QUALITY AND SAFETY CHARACTERISTICS OF HONEY PRODUCED IN DIFFERENT REGIONS OF KENYA

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2012

Quality and Safety Characteristics of Honey Produced in Different Regions of Kenya

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A thesis submitted in partial fulfillment for the degree of Master of Science in Food Science and Technology in the Jomo Kenyatta University of Agriculture and Technology

2012

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

This thesis is dedicated to my loving parents, Dr Macharius R. Olina and Mrs.Trufena Kerubo Orina, with love.

ACKNOWLEDGEMENT

My sincere gratitude to the Almighty God for enabling me undertake this course successfully and blessing my life abundantly.

With heartfelt gratitude I wish to acknowledge my supervisors Prof. G.M Kenji, Dr. A. Onyango and Dr .J. Maina, for their guidance, time, critical comments, suggestions and the encouragement they provided throughout the period of my studies. I also wish to thank Jomo Kenyatta University of Agriculture and Technology Management for providing financial support for this project and for the study leave during the two year period. I also appreciate all the help provided by the technical staff in the Department of Food Science and Technology and Department of Horticulture laboratories, especially Mr. Paul Karanja for assistance during the laboratory experiments.

I thank my darling husband, Nixon Mageka for his patience, understanding, prayers and love, not forgetting his unending moral support especially through the hard times, it would not have being easier without you, God bless. I also thank my family, friends and colleagues (Abraham Luvonga and Evans Makori) for their time and moral support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDICES

ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
AOAC	Association of Official Analytical Chemists
DDE	Dichlorodiphenylethane
EU	European Union
g	grams
GC	Gas Chromatography
HMF	Hydroxymethylfurfural
HPLC	High Performance Liquid Chromatography
KEBS	Kenya Bureau of Standards
Km	kilometers
ml	Milliters
µg	micrograms
MRL	Maximum Residue Limits
MS	Mass spectrometry
N	Normality
n	sample size
RID	Refractive Index Detector
ppm	parts per million
ppb	parts per billion
SPE	Solid Phase Extraction

$\mathbf{t_R}$	retention time
USA	United States of America
WHO	World Health Organization

ABSTRACT

Honey possesses numerous nutritional, healing and prophylactic properties. Honey has the image of being natural, healthy and clean. However, currently honey is produced in an environment polluted by contaminants from different sources. In order to have a beneficial effect, honey must be free of any contaminating agents. Heavy metals, chlorinated and phosphorous containing pesticides as well as medicinal substances of veterinary use are considered among the important potential harmful polluting agents. The extent of contamination of honey samples on sale in various important beekeeping zones of Kenya was evaluated. These zones include Mwingi, Kitui, Ntubo, Tharaka, Embu, Mbeere, Timboroa, Turbo, Malaba forest, Lenana forest, Thika Kakuzi, Kakamega forest and Taita Taveta. Parameters analyzed included moisture content, hydroxymethylfurfural (HMF), reducing sugars and sucrose content, free acidity, ash content, heavy metals (cadmium, lead, zinc, copper), residues of oxytetracyline, tetracycline, organochlorine and organophosphorous pesticides. Average constituent values were: Moisture (15.27-20.29%), HMF (2.69-263.36 mg/kg), pH (3.62-4.52), Free acidity (17.22-43.0 meq/kg), Total reducing sugars (63.24-73.34%), Ash content (0.05-0.3%), Sucrose (0.172-16.15%). Cadmium (0.01-0.05 mg/kg), Lead (0.01-0.05 mg/kg), Copper (0.07-0.24 mg/kg) and Zinc (1.01-2.10 mg/kg). Most of the samples had constituent levels within the limits sets by the Codex Alimentarius, indicating that most farmers' harvested ripened capped honey and that generally honey was stored under suitable conditions. There was significant difference in the honey samples from different

regions, this was expected. However, some samples had values of sucrose (11.16-15.47%) and HMF (59.03-263.36 mg/kg) way beyond the set limits of not more than 5% for sucrose and 40 mg/kg for HMF respectively, indicating adulteration of the honey. Most of the honey samples had tetracycline and oxytetracycline residues below the limit of detection which was set at 0.005 µg/ml. This showed that most farmers did not use drugs to treat their bees and if they do they are administered. Chlorinated and phosphorous containing pesticides analyzed included; Aldrin, pp-DDE, Endosulfan, Lindane, Dicofol and Chloropyrifos. The limit of detection was in the range of 0.002-0.008 µg/ml. No pesticide residues were detected in all the samples analyzed from different regions, indicating that the bees collected their nectar and water in pesticide-free environments. It is recommended that regulatory mechanisms for heavy metals, residues of antibiotics and residues of pesticides be put in place; this will strength honey monitoring and inspection within the industry.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND INFORMATION

Beekeeping is well established in Kenya and can be successfully carried out in about 80% of the country (Ministry of Trade & Industry, 2001). It is especially suitable in the semi-arid areas where other modes of agriculture cannot be sustained effectively. Beekeeping contributes to income as well as food security through provision of honey, beeswax and pollen as food and propolis, bee venom and royal jelly in medicine. It also contributes to seed and food production through pollination and conserves the natural environment (Ministry of Trade & Industry, 2001).

Honey can be defined as a sweet viscous liquid prepared by bees from nectar and honeydew collected from plant nectarines and processed before being stored by them for food (White, 1982). The composition and properties of honey are dependent on floral origins utilized by the bees and the climatic conditions of the area from which honey is harvested. Honey is a complex mixture, mainly composed of water, sugars (glucose, fructose, saccharose, maltose, and higher sugars), gluconic acid, nitrogenous compounds, minerals, and some vitamins (Doner, 1977).

Honey and bee products have the image of being natural, healthy and clean. During the last years, following the general trend of using what nature is directly offering, bee

products got an increasing importance as essential natural resource in promoting health, food and new therapy absolutely free from side effects of chemical medicines (Carmen *et al.*, 2001).

However, today bee products are produced in an environment polluted by different sources of contaminants. Heavy metals, chlorinated and phosphorus-containing pesticides, medicinal substances of veterinary use are considered among the important potential polluting agents (Bakan, 2002). Contamination can reach the raw materials (nectar, honeydew, pollen, plant exudates) by air, water, plants and soil and then transported into the beehives by bees (Bogdanov, 2005). Residual concentrations of contaminants in honey are highly restricted and controlled by a specialized international commission (Codex Alimentarius Commission). The quality and composition of honey can also be negatively affected by other factors such as overfeeding with sucrose and other sucrose variants, harvesting prior to maturity, unhealthy storage conditions and addition of invert sugars (Bakan, 2002; Bogdanov *et al.*, 2000; Oddo *et al*., 2004).

This study was undertaken to establish the quality and safety characteristics of honey produced in Kenya by investigating its physicochemical properties, levels of antibiotic and pesticides residues, as well as levels of heavy metals in honey obtained from different honey-producing areas.

1.2 PROBLEM STATEMENT

Studies show that the demand for residue-free honey, organic honey and other bee products continue to increase rapidly in the national, East Africa region and export market. In February 2002, the world honey market was strongly affected by a European Union (EU) ban on Chinese honey, following the identification of antibiotics in samples of Chinese honey. Since China was Europe's largest supplier of honey, this immediately led to a shortage of honey meeting EU criteria, and honey prices increased rapidly. The EU currently represents an excellent market opportunity for small producer groups in developing countries, with European and other buyers interested to buy more honey if it can meet EU criteria (Bradbear, 2009). Quality assurance in hive products is important in accessing both local and external markets. Currently, there is inadequate and inconsistent enforcement of existing standards. There is also inadequate training and poor coordination between public agencies charged with the responsibility of quality assurance (Report by Bio-Trade Company, 2009).

1.3 JUSTIFICATION

Beekeeping in Kenya is becoming an important component of today's strategies for sustainable agriculture and integrated rural development programmes. Beekeeping has a great potential for increasing income and supporting sustainable development. It is of economic importance in that it is relatively cheap, self reliant, it does not depend on importation of foreign equipment or inputs and beekeepers do not need to own land in order to keep bees. Various products such as honey, bees wax, pollen, propolis, bee

venom, royal jelly, queen bees and package bees and bee colonies can be sustainably obtained. Beekeeping is therefore considered an agro-based enterprise that is able to develop healthy linkages between biodiversity (insects and plants) and sustainable livelihood of the people through income generating hive products. Moreover, it offers comparative advantage with positive environmental consequences; bees are pollinators and many ecosystems depend on the pollination of bees for their existence and for increasing their genetic diversity (cross-pollination) (Lietaer, 2009).

Beekeeping has potential for earning substantial foreign exchange and transforming the living standard of stakeholders. With the introduction of modern beekeeping technologies (such as improved beehives and accessories, protective clothing and honey processing equipment as well as bee colony management), the industry has shown major development in various aspects and is now an important component of the livestock sector. In Kenya, honey and beeswax production is currently estimated at 14,653 and 140 metric tonnes (2007) respectively valued at Kenyan shillings 4.43 billion per annum. The country has an annual estimated honey and beeswax production potential of about 100,000 and 10,000 metric tonnes, respectively. Despite this huge potential the country is unable to meet its current local market demand for honey and beeswax which is estimated at about 15,000 metric tonnes. The deficit is met through imports (49.932 metric tonnes of honey in 2008) while the country exported 7.579 metric tonnes of honey in the same year. Importation of such products requires the enforcement of sanitary regulations to avoid sub-standard products, introduction of bee diseases and

pests and unregulated importation across the borders, which poses unfair competition to local producers. There also exists potential for the export market, but this has not been exploited due to low domestic qualities of hive products (Report by Bio Trade Company, 2009).

1.4 OBJECTIVES

Main objective:

To evaluate the quality of honey produced in different regions of Kenya with special emphasis on residues of contaminants.

The specific objectives were:

1. To determine the chemical properties of honey produced in different regions of Kenya.
2. To determine levels of heavy metals in honey produced in different regions of Kenya.
3. To determine residues of antibiotics in honey produced in different regions of Kenya.
4. To determine residues of pesticides in honey produced in different regions of Kenya.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 BEEKEEPING IN KENYA

Beekeeping in Kenya is a tradition that started time immemorial with traditional log hives playing an important role in the production of honey. Honey has been used in medicine, as food and food preservative, in cultural ceremonies and brewing traditional drinks. Out of the 42 tribes in Kenya, the most prominent communities in the beekeeping activity are the Kamba, Kalenjin, Meru, Maasai, Turkana, Kikuyu and Boran (Njoroge and Kioko, 2006).

