

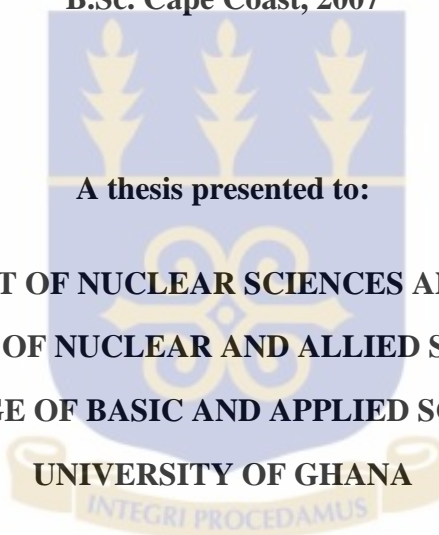
**STUDIES OF THE EFFECTS OF GAMMA IRRADIATION ON THE MICROBIAL LOAD
AND PHYSICOCHEMICAL PROPERTIES OF GHANAIAN HONEY FROM THE
ASHANTI, BRONG AHAFO AND GREATER ACCRA REGIONS**

By

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DECLARATION

I, David Ansah Larbi, hereby declare that the work contained in this thesis is the result of an authentic research carried out under the guidance of the supervisors (Dr. Dennis Adotey and Dr Charles Klutse). This work has not been submitted for a degree or diploma at any other higher educational institution. To the best of my knowledge and belief, the thesis contains no materials previously published or written by another except where due reference is made.

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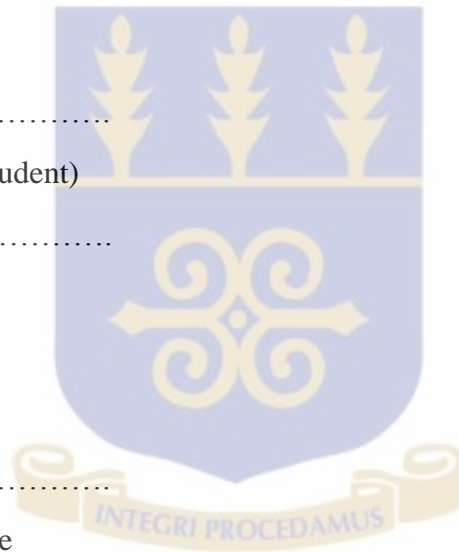
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Date:

DEDICATION

This work is dedicated to my Father, **Mr. Albert Ansah Larbi** (of blessed memory), and my **Beloved** Mother, **Madam Theresah Marteki Kordi**.

To my four (4) beloved brothers, God Bless You.



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I salute Mr. Elvis Djam Miensah for the great sacrifice he made to make this journey of higher education possible and successful. God bless him. I pray that the years ahead bring us more success than we can ever imagine and may the Lord grant us the wisdom to identify those success paths, the courage to choose them and a long healthy life to enjoy and live a life pleasing to God.

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In the event of these acknowledgements, failing to mention the names of any particular person(s), I offer my sincerest apologies.

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LIST OF ABBREVIATIONS

A. M. I	American Meat Institute
ASH	Ashanti Region
BA	Brong Ahafo Region
BP	Baird Parker agar
CFU	Coliform Unit
EC	European Commission
EMB	Eosin Methylene Blue agar
EU	European Union
GAR	Greater Accra Region
ISO	International Standards Organisation
MERCOSUR	Mercado Comum del SUR (Common Market of the South)
PA	Perfringens agar
PCA	Plate Count Agar
RTC-GAEC	Radiation Technology Centre, Ghana Atomic Energy Commission
USFDA	United States Food and Drugs Administration
XLD	Xylose Lysine Deoxycholate agar

ABSTRACT

Honey is a universal product with a wide usage throughout the life spectrum. It is a substitute for sugar for many people, an energy booster, helps maintain the blood sugar level and research has shown that it has the potential for cancer prevention. The high sugar concentration of honey and its low pH gives honey antimicrobial properties and makes it difficult for microorganisms to grow. However, research has provided evidence of the presence of microbes in honey. The microbes are introduced into honey through primary and secondary sources. The primary sources are due to honeybee foraging activities resulting in the transfer of pollen and other microbial species in the air, soil and plants into the product before it matures. The secondary sources of contamination are due to the harvesting process, materials used for harvesting and storage of the honey; as well as the method used in extracting and treating honey before it is sold to the consumer. Accordingly, it is imperative that the quality of honey on the Ghanaian market is monitored regularly to provide data on the microbial load. This study endeavours to ascertain the presence of microorganisms in Ghanaian honey, the sources of microbial contamination and the effect of gamma radiation on the microbial load as well as the physico-chemical properties of honey. Ninety (90) honey samples were collected from three regions; Brong Ahafo, Ashanti, and Greater Accra [Thirty (30) from each region]. Sampling was conducted using the farmer-to-retailer route, that is, honey was sampled directly from the beehive with the comb before the farmer harvested, samples were taken after the farmer had harvested and treated and finally honey was sampled from retailers who buy directly from the farmer. The effect of gamma radiation on the microbial load was studied using a ^{60}Co source gamma irradiation facility at doses

of 20 kGy, 30 kGy and 40 kGy on the presence of microbes and the physicochemical properties (pH, Reducing Sugar, Apparent Sucrose and Ash content) of honey. The honey samples were analyzed for the presence of Total Viable Counts, *Faecal* sp., *Clostridium* sp., *Staphylococcus* sp., and *Salmonella* sp. The Plate Count Method of microbial analyses was used to determine the microbes. For the Total Viable Count, Plate Count Agar was the media used; Eosin Methylene Blue agar for *Faecal* sp., Perfringens Agar for *Clostridium* sp., Xylose Lysine Deoxycholate Agar for *Salmonella* sp., and Baird Parker Agar for *Staphylococcus* sp. The samples were also analyzed for Reducing Sugar and Apparent Sucrose using Titrimetry. pH was determined with a pH meter and Ash Content was determined by Gravimetry. The pH values obtained for the Honey Comb samples were in the range (3.6 – 3.9), and the pH for the Retail samples were in the range (4.9 – 5.6). Microorganisms were not detected in about 70% of the honey sampled directly from the honey comb and the mean microbial count in the remaining 30% were within the range 30% - 35% whereas all the honey sampled from the retailers were contaminated with microbes. The mean microbial counts in the retailer samples were 148 CFU/g, 183 CFU/g and 271 CFU /g for Ashanti, Brong Ahafo and Greater Accra Regions respectively. These values were significantly higher than the required maximum relative to the MERCOSUR (Mercado Comun del SUR) standard (\leq 100 CFU/g). The low level of microbial detection in the Honey Comb samples (30 – 35 CFU/g) could be due to their relatively low pH levels (3.6 – 3.9) compared to the retailer samples with pH within 4.9 – 5.6. The ash content of all the honey sampled and analyzed were within the required standard with an average of 0.16% in the honey comb samples and average of 0.62% for the retail samples. The apparent sucrose concentration (in

percentage) in honey sampled from the retailers were within the range 22% - 33% which is beyond the required maximum as stipulated by the CODEX Alimentarius Commission ($\leq 10\%$). From the study, it was realized that a 20 kGy gamma radiation dose was enough to denature the microbes as well as preserving the essential qualities of the honey. To ensure good quality honey on the Ghanaian market, it is recommended that honey meant for human consumption should undergo gamma irradiation (cold pasteurization).

CHAPTER ONE

1. INTRODUCTION

1.1 Background

Honey is a food product consumed by a wide range of people throughout the life spectrum as a result of its high nutritive value. It is essentially composed of reducing sugars hence a major source of energy. It is used as a substitute for sugar by a majority of people of all ages, helps in digestion and removal of free radicals from the body, among other benefits. Besides sugars, honey also contains proteins, organic acids, amino acids, vitamins and lipids making it a rich source of other nutrients (White *et al.*, 1975).

Research has shown that honey has the potential to prevent cancer (Beretta *et al.*, 2007) and can also be used to cure some eye defects (Kwapong *et al.*, 2013). Economically, honey is a product of international value serving as a source of foreign exchange for many countries including Ghana and contributing significantly to the gross domestic production. It is also a major source of livelihood for many people who are into apiculture. But honey is only as good as its quality and honey quality cannot be judged just by its physical appearances. For these reasons, there is the need to ensure that honey is free from microorganisms and that it is wholesome for human consumption.

Honey is a flavourful product which is consumed globally as a highly nutritive value food. It is essentially composed of a complex mixture of carbohydrates (glucose and fructose account for nearly 85% - 95%, the rest being sucrose) and other minor substances such as organic acids, amino acids, proteins, minerals, vitamins and lipids (White, 1975).

Due to its high sugar concentration, high osmotic pressure and low pH, it is difficult for microorganisms to grow in honey. However, research has shown that microorganisms have been detected in honey. Some of these microbes include pollen, moulds and yeasts, as well as the spores of *Clostridium* sp. and *Bacillus* sp. (Snowdon *et al.*, 1996).

There are two main sources of microorganism contamination: primary sources include pollen, digestive tracts of honeybees, dust, air, soil and nectar; secondary sources are those arising from animals such as some rodents, insect, etc. that may visit the beehive while honey is maturing. Secondary sources of contamination are manipulation by people including food-handlers, cross-contamination, equipment and buildings where harvested honey is stored. (Snowdon *et al.*, 1996).

The microorganisms have been found to be inactive in honey but when transferred into a living host through ingestion, it could present different results. Sulfite-reducing *Clostridium* is an indicator organism, whose presence in honey provides evidence of contamination or pollution (Snowdon *et al.*, 1996). The presence of spores of *Clostridium* is especially dangerous for infants and small children (Centorbi *et al.*, 1999). Botulism is a *neuroparalytic* disease caused by *Clostridium botulinum* which could survive in honey and be transferred into an infant if honey is used as a substitute for sugar in foods.

Reports of *anaphylactic shock* in people who eat raw honey with allergens (pollen) in it have been recorded in some North American and European countries. At its worst, anaphylactic shock could cause breathing difficulties, low blood pressure, dizziness, fainting, heart failure, weakness, sweating, nausea, vomiting and a prickling sensations in the brain. (Bartkowski, 2014). Symptoms of less severe allergic reactions that may result from eating raw honey include itching, puffy skin, and rash.

Honeybees obtain their nectar from flowers of different types of plants including flowers of *Rhododendron* – a common garden flower. The nectar of flowers in this group contains a substance called *grayanotoxin*, according to a publication in the American Food and Chemical Toxicology journal (Koka *et al.*, 2007). It is explained in this article that, *grayanotoxins* are chemicals that are toxic to the nervous systems, they prevent nerve cells from functioning effectively. During foraging activities of the honey bees, pollen grains stick to the bodies of the bees, transferred into the honey comb and mature as part of the honey. These pollen cause allergic reactions in people when exposed to the pollen-contaminated honey.

1.2 Ghanaian honey

The main type of honey produced in Ghana is multifloral honey because bee foraging is not controlled by the beekeepers. The honey appears dark brown and looks thicker than most imported honey. Honey is used in various ways but mostly as a substitute for sugar in most beverages in Ghana. The honey industry is most recognized by the indigenous people and is gradually gaining wider usage locally. It is the belief among Ghanaians that, raw untreated honey from the farm is the finest, hence most people do not buy honey from supermarkets or shops but rather prefer to purchase from honey hunters and the honeybee farmers directly.

The common perception is that honey sellers adulterate the honey with burnt sugar and other additives to increase quantity thereby maximizing profit from sales. Unfortunately, there is no evidence to back these beliefs. Adulterated honey is said to be common in the local market. The consequences thereof are unknown to the consumer and the retailer. How are the chemical qualities of honey affected? How do the microbes react to the

added ingredient? Is the heat enough to kill the microorganisms? Data on the physico-chemical properties and its microbiological quality of Ghanaian honey is scarce and almost non-existent.

For health and other reasons, honey sold around the world must meet the international quality criteria. Internationally, honey quality criteria are specified in regular standards, compiled in Codex Alimentarius Standard.

1.3 Research Problem

Honey has high sugar concentration, high osmotic pressure and low pH, therefore it is difficult for microorganisms to grow in it. Notwithstanding, research has shown that microorganisms have been detected in honey. There is scarcity of published information on the microbiological properties of Ghanaian honey; and very limited information on the physicochemical characteristics of Ghanaian honey.

1.4 Research Objectives

1.4.1 Main Objective

The main objective of this research is to investigate the presence of microorganisms in Ghanaian honey and to assess how to improve on the quality of Ghanaian honey through cold pasteurization (Gamma irradiation).

1.4.2 Specific Objectives

- i. to ascertain the major source of contamination of honey in the selected regions of Ghana
- ii. to assess the wholesomeness or otherwise of some Ghana-made honey with respect to microorganisms

- iii. to evaluate the effects of gamma radiation (Cold pasteurization) on the natural properties of Ghanaian honey.
- iv. to examine the possibility of preserving Ghanaian honey based on the use of gamma irradiation from a ^{60}Co source.

