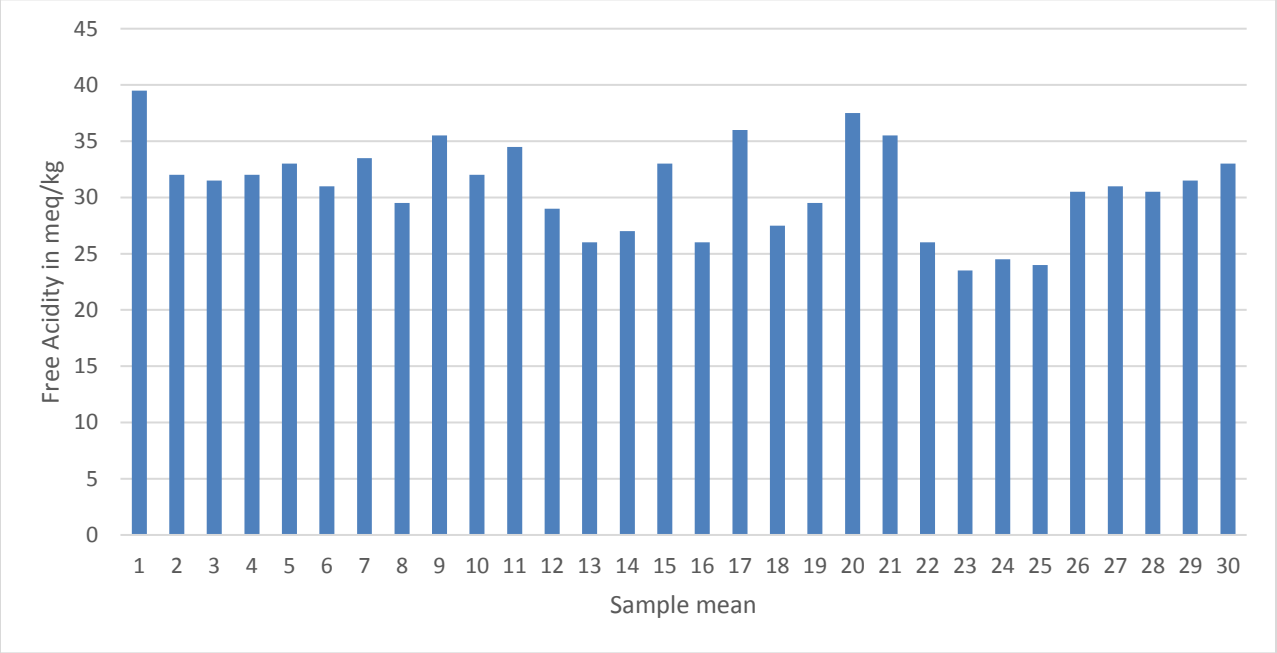

Figure 4.4: Free acidity in Kitui samples

The mean free acidity for Baringo samples was 29.67 ± 5.38 milliequivalents/Kg while the range was 23 to 38.5 meq/Kg. Samples from Kitui gave a higher free acidity mean of 30.85 ± 4.27 and a range of 24 to 39.5. All these values were within the KEBS recommended values of a maximum of 50 milliequivalents/Kg. Other measurements by Muli, et al., 2007, from ICIPE reported the free acidity range of 18.00 to 71.85 milliequivalents/ Kg for honey samples from Mwingi.

Studies conducted by Nganga, et al., 2013, showed the free acidity range to be 10.00 to 36.67meq/Kg. The studies were conducted for various honey samples obtained randomly in Nairobi supermarkets.

The results obtained in current study therefore indicate that all the honey samples are of good quality.

4.5 Sugar Measurements in Honey Samples

In general, sugar content in honey includes reducing sugars; fructose and glucose (min 60%) and non-reducing sugars; sucrose and maltose in small amounts, usually 5% or below (Commission, 2001) (Standards, 2016). A high proportion of non-reducing sugars is an indication of adulteration.

Figures 4.5 and 4.6 show the variations of sugar levels in honey samples from Baringo and Kitui honey samples. The summarized graphs were generated from results of sugar levels values recorded in appendix 5 and 6. A chromatogram of the HPLC sugar analysis is shown in appendix 7.

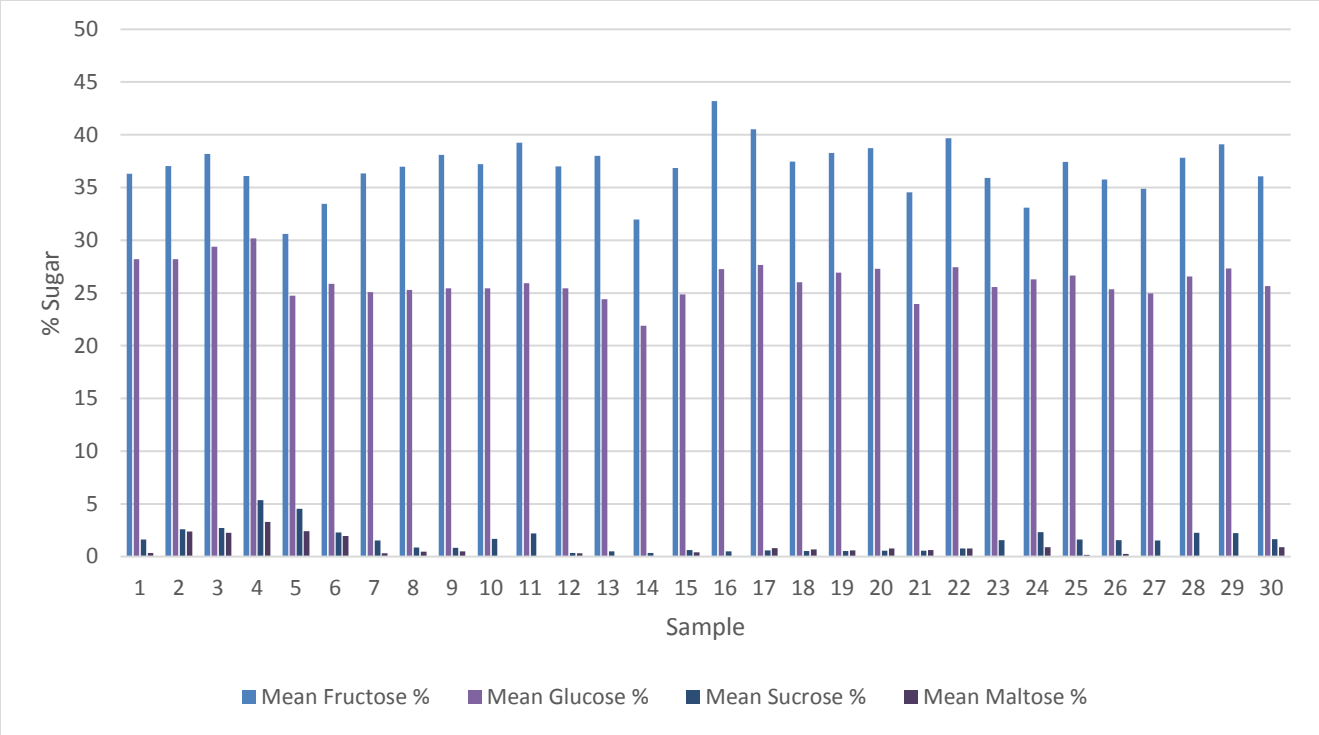

Figure 4.5: Sugar content for Baringo honey samples

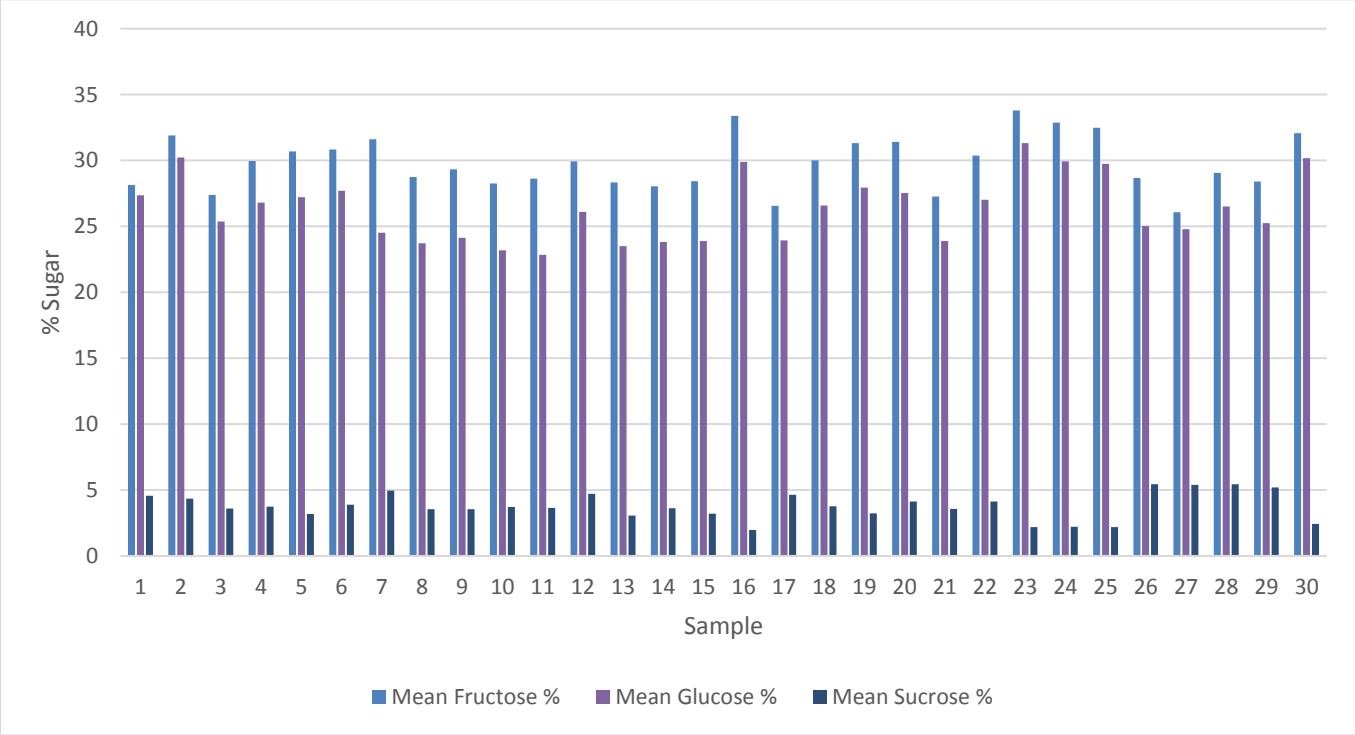

Figure 4.6: Sugar content for Kitui honey samples

Honey from Baringo had a high amount of reducing sugars; fructose (36.86%), glucose (26.17%) and very low amount of non-reducing sugars (<5%). The total mean percentage of sugars for Baringo samples was 65.29%.