About 80% of the country is suitable for beekeeping. The estimate production of honey is 80,000-100,000 metric tones and 10,000 tonnes of beeswax (Ministry of Trade and Industry, 2001). It is especially suitable in the semi-arid areas where other modes of agriculture are not very possible. These areas are dominated by acacia trees, which are known to produce sweet honey. Production areas are categorised into three depending on the production potential. These include; high potential areas (Eastern and Rift Valley Regions); medium potential (Coast, Nyanza and Western regions); low potential regions (Central, Nairobi, and North Eastern regions), (International Trade Centre, 2004).

Beekeeping has a very important role to play in the development of Kenya and other African countries. The sector has the potential to contribute positively to income

generation, alleviate poverty and serve as food security. Beekeeping also provides gainful employment both directly and indirectly. Apiculture contributes to seed and food production by facilitating pollination of plants and conserves the natural environment promoting agroforestry (International Trade Centre, 2004).

The types of beehives used in bee keeping in Kenya are the log, top-bar and langstroth hive. It is estimated that there are about 1.3 million log hives, 130,000 top-bar hive and just-over 30,000 langstroth hives in use in Kenya. It is known that the productivity of the log hives, which unfortunately comprise of over 95% the hives, is highly inefficient. This explains why honey production is very low and of inconsistent quality. The beehives (log, top-bar and langstroth) use the local raw materials for their production. There is need to encourage use of the high yielding beehives so as to enhance productivity (MacOsore *et al.*, 2005).

Honey production is seasonal depending on weather changes, there are two seasons. The first high season for honey production is between March and August and the second season is between September and January (International Trade Centre, 2004). Honey and honey products production is predominantly done by the small scale farmers in the arid and semi arid areas of Kenya where other forms of agriculture cannot be sustained unless heavy investments on water systems is done. Apiculture farmers sell their products to local traders who consolidate beehive products on behalf of the processors. Alternatively, some farmers have formed organised groups (cooperatives and/or self-

help groups) which deal directly with the processors. Where this has happened, increased honey production has been witnessed (International Trade Centre, 2004).

There are many different species of bees in the world-most of them solitary or live alone. A few species of bees are kept to produce honey. In Kenya, the most important species is called the honeybee (*Apis mellifera)*. Within this species there are a number of races of bees in Kenya which have their own particular characteristics. We have *Apis mellifera scutellata* (plains regions), *Apis mellifera monticola* (mountain region), *Apis mellifera yeminitica* (nubica) (North Eastern region) and *Apis mellifera littorea* (coastal region) (National Farmers Information Service (NFIS), 2008).

Production inputs required in the honey production are the processing equipment, which are required during harvesting, processing and packing. Before harvesting there are no inputs required since the bees are not fed commercially (International Trade Centre, 2004). After harvesting, honey is refined through sieving to separate pure honey from the wax and other impurities. Then depending on the customer preferences pure honey is packed either for shelf to be sold as table honey or in bulk for industrial use. Equipments used during processing include centrifuge extractor, honey strainers and beeswax making machine (MacOsore *et al*., 2005). It is important to note that the technology available and in use in the beekeeping is not modern. Most farmers use traditional methods and equipment. For example most use fire smokers that have the effect of tainting the taste of honey with smoke. Thus there is need for the industry to invest with

a view to improving technology in use and equipment. This will go a long way in enhancing the quality of honey products (International Trade Centre, 2004).

Other equipments that are used in processing include controlled temperature honey warmers, sample tanks straining equipments and settling and bottling containers. Imported inputs include packaging machines; homogeniser and honey testing equipments; and candle making machines (MacOsore *et al.*, 2005).

The colour of pure honey from Kenya varies from light to dark amber depending on the location of production and the types of trees in the locality. Kenya's honey is mostly produced where acacia trees abound and is renowned for its sweetness (National Farmers Information Service (NFIS), 2008).

2.2 HONEY COMPOSITION AND ITS USES

2.2.1 What is honey?

Honey is the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature (Codex Alimentarius Standard, 1981).

Honey sold should not have added to it any food ingredient, including food additives, nor shall any other additions be made other than honey. Honey shall not have any objectionable matter, flavor, aroma, or taint absorbed from foreign matter during its processing and storage. The honey should not have begun to ferment or effervesce. No pollen or constituent particular to honey may be removed except where this is unavoidable in the removal of foreign inorganic or organic matter (Codex Alimentarius Standard, 1981).

Honey is the most important product of beekeeping. Honey is food for man; is a useful source of high-carbohydrate food, and usually contains a rich diversity of minor constituents (minerals, proteins, vitamins and others), adding nutritional variety to human diets. Honey is widely used as a source of sugars for making honey wines and beers, and in the manufacture of many secondary products: breakfast cereals, bakery goods, and a multitude of other value-added products (Bradbear, 2009). It is used to preserve food. In many countries, honey is regarded more as a medicine or special tonic, rather than as an every-day food. Honey does have medicinal properties that are acknowledged increasingly by modern medicine. It is applied on wounds, burns and taken as syrup for coughs. In livestock, it is used to treat wounds (lesions) resulting from foot and mouth disease, foot rot (MacOsore *et al*., 2005).

Honey may be categorised according to its origin, the way it has been harvested and processed, and its intended use as shown in Table 2.1.

Table 2.1: Honey Categories

Category	Type	Description
Origin	Blossom honey	Obtained predominantly from the nectar of flowers (as opposed to honeydew honey).
	Honeydew honey	Is produced by bees after they collect 'honeydew' – secretions of insects belonging to the genus *Rhynchota*, which pierce plant cells, ingest plant sap and then secrete it again. Honeydew honey colour varies from very light brown or greenish to almost black, and is an important type of honey for producers in coniferous forest areas of Central and Eastern Europe
	Monofloral honey	Is where the bees have been foraging predominantly on one type of plant, and is named according to that plant. Common monofloral honey types are clover, Acacia, lime (linden) and sunflower honey. Monofloral honey is priced more highly than polyfloral honey. Light, monofloral honeys like orange blossom or Acacia – because they look so attractive – always obtain higher prices than blends of honeys.
	Multifloral honey (also known as polyfloral)	Has several botanical sources, none of which is predominant, for example, meadow blossom honey and forest honey
Processing	Comb honey	A piece of honeycomb, as produced by the bees, where the beekeeper has done no processing to separate the honey from the beeswax. The beeswax comb, as well as the honey, is edible.
	Strained honey	Is honey obtained by straining honeycombs, to separate the honey from the beeswax.
	Chunk honey	Is a jar of liquid honey inside which is placed a piece of comb honey. This can look very attractive. It is important that the liquid honey is a type that is very light and clear, and will not granulate over a long period. Honeys from Acacia and *Robinia pseudoacacia* are often used for this.
	Extracted honey	Is honey obtained by centrifuging honeycombs
	Pressed honey	Is extracted by pressing honeycombs with or without the application of moderate heat
	Crystallised or granulated honey	Is strained honey that has crystallised.

	Creamed honey	honey is strained honey that has been seeded to start crystallisation and then stirred to produce a honey of uniform, soft consistency
Intended use	Table honey	Means honey intended for consumers, to be eaten directly or as a natural sweetener for drinks or in cooking.
	Industrial or bakers' honey	Is honey that does not meet fully all the criteria for table honey, for example, the hydroxymethylfurfural (HMF) content may be higher than 40 mg/kg, although the regulations allow some exceptions. This may be because it has been heated too much, or it naturally has a high HMF, and is therefore regarded, according to the EU criteria, to be of lower quality than table honey. In this case, it still qualifies for use in the food industry, for the manufacture of bakery goods, confectionery, breakfast cereals, sauces, tobacco, and products such as honey-roasted nuts and pharmaceutical products. About 20 percent of honey on the world market is classified as bakers' honey.

Source: Bradbear, 2009

2.2.2 Honey composition

Honey is a mixture of different types of components as shown in Table 2.2, which bring out its unique sensory attributes.

Sugars account for 95 to 99% of honey dry matter. The majority of these are the simple sugars; fructose and glucose, which represent 85-95% of total sugars. Generally, fructose is more abundant than glucose. This predominance of simple sugars and particularly the high percentage of fructose are responsible for most of the physical and nutritional characteristics of honey, but the minor constituents - such as flavoring materials, pigments, acids, and minerals - are largely responsible for the differences among individual honey types (Bogdanov *et al*., 2008).

Small quantities of other sugars, such as disaccharides (sucrose, maltose and isomaltose) and a few trisaccharides and oligosaccharides are also present. Though quantitatively of minor importance, their presence can provide information about adulteration and the botanical origin of the honey (Doner, 1977).

Water is quantitatively the second most important component of honey. Its content is critical, since it affects the storage of honey. Only honeys with less than 18% water can be stored with little to no risk of fermentation. The final water content depends on a number of environmental factors during production such as weather and humidity inside the hive, but also on nectar conditions and treatment of honey during extraction and storage (Doner, 1977).

Among the minor constituents, organic acids are the most important and of this gluconic acid, which is a by-product of enzymatic digestion of glucose, predominate. The organic acids are responsible for the acidity of honey and contribute largely to its characteristic taste. Others are formic acid, lactic acid and malic acid (Doner, 1977). The color of honey varies from nearly colorless to dark brown. The consistency can be fluid, viscous or partly to entirely crystallized. The flavor and aroma vary, but are derived from the plant origin (Codex Alimentarius Standard, 1981).