1.5 Relevance and Justification

Honey is a product used as a sweetener in beverages for both adults and children. It is also used as a spread on bread, eaten raw. Besides these uses, honey is used in the treatment of wounds and eye defects (Kwapong *et al.*, 2013) and has some other medicinal applications.

For the above and many other reasons, it is very important to investigate and understand the quality of honey. Unwholesome honey (honey that is contaminated with microbes and other impurities) could be dangerous when used in treating wounds, and as an antibiotic for treating eye infections.

CHAPTER TWO

2.0 REVIEW OF LITERATURE

At 5,000 Euros (US \$6,800) per kilogram, ‘Elvish’ honey from Turkey was the most expensive in the world as at the time of this study (Sumitra, 2014). The special honey is extracted from a 1,800-meter-deep cave in the Saricayir valley of Artvin city, north-eastern Turkey. Elvish honey is expensive because it is naturally produced. The mineral-rich cave enhances the honey’s quality, adding to its value (Sumitra, 2014). The first kilogram of the honey was sold for 45,000 Euros at the French Stock Exchange in the year 2009. In the year 2010, Pharmacists from China bought another kilogram at 28,000 Euros. Currently priced at 5,000 Euros a kilogram in 2014, it is sold in bottles of 170 g and 250 g (Sumitra, 2014).

The Sidr honey from the Wadi Do’an valley, in Yemen, has also been admired internationally as a much cherished product with rich medicinal features. It is believed to cure numerous illnesses, enhances sexual power, and would contribute to finance fundamentalist organizations. (Al-Ghariba, 2009).

Honey from many other countries especially the European and the North American regions are much desired in the Ghanaian market. Locally produced honey has relatively little recognition among the ‘elite’ because it is believed that the product is adulterated after harvesting from the beehive. Also there is insufficient information on the physicochemical and microbial qualities of the locally produced honey.

2.1 Honey and Its Qualities

Honey has a versatile application in medicine and nutrition due to its rich physical and chemical characteristics, as well as its antimicrobial characteristics. The quality of honey is determined by its sensorial, chemical, physical and microbial features. The physicochemical quality criteria of honey are well specified by the European Commission's Directive 2011/110 (European Union, 2001). The major criteria of interest are moisture content, electrical conductivity, ash content, reducing and non-reducing sugars, free acidity, diastase activity and Hydroxymethylfurfural (HMF) (Susana, *et al.*, 2010)

Many authors have published on honey's physicochemical and sensorial quality (Gupta *et al.*, 1992), the chemical identification of certain compounds in honey from different sources and regions (Martos *et al.*, 2000) and, in recent years, studies on the antimicrobial properties of honey (Taormina *et al.*, 2001).

2.1.1 Physical and Chemical Properties

Honey contains sugars such as fructose, glucose, sucrose, other carbohydrates, minerals, proteins with less than 20% of water. This makes honey a concentrated sugar solution with high osmotic pressure, making the growth of any microorganisms difficult. (Voidarou *et al.*, 2011). Fig. 2.1 is a chart showing the percentage composition of the constituents of honey whereas Fig. 2.2 shows the structural formulae of the main sugars in honey.

explained the complexity of the minor sugars of honey. Invertase becomes less active when honey is ripened yet remains in the enzyme and retains its activity for some time. This does not totally eliminate the sucrose content of honey.

Diastase, also known as amylase, is responsible for the breakdown of starch to its constituent compounds nectar – which is the primary raw material for honey – has no starch, hence the presence of diastase in honey is not clear. It has been found to be present in varying amounts in almost all honey samples and can be quantified. It has been used as a honey-quality yardstick in several European countries therefore has probably had the greatest attention some time past.

Glucose oxidase is an enzyme which converts dextrose to a related material, a gluconolactone, which in turn forms gluconic acid, the principal acid in honey. In addition, glucose oxidase forms hydrogen peroxide during its action on dextrose, which has been shown to be the basis of the heat-sensitive antibacterial activity of honey.

Other enzymes like catalase and acid phosphatase have been found to be present in honey. All these honey enzymes can be destroyed or weakened by heat. (White *et al.*, 1980)

2.2 Microorganisms in Honey

Honey is a product of minimum levels of microorganisms which are attributed to the natural properties and to industry control (Snowdon and Cliver, 1996). According to (Snowdon *et al.* 1996), the microorganisms most common and primarily found in honey include yeast, moulds, *Clostridium sp.* These are microbes always present in the air.

Microbial contamination studies on honey are rare and are essentially devoted to *Clostridium botulinum* (Finola *et al.*, 2007).

The presence of *C. botulinum* spores in honey was reported for the first time in 1976 (Huhtanen *et al.*, 1981). Since then, the many studies that have been conducted around the world have shown only the presence of the spores. Honey does not contain the botulinum toxin but the spores theoretically can build the toxin after digestion. Cases of infant botulism after ingestion of honey have been reported in recent years and this has been attributed to *Clostridium botulinum* spores present in honey. These findings have led the health authorities of some countries (for example United States and United Kingdom) to restrict the use of honey in the diet of children until after a year of birth. (Bogdanov, 2009). With respect to yeast, honey contains naturally different osmotolerant yeast, which can cause undesirable fermentation. Osmotolerant yeasts can particularly develop in honeys with high moisture content. (Bogdanov, 2009).

Clostridium (botulinum and perfringens)

Many countries have research documents of honey infested with spores of *Clostridium sp.* In Argentina, *Clostridium botulinum* type A was detected in two (2) out of one hundred and seventy-seven honey samples of rural producers (de Centorbi *et al.*, 1997). In Brazil, six (6) out of eighty-five (85) honey samples analyzed were found positive for *Clostridium botulinum* (7.06%) and identified as producers of type A, B and D toxins (Ragazani *et al.*, 2008).

In California, methods for the isolation of *Clostridium botulinum* from honey samples were described in 1979. Out of ninety (90) samples, a total of nine (9) tested positive for *Clostridium botulinum*; six (6) of the positive samples had been fed to babies who

developed infant botulism. (Midura *et al.*, 1979). Also in California, out of forty-one (41) hospitalized patients, 12 had been fed honey prior to onset of constipation and, worldwide, honey exposure occurred in twenty-eight (28) of seventy-five (75) hospitalized cases. (Arnon *et al.*, 1979). Honey consumption was associated with 15% of the reported cases of infant botulism to Centers for Disease Control and Prevention. (Midura *et al.*, 1979). Up to 25% of the honey products in the US contain spores of *Clostridium botulinum* (Jimenez *et al.*, 1994).

Type A, B, and C, *Clostridium* spores were found in three (3) out of the fifty-six (56) samples of sugar for apiculture. Type A spores were detected in samples of some raw sugar and molasses and also in two (2) out of forty-one (41) samples of brown sugar lump. (Nakano *et al.*, 1990). Since these are raw materials for honey production, the presence of these contaminants may be transferred into the honey by the bees.

2.3 Sources of Microbial Contamination

The sources of contamination of honey could be primary, such as the digestive tract of honeybees, pollen and nectar. Contaminations from these sources are quite difficult to remove. There are also secondary sources such as, people who handle the honey during harvesting, processing and packaging. Contamination from these sources are not as difficult to eliminate as the primary sources (Susana *et al.* 2010).

2.4 Decontamination

There are various types of food decontamination processes many of which include introducing substances into the media to be pasteurized, a few of which have been discussed below. From literature, the most common method used in honey is cold pasteurization (gamma irradiation).

2.4.1 Addition of Organic Acids

This method of decontamination involves the addition of organic acids such as acetic, citric, propionic, ascorbic, formic to the substance. This method is common in the decontamination of meat (animal carcasses and subprimals). The most common types of acids used are acetic and lactic acids, both are recognized as safe compounds by the United States Food and Drugs Administration (USDA, 1996). Using this method in pasteurizing honey can affect the quality of the product because introducing an acid will increase the acidity of honey.

2.4.2 Steam Pasteurization

This technology uses superheated steam from potable water which is natural and non-toxic substance. This amount of heat may damage the physicochemical parameters required of natural honey and render the product unwholesome or perhaps reduce the commercial value of honey.

2.4.3 Ozonation

Ozone (O₃) is a soluble and unstable blue gas commercially produced by passing electric charges or ionizing radiation through air or oxygen. It is a powerful compound used to inactivate bacteria through its oxidizing properties, and was the first oxidizing agent to be used for disinfection of water. Ozonation was found to be effective for the inactivation of bacteria (e.g. *E. coli*, *S. typhimurium*, and *Pseudomonas fluorescens*) (Burleson *et al.*, 1975). The use of this technology, though may be effective in destroying the microbiological contaminants, could also alter the user quality of honey.

2.4.4 Irradiation Pasteurization (Cold Pasteurization)

This process involves the emission of an intense pulse of energy from a gamma radiation source (e.g., Cobalt 60) or from an electrical source (e.g., electron beam accelerator) that penetrates the honey and destroys microbes. It is a technology used to sterilize meat American Meat Institute (A.M.I., 2000).

The technology is such that, the energy passes through the product, leaving very little to no trace in the product. Hence there is no fear of contamination or radioactive honey to be sold to the public, since the honey sample is not injected with a radioactive material.

Extensive research has been conducted by numerous scientists and researchers in many different countries in several aspect of the honey quality criteria. Some of the aspects studied include the geographical and botanical origin, physicochemical qualities, microbiological qualities, antimicrobial qualities, elemental composition and so on.

Anklam (1998), analyzed some samples of honey for Hydroxymethylfurfural (HMF), moisture content, and enzyme activity. These were aimed at determining the origin of the honey samples (geographically and botanically) as part of the conclusion in this research. Whereas parameters such as HMF, residues, and enzymes activity do lead to information regarding honey's botanical and/or geographical origin, others such as aliphatic organic acids, amino acids, and aroma compounds can lead to the determination of the botanical origin of honey, whiles amino acids, flavonoids, minerals and trace elements, oligosaccharides etc. can assist in determining the geographical origin of honey.

Susane *et al.* (2010), analyzed some commercial honey samples from Portugal for their physicochemical, microbiological and antimicrobial properties. The research recorded the following results shown in Table 2.1 for some of the parameters analyzed and

Table 2.1 Results of physicochemical quality of honey

Parameters	Honey 1	Honey 2	Honey 3	Honey 4	Honey 5
Ashes (%)	0.35 ± 0.02a	0.23 ± 0.01b	0.25 ± 0.01b	0.07 ± 0.01c	0.16 ± 0.02d
Reducing sugars (%)	67.7 ± 0.5a	73.7 ± 0.4a	71.4 ± 0.6a	71.0 ± 0.4a	71.8 ± 0.3a
Apparent sucrose (%)	3.4 ± 0.5a	6.7 ± 0.2b	9.7 ± 0.2c	6.6 ± 0.3b	3.8 ± 0.2a

The letters (**a**, **b**, **c**, **d** and **e**) represents which honeys are different by Tukey test with significance of $p = 0.05$.

Table 2.2: Results of Microbial quality of honey as reported(Ref: Susane *et al.*, (2010))

Honey Sample	Aerobic mesophiles (cfu/g)	Moulds and yeasts (cfu/g)	Fecal coliforms (MPN)	Sulphite-reducing clostridia (in 0.01 g)	Salmonella (in 25 g)
1	<10	<10	<1	Negative	Negative
2	<10	1.3×10 ¹	<1	Negative	Negative
3	<10	<10	<1	Negative	Negative

The research concluded that the parameters determined met the internationally accepted standards for honey, and that the honey from Portugal was good.

Sereia *et al.*, (2013) analyzed for the microbiological quality in 11 organic and 6 non-organic honey samples of Africanized honeybees harvested from islands of the triple frontier (Sao Paolo, Parana and Mato Grosso do Sul states) and from the state of Parana, Brazil. After inoculation and incubation at 35 °C – 45 °C, results for coliform were

<3.0 CFU/g. results for moulds and yeast were between a minimum of 1.9×10^2 and maximum of 1.1×10^3 for organic honey and a minimum of 1.8×10^1 and maximum 2.5×10^2 CFU/g for non-organic honey.

In a study conducted in Greece to determine the antibacterial activity of different honeys against pathogenic bacteria, about 60 samples of honey from various botanical origin were analyzed for their antimicrobial activities against 16 clinical pathogens and their reference strains. The research concluded that all the samples were positive for antimicrobial activity. (Voidarou *et al.*, 2011)

2.4.5 Irradiation Pasteurization of Honey

Cheorun *et al* (2005), irradiated four kinds of honey samples with a Co – 60 source gamma irradiator varying the doses at 0 kGy, 5 kGy and 10 kGy. They pre-analyzed the honey samples for aerobic bacteria count, yeasts and molds, coliform, and *clostridium* spp. After the irradiation, the coliform bacterial, yeast and molds counts were below detectable limits ($\leq 10^1$ CFU/g) in all the samples. Two of the types of honey samples they analyzed (acacia and polyfloral flower honey) were completely sterilized by a 5 kGy irradiation, and the native honeys showed decreased populations of the total aerobic bacteria and *Clostridium sp.* with a 5 kGy irradiation.