Kitui honey had 29.8% fructose and 26.33% glucose, almost in equal proportions unlike those from Baringo. The sucrose content was higher than that of Baringo and it was a total mean percentage of 3.77%. No maltose was detected from honey samples from Kitui. The total sugars for Kitui amounted to 59.9%.

The studies done by Muli et al., 2007 found the mean amount of fructose and glucose to be 66.50 ± 3.25% for Baringo and 65.00 ± 0.48% for Mwingi. They found the level of sucrose to be 0.90 ± 0.42% for Baringo samples and 2.23 ± 0.48% for Mwingi samples.

Results obtained in this study, indicate that the total aggregate of glucose and fructose for Baringo samples slightly higher than that of Kitui samples. This is in agreement with findings by (Muli, et al., 2007, from ICIPE for samples from Mwingi and Baringo. However, for Argentine honey, the total sugars were found to be 82.1 ± 0.7% for unifloral honey and 82.0 ± 1.2% for multifloral honey (Enrique, et al., 2014). These values are higher than the total sugars found in this study which was 65.29 ± 4.29% for Baringo samples and 59.9 ± 3.81% for Kitui samples.

4.6 Hydroxymethylfurfural (HMF)

Content of HMF is the quality indicator of honey freshness and excess heating. Excess heating destroys some of the important enzymes and flavonoids which give honey its unique taste and flavor. Fresh honey may contain little amounts of HMF but the amount increases with prolonged storage periods. In the international market, a value higher than 40 mg/Kg would indicate honey deterioration or longer storage periods of time and therefore unacceptable.

Figures 4.7 and 4.8 show the variations of HMF levels in honey samples from Baringo and Kitui honey samples. The summarized graphs were generated from results of HMF levels values recorded in appendix 8 and 9. Appendix 10 is a chromatogram of the HPLC, HMF analysis in this study. Figure 4.9 indicates the variations of HMF levels in both regions.