Table 2.2: Honey Composition in g/100 g and pH

Component	Average	min.–max.
Water	17.2	15–20
Monosaccharides		
Fructose	38.2	30-45
Glucose	31.3	24-40
Disaccharides		
Sucrose	0.7	0.1–4.8
Others	5.0	2-8
Trisaccharides		
Melezitose	< 0.1	
Erlose	0.8	0.5–6
Others	0.5	0.5–1
Undetermined		
Oligosaccharides	3.1	
Total sugars	79.7	
Minerals	0.2	0.1–0.5
Amino acids, proteins	0.3	0.2–0.4
Acids	0.5	0.2–0.8
pH-value	3.9	3.5–4.5

Source: Bogdanov *et al.*, 2008

Minerals are present in very small quantities as shown in Table 2.3, potassium being the most abundant. Mineral/ash content contributes 0.17% by weight: Darker honeys have been shown to be substantially richer in minerals than lighter honeys, particularly potassium, chlorine, sulfur, sodium, iron, manganese and magnesium (Hermosin *et al.*, 2003; Airola, 2001). Other trace elements include nitrogenous compounds, among them

enzymes originating from salivary secretions of the worker honeybees. They have an important role in the formation of the honey. Their commercial importance is not related to human nutrition, but to their fragility and uniqueness. The main enzymes in honey are invertase (saccharase), diastase (amylase) and glucose oxidase (Doner, 1977). Traces of other proteins, enzymes or amino acids as well as water-soluble vitamins are thought to result from pollen contamination in honey.

Although honey is very unique in composition, the components are also very variable owing to geographical differences, climate and other factors. This fact makes it difficult to establish cases of adulterated honey. However, some specific components in honey, due to their specific occurrence, make it possible to determine whether honey has been tempered with (Bogdanov *et al.*, 2008).

Table 2.3: Mineral and Vitamin composition in Honey.

Component	Amount in mg/100g
Minerals	
Sodium (Na)	1.6–17
Calcium (Ca)	3–31
Potassium (K)	40–3500
Magnesium (Mg)	0.7–13
Phosphorus (P)	2–15
Zinc (Zn)	0.05–2
Copper (Cu)	0.02–0.6
Iron (Fe)	0.03–4
Manganese (Mn)	0.02–2

Chromium (Cr)	0.01–0.3
Selenium (Se)	0.002–0.01
Vitamins (mg)	
Phyllochinon (K)	ca. 0.025
Thiamin (B1)	0.00–0.01
Riboflavin (B2)	0.01–0.02
Pyridoxin (B6)	0.01–0.32
Niacin	0.10–0.20
Panthothenic acid	0.02–0.11
Ascorbic acid (C)	2.2–2.5

Source: Bogdanov *et al.*, 2008

2.3 HONEY CONTAMINANTS

2.3.1 Heavy metals in honey

Honey is a good source of major and trace elements needed for humans body. Their presence in human food is very important, but if they exceed safety levels, they will have adverse effect (Codex Alimentarius, 1993). Honey contains minerals such as calcium, iron, zinc, potassium, phosphorus, magnesium, selenium, chromium and manganese (Alkathiri & Khanbash, 1996; Murray *et al.,* 2001). Some of the trace elements present in honey are shown in Table 2.4. Emma and Susana (2006) reported that heavy metals in honey are of interest not only for quality control, but can be used as an environmental indicator. Heavy metals in honey can characterize the level of soil, plant, and air pollution.

Honey bees readily fly up to 4 km in all directions from their apiary and thus have access to an area of about 50 km^2. They are such a best small sampler that can be used in geochemical exploration. The bee honey has been used as monitors of a variety of environmental contaminants, including heavy metals (Holland and Turekian, 2003), low level radioactivity and pesticides (Nozal *et al.*, 2005, Bogdanov *et al*., 2007 and WHO, 1975).

Table 2.4: Trace Elements in Honey

Element	mg/100 g	Element	mg/100 g
Aluminium (Al)	0.01–2.4	Lead (Pb)*	0.001–0.03
Arsenic (As)	0.014–0.026	Lithium (Li)	0.225–1.56
Barium (Ba)	0.01–0.08	Molybdenum (Mo)	0–0.004
Boron (B)	0.05–0.3	Nickel (Ni)	0–0.051
Bromine (Br)	0.4–1.3	Rubidium (Rb)	0.040–3.5
Cadmium (Cd)*	0–0.001	Silicon (Si)	0.05–24
Chlorine (Cl)	0.4–56	Strontium (Sr)	0.04–0.35
Cobalt (Co)	0.1–0.35	Sulfur (S)	0.7–26
Floride (F)	0.4–1.34	Vanadium (V)	0–0.013
Iodide (I)	10–100	Zirconium	0.05–0.08

* Elements regarded as toxic, can be partially of man-made origin. Source: Bogdanov *et al*., 2008.

It is important to take in account the type of equipment used to produce honey as well as the quality of the equipment used to store honey after harvesting as also possible sources of honey contamination with heavy metals. Contact with stainless steel surfaces during harvesting, processing and/or preparation of honey for the market, can generate high

chromium content, due to the corrosive effect of honey acidity. Likewise, storing honey in galvanized containers can be a source of zinc contamination (González *et al*., 2000; Bogdanov *et al*., 2003). The amount of Zn in honey depends on the geographical location of apiary, acidity of the ground and particularly on the instrument used in apiaries, centrifuge and storage of honey, transport utilities and technologies process. Volume of Pb on the other hand depends on the location of apiaries since it is obvious that internal combustion engines are the main source of contamination with Pb (Birute *et al.*, 2006).

Lead (Pb) and cadmium (Cd) are considered the principle toxic heavy metals and are thus most frequently studied. Lead, contained in the air and originating mainly from motor traffic can, contaminate air and then directly nectar and honeydew. Generally, lead is not transported by plants. On the other hand, Cd originating from metal industry and incinerators is transported from soil to plants and can then contaminate nectar and honeydew. Only a small portion of Cd might reach honey by air, mainly in the vicinity of incinerators. Other heavy metals like mercury (Hg) and nickel (Ni) have been much less frequently studied. Worldwide there are no specific Maximum Residue Limit (MRL) levels for these heavy metals in honey, (Bogdanov, 2005).

Heavy metals are toxic because they cause DNA damage and their carcinogenic effects in humans are caused by their mutagenic ability (Baudoiun *et al.*, 2002). A very important biological property of metals is their tendency to bioaccumulate (Bousquet *et*

al., 1984; Shukla *et al*., 2007). A potential threat is that heavy metals are not readily degradable and without intervention may progressively bioaccumulate in the body. These toxic elements may therefore constitute pollutant-induced harm. While apparently uncommon, allergies to honey have been reported and can involve reactions varying from cough to anaphylaxis (Kiistale *et al.,* 1995). Other symptoms may include dizziness, nausea, vomiting, convulsions, headache, palpitations and death in some cases (Bogdanov *et al*., 1999). Additionally, long term ingestion of honey containing heavy metals such as Cu and Fe may lead to significant reactions including gastrointestinal disorders (Salem, 1982).

2.3.2 Residues of antibiotics in honey

In the recent past, news about "contaminated honey" has been distributed by the mass media in the European countries. The most recent example is the news about antibiotic-contaminated honey. Antibiotics such as tetracyclines have been widely used for treating diseases in animal including bees (Li, *et al*., 2008). The occurrence of antibiotic residues in human food arising from its veterinary use is a cause of concern to consumers worldwide, because of possible toxic or allergic reactions and the possibility that pathogenic organisms could become resistant to these drugs (Pena *et al*., 2005). Tetracyclines are used against a wide range of gram negative and gram positive micro-organisms, including some anaerobes and have been widely used for the treatment of infectious diseases and as an additive in animal foodstuffs (Goodman, *et al*., 1985).

The food contamination problem in beekeeping centers around the use of sulphonamides and antibiotics for the control of bacterial honeybee diseases such as American foulbrood and European foulbrood (Spivak, 2000). American Foulbrood, a bacterial (*Paenibacillus larvae* subsp. *Larvae)* disease of honey bee (*Apis mellifera* L.) brood, is spread to all continents where there are honey bees (Matheson, 1993). The pathogens produces extremely environmentally stable spores and once clinical symptoms of diseased brood are visible to the beekeeper, infected colonies are likely to succumb to the disease if left untreated (Hansen and Brodsgaard, 1999). Because of the severity of American foulbrood, its control is often regulated by law and many countries require the destruction of infected colonies. Where destruction of visibly infected colonies is not required, antibiotics are often used both therapeutically and as a prophylaxis (Shimanuki, 1997). The latter approach for disease control is now in jeopardy because of a widespread tolerance of the pathogen to the most widely used antibiotic formulations (Miyagi *et al.,* 2000).

The causative agent of European foulbrood (EFB) is *Melissococcus pluton*. This disease is quite different from American Foulbrood, and is less dangerous since it is less contagious, and colonies with EFB can be treated and cured of the disease. The smell of EFB is distinct from that of American Foulbrood. EFB affects mainly unsealed brood. Some beekeepers burn and destroy the colony and the hive. However, worker bees remove diseased larvae outside the hive and a colony with EFB can sometimes survive without intervention from the beekeeper. Strong colonies are more resistant to this

disease. The disease will be more common in small colonies that are stressed, for example, colonies belonging to migratory beekeepers, and those that are short of water. Some beekeepers treat bees with antibiotics such as *Terramycin* (tetracycline), which merely suppress the bacteria population – the antibiotic is mixed with sugar and spread on to the colony or diluted with syrup and sprayed on to the colony with a six-week post-application interval prior to harvesting the honey (Bradbear, 2009). If bees are continuously fed antibiotics, the symptoms of the disease will never show. The use of antibiotics in this way is not an environmentally sound procedure and is banned by law in many countries. Excess use of antibiotics allows them to enter the food chain and risks selecting resistant disease-causing organisms within the human population, thus making these compounds useless in controlling important human diseases (Bradbear, 2009).

Beekeeping in Africa is still largely based on traditional hives or hives where combs are not stored and/or changed between colonies. In Kenya, for example, it is estimated that only approximately 10% of the bee colonies are found in moveable frame hives, most of them in Kenya Top Bar Hive (Mbae, 1999). As the introduction of modern langstroth equipment increases in East-Africa and elsewhere, it is of particular interest to know if *P. larvae* subsp. *larvae* is present in regions where transition from traditional beekeeping to modern methods is actively taking place. If the pathogen is present in the bee population it may surface later partly because of new management practices (Ingemar & Suresh, 2003).