Bera *et al.*, (2009), irradiated seven different types of honey samples collected from apiaries, honey depots and commercial establishments at Sao Paulo state. The samples were irradiation with a Cobalt -60 source gamma irradiator at 10 kGy, their main objective was to find out how each of the honey samples reacted to the radiation dose. They conducted physical and chemical analyses samples after irradiation; (including Hydroxymethylfurfural (HMF), sugars, pH, free acidity). According to their report –

published in the Radiation Physics and Chemistry journal in the year 2009, the physical and chemical results obtained for honeys irradiated at 10 kGy were within the official Brazilian regulations for honeys.

Molan *et al.*, (1996) researched on the effects of gamma-irradiation on the antibacterial activity of honey. They took two honeys with antibacterial activity due to enzymatically-generated hydrogen peroxide and three Manuka honeys with non-peroxide antibacterial activity. The samples were seeded with spores of *Clostridium perfringens* and *Clostridium tetani* and irradiated with 25 kGy of gamma energy. Sufficient sterility was achieved.

(Hussein *et al.*, 2014) in the Univ. of Kebang., studied the effects of gamma radiation on two types of Malaysian honey; Gelam and Nenas were studied. They irradiated both samples at a dose of 25 kGy in a cobalt-60-source irradiator. Properties examined included; pH, moisture, acidity, color and sugar content and also vitamins C and E, and HMF, both before and after irradiation. They observed increases in the values of color intensity and vitamin C after irradiation for both honey types. Besides these, the other parameters did not record any significant changes in values. (Hussein *et al.*, 2014).

2.5 Work Done in Ghana

Physicochemical Properties of some Ghanaian Honey

An article published by an employee of the Food Research Institute, (a subsidiary of the Council for Scientific and Industrial Research in Ghana), analyzed some honey samples for some physical and chemical parameters (Ankrah, 1998). With respect to microbial quality, there is scarce publication. This research was conducted with five honey samples bought from the market and analyzed for moisture content, ash, reducing sugars, sucrose,

nitrogen, calcium and iron contents. The sample size was relatively small, sample area was Accra, quite narrow for effective comparison, and no information on microbial contamination or types of microbes that may be found was reported.

Stingless bee Honey

Kwapong *et al.*, (2013), conducted a research on the treatment of eye infections with stingless bee honey. They isolated pathogens collected from eyes of people infected with eye problems, compared the sensitivity of these pathogens to eight standard antibiotics, used in the treatment of eye infections in Ghana, and also with stingless bee honey. The objective was to find out antimicrobial capability of stingless bee honey. This research didn't focus on the sting-bee honey (perhaps the most consumed type of honey in the country), it didn't focus on the physicochemical properties of the honey, and neither did it focus on the possibility of microbes in the honey and the types of microbes, if any, in the honey (Kwapong *et al.*, 2013).

Knowledge of the microbial quality of honey would be an advantage to the use of honey in treatment of eye infections. Without this knowledge, honey contaminated with pathogens could be dangerous when used medically and complicate the defect rather than the intended positive use as a cure. It is therefore very important to verify that honey is significantly free of microorganisms for medicinal and other purposes and this is what the main objective of this research is. This research would provide reliable data on the use of gamma radiation to decontaminate honey produced in Ghana.

CHAPTER THREE

3.0 METHODOLOGY

3.1 SAMPLING LOCATIONS

This research was conducted in Ghana, West Africa, in three (3) regions; Brong Ahafo, Ashanti and Greater Accra Table 3.1 and Fig. 3.1 show the regions and sample locations, and the map of the locations respectively. Two (2) of the regions – Brong Ahafo and Ashanti – are believed to be among the largest honey production centres in the country (Alaazi *et al.*, 2010). Brong Ahafo has an estimated 2188 apiaries, the Ashanti has 2243 apiaries and the Greater Accra region has 1536 apiaries (Alaazi *et al.*, 2010).

Table 3.1: Sampling Locations

REGION	TOWNS
Ashanti	Aboaso, Agona, Ahwiaa, Asenua, Fawode, Jamasi, Mampongten, Nkwanta, Ntonso, Pankrono
Brong Ahafo	Atebubu, Berekum, Drobo, Dumasua, Fiapre, Kintampo, Mantukwa, Nsoatre, Tanoso and Techiman
Greater Accra	Ablekuma, Adenta, Afienya, Akweteyman, Amasaman, Awoshie, Ayi Mensah, Oyibi, Pokuase and Weija

The sampling locations were small towns with relatively low vehicular and construction activities compared to the major cities in the regions. The beehives are mostly located away from residences or places where human activities are predominant to protect the residences and also to ensure minimal human influence on bee foraging.

The sampling started in August, 2014. From August through to December, 2014, samples were taken from the sample points in the Ashanti and Brong Ahafo Regions. From January through to March, 2015, samples were taken from the Greater Accra Region. Sampling was conducted with the available resources, (period within which to complete the project, available beekeepers and finance) and most of the places visited were rural communities with some navigational shortcomings. Considering the complex nature of the sampling process and the locations, sampling within the same month proved quite difficult with the available resources. These informed the decision to spread the sampling across this period.

3.2 QUESTIONNAIRE ADMINISTRATION

Based on the objectives of this study, a questionnaire was designed (a sample of which can be found in Appendix 1). This was to help find out how the activities and apiculture practices of the beekeepers contribute to the contamination of honey. To those who could neither read nor write, oral interviews were conducted based on the questionnaire.



Fig. 3.1: Map of Ghana showing sampling locations

3.3 SAMPLING

The samples were taken along the farmer/hunter-to-trader route. The first set of samples was essentially honey taken directly from the honey comb prior to the farmer harvesting for extraction and packaging (Fig. 3.2 and Fig. 3.3). The next set of samples was honey which had been harvested, processed and ready for selling to the retailers.



Fig. 3.2: Honey combs harvested for honey extraction at one of the farms visited

The last set of samples was taken from the retailers, only the retailers who buy from the farmers sampled were considered (Fig. 3.4). This was efficient because the retailers personally assisted in locating the farmers from whom they obtain their stock. Some of the samples were obtained from honey hunters. At each sampling location, three (3) samples were taken and in all, 30 samples were taken from each region. A total of 90 samples were collected for this research. Prior to the analysis, the honey samples were

stored in clean plastic bottles and tightly covered to prevent external contaminants as much as possible.



Fig. 3.3: Sieving of honey after extraction from honey comb by Farmers

The samples were systematically coded based on the regions, sampling site and collection date (Fig. 3.4a and Fig. 3.4b)



3.4a: Labeled with initials of sample locations



3.4b: Number codes indicate type of sample

Fig. 3.4: Samples labeled and packed for transportation to the laboratory at GAEC



Fig. 3.5: Honey on sale by the road side at Adenta

They were then sealed in transparent polyethene bags (to prevent, as much dust particles as possible, from the bottled samples) and packed into paper boxes for transport to the laboratory. At the laboratory, the samples were removed from the transparent polyethene bags, kept in clean shelves at room temperature, still in their original packages, prior to analysis. The temperature conducive for microbial growth varies with respect to the type of microorganisms. Mesophyllic bacteria grow best within 30 °C to 37 °C, therefore keeping the honey samples at room temperature and sometimes lower (when air

conditioning is available) could inhibit microbial growth. Care was taken to prevent, as much as possible, contamination while in the storage shelves.

For each sampling point, a control was added. The control consisted of an empty bottle which was treated the same way as the sampling bottles except that honey was not added.

All the plastic containers for the sampling were pre-washed and steam sterilized.

3.4 ANALYSIS OF HONEY SAMPLES

The flow diagram (Fig. 3.6) illustrates a general overview of the analyses performed on the honey samples;

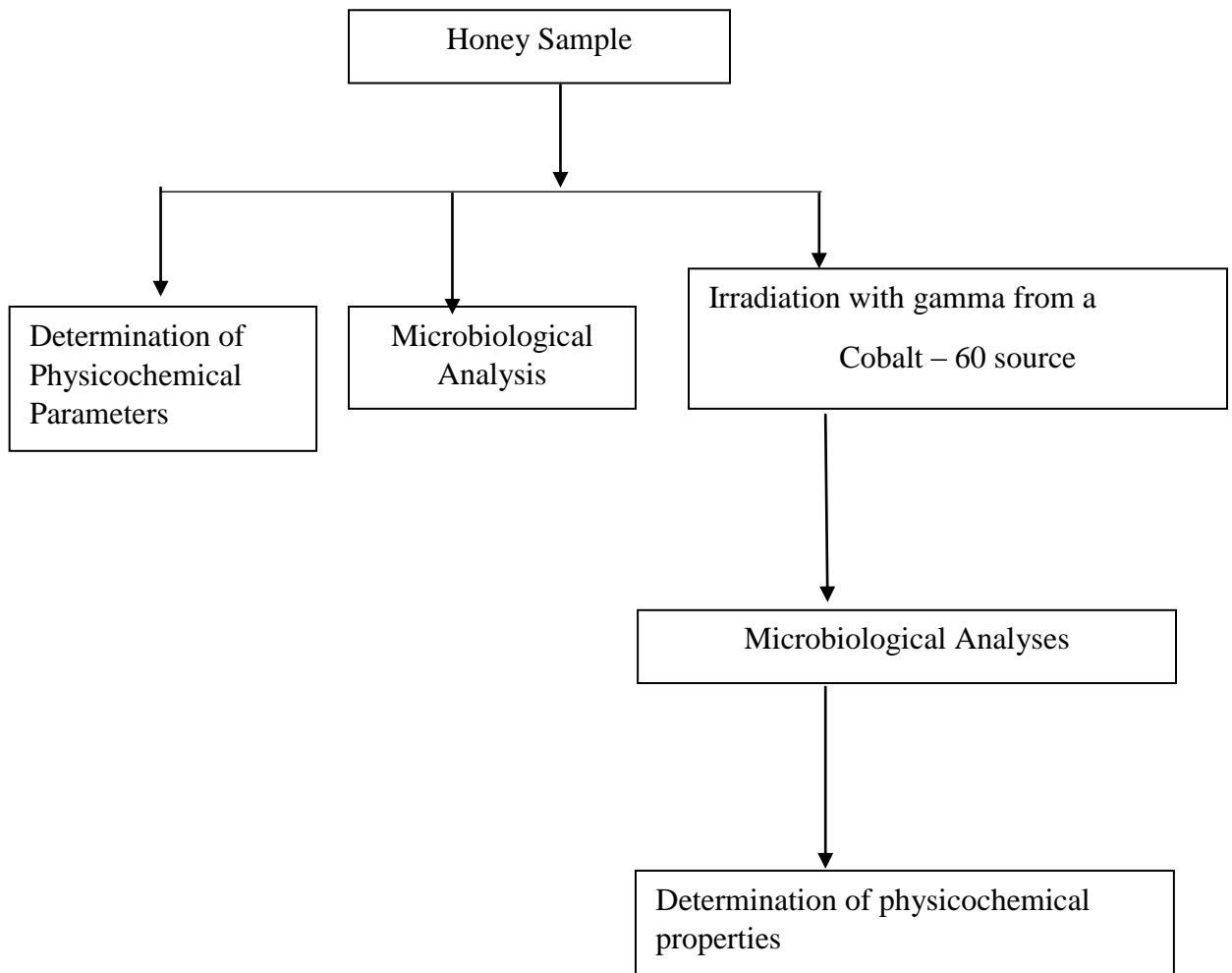


Fig. 3.6: Flow diagram of analyses

3.4.1 Microbial Analysis

The microorganisms in the honey samples (farmer – to- trader route) were determined using the Colony Count Technique (ISO 4832: 2006 – Microbiology of food and Animal Feeding Stuffs – Horizontal Method for Enumeration of Coliform). This was followed by decontamination of the honey samples by irradiation with gamma rays using a cobalt 60 source.

The decontaminated honey samples were then subjected to microbial analyses to estimate the degree of decontamination. The detailed experimental procedure are presented below.

The samples were analyzed for Total Viable Counts, *Faecal* sp., *Clostridium* sp., *Staphylococcus* sp., and *Salmonella* sp. These are common causative microorganisms of foodborne illness and also they are indicator organisms showing the unhygienic processing, package and other treatment of food. The total viable count estimates all possible aerobic and anaerobic microbes present in the samples.

Apparatus

Colony counter

Incubator

Steam Sterilizers

Laminar chamber

All the glassware were sterilized prior to the analysis. The glass petri dishes (Fig. 3.7) are packed in canisters – 13 pieces per canister – and sterilized in a hot air oven for 2 hours.



Canisters for sterilizing Petri dishes

Fig. 3.7: Petri dishes prepared for sterilization

Media used

Total viable count	Plate Count Agar
<i>Faecal</i> sp.	Eosin Methylene Blue agar
<i>Clostridium</i> sp.	Perfringens agar
<i>Staphylococcus</i> sp.	Baird Parker agar
<i>Salmonella</i> sp.	Xylose Lysine Deoxycholate agar

3.4.1.2 Preparation of Agar

The method of preparation was the same for each agar. About 22 g of the agar powder was weighed into a 0.5 L Erlenmeyer flask based on manufacturer's instruction. About 50 mL aliquot of distilled water was added to the content in the flask and then shaken gentle to allow for mixing. When it has blended, more of the distilled water was then added to the content to reach the 0.5 L mark (Marshall, 1993).