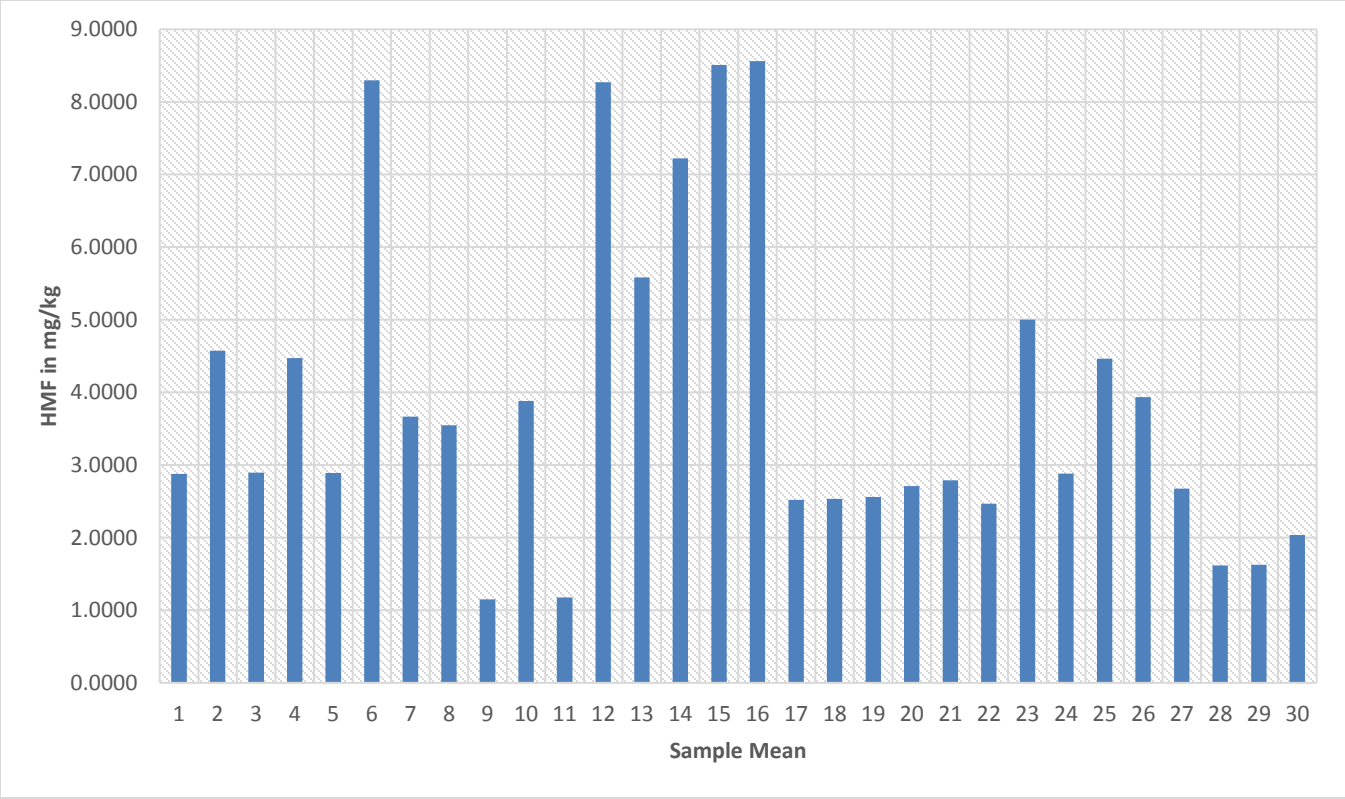

Figure 4.7: HMF levels in Baringo samples

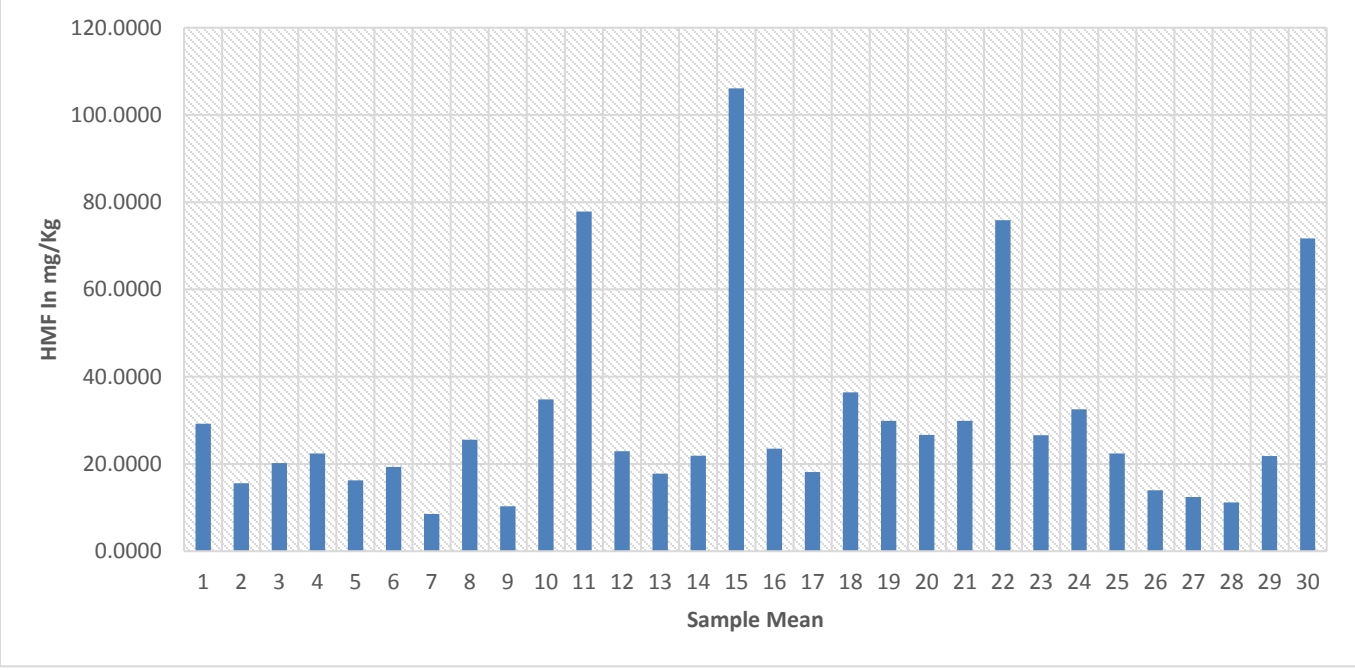

Figure 4.8: HMF levels for Kitui samples

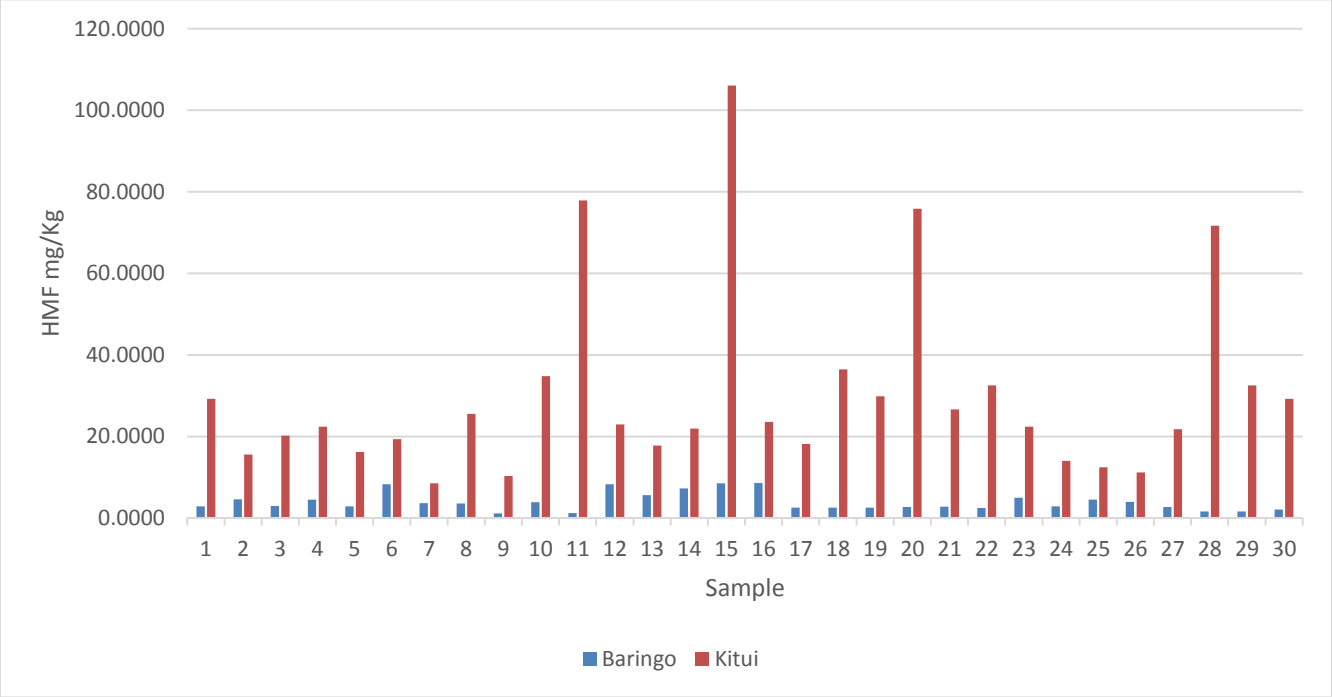

Figure 4.9: Variation of HMF levels for Baringo and Kitui samples

The samples from Baringo showed the lowest HMF values with a mean of 3.91 ± 0.18, an indication of a high quality of honey and fresh.

Samples from Kitui had a mean HMF value of 30.05 ±3.55 mg/Kg, with a range of 6.52 mg/Kg and 114.06 mg/Kg, over eight times in samples from Baringo. This trend is replicated with results of studies done by Muli, et al., 2007, who determined the HMF levels to be 3.70 ± 0.51 and 29.20 ± 5.35 for Baringo samples and Mwingi samples, respectively.

4.7 Trace Elements of Honey from Baringo and Kitui

Worldwide, there are a number of researches being done to determine the mineral content in honey and for its properties. There are varying reasons why these studies are being conducted and this includes; honey quality, dietary supplementation, health concerns, therapeutic purposes and environmental concerns. Getting the level of trace elements in honey will inform all these reasons depending on the results obtained.

The comparative data for the trace element in honey using the ICP-OES were in good agreement with the results of EDXRF. A similar comparison was done to analyze trace elements in water using TXRF and ICP-OES and the results were not significantly different (Ralitsa, Detcheva, Karadjov, Jordanov, & Ivanova, 2013).

Figure 4.10 - 4.11 shows the various trace element concentrations for each of the regions. Figure 4.12 shows the variations of the trace elements in both regions. These were generated from data in appendix 11 and 12.