In general, two types of drugs, tetracycline and sulphonamides are frequently used, although during the recent years other antibiotics like chloromphenol, streptomycin and nitrofuran have been recognized to be administered to colonies by beekeepers. The application of the law in relation to these antibiotics is not harmonized across all member states of the European Union. The Commission of the European Union laid down the procedure for establishing maximum residue limits (MRLs) of veterinary medical products in foodstuffs of animal origin (Council Regulation (EEC), 1990). However, no MRLs have been fixed for using with bee products.

The EU is by far the world's largest market for honey. Moreover, it is not self-sufficient but needs to import very large amount of honey from countries outside the EU. In 2002, Chinese honey was banned by the EU. The honey ban was imposed because chloramphenicol was found in the honey, which can be dangerous for certain people. The ban on Chinese honey was lifted in 2004. However, EU importers have become very reluctant to buy Chinese honey. The reputation of Chinese honey has been severely damaged and the recovery of supplies of Chinese honey for the EU will depend on improvements to the quality control system applied by Chinese exporters (CBI Market Survey, 2009). The increasing demand for residue-free honey opens opportunities for honey producers in the developing countries like Kenya to export their honey thereby improving the livelihood of beekeepers.

2.3.3 Residues of pesticides in honey

Pesticides are used for public health, animal and plant protection purpose. Pesticides marketed in Kenya include insecticides, fungicides, nematicides and miticides. Most of the pesticides are used in agriculture sector to control livestock, plant pests and diseases. For public health purposes pesticides are used to control mosquitoes and tsetse flies which are disease vectors. These pesticides are largely synthetic compounds which kill or deter the destructive activity of the target organism. Unfortunately, these compounds possess inherent toxicities that endanger the health of the farm operator, consumer and the environment (Ministry of Tourism, Trade & Industry, 2006).

The use of pesticides has certainly increased agricultural production, and improved longevity and quality of life. Coupled with these successes are a number of side effects. Pesticide use is still indispensable in Kenya in the area of agricultural production and public health vector control. However, the toxicity of these compounds and their presence in the environment pose grave issues that obliges the development of methods that will increase agricultural productivity and disease vector control with minimal environmental contamination and side effects to non-target species (Musa *et al*.,2011).

Honeybees are the main pollinating agents for numerous plants and fruit trees and hence, play a key role in agriculture and more generally in the maintenance of ecological biodiversity. They are the most affected farm animals by pesticides (Robert, 2006). Persistent pesticide use in agriculture can theoretically contaminate bee products.

Honeybees may be poisoned when they feed on nectar or pollen contaminated by pesticides. Bees may also be poisoned when they fly through a cloud of pesticide dust or spray or walk on treated parts of plant. Sometimes, colonies in the hives can be directly affected, but most commonly only field bees are killed or have their physiological functions altered, (Robert, 2006).

According to Nyamu (2008), there is increasing use of chemicals and pesticides among Kenyan farmers. The government is focused on boosting crop yields yet there is lack of regulation and information to guide users on the use, health and environment hazards of the pesticides. In rural areas, the main concern is the indiscriminate use of pesticides. Certain pesticides used extensively in small-scale agricultural activities are so lethal that their use is either banned or is being phased-out in countries such as Canada. Kenya's importation and use of agrochemicals has more than tripled in the last decade, but majority of farmers do not handle the hazardous chemicals safely (Nyamu, 2008).

Previous research conducted on sediments, micro-invertebrate organisms from both marine and freshwater ecosystems in Kenya have continually revealed contamination by pesticides (Barasa, 1998; Everaarts *et al*., 1997; Getenga *et al*., 2004; Mugachia *et al.*, 1992; Munga, 1985; Wandiga *et al.*, 2002). Levels of pesticide contamination at the top of the food chain in the basin have been exhibited by presence of residues in the cow and human milk, and bird eggs (Kahunyo *et al.*, 1986; Kanja, 1988; Kituyi *et al.*, 1997; Wandiga and Mutere, 1988).

Pesticide use in Kenya is already one of the highest in sub-Saharan Africa with a market share of approximately USD 40.4 million by 2003 (Gonzalez *et al*., 2003). However, several chemical contaminants from the agricultural fields, comprising of pesticides and other agrochemicals have been reported in the drainage systems and are likely to jeopardise the quality of the water bodies that support the fishery industry and are used for domestic human consumption. The use of the pesticides poses a great challenge to the country to develop satisfactory techniques that can combine optimal agricultural productivity and environmental protection (Musa *et al.*, 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY DESIGN

The study was divided into three parts: i) collection of samples, ii) proximate analysis of the samples iii) analysis of pesticide residues, antibiotics and heavy metals.

3.2 STUDY SITE

The honey samples were obtained from identified beekeepers and honey traders in various important beekeeping zones in Kenya. Sample size of each region was determines according to the honey production potential of the region. The regions include; Mwingi (n=14), Kitui (n=10) Mbeere (n=5), Timboroa (n=3), Turbo (n=3), Taita Taveta (n=5), Kakamega forest (n=3), Malaba forest (n=3), Tharaka (n=5), Ntubo (n=3), Embu (n=3), Lenana forest (n=2) and Thika Kakuzi (n=3). Samples were collected during the two seasons of honey harvesting that is between September 2009 and January 2010 and March 2010 and August 2010. The honey samples were collected within one month after harvesting and stored at room temperature ($25 \pm 2\ ^{0}C$) away from direct sunlight and analyzed within two weeks.

3.3 ANALYSIS OF CHEMICAL PROPERTIES

3.3.1 Determination of moisture content of honey

Five grams of each sample was weighed and placed into a pre-weighed aluminum drying dish. The samples were dried to constant weight in an oven (Model WFO-1000ND, Tokyo Rikakikai CO., LTD) at 105 ^{0}C for 4 hours under vacuum. (AOAC, 1995).

Percent moisture was calculated as follows:

$$\frac{\text{(Weight of dish + sample before drying) – (weight of dish + sample after drying) * 100}}{\text{Weight of the sample}}$$

3.3.2 Determination of ash content

Five grams of each honey sample was separately weighed out into a porcelain crucible previously ignited and weighed. Organic matter was charred by igniting the sample on a hot plate in the fume cupboard. The crucibles were then placed in a muffle furnace (Model KL-420, Toyo Seisakusho Co.LTD) and maintained at 550 ^{0}C for 6 hours. They were then cooled in a desiccator and weighed immediately (AOAC, 1995).

The Ash was calculated on a percent scale as:

$$\text{Ash (\%)} = \frac{\text{(weight of crucible + ash)-(weight of empty crucible)*100}}{\text{Sample weight}}$$

3.3.3 Determination of pH and free acidity

Ten grams of each honey sample was dissolved in 75 ml of distilled water in 250 ml beaker, stirred with magnetic stirrer, pH meter (Model 20, Denver Instrument Company) electrode was immersed in the solution and pH was recorded. The solution was titrated with 0.1N NaOH solution until a pH of 8.3 was attained (AOAC, 1995).

$$\text{Free acidity (miliequivalent/kg)} = \frac{(\text{ml of 0.1N NaOH used for sample} - \text{ml blank}) * 50}{\text{Weight of sample (g)}}$$

3.3.4 Determination of hydroxymethylfulfurals (HMF)

Spectrophotometer-UV-VIS (UV-1601 PC model, Shimadzu Corp., Kyoto, Japan) was used to measure Absorbance at 284 and 336 nm. Carrez solution I was made by dissolving 15 g of $K_4Fe(CN)_6.3H_2O$ in 100 ml of distilled H_2O. Carrez solutions II was made by dissolving 30 g of $Zn(CH_3COO)_2$ in 100 ml of distilled H_2O. Sodium hydrogen sulphite solution (0.20%) was made by dissolving 0.20 g of $NaHSO_3$ in 100 ml of distilled H_2O (freshly prepared).

Five grams of each honey sample was weighed accurately in a small beaker and transferred with 25 ml of H_2O to 50 ml volumetric flask. 0.5 ml of Carrez solution I was added then mixed, 0.5 ml of Carrez solution II was also added and mixed then diluted to volume with H_2O. The mixture was filtered through filter paper, the first 10 ml filtrate was discarded. 5 ml of the filtrate was pipette into each of two test tubes. 5 ml H_2O was

added to one tube (sample) and 5 ml $NaHSO_3$ solution to other (reference). The reactants were mixed well (using vortex) and the absorbance of samples was determined against reference at 284 and 336 nm in one centimeter cells (AOAC, 1995).

mg (HMF)/100g honey = (A284-A336)*14.97*5/ weight of sample (g)

3.3.5 Determination of sucrose by High Performance Liquid Chromatogram (HPLC)

Five grams of each honey sample were weighed into a small beaker and transferred to a 50 ml volumetric flask with 25 ml H_2O. Acetonitrile was added to volume immediately and then followed by filtration through 0.45µm microfilter. 2.5 grams of sucrose was weighed into a 50 ml volumetric flask and made to volume with acetonitrile: water (50:50 v/v) this was the standard solution. Serial dilutions were made from the stock solution containing the sugars. (AOAC, 1995).

Fifty microliter (µl) of the standard solution and sample solutions were injected into the HPLC (model C-R7A plus Shimadzu Corp., Kyoto Japan) fitted with NH_2P ,250 × 4.6 mm, 5 µm columns. At an oven temperature of 30 ^{0}C and equipped with RI detector (Model RID-6A, Shimadzu Corp., Kyoto, Japan). The mobile phase was Acetonitrile: Water (75:25) flowing at the rate of 1.0 µl per min.

3.3.6 Determination of Total Reducing Sugars by Lane-Eynon method

Fehling solution 1 was made by dissolving 34.639 g of $CuSO_4.5H_2O$ in distilled H_2O and made up to 500 ml and then filtered. Fehling solution 2 was made by dissolving 173 g of sodium potassium tartarate and 50 g of NaOH in distilled H_2O and made up to 500 ml**.** Methylene Blue solution was made by dissolving 1 g of methylene blue reagent in 100 ml of distilled H_2O.

One grams of honey was weighed and diluted with distilled water to 250 ml in a volumetric flask. 5 ml of each Fehling solution 1 & 2 were added in a conical flask. 15 ml of each sample sugar solution was added from the burette and mixed well. The mixture was then heated to boil. 4 drops of methylene blue was added. Without removing the flame, titration was completed when the indicator was decolorized with sample solution, this was the preliminary titration. In the final titration, 5 ml of Fehling solution 1& 2 were added in a conical flask. The sample solution with volume 1 ml less than the volume required in the preliminary titration was added. The mixture was boiled for 2 minutes then 4 drops of methylene blue were added. Without removing the flame, titration was completed when the indicator was decolorized with sample sugar solution (AOAC, 1995).