The prepared media were then sterilized at 100 °C for about thirty (30) minutes allowing the mixture to further melt and enhance blending. The media was tempered in a water bath to a temperature of 45 °C. This was done while ensuring that the water level in the bath was about 1 cm above the level of the medium in the bottle.

3.4.1.3 Inoculation and Incubation

The working surface of the laminar chamber (Fig. 3.8) was cleaned with 70% isopropyl alcohol. Duplicate inoculations were done for each sample, hence each duplicate petri dish was clearly marked per sample per microbe analyzed. About 10 g of each honey sample was weighed and homogenized in 90 mL of peptone water solvent by agitating in dilution bottle for about 10 seconds. About 1ml aliquot of the prepared sample was pipetted into five other 10 mL bottles with peptone water.

A cotton-coated piece of plastic rod was used to wipe the inner surface of the control plastic bottle and the content dissolved in 5 mL of peptone water which was then transferred into a labeled Petri dish for inoculation with Plate Count Agar and incubation. This was done for each control taken at the sample points visited. The controls were taken mostly at the honey processing areas of the honey farmers.

About 1 mL aliquot of the prepared sample was pipetted into the labelled Petri dishes and 15 mL of the prepared agar was added to the Petri dishes. This was repeated for each of the agar for the various plate, counts that is, Perfringens agar, Baird-Parker agar, eosin methylene blue agar, Xylose Lysine Deoxycholate agar. These transfers and sample dilutions were done in a laminar chamber where temperature was controlled at 45 °C. The agar were transferred within 15 minutes after the sample mixtures were pipetted into the petri dishes. For each sample inoculated for incubation, a blank Petri dish (without

sample) was inoculated with plate count agar to verify if the pre-sterilized Petri dishes were free of any possible microorganisms which could influence the results of the analysis.

The sample-agar mixtures in the Petri dishes were gently swirled to mix thoroughly, left for 15 minutes to settle, after which 4 mL of the respective agar were added to the mixtures as a second layer and left to solidify. This was to ensure that the entire sample was well covered with the agar. The inoculated samples were then incubated (Fig. 3.9a and Fig. 3.9b) at 37 °C for 48 hours, monitoring the changes every 24 hours until the incubation time had elapsed.

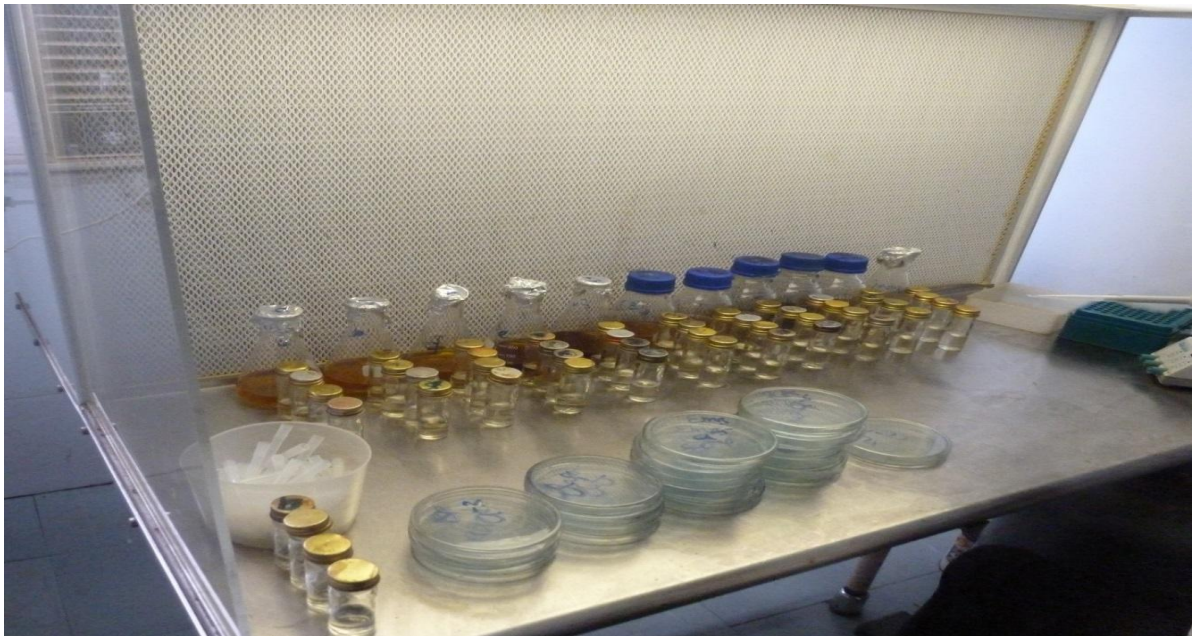


Fig.3.8: Laminar Chamber with prepared samples and labeled Petri dishes at the Microbiology Laboratory at GAEC



Fig.. 3.9a: Incubator

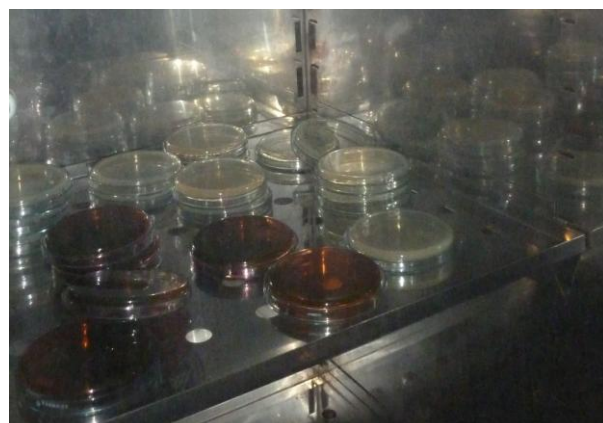


FIG.. 3.9b: incubator with inoculated samples

Fig. 3.9: Inoculated Samples were incubated

Counting of colonies

Colonies were counted immediately after the incubation period. A colony represents cells that happen to be well separated on the plate and so can be distinguished after growth, it could be one cell or several thousands (Sutton, 2006). Petri dishes (plates) which have microbial growths up to at least 30 colonies or at most 300 colony units formed per plate are the most favored in colony counting, sometimes the range could be 25 to 250 CFU/plate, and these were selected for counting (Sutton, 2006). For plates that contained colonies that spread out, a representative portion of the plate was selected for counting, that is if a quarter of the plate was counted for colonies, it was estimated that the colonies formed on the plate were four times the count values for the quarter portion.

Counts were calculated using the formula (ISO 4832 formula for microbial count):

$$N = \frac{(C1 + C2 + C3)}{v} \times d$$

Where C1, C2, C3 = count values for the triplicate plates

V = volume of sample on each plate

d = dilution factor for the plates counted

N = the Colony Forming Unit (CFU) per gram or mL of product.

Count results were rounded off to two (2) significant Figures

3.4.2 Determination of Physicochemical Parameters.

To assess the quality of honey, the physicochemical parameters (pH, Apparent Reducing sugar, Apparent Sucrose, Ash content) of sixty honey samples were determined. The results were compared to International Food Standards for honey as stated in the CODEX Alimentarius standards for the quality of Honey. The samples selected were the honey comb samples (S1) and the consumer samples (S3) from all the three regions. The samples taken after harvesting and treating were not subjected to any physical and chemical test because it was assumed there would be no significant alteration to the physicochemical properties during percolation and sieving. The physicochemical tests were conducted before and after 'decontamination' by gamma radiation so as to determine how they are affected by the high energy photons during gamma-irradiation.

The quality of honey depends on several parameters. Some of these parameters include, concentration of reducing sugar and sucrose, pH and percentage ash. Good quality honey, by CODEX Alimentarius Standards, honey must have not less than 60% reducing sugars and not more than 10% sucrose.

3.4.2.1 Ash Content

Principle

The ash content is a quality criterion that determines the origin of honey. It can also be used to determine the concentration of organic and inorganic acids, and the presence of suspended solids in honey (Bogdanov, 2009).

Methodology

The Ash dish was heated in an electrical furnace at 600 °C and was subsequently cooled in a desiccator to room temperature and weighed to the nearest 0.001 g (M_2). Exactly 5g of the honey sample was weighed to the nearest 0.001 g into the ash dish that had been prepared (M_0). Two drops of olive oil were added to the sample. Ashing was commenced at a temperature of 350 °C and progressively increased to maximum temperature of 600 °C. The heating was done for an hour. The ash dish was then cooled in the desiccator and weighed (M_1).

The ash proportion (W_A) in $\left[\frac{g}{100} g\right]$ honey was calculated using the formula below as stipulated by the International Honey Commission;

$$W_A = \frac{M_1 + M_2}{M_0} \times 100$$

M_0 = Weight of sample

M_1 = Weight of ashed sample on dish

M_2 = Weight of pre-ashed dish without sample

3.2.4.2 Reducing Sugar

‘Apparent reducing sugars’ are the sugars which reduce a Fehling’s solutions from blue to brick red under specified conditions. (Bogdanov, 2009). Honey is primarily composed of reducing sugars (85% to 95%) and apparent sucrose (5% to 10%). The high sugar concentrations give honey its osmotic pressure which helps to inhibit the growth of microorganisms.

Table 3.2: Apparatus and reagents for reducing sugar and sucrose determination

Apparatus	A 50 mL burette, Erlenmeyer flask, electric heater
Chemicals and Reagents:	Fehling solution A (7% CuSO ₄) and Fehling solution B (25% KOH); 0.2% Methylene Blue Indicator

The method employed in this research was a modification of the Lane and Eynon procedure. This procedure involved the reduction of Soxhlet’s modification of Fehling’s solution by titration at boiling point against a solution of reducing sugars in honey.

About 50 mL of 1% sample (prepared by diluting 2 g of honey in 200 mL distilled water), were placed into a burette as the titrant (Fig. 3.10a and Fig. 3.10b). In the preparation of the analyte, 10 mL each of Fehling solutions A and B with 8 mL of distilled water were transferred into a 250 mL conical flask (Fig. 3.10c and Fig. 3.10d)

This was followed by the addition of two drops of 0.2% Methylene Blue Indicator. The mixture was heated until it started boiling. Titration started at this point until the initial colour of blue changed to brick red. Titration was stopped and the volume of titrant noted and recorded (Fig. 3.10e and Fig. 3.10F)

The % Total Reducing Sugar was calculated using the equation:

$$\% \text{ total reducing sugar} = \frac{2 \times 10.2}{\text{titre value} \times \text{mass of sample}} \times 100$$

10.2 = Glucose factor

3.2.4.3 Apparent Sucrose

To determine the apparent sucrose, the concentration of invert reducing sugar was determined. This was done by measuring about 10 mL of 6.34 M HCL and 50 mL of 1% honey and transferred into a conical flask and kept on a water bath at 60 °C for 20 – 30 minutes. The sample was cooled and neutralized with a 5 M NaOH_(aq) to pH of 7 (the pH was monitored with the pH meter). The samples were transferred into a burette. About 10 mL each of Fehling solution A and B and 8 mL water were transferred into 250 mL flask and heated till the solution started boiling. Titration started at this stage after the addition of two drops of 0.2% Methylene Blue Indicator and titration was completed while the solution was boiling. At the endpoint, the blue colour of the solution changed to brick red.

Calculations

The % Reducing Sugar was calculated using the formular

$$= \frac{2 \times 10.2}{\text{titre value} \times \text{mass of sample}} \times 100$$

From the results obtained, percentage sucrose was calculated for by;

$$\text{Percent sucrose} = \% \text{ sugar (after inversion)} \times 0.95$$

The methods are the 'Harmonised Methods of the International Honey Commission for honey quality analyses.



FIG. 3.10a: Fetching sample for weighing



FIG. 3.10b: Weighing of sample



FIG. 3.10c: Preparation of analyte for titration



FIG. 3.10d: Pre-heating of analyte for titration



FIG. 3.10e: Thermal Titration



FIG. 3.10f: Thermal Titration

FIG.. 3.10: Determination of Reducing Sugar and Apparent Sucrose Concentrations

pH

Honey is a product of high acidity with pH values ranging from 3.6 to about 6.5 for different types of honey (Bogdanov, 2009). The low pH also inhibits the growth of microorganisms in honey therefore contributes to the long shelf life of the honey product.

The pH meter was calibrated using buffer solutions of pH 3.7 and 9.0. About 10 g aliquot of the sample were weighed into 75 mL of carbon dioxide-free water in a 250 mL beaker. The solution was stirred to homogenize and the pH electrode was immersed in the solution and the pH recorded.

3.4.3: Sterilization of Honey by Irradiation with gamma rays

Principle

Gamma irradiation is a physical method of sterilization or decontamination where products are exposed to gamma rays. The gamma rays, which are a form of electromagnetic radiation of very short wavelengths, acts as a source of ionizing energy that destroyed bacteria and pests.