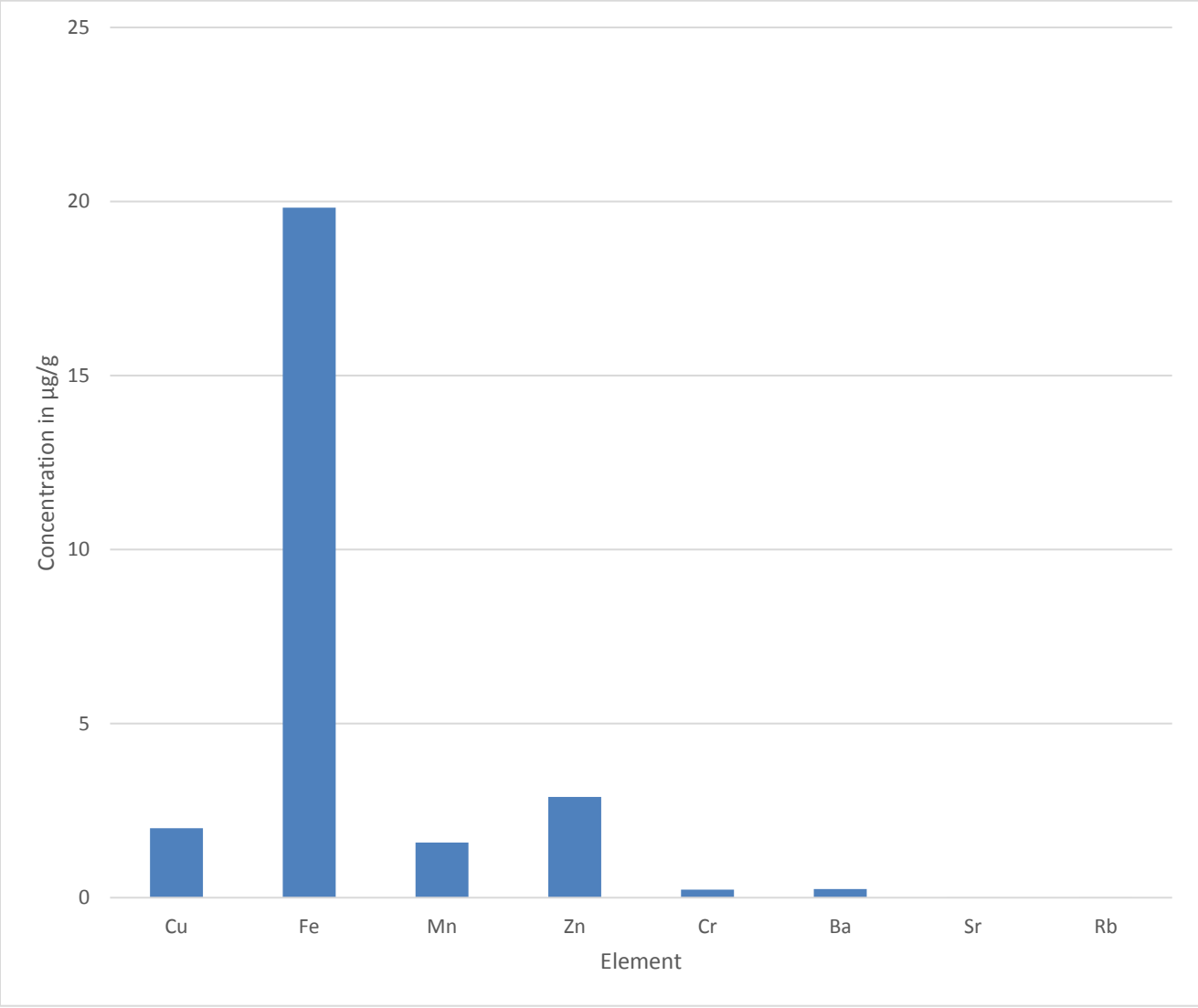

Figure 4.10: Trace elements in Baringo honey samples

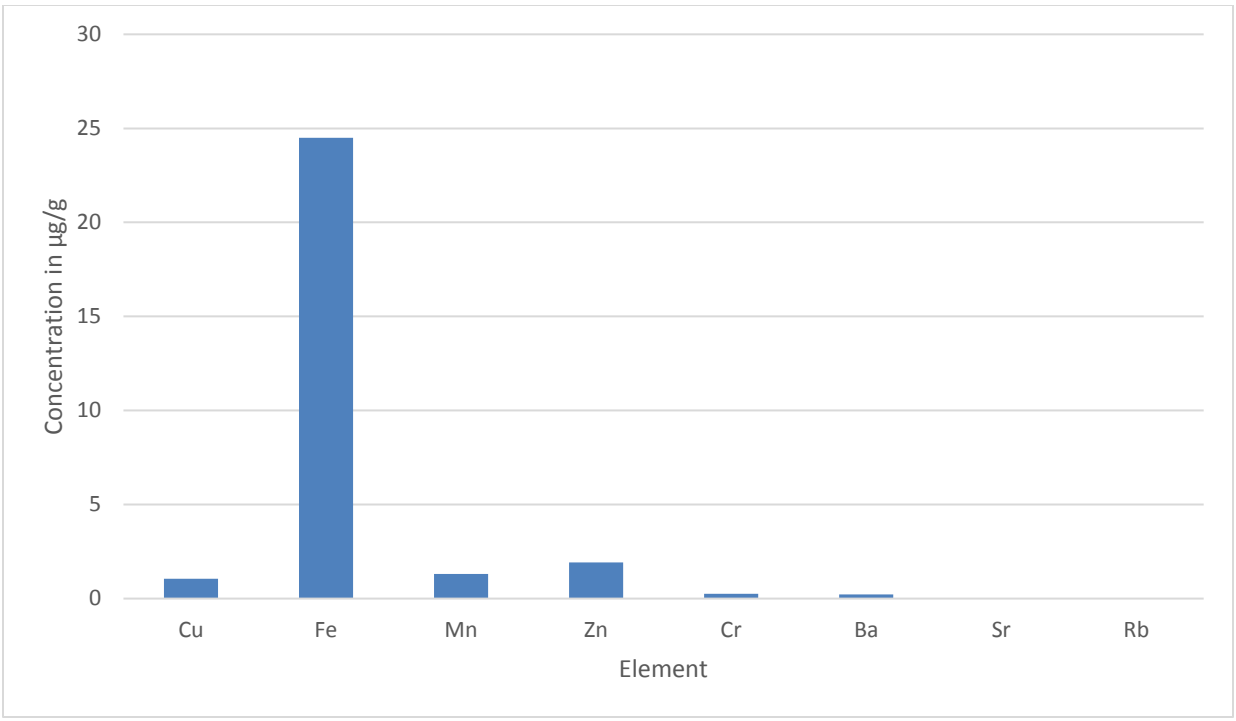

Figure 4.11: Trace elements in Kitui honey samples

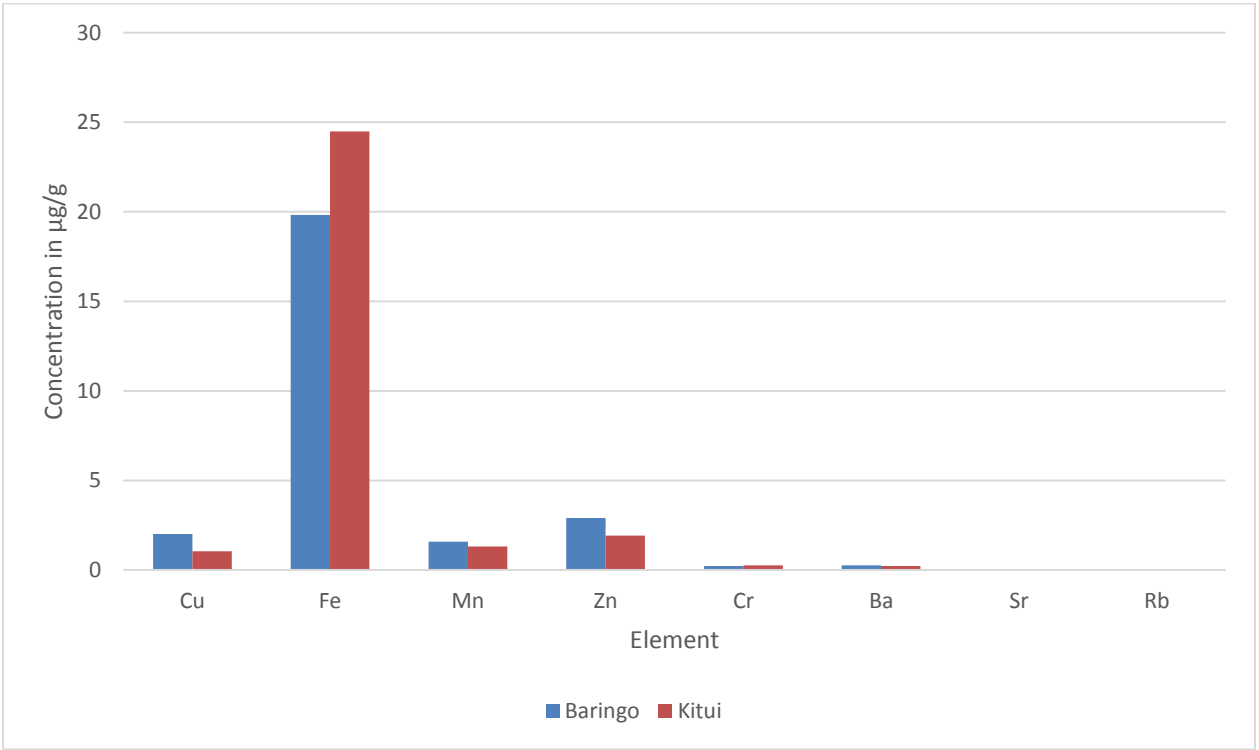

Figure 4.12: Trace elements for Baringo and Kitui honey samples

4.7.1 Iron (Fe)

Iron was one of the most prominent metals of all the trace elements and it was present in all the honey samples studied. For Baringo samples, the average amount for Fe was 19.82 ± 2.74 µg/g. This average value is a bit lower than that of Kitui samples. The maximum and the minimum levels of Iron for Baringo samples were found to be 82.78 µg/g (Molo River 1) and 5.2 µg/g (Junction 413) respectively. For Kitui samples, Fe was detected in all the samples and it was the most prevalent metal. The average amount of Iron for Kitui samples was 24.49 ± 3.38 µg/g which is a bit higher than the value for Baringo samples. The highest amount for Iron was 97.63µg/g and the lowest amount was 6.45 µg/g. These samples were Kitui 404 and Kitui 405 respectively. Studies done by Wamwangi, 2012, showed the amount of Fe to be ranging from 8.6 µg/g to 15.6 µg/g. The mean was 10.7 µg/g. The study was done to determine the trace elements in raw honey and processed honey and Wamwangi, 2012, found that raw honey had a lower level of Fe than processed honey.

Fredes & Montenegro, 2006, determined heavy elements in Chilean honey using ICP-OES and found the amount of iron to be 3.13 ± 1.44 µg/g. This is a bit lower than the results of this study. Enrique, et al., 2014, found Fe level in Argentine honey to be 3.57 µg/g which is also lower than the levels found in this study.

4.7.2 Manganese (Mn)

All honey samples studied showed presence of Mn. The average amount of Manganese for Baringo samples was found to be 1.58 ± 0.22 µg/g which is slightly higher than that of Kitui samples which had a mean of 1.30 ± 0.52 µg/g. The range was between 12.32 µg/g and 0.45 µg/g. This value is slightly lower compared to Argentine honey determined by Marcelo et al., 2014 which was found to be 2.61 µg/g.

Unlike samples from Baringo which showed Mn in all the samples, Kitui had four sample whose Mn levels were below detection. The mean levels for manganese was found to be 1.30 ± 0.52µg/g. The range was 0 ppm to2.87 µg/g. This is because some few samples had very low levels of Mn that were below the detection limit. Wamwangi, 2012, did some studies on Kitui honey and it was determined to have a mean of 7.8 ± 2.3 ppm for Mn. This value is slightly higher than the one found in this study.

4.7.3 Copper (Cu)

Copper showed wide variation from one sample to another and in some cases no copper was detected. The average amount of copper of 1.99 ± 0.38 µg/g obtained for Baringo samples was slightly higher than that of Kitui honey samples. The concentration levels for copper ranged from 0ppm to 5.89 µg/g. Overall, the mean value of copper (1.99 ± 0.38 µg/g) was higher compared to a mean of 0.29 µg/g for Argentine honey done by Enrique, et al., 2014.

The average value for copper for Kitui samples was 1.04 ± 0.16 µg/g. The range was 0 µg/g to 7.19 µg/g. This value is a lower compared to the findings of Wamwangi, 2012, who did some studies on Kitui raw and processed honey and found a mean of 10.3 µg/g for Cu and a range of 7.7 µg/g to 14.5 µg/g.

4.7.4 Zinc (Zn)

Zinc was prevalent and showed variations amongst the samples. The mean levels of zinc for Baringo samples were 2.89 ± 0.77 µg/g, these values are higher than those of Kitui. The maximum level of Zinc for Baringo samples was 17.40 µg/g and the minimum value was 0.31 µg/g. The mean was slightly higher than those of Argentine honey by Enrique, et al., 2014 who found a mean of 1.17 µg/g.

Zn was also detected in most of the samples from Kitui and the mean was found to be 1.92 ± 0.41 µg/g. The average value was lower than the value in Baringo samples. The range was between 0 µg/g and 11.50 µg/g. Some studies were done for trace elements of Kitui honey by Wamwangi, 2012 and the mean for Zinc was 6.3 µg/g. The range was 3.5 µg/g to 13.8 µg/g. These values are slightly higher than the findings of this study.

4.7.5 Chromium (Cr)

For Baringo samples, the levels of Chromium were at an average of 0.22 ± 0.07 µg/g and a range of 0ppm to 1.29 µg/g. On average, the levels of this metal were almost the same as those of Kitui samples. Enrique, et al., 2014 found a mean of 0.03 ± 0.02 µg/g for Cr. This is a bit lower compared to the results of this study for Chromium.

For Kitui samples, the average value of Chromium was found to be 0.25 ± 0.09 µg/g which was almost the same as that of Baringo. The maximum and minimum values were 0 µg/g and 0.49 µg/g

respectively. The maximum value was found to be higher (about twice) compared to that of Baringo. Fredes & Montenegro, 2006, found Chilean honey to have the mean for Cr to be 0.07 ± 0.03 µg/g. Enrique, et al., 2014 found a lower mean of 0.03 ± 0.02 µg/g. These values are lower compared to what was found in this study.

4.7.6 Barium (Ba)

Barium had an average of 0.25 ± 0.09 µg/g for Baringo samples and a range of 0 µg/g to 0.85 µg/g. The average value was almost the same as that of Kitui samples. Studies done by Batista, et al., 2012 for Brazilian honey found a mean value for Ba as 374 ± 440 ng/g.

Kitui samples showed a lower average value of 0.22 ± 0.08 µg/g and a range of 0 µg/g to 0.59 µg/g. Studies done on Brazilian honey by Batista, et al., 2012, found a mean value for Ba as 374 ± 440 ng/g.

4.7.7 Strontium (Sr)

The highest amount of Strontium in honey samples from Baringo was 0.12 µg/g. Most of the samples, however, did not indicate any amounts of Sr at all. Studies done by (Fredes & Montenegro, 2006) found mean levels of Sr in Chilean honey to be 2.39 ± 5.26 mg/Kg and the range was between 0.01 and 23.06 mg/Kg. This value is higher than the those of this study.

For Kitui samples, the amount of Sr highly varied from one sample to another. Most of the samples had 0 µg/g for Strontium but the maximum value was at 0.23 µg/g. Some studies done on the elements of Chilean honey by Fredes & Montenegro, 2006, found a higher value for Sr with a mean of 2.36 ± 5.33 µg/g.

4.7.8 Rubidium (Rb)

The range of Rubidium for Baringo samples was 0 ppm to 0.15 µg/g. However, Rb was not detected in majority of the samples.

The highest value for Rubidium from Kitui samples was at 0.18 µg/g. Most of the other samples did not show detectable amounts of Rb. Wamwangi, 2012, did some analysis on raw honey for Kitui and found a range of 1.5 µg/g to 3.7 µg/g and a mean of 2.6 µg/g which is slightly higher than the ones found in this study.

4.7.9 Other Trace Elements

The other trace elements that were not found in the honey samples from Baringo county include As, Cd, Co, Hg, Sn, Mo, Ni, and Ti. Studies conducted by Enrique, et al., 2014, found low amounts of these elements.

Honey samples from Kitui did not have Mo, Ni, Ti, As, Cd, Co, Hg and Sn. Most studies done find very low amount of these metals in honey.