Total reducing sugar content (%) = a * f * V/b * 1/1000 * 1/S * 100

Where:

a = Lane- Eynon factor as mg of reducing sugar corresponding to the milliliters of sample sugars solution required from the table.

B= ml of the sample sugar required

f= Factor of Fehling solution 1

S= weight of sample taken (g).

V= volume of the sample sugar solution prepared.

3.4 DETERMINATION OF HEAVY METALS BY ATOMIC ABSORBANCE SPECTROMETRY (AAS).

Minerals were determined by acid digestion according to the method described by Gupta (1999). 1 gram of honey sample was placed in 100 ml conical flask. To this 20 ml of acid mixture (three parts HNO_3 with one part $HCLO_4$) was added. The flask was placed on hot plate in a digestion chamber. Then, the flasks were heated until production of red NO_2 fumes ceases. The content was further evaporated until the volume was reduced to about 3 to 5 ml but not to dryness. Completion of digestion was confirmed when the liquid became colorless. After cooling the flask, 20 ml of distilled water was added and the solution filtered through Whatman No.1 filter paper and transferred to 25 ml flask. It was then made up to the mark with deionized water.

The levels of Pb, Cd, Zn, and Cu were determined by atomic absorption spectrophotometer (AAS) using standard methods. Working standards ranged from 0-1 ppm for cd and Pb and 0.5-2.5 ppm for Zn and cu were prepared from the standard

solution by serial dilution. Each standard was aspirated into the AAS (Model AA-6200, Shimadzu, Corp., Kyoto Japan) and its respective absorption was recorded to prepare a standard curve. The same procedure was applied for the prepared sample solutions for each extract and results recorded. The mineral concentrations were calculated from the standard curve.

3.5 DETERMINATION OF RESIDUES OF TETRACYCLINE AND OXYTETRACYCLINE

Three grams of each honey sample were weighed into centrifuge tube. Fifteen ml of McILvaine buffer (pH 4.0) with 0.1 $molL^{-1}$ Na_2EDTA were added and the mixture vortexed until the honey dissolved completely. The solid phase extraction (SPE) cartridge was conditioned with 5 ml of methanol and 5 ml of McILvaine buffer (pH 4.0) containing 0.10 mol L^{-1} Na_2EDTA. After conditioning, 5 ml of sample were allowed to pass through the cartridge followed by 2.5 ml of McILvaine buffer (pH 4.0): methanol (85:15 v/v) and 2.5 ml of water. The cartridge was dried for 2 minutes by aspiration and another washing step with 2.5 ml acetonitrile was done. Cartridge was dried again for one minute and analytes were eluted with 3.0 ml of ethyl acetate: methanol (75:25 v/v). The elution mixture was evaporated until dryness under gentle nitrogen flow in a water bath (30-35 ^0C) and the residue was dissolved in 1 ml methanol: water (15-85 v/v) (Gustavo, *et al.*, 2010).

Twenty µl of the final extract were injected onto the HPLC system (Shimadzu VP series (Japan)) which was equipped with binary LC-10AD pump, DGU-14A degasser, CTO-10AVP column oven set at 35 ^{0}C and SPD-10A UV-VIS detector set at 360 nm wavelength. The separation was performed on an Inertsil ODS-2 column. The mobile phase was 0.01M oxalic acid, acetonitrile and methanol (16:3:2) flowing at 0.6 ml/min. The authentic standards were hydrochloride salts of tetracyclines and oxytetracycline. Tetracycline and oxytetracycline stock solution (100 µg/ml) were prepared in mobile phase, serial dilutions (0.1-1 ppm) were then made.

3.6 ANALYSIS OF RESIDUES OF PESTICIDES

Five grams of each honey sample was mixed with 50 ml of water and agitated by a stir bar for 10 minutes. C18 cartridges were preconditioned by passing 10 ml of methanol and 10 ml of water with the aid of a vacuum pump to avoid dryness. The sample was passed through the solid phase and the retained pesticides eluted by passing first 10 ml of ethyl acetate, followed by 4 ml of methanol and then 1 ml of dichloromethane. The eluate was evaporated to 0.5 ml using a gentle steam of nitrogen and reconstituted with methanol to 1ml, obtaining a final extract in 100% methanol. One µl was injected into the GC-MS system.

GC analysis was carried out on a Finnigan GC-8000 series, interfaced with a voyager EI-MS detector (CE Instrument, Milan, Italy). Column used was a fused silica capillary column (30m*0.25mm I.D., 0.25µm) with chemical bonded phases DB-5. The injector

temperature was 220 ^{0}C and the detector one was 280 ^{0}C. Sample was injected in the splitless mode, and the splitless was opened after 60 seconds. The oven temperature was as follows: initial temperature of 150 ^{0}C held for 1 min, increased to 230 ^{0}C at 3 ^{0}C min^{-1}, held for 15 min and then increased to 250 ^{0}C at 3 ^{0}C min^{-1} and held for 15 min. the MS ionization potential was 70eV and the temperatures were as follows: ion source 250 ^{0}C, transfer line 200 ^{0}C and analyzer 230 ^{0}C (Cristina et al., 2003).

3.7 DATA ANALYSIS

Data were assessed using Analysis of Variance (ANOVA) with the Statistical Analysis Software (SAS) statistical package (Snedecor and Cochran, 1987). Mean comparison for treatments were made using Duncan's Multiple Range Tests (Steel and Torrie, 1980). The mean values were reported with standard deviations (S.D) of the means.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 CHEMICAL PROPERTIES OF HONEY FROM DIFFERENT REGIONS

4.1.1 Moisture content, pH, acidity and ash of honey from different regions

The means for the quality variables analyzed (moisture, pH, acidity and ash) in honey from different regions are summarized in Table 4.1. The moisture content of the honey samples from various locations ranged from 15.27-20.29% meaning that all the samples had moisture content within the limit allowed by the Codex, Council of the European Union (EU) and Kenya Bureau of Standards (KEBS) of ≤ 21%. The pH of the honey samples ranged from 3.62-4.52; this was also within the acceptable limit of ≤ 5.6 (Kirkwood *et al.*, 1960). The maximum acceptable limit for free acidity of honey is ≤ 50 meq/kg; all the samples were within this acceptable limit. The ash content of honey from various locations ranged from 0.05-0.30% and was within the acceptable range of 0.04-0.93% (White, 1975; Kirkwood *et al*., 1960).

There was a significant ($p < 0.05$) difference in moisture between honey from different locations. Kakamega (20.26%), Embu (20.29%) and Kitui (19.76%) honeys had the highest moisture content while Lenana (15.27%), Timboroa (15.75%) and Thika (15.64%) honeys had the lowest values. There was a significant difference at $p < 0.05$ in pH, free acidity and ash content between honeys from different regions. Honey from

Ntubo, Thika, Kitui and Turbo had the highest pH and were not significantly different from each other. Honey from Embu had the highest content of free acidity and was significantly different from Lenana, and Kakamega which had lowest free acidity. Mwingi had the highest ash content of 0.3% while Taita Taveta lowest (0.05%).

Moisture content is the criterion that determines the capability of honey to remain stable and resist spoilage by yeast fermentation. High moisture content increases the probability/risk that the honey will ferment upon storage. The final moisture content of a honey sample depends on a number of environmental factors during production such as weather, humidity amounts inside the hive, nectar conditions and treatment of the honey during storage and extraction (Muli *et al*., 2007). All the honey samples from various locations had moisture content that were within the acceptable range, an indication that most farmers harvested ripened capped honey and that generally honey was stored under suitable conditions. The differences in the means of the moisture content of honey from the various locations were expected due to difference in environmental conditions and nectar source, they were comparable to those obtained by Muli *et al* (2007). The means of moisture were also comparable to Ugandan honey as reported by Kugonza and Nabakabya (2008). However, they were low compared to Tanzanian honey whose moisture content ranged from 21.6-22.7% as reported by Andrews *et al*., 2004.

Table 4.1: Moisture, pH, free acidity and ash content of honey from different regions

Area	Moisture (%)	pH	Free acidity (meq/kg)	Ash (%)
Mwingi (n=14)	18.38^{b}±1.63	4.09^{c}±0.13	34.92^{ab}±3.20	0.30^{b}±0.04
Ntubo (n=3)	15.87^{e}±0.03	4.45^{a}±0.02	25.33^{bcde}±1.53	0.18^{cd}±0.01
Tharaka (n=5)	17.91^{bc}±1.76	4.02^{cd}±0.08	27.67^{bcd}±4.89	0.14^{cd}±0.05
Embu (n=3)	20.29^{a}±0.20	3.70^{e}±0.01	43.0^{a}±2.0	0.11^{de}±0.14
Mbeere (n=5)	16.82^{cd}±1.99	3.97^{cd}±0.01	35.0^{ab}±3.5	0.18^{cd}±0.01
Timboroa (n=3)	15.75^{de} ± 0.06	3.82^{de}±0.01	20.67^{cde}±0.58	0.09^{de}±0.01
Turbo (n=3)	16.71^{cde}± 0.03	4.40^{ab}±0.01	17.67^{de}±0.58	0.13^{cde}±0.03
Malaba (n=3)	16.35^{cde}± 0.08	4.17^{bc}±0.08	18.33^{de}±1.15	0.12^{cde}±0.02
Lenana (n=2)	15.27^{e}±0.20	4.18^{bc}± 0.01	14.33^{e} ± 0.58	0.17^{cd}±0.03
Thika Kakuzi (n=3)	15.64^{e} ± 0.60	4.52^{a}±0.01	22.0^{cde}±1.0	0.16^{a}±0.03
Kitui (n=10)	19.76^{a}± 0.43	4.35^{ab}±0.30	29.0^{bc}±5.80	0.08^{c}±0.02
Kakamega (n=3)	20.26^{a}± 0.72	3.87^{de}±0.07	17.22^{e}±2.54	0.19^{e}±0.10
Taita Taveta (n=5)	15.94^{e}±0.33	3.62^{e}±0.03	36.67^{ab}±1.21	0.05^{e} ±0.01
Quality standards				
EU	≤ 21	≤5.6	≤ 50	0.6
Codex	≤ 20	≤5.6	≤ 50	0.6
KEBS	≤ 20	≤5.6	≤ 50	0.5

Value = Mean ± S.D. Means of the same parameter followed by the same letter are not significantly different ($p < 0.05$). S.D= Standard deviation. n=sample size

The significance of pH at acidic range in foods cannot be overemphasized. The low pH of honey inhibits the presence and growth of microorganisms. These parameters have great importance during the extraction and storage of honey, as they influence the texture, stability and shelf life of honey (Terrab *et al*., 2002). Therefore they prevent the honey samples from constant infection by various species of micro-organisms and thus help to ensure constant shelf life for the honey samples (Lawal *et al.,* 2009). It is important to note that the pH of honey does not directly reflect the total acid content, but rather reflects the buffering action of the inorganic cation constituents on the organic acids present (Sief and Elfadil, 2009). The acidic pH of all honey samples from various locations indicates they have a good and stable shelf life. The difference in the means of pH from various locations was due to the difference in environmental conditions including the source of nectar. The means of pH were low compared to honey from Nigeria which ranged from 4.67-5.14 and Tanzanian honey (4.20-4.87) as reported by Lawal et al., 2009 and Andrew et al., 2004 respectively.