Instrumentation

The Gamma Irradiation facility is a ^{60}Co (category IV wet storage gamma radiator) with an initial source strength of 50 kCi. It was manufactured and installed at the Gamma Irradiation facility of the Radiation Technology Centre, Ghana Atomic Energy Commission in Accra, by the 'Isotope Company Ltd' a company based in Hungary, in 2010 (RTC-GAEC).

The cobalt 60 (^{60}Co) source has a current strength of 26 Kci with a dose rate of 1.4 kGy/hr around the shroud.

Packaging of the honey samples for Irradiation

About 50 mL of the honey samples were packaged in 100 mL polyethylene bottles for irradiation. Twenty (20) bottles each per dose rate were secured with a masking tape and the samples were transferred into the gamma chamber (FIG.3.11) by the pneumatic transfer system attached to the facility. The samples were then irradiated with gamma radiation from a Co-60 source at varying doses of 20 kGy, 30 kGy and 40 kGy. The doses were varied in the control room (FIG. 3.12)

The Ethanol Chlorobenzene (ECB) dosimeters were stuck to the samples, and were together subjected to gamma radiation for half the predetermined period for each dose (this period is the time taken to completely irradiate the samples at each dose. At a dose rate of 1.4 kGy/hr., irradiating at 20 kGy will take approximately 14 hours to complete. The honey samples were then turned through 180 degrees and then subjected to gamma radiation for the other half of the predetermined period to ensure homogenous distribution of the dose delivered under the same conditions.

The ECB dosimeters were removed from the honey samples after the irradiation period. The absorbed dose was then determined using a calibrated readout instrument (High Frequency Dosimeter System, Model 2131, version 2.5, and produced by SENSOLAB LTD).

The expected and absorbed/delivered doses for the Honey samples are as follows;

Table 3.3: Absorbed and Expected doses

Expected dose(kGy)	Delivered dose(kGy)
20	20.83
30	33.08
40	41.64

Uniformity ratio: 1.09



FIG. 3.11: Honey samples in irradiation chamber



FIG. 3.12: Radiation Monitoring Room

CHAPTER FOUR

4.0 Results and Discussion

All foods including honey should be free of microorganisms. The honey samples were analyzed for microorganisms to ascertain whether the treatment given to the product in the percolation, sieving and packaging processes affected its quality before it finally reaches the consumer. The microorganisms considered in this research are very common microbes frequently encountered where food products are treated in conditions suspected to be unhygienic.

Staphylococcus sp., are common on man's respiratory passages, skin and superficial wounds. Although they can be denatured by the heat of cooking yet the toxins produced by these microorganisms can resist heat and may not be destroyed when in the human alimentary causing food poison (Wagner, 2008). Since honey is used raw, it will be dangerous to have these microbes present in it.

Data from this research will surely help improve the quality of Ghanaian honey, so that it can compete strongly with that on the international market.

4.1 Responses from Interviews (Through Questionnaire Administration)

Fig. 4.1 to Fig. 4.4 are graphical representations of some of the responses of the beekeepers regarding honeybee farming and its practices. Majority of the beekeepers (20%) were within the ages 51 – 60 years (Fig. 4.1). For a greater percentage of the age group of 60 years and above, beekeeping was a full time business whereas those below 60 years are mostly crop and livestock farmers who keep bees as part of their business.

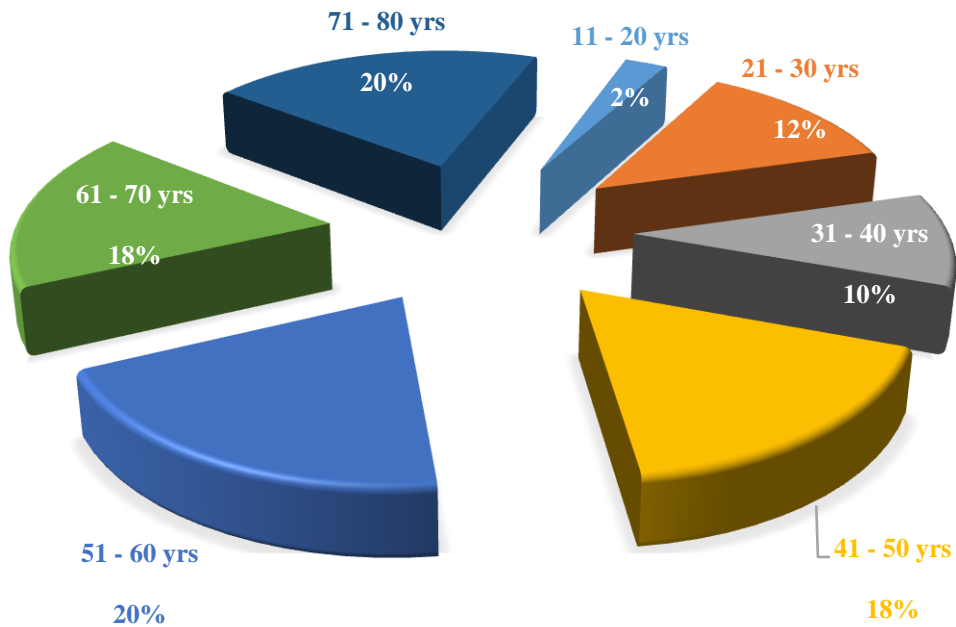


Fig. 4.1: Age distribution of beekeepers for all three (3) Regions

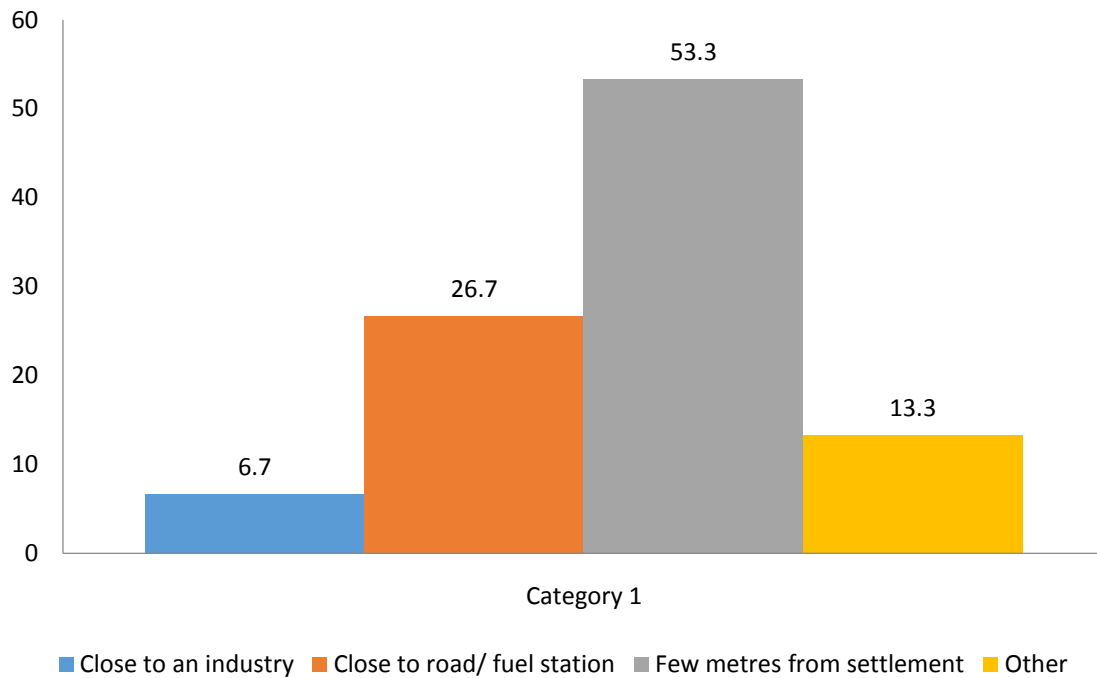


Fig. 4.2: Location of Apiaries

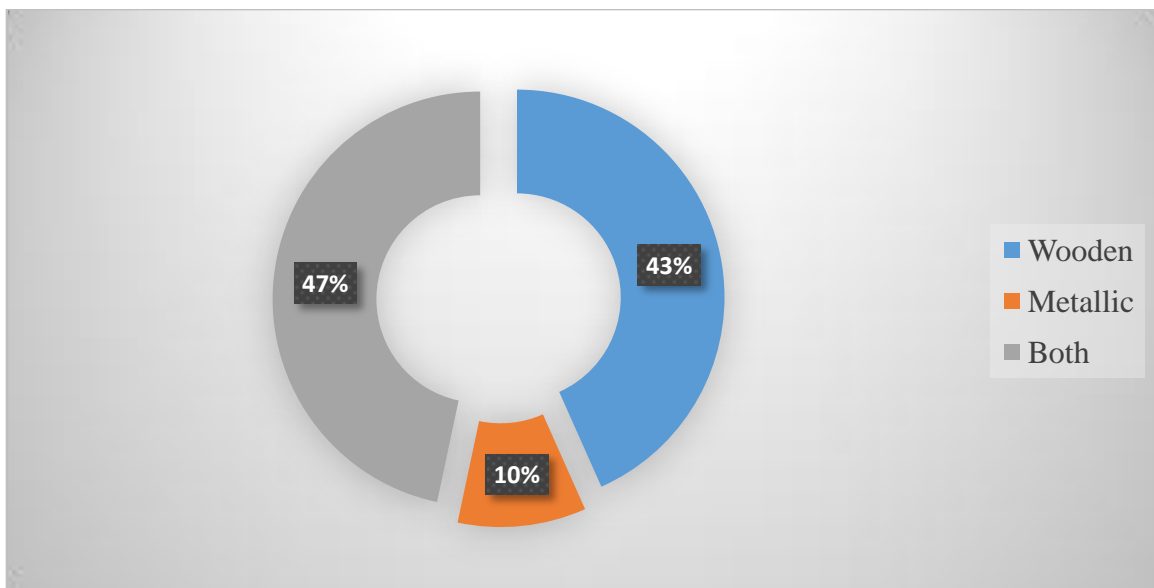


Fig. 4.3: Extraction Equipment

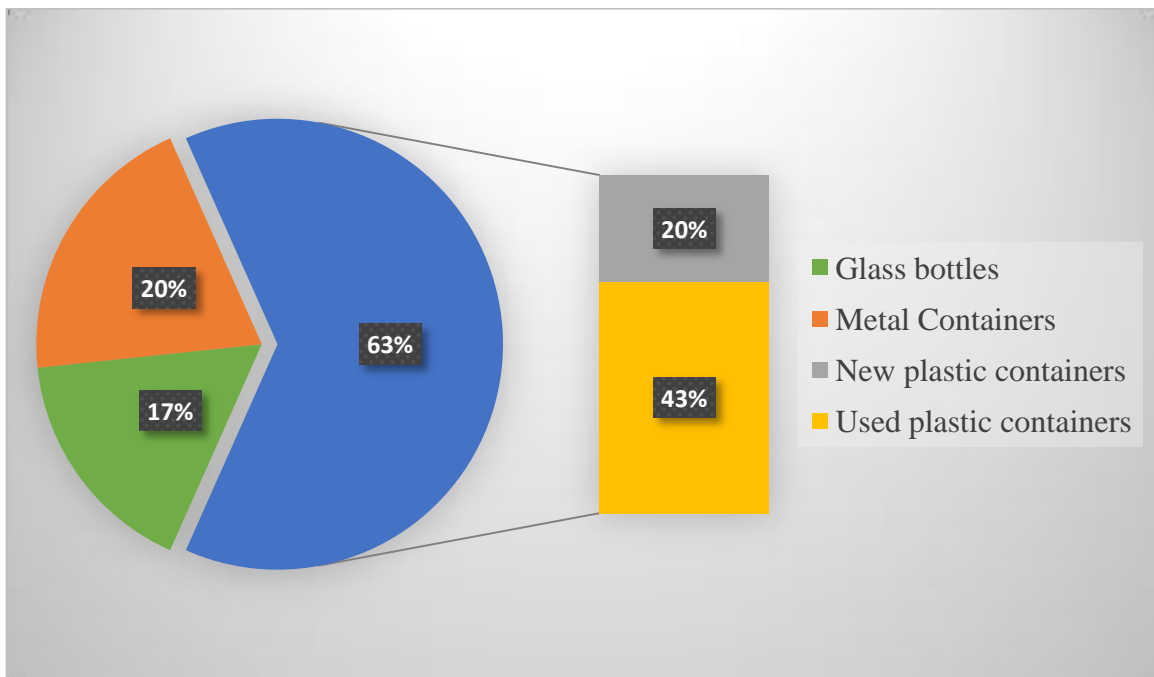


Fig.4.4: Storage containers for Honey

Most of the apiaries (about 53.3%) were located away from places of residence as shown in Fig. 4.2. There were some apiaries that were located not far away from fuel stations and about 20% of the apiaries were located close to quarries, wood processing factories, and places where (human activities) were quite common. 47% of the beekeepers used metallic and wooden ladles to scoop the honey from its comb (Fig. 4.3). For others, they keep the combs in containers over a period to get the honey out of the combs by gravity.