Most studies show that honey has a wide variety of positive nutritional and health effects. The studies also show that the positive effects can be maximized if honey is consumed at higher doses of 50 to 80 g per intake (Bogdanov, et al., 2008).

4.8 Statistical Analysis

Student's t-test was used to compare the two-independent means of the honey sample distribution to sought information of whether there was a significant difference between the two data sets of Baringo and Kitui.

Table 4.3 show the results of the student t distributions for all the parameters analyzed in this study. The data in appendix 1 – 12 was used to generate the t_{cal} values.

Table 4.3: Statistical analysis for differences in parameters

Parameter	**t_{cal}**	**t_{tab}**	**Implication**
pH	5.38	2.045	Significant
Free Acidity	1.33	2.045	Not significant
Sugars	5.35	2.045	Significant
HMF	7.83	2.045	Significant
Cr	0.93	2.045	Not significant
Zn	2.56	2.045	Significant
Ba	2.06	2.045	Significant
Fe	1.52	2.045	Not significant
Mn	1.77	2.045	Not Significant
Cu	2.87	2.045	Significant

The honey samples from Kitui and Baringo had significant differences in pH, sugars, HMF, and to some extent a few trace elements; Zn and Cu.

CHAPTER FIVE

RECOMMENDATIONS AND CONCLUSIONS

5.1 Conclusions

The total sum of glucose and fructose proportions in nectar-based honey should not be less than 60 g/100g according to the codex Alimentarius, 1999. As for honey dew honey, it should not be less than 45 g/100g. The honey samples from Baringo had a total of 63.13% for glucose and fructose but samples from Kitui had results averaging 56.13% for the sum of two sugars. This indicates that Kitui honey may be a result of blended nectar honey and honeydew honey in origin. Some other samples from Kitui had sucrose levels higher than 5% which is above the recommended level. This is an indication of deliberate adulteration also indicated by higher HMF levels (> 100 mg/Kg). Samples from Baringo had very low levels of sucrose (mean 1.56%) and HMF (mean 3.91 mg/Kg), therefore, of high quality.

The mean pH for Baringo was 3.65 ± 0.28 and that of Kitui samples was 3.46 ± 0.31. The variations in pH may be due to the different flower species in the regions and therefore making some samples more acidic than others.

The following elements As, Cd, Hg, Co, Sn, Mo, Ni, Ti and Pb were not present in both honey samples of Baringo and Kitui. The other metals including; Cu, Zn, Ba, Mn, Cr, Fe, Sr and Rb were present in both samples from Kitui and Baringo. The samples from Baringo had an average of 1.99 ± 0.38 µg/g for Copper, 19.82 ± 2.74 µg/g for Iron, 1.58± 0.22 µg/g for Manganese, 2.89 ± 0.77 µg/g for Zinc, 0.22 ± 0.07 µg/g for Chromium and 0.25 ± 0.09µg/g for Barium. While those from Kitui samples, the average values were 1.04 ± 0.16 µg/g for copper, 24.49 ± 3.38 µg/g for Iron, 1.30 ± 0.52 µg/g for Manganese, 1.92 ± 0.41 µg/g for Zinc, 0.25 ± 0.08 µg/g for Chromium and 0.22 ± 0.08 µg/g for Barium. Statistics show that there is a significant difference in all the parameters analyzed except free acidity, Fe, Mn and Cr which did not have significant differences. This may be explained by the fact that both Baringo and Kitui have almost the same climatic and vegetation conditions; semi-arid. The honey from both regions are not contaminated by potential farming activities like insecticides or heavy traffic and industries.

5.2 Recommendations

1) There is need for the apiculture industry to harmonize all the honey processing processes, promote food labelling of apiculture products.
2) Research needs to be done on both honey and soil where the vegetation containing the nectar grows so as to compare between the minerals in honey and in the soil to see whether there is a relationship between them. This is because the metals or other contaminants can be taken up from the soil, water or use of fertilizers.
3) There is need to do more research on the heavy metals of honey from the urban areas and its surroundings in order to determine the level of pollution from industrialization and heavy traffic.

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Appendix 1: pH Measurements for Baringo samples

Sample	Weight 1 (g)	Weight 2 (g)	pH
Esageri 5	9.9998	10.0000	3.8 ± 0.04
Junction 402	9.9997	9.9998	4.0 ± 0.11
Molo River 1	10.0002	9.9998	4.0 ± 0.06
Junction 403	9.9999	10.0002	3.8 ± 0.17
Junction 401	10.0002	10.0000	3.9 ± 0.08
Esageri 1	10.0003	10.0002	3.4 ± 0.26
Esageri 3	10.0002	10.0002	3.5 ± 0.27
Esageri 4	9.9998	10.0000	3.9 ± 0.11
Esageri 2	9.9998	10.0002	3.9 ± 0.09
Junction 404	9.9998	10.0003	3.8 ± 0.17
Junction 413	9.9998	10.0002	3.6 ± 0.41
Junction 414	10.0002	10.0003	4.0 ± 0.05
Molo River 2	10.0001	10.0003	4.0 ± 0.2
Molo River 3	10.0002	10.0002	4.0 ± 0.05
Junction 425	9.9998	10.0003	3.7 ± 0.22
Junction 421	10.0000	10.0003	3.5 ± 0.2
Junction 422	10.0001	10.0000	3.7 ± 0.2
Junction 423	10.0002	10.0003	3.9 ± 0.08
Junction 412	10.0003	9.9999	3.8 ± 0.16
Junction 424	10.0001	10.0001	3.9 ± 0.04
Muserechi 1	10.0001	10.0002	3.7 ± 0.07
Muserechi 2	10.0000	10.0003	3.6 ± 0.08
Muserechi 3	10.0002	10.0003	3.9 ± 0.02
Muserechi 4	10.0000	9.9998	3.2 ± 0.26
Muserechi 5	9.9999	10.0002	3.9 ± 0.09
Muserechi 6	9.9998	9.9999	3.3 ± 0.06
Junction 411	10.0003	10.0002	3.4 ± 0.09
Junction 415	9.9999	10.0003	3.2 ± 0.04
Junction 416	10.0002	10.0003	3.3 ± 0.06
Junction 417	10.0003	10.0003	3.2 ± 0.05
Mean			**3.64 ± 0.28**

Appendix 2: pH Values for Kitui Samples

Sample	Weight 1	Weight 2	pH
Kitui 401	9.9998	10.0004	3.9 ± 0.2
Kitui 402	10.0003	10.0000	3.3 ± 0.04
Kitui 403	10.0003	10.0003	3.2 ± 0.07
Kitui 404	10.0002	9.9999	3.3 ± 0.03
Kitui 405	10.0003	10.0001	3.3 ± 0.1
Kitui 406	9.9999	9.9998	3.2 ± 0.06
Kitui 407	10.0003	9.9999	3.5 ± 0.09
Kitui 408	10.0003	10.0002	3.1 ± 0.12
Kitui 409	10.0003	10.0003	3.6 ± 0.04
Kitui 410	10.0001	9.9998	3.3 ± 0.05
Kitui 201	10.0000	9.9999	3.6 ± 0.06
Kitui 202	9.9998	10.0001	3.8 ± 0.04
Kitui 203	10.0003	10.0001	3.1 ± 0.1
Kitui 204	10.0001	9.9999	3.2 ± 0.13
Kitui 205	10.0003	9.9998	3.4 ± 0.03
Kitui 206	10.0003	10.0001	3.6 ± 0.05
Kitui 207	10.0002	9.9998	3.7 ± 0.04
Kitui 208	10.0002	10.0001	2.9 ± 0.12
Kitui 209	9.9998	10.0001	3.5 ± 0.07
Kitui 210	10.0001	9.9998	3.9 ± 0.04
Kitui 101	10.0003	10.0001	3.7 ± 0.08
Kitui 102	10.0003	10.0001	3.4 ± 0.09
Kitui 103	10.0003	10.0002	3.7 ± 0.05
Kitui 104	10.0000	9.9999	3.8 ± 0.06
Kitui 105	9.9998	10.0001	3.9 ± 0.1
Kitui 301	10.0002	10.0002	3.3 ± 0.09
Kitui 302	10.0003	10.0001	3.5 ± 0.08
Kitui 303	9.9999	10.0002	3.2 ± 0.12
Kitui 304	10.0003	9.9999	3.4 ± 0.07
Kitui 305	10.0001	9.9999	3.5 ± 0.06
Mean			**3.46 ± 0.31**

Appendix 3: Free Acidity Measurement for Baringo samples

Sample	Free Acidity (meq/Kg)
Esageri 5	32.5 ± 7.7
Junction 402	30.5 ± 7.7
Molo River 1	38.5 ± 0.7
Junction 403	28 ± 1.4
Junction 401	28.5 ± 0.7
Esageri 1	29.5 ± 3.5
Esageri 3	23.5 ± 0.7
Esageri 4	28.5 ± 0.7
Esageri 2	33.5 ± 6.3
Junction 404	31.5 ± 7.7
Junction 413	29 ± 2.8
Junction 414	32.5 ± 2.1
Molo River 2	38
Molo River 3	37.5 ± 0.7
Junction 425	27 ± 1.4
Junction 421	31.5 ± 3.5
Junction 422	27 ± 4.2
Junction 423	34 ± 7.1
Junction 412	34.5 ± 6.3
Junction 424	33 ± 7.1
Muserechi 1	23
Muserechi 2	23 ± 2.8
Muserechi 3	24.5 ± 0.7
Muserechi 4	23 ± 1.4
Muserechi 5	28 ± 1.4
Muserechi 6	22
Junction 411	23
Junction 415	31.5 ± 0.7
Junction 416	32
Mean	**29.67 ± 5.38**