The predominant acid in honey is gluconic acid, a derivative of dextrose (Stinson *et al.*, 1960). The gluconic acid present in all honey originates from activity of glucose oxidase, added by bee during ripening (White and Subers, 1963), with some contribution from bacteria action during ripening (Ruiz-Argueso and Rodriquez-Navarra, 1973). Acidity is responsible for the taste of honey; however excessive acidity is undesirable because it leads to a sour-off-taste and running texture (Lopez *et al.*, 1996). A high level of acidity in honey is associated with fermentation resulting in alcohol and subsequently

into acetic acid. Acidity is promoted by high yeast cells count and increased moisture content (White, 1982). This promotes yeast proliferation, accelerating fermentation and acid production (Considine and Considine, 1986). The difference in the means of free acidity of honey from different location was expected, values of free acidity obtained in this study were lower compared to what was obtained by Muli *et al*. (2007) and Kugonza & Nabakabya (2008), which ranged from 28.16-71.85 meq/kg and 39.5-56.2 meq/kg respectively. This difference in free acidity was due to source of nectar and climatic condition. In addition, the considerable variation in the amount of acids in honey perhaps reflects the time required for nectar to be completely converted into honey under differing conditions of the environment, colony strength and sugar concentration of the nectar (Muli *et al*., 2007). From the result, the honey from different location was in good condition and there was no fermentation of the honeys.

Certain nitrogen compounds, minerals, vitamins, pigments and aromatic substance contribute to ash content of honey (Ghazal *et al*., 2008). Codex Alimentarius Commission Standards (2001) for honey, proposed ash content not more than 0.6% for normal honey (Ghazal *et al.,* 2008). The ash content from various location showed that samples obtained from Mwingi had the highest value of 0.3% while Taita Taveta had the lowest values of 0.05%. These values are low compared to Nigerian honey which ranged from 0.60-0.84% as reported by Lawal *et al.,* 2009. The difference in the ash content between honeys from different locations could be attributed to the floral origin and the materials gathered by the bees during foraging. The variation was also apparently due to

many factors such as difference in soil and atmospheric conditions as well as in the type and physiology of each plant. However, in all the samples from various locations, the ash content was within the acceptable range.

4.1.2 Hydromethylfurfural (HMF), Total reducing sugars and sucrose content in honey from different regions

There was a significant ($p < 0.05$) different in HMF between honeys from different locations. The HMF from various locations ranged from 3.94-263 mg/kg, with Taita Taveta having the highest value of 263 mg/kg and Mwingi the lowest (3.94 mg/kg) as shown in Table 4.2. These results were consistent to what was obtained by Muli *et al*., 2007. Reducing sugars ranged from 63.23-73.34% as shown in Table 4.2, honeys from Taita Taveta and from Thika Kakuzi were below the acceptable minimum limit of 65%. These results were consistent with the results obtained by Muli *et al.,* 2007. Mwingi honey had the highest amount of reducing sugars while Taita Taveta honey had the lowest at 63.23%. There was a significant difference at $p < 0.05$ in reducing sugars among the honeys from various locations.

Sucrose values ranged from 0.17-16.15% with most locations having their sucrose value above the maximum acceptable value of 5% (White, 1975). These results were very high compared to what was obtained by Muli *et al*., 2007. There was a significant difference ($p< 0.05$) in sucrose among the honey produced in the various locations with Tharaka

honey having the highest amount of sucrose at 13.81% and Mwingi honey had the lowest at 0.37%.

The HMF content is widely recognized as a parameter of honey samples freshness and tends to increase during processing and/or aging of the product. HMF in honey is formed from carbohydrates, mainly from fructose, which is thermally more labile than saccharose and glucose. Fructose disintegrates at approximately 60 ^{0}C (Belitz and Grosch, 1992). Its formation is a natural process because honeys are acidic, but temperature accelerates the process and the HMF concentration increases according to storage duration and also according to beekeeping practices (Jeanne, 2005). HMF value is virtually absent or very low in fresh honey and is high in honey that has been heated, stored in non-adequate conditions or adulterated with invert syrup (Nozal *et al*, 2001). Chemical properties of honey such as pH, mineral content and total acidity also affect HMF content. The presence of organic acids also favours the production of HMF because it's an acid catalysed dehydration of hexose sugars (Kalabova, 2003). Another factor that can increase the HMF level of honey in bee hive is the tropical climate. Hot weather can increase the HMF levels of honey in the bee hive. Consequently the Codex Alimentarius (2001) and International Honey Commission (2002) set maximum concentration of HMF to 40 mg/kg for honey from non-tropical regions and 80 mg/kg for honey from tropical regions (Said and Puripast, 2010).

Table 4.2: Total reducing sugars, Sucrose and HMF content in honey from different regions

Area	HMF(mg/kg)	Total Reducing sugar (%)	Sucrose (%)
Mwingi (n=14)	$3.94^{e}\pm0.29$	$73.34^{ab}\pm2.77$	$0.37^{d}\pm0.26$
Ntubo (n=3)	$95.03^{cd}\pm0.07$	$67.28^{de}\pm2.66$	$11.16^{abc}\pm2.23$
Tharaka (n=5)	$81.02^{cde}\pm1.20$	$70.95^{abcd}\pm3.98$	$13.81^{a}\pm0.72$
Embu (n=3)	$79.76^{cde}\pm0.26$	$72.98^{abcd}\pm0.81$	$5.84^{bcd}\pm0.84$
Mbeere (n=5)	$59.03^{de}\pm0.19$	$65.20^{a}\pm0.91$	$16.15^{a}\pm1.79$
Timboroa (n=3)	$6.11^{de}\pm 0.10$	$67.37^{de}\pm4.55$	$0.17^{d}\pm0.07$
Turbo (n=3)	$6.33^{de}\pm 0.14$	$69.76^{abcde}\pm0.21$	$0.38^{d}\pm0.03$
Malaba (n=3)	$8.51^{cde}\pm 0.28$	$68.53^{cde}\pm2.32$	$0.21^{d}\pm0.04$
Lenana (n=2)	$2.69^{e}\pm 0.06$	$69.02^{bcde}\pm3.29$	$0.34^{d}\pm0.03$
Thika Kakuzi (n=3)	$5.48^{e}\pm0.14$	$64.94^{e}\pm5.58$	$4.47^{cd}\pm0.40$
Kitui (n=10)	$134.8^{c}\pm1.9$	$68.21^{de}\pm2.20$	$13.75^{a}\pm2.92$
Kakamega (n=3)	$218.31^{a}\pm3.85$	$68.97^{cde}\pm1.51$	$15.47^{a}\pm0.84$
Taita Taveta (n=5)	$263.36^{b}\pm3.29$	$63.23^{e}\pm2.46$	$11.78^{ab}\pm0.81$
Quality standards			
EU	≥ 40	≤ 5	≤ 65
CODEX	≥ 40	≤ 5	≤ 65
KEBS	≥ 40	≤ 5	≤ 65

Value = Mean ± S.D. Means of the same parameter followed by the same letter are not significantly different ($p < 0.05$). S.D= Standard deviation. n=sample size

High levels of HMF also suggest the possibility that natural honey has been adulterated with invert syrup, prepared from sucrose by acid hydrolysis. Acid-inverted invert syrup invariably contains high amounts of HMF (Doner, 1977). Honey bees can be fed with various food stuffs to supplement inadequate supplies of pollen or honey. Kerkvliet and Meijer (2000) reported that honey adulterated with 50% cheap fructose syrup contains HMF twice as high as pure honey. Extremely high (>500 mg/kg) HMF values demonstrates adulteration with invert syrup (Coco *et al*., 1996).

Many countries including Kenya have set the national limit for HMF content in honey at 40 mg/kg. More than half of the honey samples from various locations analyzed had HMF values higher than the acceptable limit (≤ 40 mg/kg). Taita Taveta had the highest value of 263.35 mg/kg, Kakamega 218.31 mg/kg while areas like Mwingi had the lowest value of 3.94 mg/kg. These high values of HMF in the honey samples are an indication of temperature abuse during processing. Fresh honey is usually heated in order to facilitate processing and to maintain good quality. However, excessive heat treatment leads to formation of HMF and reduce honey quality. According to Rodgers (1979), honey with HMF exceeding 100 mg/kg is due to decay of simple sugar caused by temperature above 75 ^{0}C and/or prolonged storage while HMF above 150 mg/kg is an indicator of honey adulteration with commercial invert sugar. Kakamega honey for instance, had high HMF value of 218.31 mg/kg, high moisture content of 20.26% and a high sucrose level of 15.47%, this indicates that in addition to heating of the honey there is addition of sucrose syrup. This could also be true to Kitui honeys. However Taita

Taveta and Ntubo honeys had high HMF values but lower moisture contents compared to Kakamega though they also had high sucrose content this would be indicate that heating of the honey during processing and no addition of sucrose syrup. The difference in HMF in honey from different regions could be due to the different methods adopted by the farmers for extraction and storage of honey.