The product, after extraction from the combs, was sieved and finally stored in containers made of a range of materials. About 63% of the retailers used plastic containers of which 43% of these containers were pre-used water bottles. 17% also used glass bottles of different products ranging from beer to soft drinks for storage and sale of honey (Fig. 4.4).

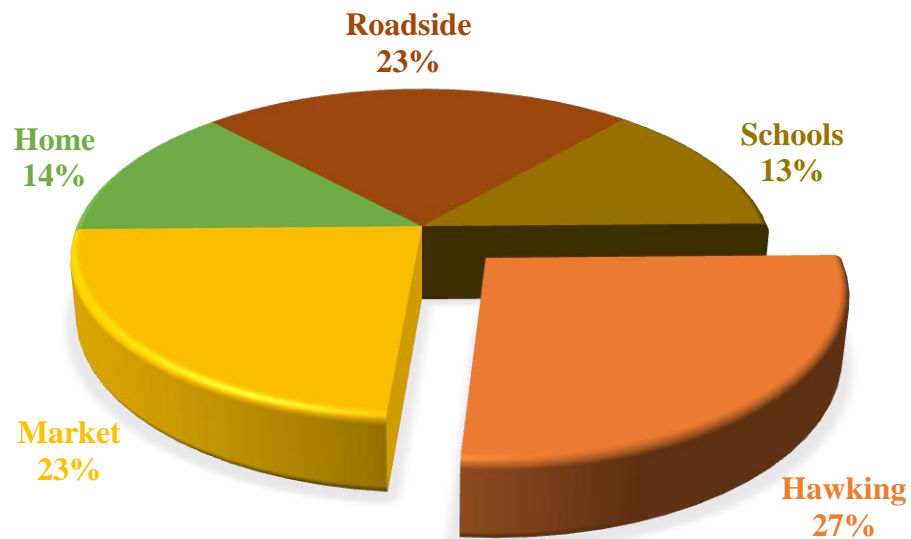


Fig. 4.5: Places where Honey is sold

Mostly, the retailers carry honey around from house-to-house, workplaces and so on, for sale. From Fig. 4.5, 27% of the retailers sell their honey by hawking and only a few retailers (about 14%) sell theirs in their homes and these have their customers who trust their honey, and come to the house to purchase. A combine 46% of the product are sold on markets and roadside, most travelers stop their cars to purchase honey before continuing their journey.

These methods of extraction, treatment and sale, all contribute to the exposure of honey to contamination. A sample table of detailed responses from the administered questionnaire is presented in appendix 8.

4.2 Results of Microbiological Analyses

Total viable count values for honey ranged from 0 to several thousand per gram. This variation in bacterial counts may be due to the type of sample (Honey comb samples, finished or retailed), the freshness of the honey, the time of harvest and the analytical techniques used (Snowdon and Cliver, 1996). The microbial quality of honey in this research will be compared with the publication by Josiane *et al.*, (2010), who used the MERCOSUR GMC, number 15/94 technical rules for identity fixation in honey quality, approved by ordinance number 367 from September 4, 1997. This states that honey may contain a maximum of 100 CFU/g.

Fig. 4.6 is a graphical representation of the results obtained for Microbial Counts in honey sampled in the Brong Ahafo Region. The microbial count values in about 80% of the honey samples were below the MERCOSUR stipulated maximum (100CFU/g) for wholesome honey. However there were samples from a few of the locations where the microbial counts were significantly above this level. The samples from Fiapre and

Berekum were purchased from the market. The honey from these locations were purchased from honey hunters who have specific locations where bees make their honey. They visit these locations, on their way from their farms, to extract the product to the market directly for sale. They use their farming tools to collect the honey, hence the results as shown in Fig. 3.5.

In the Ashanti Region, the apiary in Agona was located just a few meters away from a fuel station and honey extraction happens on site when harvested. The beekeepers admitted that it was a good location for marketing since people come around for refueling and to shop in the mini mart around. This could have resulted in the contamination of the samples from these locations (Fig. 4.6). The honey sampled from Jamase was purchased from a farmer who had collected some honey comb into a plastic bucket to be taken home for extraction. Honey was heat-drained from the comb by leaving it in the sun.

In the Greater Accra Region, the retailers from whom honey was sampled, were selling in markets (23%), by the road side (23%), hawking (27%) at places where vehicular activities were quite common and where most roads were untarred. In most cases, the retailers transfer the honey into smaller containers after they have bought the honey in bulk quantities. These factors could have played a pivotal role in contamination of the honey hence the relatively higher Microbial Count readings recorded in this region. (Fig.4.8).

All the honey sampled from the three regions tested negative for *Salmonella* sp.