Appendix 4: Free Acidity Measurements for Kitui Samples

Sample	Weight	Free Acidity (meq/Kg)
Kitui 401	9.9998	39.5±1.4
Kitui 402	10.0003	32
Kitui 403	10.0003	31.5±0.7
Kitui 404	10.0002	32
Kitui 405	10.0003	33±0.7
Kitui 406	9.9999	31±0.7
Kitui 407	10.0003	33.5±2.6
Kitui 408	10.0003	29.5±0.7
Kitui 409	10.0003	35.5±0.7
Kitui 410	10.0001	32
Kitui 201	10.0000	34.5±0.7
Kitui 202	9.9998	29±1.4
Kitui 203	10.0003	26±4.2
Kitui 204	10.0001	27±2.8
Kitui 205	10.0003	33
Kitui 206	10.0003	26±2.8
Kitui 207	10.0002	36
Kitui 208	10.0002	27.5±1.4
Kitui 209	9.9998	29.5±1.4
Kitui 210	10.0001	37.5±1.2
Kitui 101	10.0003	35.5±0.7
Kitui 102	10.0003	26±4.2
Kitui 103	10.0003	23.5±2.6
Kitui 104	10.0000	24.5±2.4
Kitui 105	9.9998	24±2.6
Kitui 301	10.0002	30.5±0.7
Kitui 302	10.0003	31±1.4
Kitui 303	9.9999	30.5±2.1
Kitui 304	10.0003	31.5±0.7
Kitui 305	10.0001	33
Mean		**30.85 ± 4.27**

Appendix 5: Sugar Measurements for Baringo Samples

Sample	Weight(g)	Fructose %	Glucose %	Sucrose %	Maltose %
Junction 404	5.0002	36.3 ± 1.2	28.2 ± 1.1	1.6 ± 0.06	0.3 ± 0.03
Esageri 3	5.0003	37.0 ± 1.1	28.2 ± 0.4	2.6 ± 0.08	2.4 ± 0.1
Esageri 2	5.0000	38.2 ± 1.0	29.4 ± 0.9	2.7 ± 0.07	2.3 ± 0.09
Esageri 4	5.0002	36.1 ± 0.9	30.2 ± 1.1	5.3 ± 0.09	3.3 ± 0.3
Esageri 1	5.0004	30.6 ± 1.3	24.7 ± 0.8	4.5 ± 0.08	2.4 ± 0.1
Esageri 5	4.9996	33.5 ± 0.9	25.9 ± 0.4	2.3 ± 0.05	1.9 ± 0.2
Junction 412	5.0003	36.3 ± 0.7	25.1 ± 0.8	1.5 ± 0.04	0.3 ± 0.02
Junction 423	5.0001	37.0 ± 0.5	25.3 ± 1.0	0.8 ± 0.01	0.5 ± 0.05
Junction 421	4.9998	38.1 ± 0.6	25.4 ± 0.7	0.8 ± 0.01	0.5 ± 0.06
Junction 402	5.0001	37.2 ± 1.1	25.4 ± 0.8	1.6 ± 0.07	<0.01
Molo River 1	5.0001	39.3 ± 1.2	25.9 ± 1.2	2.1 ± 0.08	<0.01
Junction 422	5.0002	37.0 ± 0.4	25.5 ± 0.9	0.3 ± 0.01	0.3 ± 0.02
Junction 425	5.0003	38.0 ± 0.3	24.4 ± 0.3	0.5 ± 0.01	<0.01
Junction 424	5.0002	31.9 ± 0.2	21.9 ± 0.6	0.3 ± 0.01	<0.01
Junction 401	5.0004	36.9 ± 0.4	24.9 ± 0.9	0.6 ± 0.01	0.4 ± 0.01
Junction 403	5.0000	43.2 ± 0.1	27.3 ± 1.1	0.5 ± 0.01	<0.01
Muserechi 6	5.0012	40.5 ± 0.3	27.7 ± 1.0	0.5 ± 0.01	0.8 ± 0.04
Muserechi 1	4.9995	37.5 ± 1.1	26.0 ± 0.9	0.5 ± 0.01	0.7 ± 0.03
Muserechi 4	5.0006	38.3 ± 1.2	26.9 ± 0.8	0.7 ± 0.02	0.6 ± 0.02
Muserechi 2	5.0003	38.7 ± 0.2	27.3 ± 0.6	0.5 ± 0.01	0.8 ± 0.05
Muserechi 3	5.0004	34.6 ± 0.4	23.9 ± 0.7	0.6 ± 0.02	0.6 ± 0.07
Muserechi 5	4.9999	39.7 ± 0.2	27.5 ± 0.5	0.7 ± 0.02	0.8 ± 0.06
Junction 417	4.9999	35.9 ± 0.7	25.6 ± 0.5	1.5 ± 0.08	<0.01
Junction 411	5.0001	33.1 ± 0.9	26.3 ± 0.6	2.3 ± 0.09	0.9 ± 0.09
Junction 416	5.0002	37.4 ± 1.3	26.7 ± 1.2	1.6 ± 0.06	0.2 ± 0.01
Junction 415	5.0003	35.7 ± 0.9	25.3 ± 1.1	1.5 ± 0.06	0.3 ± 0.04
Junction 414	5.0002	34.9 ± 1.1	24.9 ± 0.7	1.5 ± 0.04	<0.01
Molo River 2	4.9998	37.8 ± 0.7	26.6 ± 0.6	2.3 ± 0.04	<0.01
Molo River 3	5.0000	39.1 ± 0.5	27.3 ± 0.4	2.2 ± 0.05	<0.01
Junction 413	5.0002	36.1 ± 0.3	25.7 ± 0.5	1.7 ± 0.06	0.9 ± 0.06
Mean		**36.86 ± 2.59**	**26.17 ± 1.69**	**1.56 ± 0.98**	**0.70 ± 0.37**

Appendix 6: Sugar Measurements for Kitui Samples

Sample	Weight	Fructose %	Glucose %	Sucrose %
Kitui 402	5.0002	28.1 ± 0.42	27.4 ± 0.07	4.6 ± 0.76
Kitui 401	5.0001	31.9 ± 0.45	30.2 ± 0.08	4.3 ± 0.07
Kitui 403	5.0002	27.3 ± 0.76	25.3 ± 0.09	3.6 ± 0.38
Kitui 404	5.0001	29.9 ± 0.22	26.8 ± 0.10	3.7 ± 0.22
Kitui 405	5.0003	30.6 ± 0.21	27.2 ± 0.06	3.2 ± 0.34
Kitui 406	5.0000	30.8 ± 0.09	27.7 ± 0.05	3.9 ± 0.03
Kitui 407	5.0003	31.6 ± 0.08	24.5 ± 0.02	4.9 ± 0.21
Kitui 408	5.0000	28.7 ±0.06	23.7 ± 0.06	3.5 ± 0.04
Kitui 409	5.0001	29.3 ± 0.05	24.1 ± 0.03	3.6 ± 0.05
Kitui 410	5.0000	28.2 ± 0.07	23.1 ± 0.04	3.7 ± 0.09
Kitui 303	4.9999	28.6 ± 0.08	22.8 ± 0.06	3.6 ± 0.07
Kitui 305	5.0000	29.9 ± 0.06	26.1 ± 0.09	4.7 ± 0.24
Kitui 304	5.0002	28.3 ± 0.09	23.5 ± 0.07	3.1 ± 0.15
Kitui 301	5.0002	28.0 ± 0.11	23.8 ± 0.10	3.6 ± 0.16
Kitui 302	5.0000	28.4 ± 0.09	23.8 ± 0.12	3.2 ± 0.11
Kitui 210	5.0005	33.3 ± 0.04	29.8 ± 0.11	1.9 ± 0.08
Kitui 202	4.9998	26.5 ± 0.13	23.9 ± 0.09	4.6 ± 0.69
Kitui 203	4.9999	30 ± 0.32	26.5 ± 0.07	3.8 ± 0.54
Kitui 201	5.0000	31.3 ± 0.08	27.9 ± 0.06	3.2 ± 0.23
Kitui 204	4.9998	31.4 ± 0.06	27.5 ± 0.08	4.1 ± 0.71
Kitui 205	5.0004	27.2 ± 0.04	23.8 ± 0.04	3.6 ± 0.45
Kitui 206	4.9999	30.3 ± 0.07	27.0 ± 0.11	4.1 ± 0.31
Kitui 207	4.9999	33.7 ± 0.07	31.3 ± 0.12	2.2 ± 0.42
Kitui 208	5.0000	32.8 ± 0.09	29.9 ± 0.06	2.2 ± 0.23
Kitui 209	5.0003	32.4 ± 0.06	29.7 ± 0.06	2.2 ± 0.16
Kitui 105	5.0001	28.6 ± 0.07	25.0 ± 0.08	5.5 ± 0.85
Kitui 104	5.0001	26.1 ± 0.09	24.8 ± 0.06	5.4 ± 0.87
Kitui 103	5.0000	29.0 ± 0.09	26.5 ± 0.13	5.4 ± 0.65
Kitui 102	5.0002	28.4 ± 0.08	25.2 ± 0.07	5.2 ± 0.49
Kitui 101	4.9999	32.1 ± 0.11	30.1 ± 0.16	2.4 ± 0.12
Mean		**29.78 ± 2.07**	**26.33 ± 2.48**	**3.77 ± 1.02**

Appendix 7: Sugar Chromatogram

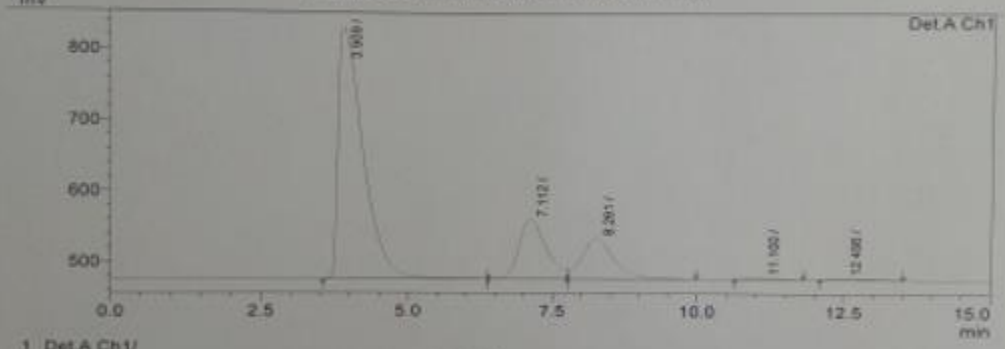
6/7/2018 12:38:41 1 / 1

==== National bee keeping Station ====
P.O BOX 34188
NAIROBI
ANALYSIS REPORT

C:\LabSolutions\Data\Project1\UON FILE71.lcd

Acquired by	: Admin
Sample Name	: Muserechi 3
Sample ID	: 2
Vail #	:
Injection Volume	: 20 uL
Data File Name	: UON FILE71.lcd
Method File Name	: sugars.lcm
Batch File Name	:
Report File Name	: Report4.lcr
Data Acquired	: 6/7/2018 12:14:12 PM