The main reducing sugars of honey are glucose and fructose. Fructose determines the hygroscopic features of honey while glucose determines the speed of honey crystallization (Dimins *et al.*, 2006). The sugar spectrum of honey depends on the sugars present in the nectar and enzyme present in the bee and nectar (Maurizio, 1959). Therefore the difference in the reducing sugars from different areas is mainly due to environmental conditions and source of nectar for the bees which is different for most regions.

Heating of honey to facilitate processing and maintain good quality also affects the amount of reducing sugars in honey. HMF formation results from acid catalyzed dehydration of hexose sugars with fructose being particularly susceptible to this reaction (Doner, 1977). This leads to decrease in reducing sugar and increase in HMF. This is evident in Taita Taveta honey where the amount of reducing sugar is 63.23% while HMF value is 263.36 mg/kg as compared to Mwingi honey which had reducing sugars at 73.34% and HMF value of 3.94 mg/kg. Therefore excessive heating affects the quality of honey by decreasing the amount of reducing sugars while increasing the HMF value.

It has been proposed that the relative amount of reducing sugars determines the honey's tendency to crystallize (Crane, 1990). In honey of good quality, the percentage of fructose should exceed that of glucose. Honey with high fructose: glucose ratio would remain liquid for longer periods because of the modification of the saturated level of glucose by the presence of the larger amount of fructose (White *et al.*, 1964). The actual proportion of fructose to glucose in any particular honey depends largely on the source of nectar (Anklam, 1998). The fructose: glucose ratio may also have an impact on honey flavor since fructose is much sweeter than glucose.

The main sugars in honey are the monosaccharides hexoses fructose and glucose which are products of the hydrolysis of the disaccharide sucrose. Invertase converts the sucrose in pure honey into fructose and glucose. Sucrose comprises a little over 1% of the honey composition. During the processing of honey, heat destroys the invertase in honey. Adulterated honey therefore contains excess sucrose and low fructose due to the loss in activity of the enzyme invertase (Lawal *et al*., 2009). Honey from certain regions had very high levels of sucrose; Mbeere honey had 16.15% and Kakamega honey 15.47%. These are three times higher than the acceptable limit of $\leq 5\%$. These high percentages could be due to addition of sugar syrups in the honey during processing. However, according to Abu-Tarbouch *et al*., (1993), high sucrose content may be a plant characteristic and is not an indication of sugar feeding to the bees. Sugar fed honey is low in water content and pH along with high sucrose content.

4.2 CONTAMINANTS IN HONEY

4.2.1 Heavy metals in honey from different regions

Table 4.3 shows the heavy metals (Cd, Pb, Cu and Zn) present honey samples from different regions under investigation. Cadmium ranged from 0.01-0.05 mg/kg which is below the limit set by KEBS and Codex Alimentarius of 0.1 mg/kg. There was a significant difference ($p < 0.05$) in cadmium value among honey samples from different regions. Honey from Ntubo had the highest amount of cadmium at 0.05 mg/kg while Embu honey had the lowest at 0.01 mg/kg. On the other hand, Pb present in honey from various regions ranged from 0.01-0.05 mg/kg, which is below the set limit of 0.1 mg/kg. There was a significant difference in Pb content among the honey samples from different locations with Malaba honey having the highest value at 0.05 mg/kg while Turbo and Thika Kakuzi honeys had the lowest values at 0.01 mg/kg each. The set limits for copper are 2.0 mg/kg according to KEBS and Codex, the samples analyzed ranged from 0.07-0.24 mg/100g, Mwingi and Kakamega honeys had the highest values of copper at 0.24 mg/kg each. There was a significant difference in the amount of copper in honey from different regions. Zinc has a set limit of 5.0 mg/kg by KEBS and the samples from various regions ranged from 1.01-2.10 mg/kg. Honey from Mwingi had the highest amount of zinc at 2.10 mg/kg while honey from Turbo had the lowest value at 1.01 mg/kg. All the samples lied within the set limit. There was a significant difference in zinc content among the honey from different region.

Table 4.3: Cadmium, Lead, Copper and Zinc in honey from different regions

Area	Cadmium (mg/kg)	Lead (mg/kg)	Copper (mg/kg)	Zinc (mg/kg)
Mwingi (n=14)	0.03^{abc}<0.01	0.03^{ab}<0.01	0.24^{a}±0.01	2.10^{a}±0.01
Ntubo (n=3)	0.05^{a}±0.01	0.03^{ab}<0.01	0.11^{cde}±0.01	1.65^{f}<0.01
Tharaka (n=5)	0.03^{abc}<0.01	0.03^{ab}<0.01	0.07^{e}<0.01	1.89^{d}<0.01
Embu (n=3)	0.01^{c}<0.01	0.04^{ab}<0.01	0.18^{abc}±0.01	1.72^{e}<0.01
Mbeere (n=5)	0.02^{bc}±0.01	0.03^{ab}<0.01	0.15^{abcde}<0.01	1.95^{c}<0.01
Timboroa (n=3)	0.02^{bc}±0.01	0.02^{ab}<0.01	0.17^{abcd}±0.01	1.71^{e}<0.01
Turbo (n=3)	0.01^{c}<0.01	0.01^{b}<0.01	0.09^{cde}<0.01	1.01^{i}<0.01
Malaba (n=3)	0.02^{bc}<0.01	0.05^{a}<0.01	0.16^{abcde}<0.01	1.35^{h}±0.01
Lenana (n=2)	0.02^{bc}<0.01	0.02^{ab}<0.01	0.14^{bcde}<0.01	1.47^{g}<0.01
Thika Kakuzi (n=3)	0.01^{c}<0.01	0.01^{b}±0.01	0.22^{ab}<0.01	2.03^{b}<0.01
Kitui (n=10)	0.02^{bc}<0.01	0.03^{ab}<0.01	0.18^{abc}<0.01	1.39^{h}<0.01
Kakamega (n=3)	0.03^{abc}±0.03	0.04^{ab}<0.01	0.24^{ab}<0.01	1.62^{f}±0.01
Taita Taveta (n=5)	0.04^{abc}<0.01	0.03^{ab}<0.01	0.08^{de}±0.01	1.87^{d}<0.01

Value =Mean ±S.D. Means on the same row followed by the same letter are not significantly different ($p < 0.05$) S.D=Standard deviation. n=sample size

Several workers have studied the trace and toxic elements in honey. Cd concentrations were lower than the values reported by Fakhimzadeh and Lodenius (2000) in Finnish

honey. The Cd values were consistent with the values reported on Sudan honey by Mohammed and Babiker (2009). Pb concentrations were very low compared to the values obtained by Mohammed and Babiker (2009) and Fakhimzadeh and Lodenius (2000). Pb values were consistent with the values obtained in Chilean honey which ranged from 0.01-0.11 mg/kg as reported by Fredes and Montenegro (2006). Chilean honey had cu concentration ranging from 0.06-2.0 mg/kg with a mean of 0.08mg/kg as reported by Fredes and Montenegro (2006); this is consistent with the results obtain in this study. Nigerian honey as reported by Achudume and Nwafor (2010) had high concentrations of copper ranging from 21.56-28.83 mg/kg. Sudan honey also had very high copper values (2.94-58.12 mg/kg) as reported by Mohammed and Babiker (2009). Zn concentrations were low compared to values reported by Mohammed and Babiker in Sudan honey which ranged from 4.86-9.61mg/kg. Honey from Nigeria had very low concentration of Zn (0.06-0.216 mg/kg) as reported by Achudume and Nwafor (2010) compared to the results obtained in this study. Chilean honey ranged from 0.01-4.73 mg/kg this is comparable with the results of this study

The quantitative and qualitative ratio of chemical elements in honey is characteristic of each blossom of the plant from each region of the country, so the total quantity of mineral materials depends on a location. It is possible to determine the origin of specific samples of honey and the environmental pollution of a region from the quantitative and qualitative ratio of heavy and rare metals in honey. Honey is a useful bio-monitor for information related to the environment where the bees live. Since honey bees readily fly

up to 4 km in all directions from their apiary and thus have access to an area of about 50 km^2 (Hoopingarner and Waller, 1993) and the bees come in contact not only with air but also with soil and water, the concentration of heavy metals in honey reflects their amount in the whole region (Ioannidou *et al.*, 2004).

Therefore, honey has been recognized as a biological indicator of environmental pollution (Przybyloswki and Wilczynska, 2001). But nevertheless determination of heavy metals in honey is of high interest mainly for quality control and nutritional aspect. High levels of metals are undesirable because of their known or supposed toxicity so that, for instance, a limit of 1 mg/kg for lead is set in some countries (Buldini *et al.*, 2001). Moreover, nectar of plants serves as potential source of exposure to metals such as lead, cadmium and copper which occur at various levels in the environment.

The analysis of the contamination of honey with heavy metals (Cd, Pb, Cu and Zn) as shown in Table 4.3 revealed the presence of these metals in minute concentrations below the permitted levels. No toxicological problems can be expected from the honey. This results show the good quality of honey analyzed in relation to the studied parameters.

4.2.2 Residues of Oxytetracycline and Tetracyclines in honey from different regions

Table 4.4: Antibiotics, retention time, limit of detection and % recovery analyzed in honey

Antibiotics	Retention time in minutes	Limit of detection in µg/ml	% Recovery
Oxytetracycline	8.48	0.005	66
Tetracyclines	9.942	0.005	72

Of all the honey analyzed there were no residues of tetracycline and oxytetracyclines detected, they were below the detection limit. The occurrence of residues of antibiotics in honey has become a major concern in the world today. In Belgium 2 out of 72 samples of honey analysed were positive of tetracylines. Twenty nine out of 98 samples (29.6%) of honey imported into Belgium market contained tetracylines (Reybroeck, 2003). In the Italian market, a total of 6.3% of all samples analyzed were positive for antibacterial drugs analyzed; in particular 6.8% of imported honey and 6.1% of honey on Italian market. Only 1.7% of local honey had antibacterial residues (Baggio *et al*., 2009). Bonvehi and Gutierreaz (2009) also reported positive results of tetracycline in 24 out of 68 samples of Spain honey analyzed ranging from 15 to 920 µg/kg. The EU, Codex and KEBS have set the tolerable limit of antibiotics especially tetracycline at not more than 1 mg/kg.