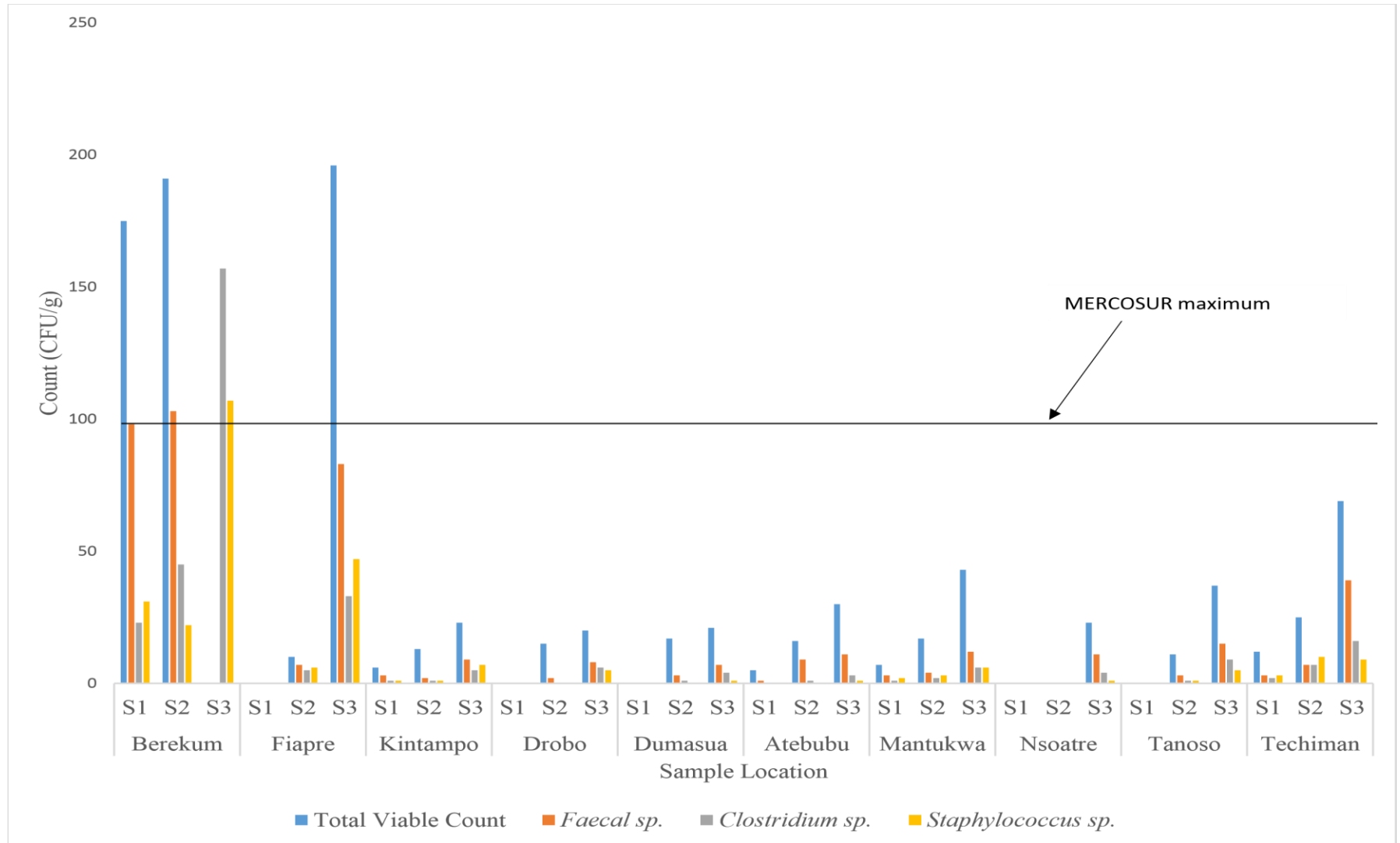


Fig. 4.6: Microbial Count in Honey Sampled from the Brong Ahafo Region

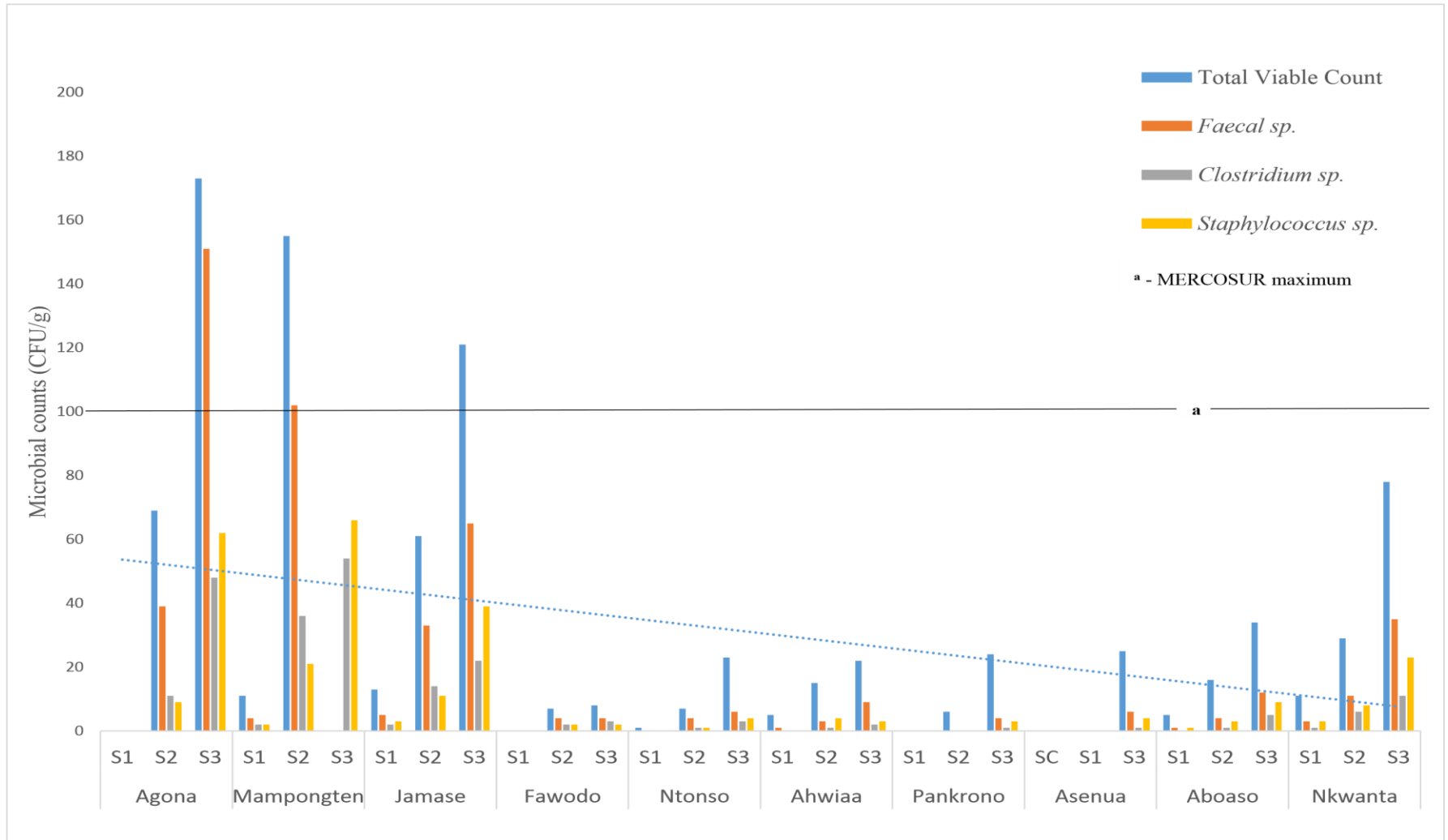


Fig. 4.7: Microbial Count per Sample in Honey sampled from the Ashanti Region

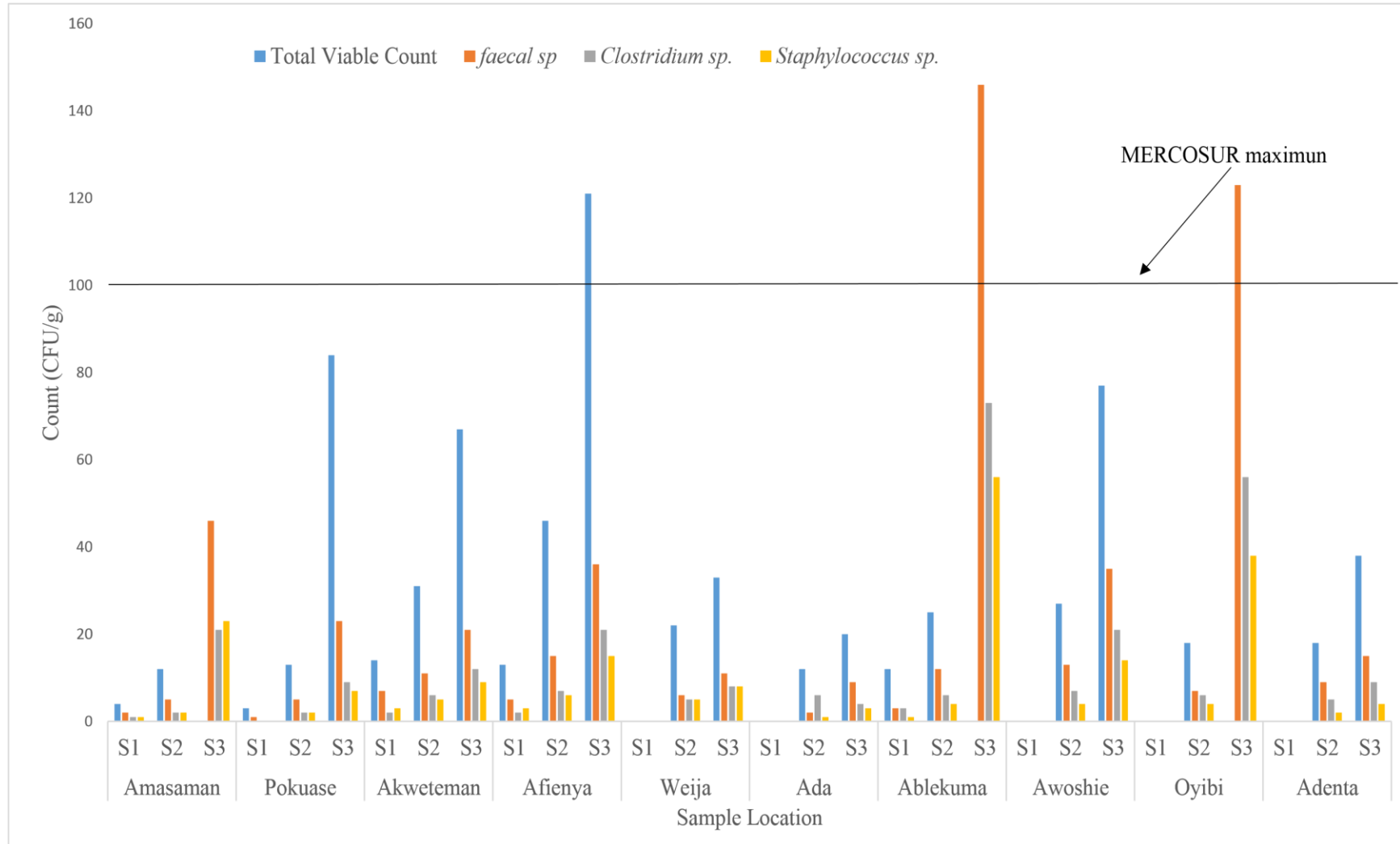


Fig. 4.8: Microbial Count per in Honey sampled in the Greater Accra region

4.2.1 Relative Microbial Counts per Region

To ascertain the major source of contamination of the honey samples, comparison of microbial count in the different samples was evaluated.

4.2.1.1 Brong Ahafo Region

Table 4.1: Microbes Detected in Samples from the Brong Ahafo Region

Microorganisms	Counts (CFU/g)					
	<u>Sample One (S1)</u>		<u>Sample Two (S2)</u>		<u>Sample Three (S3)</u>	
	Absolute	Mean	Absolute	Mean	Absolute	Mean
TVC	30	3.0 ± 1	144	16±4	1462	183±20
<i>Faecal sp.</i>	12	1.0 ± 0.4	37	5±2	895	112±10
<i>Clostridium sp.</i>	7	1.0 ± 0.3	19	2±1	186	24±3
<i>Staphylococcus sp.</i>	7	1.0 ± 0.4	20	3±1	102	13±2
<i>Salmonella sp.</i>	Negative	Negative	Negative	Negative	Negative	Negative

TVC – Total viable count; S1 – Honey comb sample, S2 – Treated, S3 – Retail sample; Mean values (Average ± St. Dev.)

The absolute values in Table 4.1, indicate the total microbial counts in samples from apiaries in this region, whereas the mean values represent the microbial count per sample. After incubation at 37 °C for 48 hours, Total Viable Count in the honey comb samples were an average of 3 CFU/g per sample analyzed, the mean values of *Faecal sp.*, *Clostridium sp.*, and *Staphylococcus sp.* were 1 CFU/g. The mean Total Viable Counts, *Faecal sp.*, *Clostridium sp.*, and *Staphylococcus sp.*, in the ‘Harvested and Treated samples’ (S2) were (5, 2, and 3) CFU/g, respectively as shown in Table 4.1. These count values were relatively higher than the microbial counts for the Honey Comb Samples. This is an indication that the local treatment methods introduce microbes in the product. Generally, the honey sampled from the honey combs and that sampled from the treated

honey from the beekeeper were low in microbial count and within the MERCOSUR maximum, except for the retail samples which recorded mean value of 183 ± 20 CFU/g for Total Viable Count. The most prominent among the microbes detected was *Faecal* sp. with mean count value of 112 ± 10 CFU/g. (Table 4.1)

4.2.1.2 Ashanti Region

Fig. 4.9, represents the mean values of the various types of microbes analyzed in each of the samples from the apiaries in the Ashanti Region. It can be observed that the Microbial Counts in the Honey Comb Samples (S1) were relatively very low per sample compared to the Extracted and Sieved Samples (S2) which could be as a result of the treatment procedure, the equipment used in collecting honey from the beehives and in sieving, and the method of harvesting from the hive. Higher number of Coliform Units were detected in the Retail Samples (S3). Generally, the microbial count values were within the MERCOSUR maximum level, except for the Retail Samples where mean values of 148 CFU/g and 110 CFU/g were recorded for Total Viable Count and *Faecal* sp. respectively

4.2.1.3 Greater Accra Region

In the Greater Accra Region, the trend was similar to those observed in the Ashanti and Brong Ahafo regions. *Salmonella* sp. was not detected in any of the samples, from the apiary, through to the samples obtained from the retailers. However, *Faecal* sp., spore forming *Clostridium* sp. and *Staphylococcus* sp. were detected in all the samples (Fig. 4.10). *Faecal* sp. was relatively higher than *Clostridium* sp. and *Staphylococcus* sp. in all the samples analyzed. The significant difference between the Total Microbial Count and the specific microbes analyzed represent the possibility of other microbes present, which were not ascertained in this research.

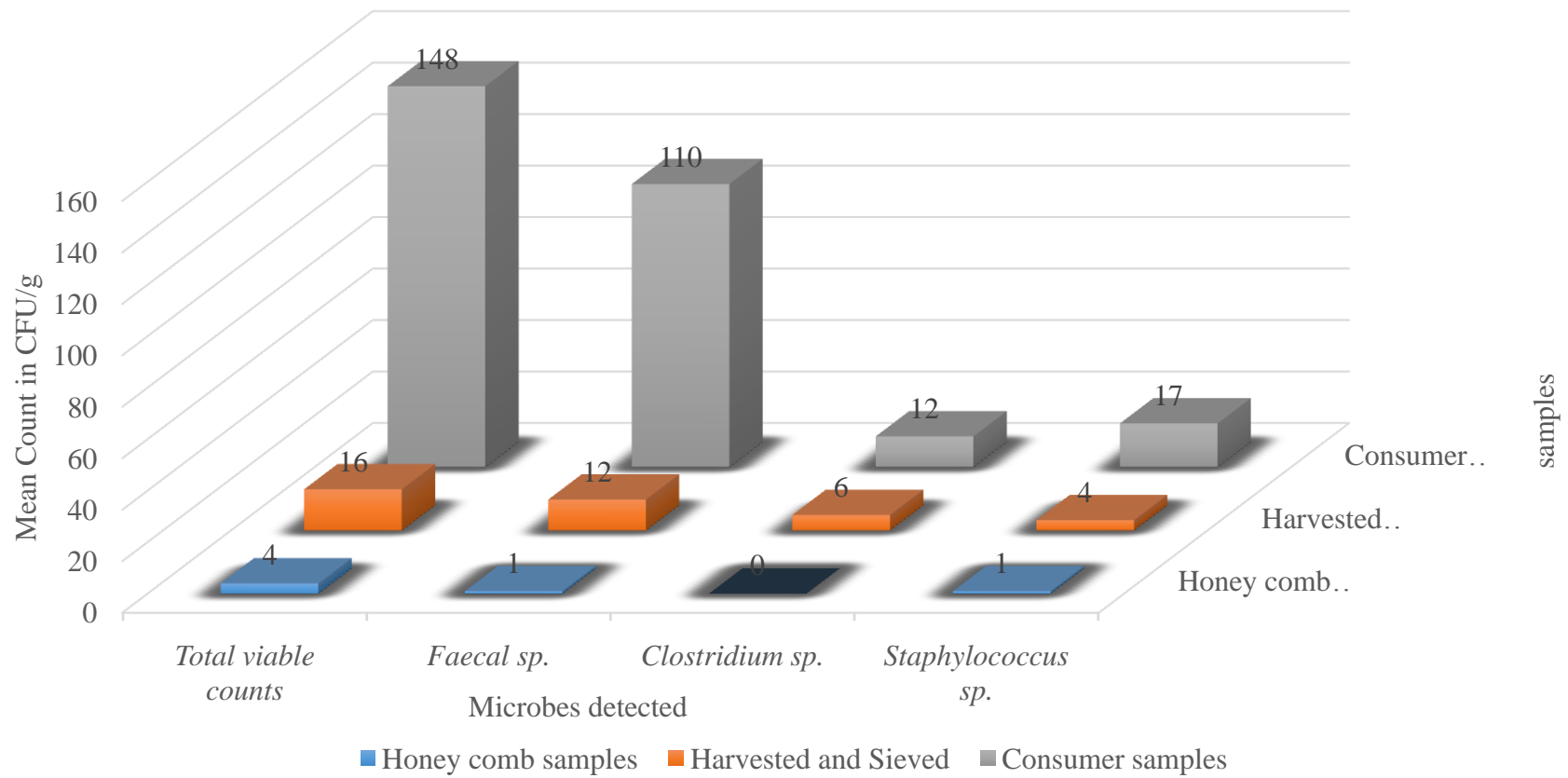


Fig. 4.9: Microbial count per honey sample in Ashanti Region

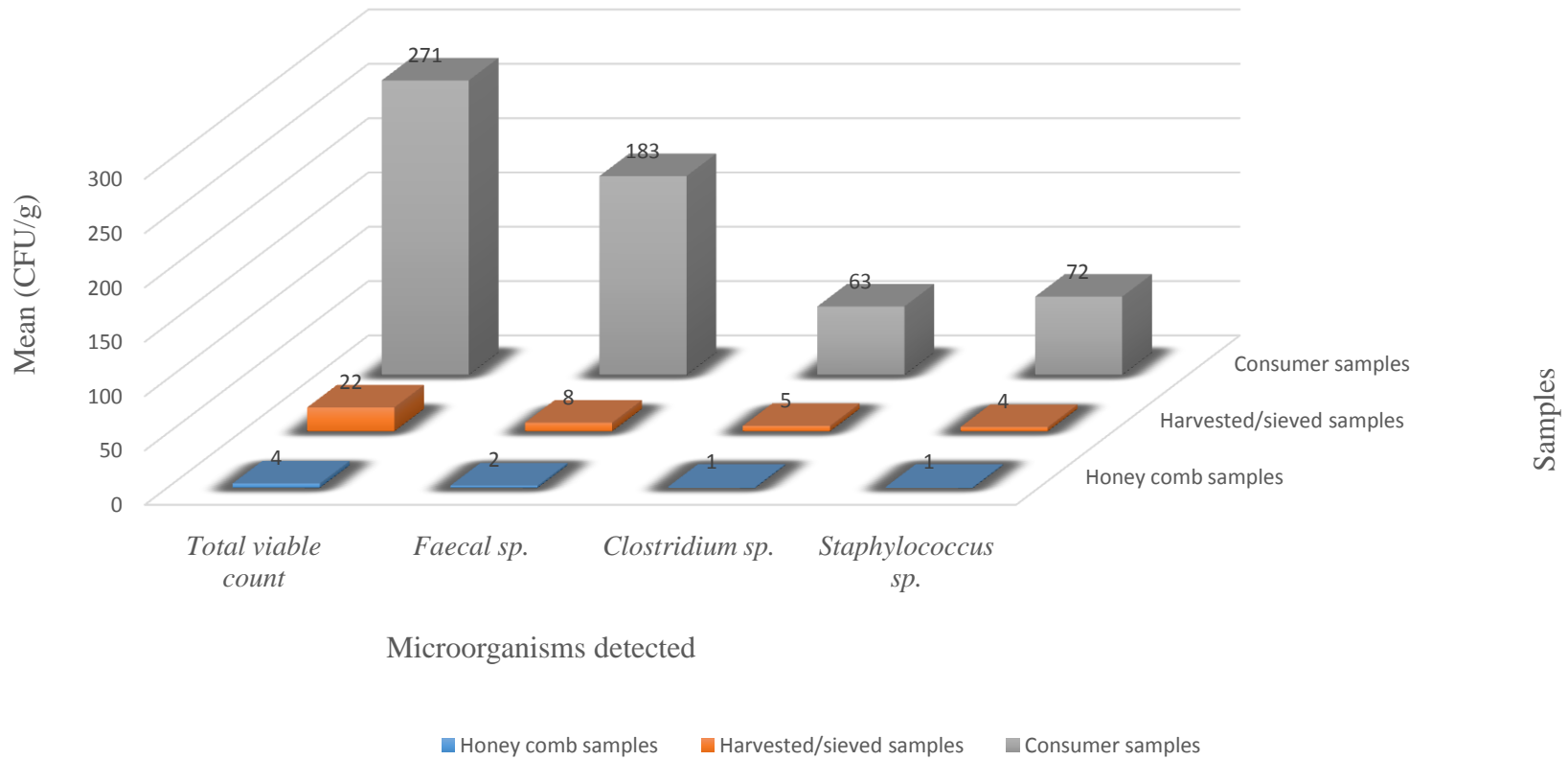


Fig. 4.10: Microbial count per sample in Greater Accra Region

4.2.2 Microbial Counts per sample per Region

4.2.2.1 Honeycomb Samples (S1)

Table 4.2: Microbial counts in Honey comb samples per sample region.