C:\LabSolutions\Data\Project1\UON FILE71.lcd



1 Det.A Ch1/

PeakTable

Detector A Ch1 285nm

Peak#	Name	Ret. Time	Area	Conc.	Units
1		3.909	10179447	0.000	
2		7.112	2797316	0.000	
3		8.261	2292779	0.000	
4		11.100	59058	0.000	
5		12.496	64291	0.000	
Total			15792891		

heoretical Plates/meth	Tailing Factor
2946.667	2.637
7418.297	0.000
7543.350	0.000
14253.987	1.143
15528.128	1.473

DONE BY Evalyne **DATE** 6th July 2018 **SIGN**

CHECKED BY JANE **DATE** 6/7/18 **SIGN** JK

Appendix 8: HMF Baringo Samples

Sample	Weight	HMF (mg/kg)
Esageri 4	5.0003	2.876 ± 0.029
Esageri 2	5.0002	4.575 ± 0.071
Esageri 3	5.0000	2.895 ± 0.006
Esageri 5	5.0003	4.471 ± 0.013
Esageri 1	5.0001	2.889 ± 0.009
Junction 417	5.0002	8.297 ± 0.140
Junction 402	5.0001	3.666 ± 0.021
Junction 404	4.9998	3.546 ± 0.061
Junction 401	4.9998	1.151 ± 0.023
Junction 403	5.0000	3.883 ± 0.017
Junction 411	5.0000	1.175 ± 0.044
Junction 412	5.0003	8.272 ± 0.269
Junction 413	4.9998	5.584 ± 0.463
Junction 414	5.0001	7.223 ± 0.189
Junction 415	5.0002	8.509 ± 0.223
Junction 416	5.0003	8.561 ± 0.101
Muserechi 1	5.0003	2.519 ± 0.085
Muserechi 2	5.0003	2.533 ± 0.043
Muserechi 3	5.0002	2.561 ± 0.049
Muserechi 4	4.9999	2.711 ± 0.099
Muserechi 5	5.0002	2.788 ± 0.013
Muserechi 6	5.0003	2.469 ± 0.027
Junction 421	5.0000	5.003 ± 0.028
Junction 422	5.0003	2.882 ± 0.016
Junction 423	5.0000	4.464 ± 0.342
Junction 424	5.0002	3.934 ± 0.131
Junction 425	5.0003	2.764 ± 0.063
Molo River 1	5.0003	1.615 ± 0.048
Molo River 2	5.0000	1.623 ± 0.019
Molo River 3	5.0003	2.036 ± 0.190
Mean		**3.91 ± 3.55**

Appendix 9: HMF Kitui Samples

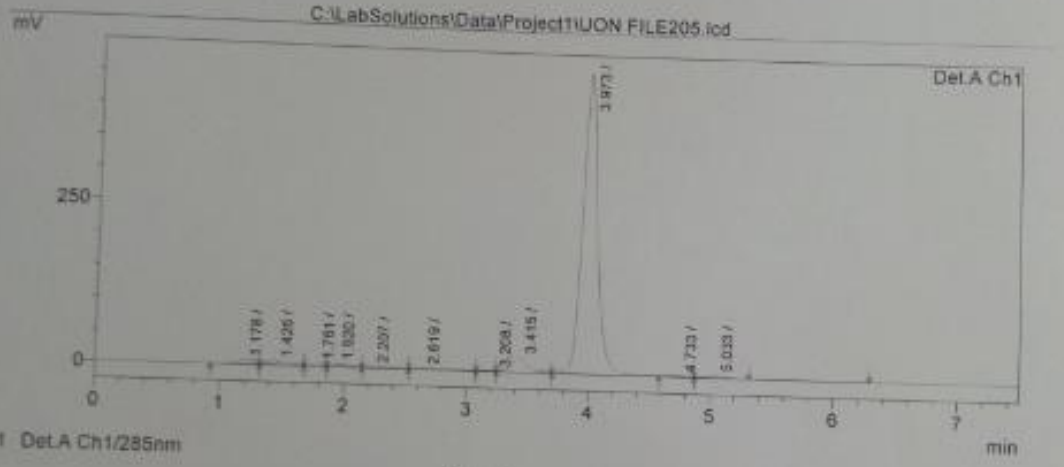
Sample	Weight	HMF (mg/kg)
Kitui 101	4.9999	29.21 ± 3.28
Kitui102	5.0003	15.56 ± 2.65
Kitui 103	4.9998	20.21 ± 0.03
Kitui 104	5.0003	22.39 ± 2.38
Kitui 105	5.0003	16.21 ± 5.83
Kitui 201	5.0003	19.31 ± 2.81
Kitui 202	4.9998	8.52 ± 2.84
Kitui 203	5.0003	25.53 ± 4.49
Kitui 204	5.0000	10.33 ± 2.86
Kitui 205	5.0001	34.82 ± 2.83
Kitui 206	5.0001	77.87 ± 11.21
Kitui 207	5.0001	22.94 ± 4.58
Kitui 208	5.0001	17.80 ± 3.45
Kitui 209	5.0001	21.88 ± 2.36
Kitui 210	5.0001	106.01 ± 11.26
Kitui 301	5.0001	23.53 ± 3.27
Kitui 302	5.0001	18.18 ± 3.23
Kitui 303	5.0001	36.44 ± 1.67
Kitui 304	5.0001	29.87 ± 3.33
Kitui 305	5.0001	26.67 ± 1.54
Kitui 401	5.0001	29.89 ± 3.15
Kitui 402	5.0001	75.86 ± 13.44
Kitui 403	5.0001	26.60 ± 3.45
Kitui 404	5.0001	32.52 ± 4.37
Kitui 405	5.0001	22.38 ± 2.34
Kitui 406	5.0001	13.99 ± 2.79
Kitui 407	5.0001	12.42 ± 3.25
Kitui 408	5.0001	11.15 ± 2.23
Kitui 409	5.0001	21.79 ± 2.14
Kitui 410	5.0001	71.72 ± 5.24
Mean		**30.06 ± 0.18**

Appendix 10: HMF Chromatogram

==== National bee keeping Station ====
P.O BOX 34188
NAIROBI
ANALYSIS REPORT

C:\LabSolutions\Data\Project1\UON FILE205.lcd

Acquired by	: Admin
Sample Name	: Kitui 102
Sample ID	: 1
Vail #	:
Injection Volume	: 20 uL
Data File Name	: UON FILE205.lcd
Method File Name	: HMF.lcm
Batch File Name	:
Report File Name	: Report4.lcr
Data Acquired	: 7/4/2018 9:22:04 AM



1 Det.A Ch1/285nm

PeakTable

Detector A Ch1 285nm

Peak#	Name	Ret. Time	Area	Conc.	Units
1		1.178	71701	0.000	
2		1.426	84119	0.000	
3		1.761	39621	0.000	
4		1.920	57436	0.000	
5		2.207	38321	0.000	
6		2.619	39717	0.000	
7		3.208	10594	0.000	
8		3.415	223303	0.000	
9		3.973	4105912	0.000	
10		4.733	12359	0.000	
11		5.033	41938	0.000	
Total			4725020		

heoretical Plates/mcn	Tailing Factor
664.090	0.000
987.981	0.000
4478.773	0.000
4226.449	0.000
316.599	0.000
4060.602	0.000
172.990	0.000
23073.019	0.000
32280.532	1.326
41524.893	0.000
36760.658	0.000