This shows that most farmers in Kenya either do not use any veterinary drugs in beekeeping or if they do, they are administered correctly thereby reducing their

occurrence in the honey. Therefore the honey analyzed was of good quality in relation to the studied parameter.

4.2.3 Residues of pesticides in honey from different regions

Retention times (t_R) of the 6 pesticides were determined individually and are presented in Table 4.5. The limit of detection (LOD) of each pesticide listed in Table 4.5 was determined from injection of the standards. Linearity was obtained for pesticides using standards ranging from 0.01µg/ml-0.1µg/ml,the 6 pesticides had r^2 of 0.998.

In all the honey samples analyzed, the listed pesticides were not detected in them. Cristina *et al.,* (2003) analysed honey samples from Portugal and Spain and found out that most were contaminated with organochlorine pesticides. The results also indicated that Portuguese honeys were more contaminated than Spanish ones. Sandra *et al*., 2007 analysed honey from Bauru in Brazil, the results indicated that most pesticides found in the samples belonged to the organohalogens and organophosphorous groups and lower levels of residues of some organonitrogen and pyretroids. Malathion residues were detected in all the samples, in a high concentration, owing to its application to control dengue mosquitoes in the area studied.

Pesticides play a beneficial role in agriculture, because they help to combat the variety of pests that destroy crops. Slow degradation of pesticides, in the environment and extensive or inappropriate use by farmers, can lead to environmental contamination of

the water, soil, air, several types of crops and indirectly humans (Hamilton & Crossley, 2004; Olkowski, 1991). Honeybees perform the vital task of pollinating agricultural crops, everyday they make an average of 10 journeys to explore roughly 7 km^2 in the area near their hives, gathering nectar, water and pollen from flowers.

Table 4.5: Pesticide class, Retention time, Limit of detection and % recovery of 6 pesticides analyzed in honey from different regions.

Pesticide	Pesticide class	Retention time (t_R) (min)	Limit of detection (µg/ml)	% recovery
Aldrin	organochlorine	11.40	0.005	85 ± 1.08
pp-DDE	organochlorine	15.60	0.002	88 ± 0.13
Endosulfan	organochlorine	16.17	0.004	79 ± 1.56
Lindane	organochlorine	13.57	0.006	86 ± 0.08
Dicofol	organochlorine	19.63	0.005	83 ± 0.35
Chlorpyrifos	organophosphate	22.83	0.008	89 ± 0.03

During the process, various microorganism, chemical products, and particles, suspended in the air, are intercepted by these honeybees and retained in the hair of their body surface or inhaled and attached to their trachea (Devillers & Pham-Delegue, 2002). Finally, a variety of materials are taken into the hive (nectar, pollen, honeydew, propolis and water) and stored. The tolerable limit set by EU, Codex and KEBS for organochlorine and organophosphorus is not more than 50 ppb. From the results the

honey samples were clean meaning the environment from which the bees collect their nectar is not polluted.

Lindane has been in long term use for seed dressing to protect crops against ants, but it is currently under restricted pesticides due to its persistence and toxicity, whereas endosulfan, dicofol and chlorpyrifol are used as insecticides (PCPB, 1998). DDE was extensively applied in aerial sprays against mosquitoes to control malaria (Mitema and Gitau, 1990), whereas aldrin, and dieldrin are used in termite control in building industry (Getenga *et al.,* 2004).The public use of these compounds is restricted (aldrin, dieldrin, lindane) in Kenya (PCPB, 1998). According to Musa *et al* (2011), high residues of lindane, endosulfan and heptachlor in the environment indicates that some farmers are still applying them illegally, and hence more strict control measures against the use of these compounds needs to be put in place. They were established by a survey and were found in the stockiest shops and used in the farms.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Quality of honey produced in the regions studied met the standard set by Codex, EU and KEBS and were free of contaminants studied. However, majority of the honey samples had HMF and sucrose values higher than the set standards. This was due to the different methods adopted by the farmers for extraction and storage of honey. These can be corrected or improved by proper training of the farmers by relevant stakeholders. Honey produced in the studied regions in Kenya is able to penetrate the EU market if proper measures are put in place.

5.2 RECOMMENDATIONS

It is recommended that regulatory mechanisms for heavy metals, residues of antibiotics and residues of pesticides be put in place; this will strengthen honey monitoring and inspection within the industry.

In addition, it's recommended that the government in collaboration with relevant stakeholders promote good beekeeping practices; this will help avoided some of the practices which result to adulteration of honey like excessive heat treatment and addition of sugar syrups. It is also recommended that the beekeepers be informed of the emerging potential for exporting honey to the EU.

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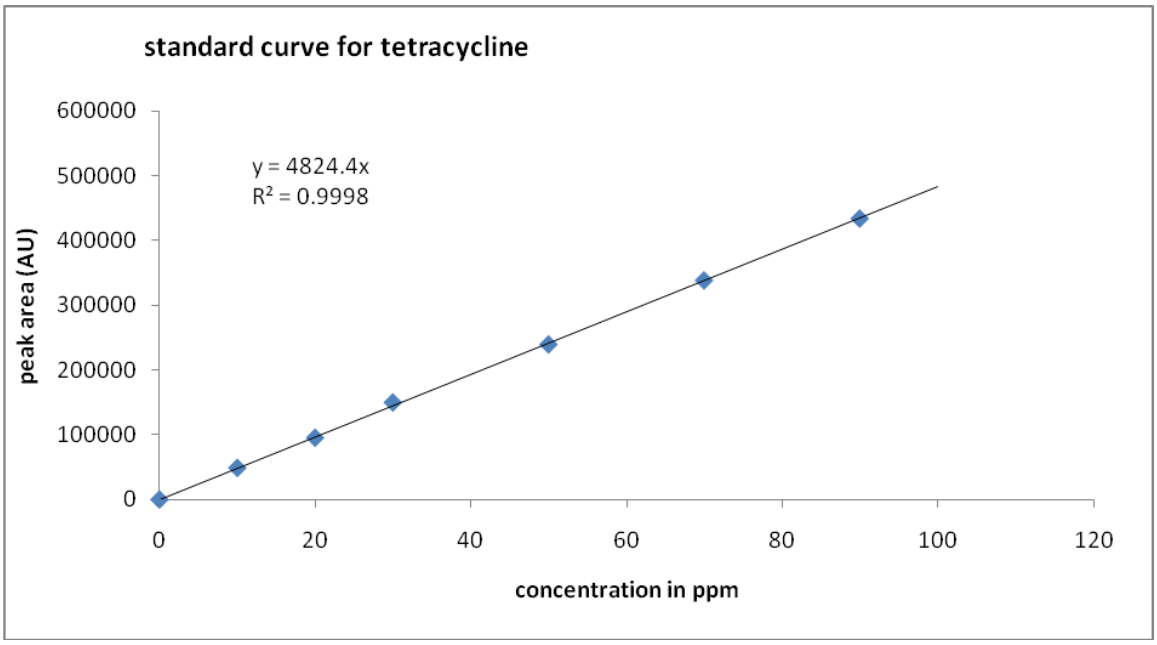
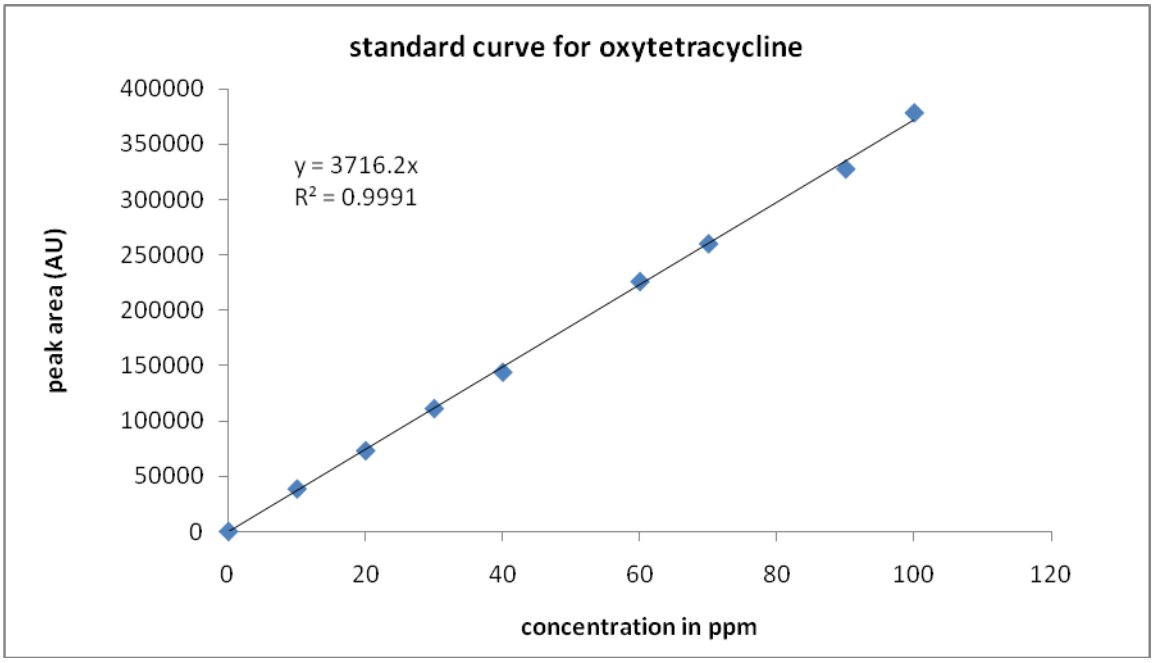
APPENDICES

Appendix 1: Standard curves of tetracycline and oxytetrecycline





Appendix 2: Calculation of heavy metals concentration

$$\text{Metals in mg/100g} = \frac{\text{Concentration in µg/ml (machine reading)} * \text{dilution factor} * 100}{\text{Weight of sample} * 1000}$$

Appendix 3: Calculation of % Recovery

$$\%\ \text{recovery} = \frac{\text{peak area of spiked honey sample with 10ppm standard}}{\text{Peak area of standard at 10ppm standard}} * 100$$