Appendix 11: Trace Element Concentration for Baringo Samples

Sample	Cu (µg/g)	Fe (µg/g)	Mn (µg/g)	Zn (µg/g)	Cr (µg/g)	Ba (µg/g)	Sr (µg/g)	Rb (µg/g)
Esageri 2	<0.01	9.3 ± 0.92	1.36 ± 0.48	2.47 ± 0.49	<0.01	0.36 ± 0.06	<0.01	<0.01
Muserechi 6	5.16 ± 1.04	23.4 ± 3.02	1.52 ± 0.40	2.57 ± 0.82	<0.01	0.56 ± 0.19	<0.01	<0.01
Muserechi 2	0.63 ± 0.05	30.1 ± 0.52	1.06 ± 0.14	9.87 ± 0.12	<0.01	0.16 ± 0.00	<0.01	<0.01
Molo River 1	<0.01	80.3 ± 3.42	11.8 ± 1.61	15.7 ± 0.83	<0.01	0.46 ± 0.25	0.12 ± 0.01	0.13 ± 0.01
Junction 403	5.21 ± 0.45	33.8 ± 2.52	1.16 ± 0.11	1.97 ± 0.36	0.21 ± 0.03	0.26 ± 0.04	<0.01	<0.01
Molo River 2	6.18 ± 0.27	15.7 ± 3.02	1.48 ± 0.09	3.17 ± 0.23	0.14 ± 0.03	0.16 ± 0.04	<0.01	<0.01
Esageri 5	7.88 ± 1.08	28.5 ± 1.92	1.59 ± 0.04	8.37 ± 0.86	0.23 ± 0.03	0.76 ± 0.04	0.11 ± 0.01	<0.01
Junction 425	8.76 ± 0.77	31.5 ± 3.32	1.28 ± 0.08	3.47 ± 0.49	0.78 ± 0.73	0.36 ± 0.09	<0.01	<0.01
Junction 413	9.04 ± 0.36	7.5 ± 3.22	0.93 ± 0.03	0.97 ± 0.5	<0.01	0.36 ± 0.12	<0.01	<0.01
Molo River 3	< 0.01	16.7 ± 1.72	1.28 ± 0.03	2.67 ± 0.19	0.34 ± 0.03	<0.01	<0.01	<0.01
Esageri 1	< 0.01	22.8 ± 0.72	0.73 ± 0.07	4.37 ± 0.16	0.28 ± 0.03	0.16 ± 0.03	<0.01	<0.01
Esageri 3	12.24 ± 1.47	11.3 ± 1.42	2.02 ± 0.16	3.27 ± 0.13	0.15 ± 0.03	0.16 ± 0.00	<0.01	<0.01
Esageri 4	13.93 ± 0.79	9.0 ± 0.52	2.73 ± 0.85	4.27 ± 0.18	0.18 ± 0.03	0.16 ± 0.04	<0.01	<0.01
Muserechi 1	14.59 ± 0.45	27.8 ± 2.12	1.82 ± 0.08	3.47 ± 0.14	0.2 ± 0.03	0.16 ± 0.00	<0.01	<0.01
Muserechi 3	15.66 ± 0.95	25.8 ± 1.32	1.17 ± 0.04	3.37 ± 0.17	<0.01	0.56 ± 0.25	<0.01	0.10 ± 0.01
Muserechi 4	16.8 ± 0.12	14.6 ± 1.82	1.38 ± 0.09	1.97 ± 0.22	0.27 ± 0.03	0.16 ± 0.03	<0.01	<0.01
Muserechi 5	17.90 ± 0.05	13.4 ± 1.72	1.06 ± 0.04	0.77 ± 0.33	0.29 ± 0.03	0.26 ± 0.07	<0.01	<0.01
Junction 411	<0.01	8.5 ± 2.32	1.71 ± 0.12	0.87 ± 0.16	0.25 ± 0.03	0.16 ± 0.02	0.12 ± 0.01	<0.01
Junction 412	19.59 ± 0.06	9.5 ± 2.32	1.01 ± 0.04	0.77 ± 0.2	0.23 ± 0.03	0.16 ± 0.03	<0.01	<0.01
Junction 414	20.67 ± 0.07	6.9 ± 0.52	1.59 ± 0.15	0.87 ± 0.09	<0.01	<0.01	<0.01	<0.01
Junction 415	<0.01	8.0 ± 0.12	0.83 ± 0.09	1.27 ± 0.16	0.18 ± 0.03	0.16 ± 0.02	<0.01	<0.01
Junction 416	<0.01	9.3 ± 0.82	0.69 ± 0.05	1.07 ± 0.16	0.24 ± 0.03	0.16 ± 0.01	<0.01	<0.01
Junction 417	23.49 ± 0.07	10 ± 0.02	1.30 ± 0.43	2.27 ± 0.22	0.22 ± 0.03	0.16 ±0.02	<0.01	0.13 ± 0.01
Junction 421	24.43 ± 0.04	24.8 ± 0.62	0.52 ± 0.14	2.57 ± 0.17	0.18 ± 0.03	0.16 ± 0.02	<0.01	<0.01
Junction 422	<0.01	22.1 ± 1.02	1.28 ± 0.06	1.37 ± 0.16	0.16 ± 0.03	0.16 ± 0.00	0.11 ± 0.01	<0.01
Junction 423	26.11 ± 0.09	16.9 ± 1.02	0.66 ± 0.09	0.97 ± 0.14	0.15 ± 0.03	0.16 ± 0.01	<0.01	<0.01
Junction 424	<0.01	13.8 ± 0.82	1.43 ± 0.19	1.07 ± 0.16	0.23 ± 0.03	0.16 ± 0.02	<0.01	<0.01
Junction 401	28.62 ± 0.09	29.8 ± 0.72	1.13 ± 0.06	1.07 ± 0.04	<0.01	<0.01	<0.01	<0.01
Junction 402	29.46 ± 0.04	17.9 ± 0.92	0.75 ± 0.23	0.87 ± 0.14	0.12 ± 0.03	0.16 ± 0.01	<0.01	<0.01
Junction 404	<0.01	15.6 ± 0.72	0.91 ± 0.05	0.37 ± 0.16	0.11 ± 0.03	0.16 ± 0.01	<0.01	<0.01
Mean	**1.99 ± 0.38**	**19.82 ± 2.74**	**1.58 ± 0.22**	**2.89 ± 0.77**	**0.22 ± 0.07**	**0.25 ± 0.09**		

Appendix 12: Trace Element Concentration for Kitui Samples

Sample	Cu (µg/g)	Fe (µg/g)	Mn (µg/g)	Zn (µg/g)	Cr (µg/g)	Ba (µg/g)	Sr (µg/g)	Rb (µg/g)
Kitui 104	6.76 ± 0.61	37.3 ± 0.61	2.6 ± 0.4	10.8 ± 0.52	0.47 ± 0.01	0.48 ± 0.049	0.21 ± 0.01	0.18 ± 0.02
Kitui 302	0.11 ± 0.08	83 ± 1.12	0.9 ± 0.07	3.1 ± 0.06	0.32 ± 0.01	0.29 ± 0.254	<0.01	<0.01
Kitui 206	<0.01	42.8 ± 0.83	1.1 ± 0.14	0.8 ± 0.11	<0.01	0.26 ± 0.028	<0.01	<0.01
Kitui 404	<0.01	93.8 ± 5.44	2.2 ± 0.09	<0.01	<0.01	0.49 ± 0.141	0.15 ± 0.02	0.16 ± 0.01
Kitui 101	0.56 ± 0.49	22.3 ± 1.45	0.8 ± 0.04	4.3 ± 0.11	0.29 ± 0.00	0.14 ± 0.035	<0.01	<0.01
Kitui 102	3.11 ± 1.15	20.1 ± 0.16	2.1 ± 0.12	2.8 ± 0.07	0.37 ± 0.01	0.15 ± 0.042	<0.01	<0.01
Kitui 103	1.15 ± 0.08	29.4 ± 0.77	1.9 ± 0.21	2.1 ± 0.14	<0.01	0.17 ± 0.056	<0.01	<0.01
Kitui 105	<0.01	18.7 ± 0.78	1.9 ± 0.03	0.9 ± 0.02	0.24 ± 0.01	0.36 ± 0.098	<0.01	<0.01
Kitui 201	<0.01	12.8 ± 0.69	1.3 ± 0.08	1.7 ± 0.05	0.28 ± 0.04	<0.01	<0.01	<0.01
Kitui 202	<0.01	13.7 ± 0.71	1.0 ± 0.06	1.6 ± 0.03	0.2 ± 0.00	0.2 ± 0.077	<0.01	<0.01
Kitui 203	0.33 ± 0.16	18.7 ± 0.61	2.0 ± 0.09	1.8 ± 0.05	0.22 ± 0.00	<0.01	<0.01	<0.01
Kitui 204	0.63 ± 0.06	11.6 ± 0.71	1.3 ± 0.07	0.9 ± 0.06	0.23 ± 0.02	0.18 ± 0.014	<0.01	<0.01
Kitui 205	<0.01	15.8 ± 0.61	1.2 ± 0.09	0.7 ± 0.04	0.21 ± 0.03	0.13 ± 0.014	<0.01	<0.01
Kitui 207	<0.01	20.6 ± 0.91	<0.01	2.3 ± 0.06	0.16 ± 0.00	0.21 ± 0.021	<0.01	<0.01
Kitui 208	0.37 ± 0.08	27.4 ± 1.12	0.6 ± 0.55	2.2 ± 0.02	<0.01	<0.01	<0.01	<0.01
Kitui 209	0.26 ± 0.04	25 ± 0.72	0.8 ± 0.06	0.9 ± 0.02	<0.01	0.27 ± 0.063	<0.01	<0.01
Kitui 210	0.68 ± 0.06	25.8 ± 3.52	1.2 ± 0.10	0.5 ± 0.05	0.24 ± 0.02	0.24 ± 0.063	<0.01	<0.01
Kitui 301	0.64 ± 0.06	23 ± 1.02	2.0 ± 0.12	<0.01	0.15 ± 0.00	<0.01	<0.01	<0.01
Kitui 303	<0.01	13.7 ± 0.62	1.6 ± 0.42	<0.01	0.16 ± 0.02	<0.01	<0.01	<0.01
Kitui 304	0.11 ± 0.01	15.2 ± 1.32	1.2 ± 0.04	0.8 ± 0.08	0.13 ± 0.00	0.14 ± 0.021	<0.01	<0.01
Kitui 305	0.13 ± 0.01	18.3 ± 0.72	0.8 ± 0.03	0.8 ± 0.0	<0.01	0.24 ± 0.042	0.15 ± 0.02	<0.01
Kitui 401	<0.01	12.0 ± 0.32	<0.01	1.3 ± 0.05	<0.01	<0.01	<0.01	<0.01
Kitui 402	0.54 ± 0.01	10.0 ± 0.12	1.1 ± 0.06	1.4 ± 0.02	0.215 ± 0.00	<0.01	<0.01	<0.01
Kitui 403	0.43 ± 0.05	8.0 ± 0.12	1.1 ± 0.03	0.9 ± 0.03	0.32 ± 0.02	0.15 ± 0.021	<0.01	<0.01
Kitui 405	0.77 ± 0.08	6.8 ± 0.43	0.9 ± 0.04	0.7 ± 0.04	0.315 ± 0.00	0.16 ± 0.028	<0.01	<0.01
Kitui 406	0.46 ± 0.00	10.0 ± 0.13	<0.01	1.7 ± 0.03	0.2 ± 0.00	0.22 ± 0.056	<0.01	<0.01
Kitui 407	<0.01	17.2 ± 0.83	1.4 ± 0.29	<0.01	0.33 ± 0.01	<0.01	<0.01	<0.01
Kitui 408	<0.01	25.8 ± 3.53	1.3 ± 0.16	2.5 ± 0.16	0.38 ± 0.00	0.12 ± 0.021	<0.01	<0.01
Kitui 409	0.51 ± 0.04	27.7 ± 0.73	1.2 ± 0.15	1.9 ± 0.07	0.29 ± 0.01	0.13 ± 0.021	<0.01	<0.01
Kitui 410	0.49 ± 0.02	28.6 ± 1.83	0.7 ± 0.24	0.7 ± 0.04	0.27 ± 0.01	0.15 ± 0.042	<0.01	<0.01
Mean	**1.04 ± 0.16**	**24.49 ± 3.38**	**1.30 ± 0.52**	**1.92 ± 0.41**	**0.25 ± 0.09**	**0.22 ± 0.08**		