Microorganisms	Counts (CFU/g)					
	B/A		ASH		GAR	
	<u>Absolute</u>	<u>Mean</u>	<u>Absolute</u>	<u>Mean</u>	<u>Absolute</u>	<u>Mean</u>
Total aerobic count	30	5.0±2	35	4.0±1	35	6.0±2
<i>Faecal sp.</i>	12	1.0±0.3	10	1.0±0.3	14	2.0±0.4
<i>Clostridium sp.</i>	7	<1	3	<1	9	1.0±0.02
<i>Staphylococcus sp.</i>	7	<1	7	<1	9	1.0±0.03

Table 4.2 represents the average Microbial Counts in Coliform Units per gram of sample (CFU/g) counted in honey comb samples after incubation at 37 °C for 48 hours. The mean value for Total Viable Count was less than 10 CFU/g for all the sample locations, whereas the mean values for *Faecal sp.*, *Clostridium sp.* and *Staphylococcus sp.* in these samples were less than 5 CFU/g. Comparing these values with the MERCOSUR standards, they are reasonably below the allowed maximum of 100 CFU/g. The honey sampled directly from the beehive are relatively wholesome for human consumption.

Fig. 4.11 represents the distribution of microbial contaminants in Honey Comb samples per region. It is observed that the highest mean recorded per Honey Comb sample was the samples from the Greater Accra Region. It is worth mentioning that though the limit of microbes detected in these samples were significantly below harmful limits for honey relative to the MERCOSUR maximum.

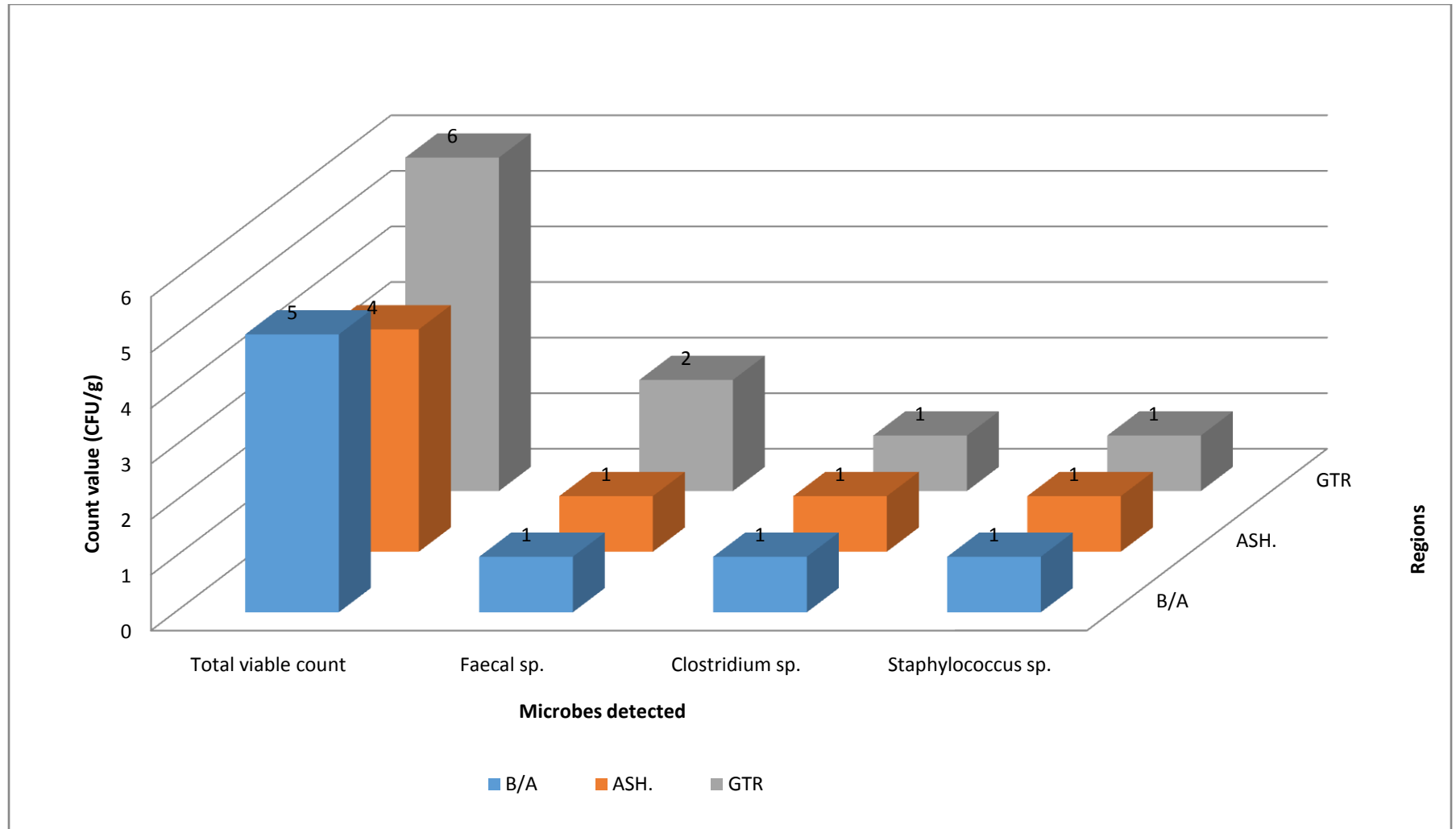


Fig. 4.11: Mean Microbial Count in honey comb samples per region

4.2.2.2 Sieved Samples (S2)

FIG.. 4.12 below represents data for the mean microbial counts in the Extracted and Sieved (S2) samples. The Mean Value (i.e. the Microbial Count per sample) for the Total Viable Count for the Brong Ahafo, Ashanti and Greater Accra Regions were respectively 16, 16 and 22 CFU/g. These values fall within 10 to 10^2 CFU/g as reported by Snowden and Cliver (1996), and Maria *et al.*, (2010), hence Honey treated by the beekeeper can be deemed wholesome in terms of microbial quality. The Mean Values for *Faecal* sp., *Clostridium* sp., and *Staphylococcus* sp. for each sample in all the sample areas were below 10 CFU/g.

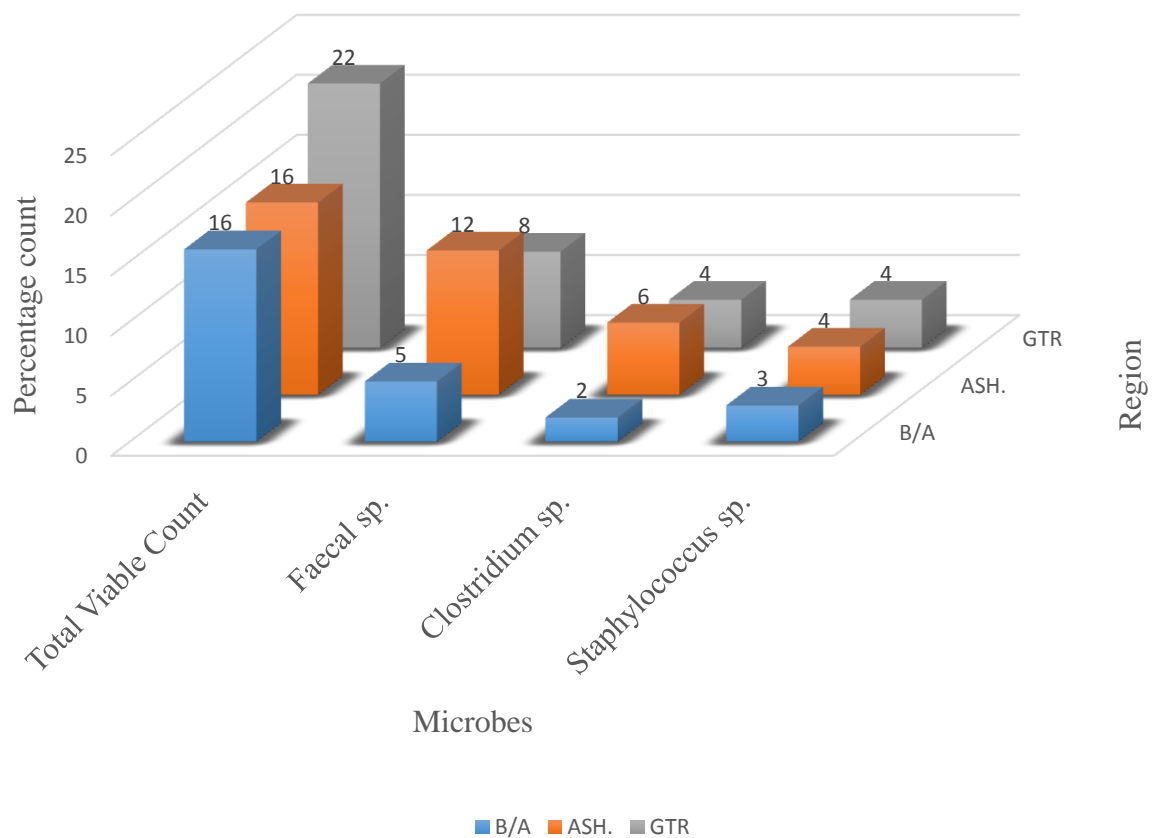


FIG.. 4.12: Average count of microbes detected in the treated samples per region

4.2.2.3 Consumer (Retail) Samples (S3)

Table 4.3: Microbial count in Retailer Samples per sample location.

Microorganisms	Counts (CFU/g)					
	B/A		ASH		GAR	
	<u>Absolute</u>	<u>Mean</u>	<u>Absolute</u>	<u>Mean</u>	<u>Absolute</u>	<u>Mean</u>
TVC	1462	183±16	1332	148±12	2440	271±18
<i>Faecal sp.</i>	895	112±10	992	110±9	1465	183±15
<i>Clostridium sp.</i>	186	24±4	196	25±4	425	53±5
<i>Staphylococcus sp.</i>	102	13±3	153	17±5	577	72±6
<i>Salmonella sp.</i>	Negative	Negative	Negative	Negative	Negative	Negative

TVC – Total Viable Count

In Table 4.3 above, it is observed that the mean microbial count for the Retailer Samples (183, 148 and 271) CFU/g for B/A, ASH and GAR, respectively, were relatively higher than the mean microbial counts of honey sampled directly from the honey comb (S1) as well as honey sampled from the farmer after harvesting, extracting and sieving (S2). The count values were significantly higher than the MERCOSUR maximum standard of 100 CFU/g for all the sample locations. This indicates that honey sampled from the retailers were contaminated. The honey sampled from the Greater Accra Region had the highest mean microbial count for Total Viable Count.

4.3 Results of Physicochemical Analyses of the Honey Samples

Fig. 4.13, Fig. 4.14 and Fig. 4.15 represent results from the physical and chemical parameters analyzed for a selected 60 samples comprising the Honey Comb Samples (S1) and the Retail Samples (S3) from all the 90 sample locations. It is part of the hypothesis of this research that the harvested and processed samples (S2) would have no significant difference in terms of reducing sugar and apparent sucrose concentrations, ash content and pH. However, should there be any significant change to honey in terms of these qualities, it would happen during the products' transition to the consumer.

4.3.1 Pre-Irradiation

The physicochemical analyses were conducted to identify any significant changes occurring as a result of the dose of gamma radiation used in decontamination. It is the high concentration of reducing sugars and the low pH (acidic nature) of honey that makes it difficult to support microbial growth, in case these parameters alter, the shelf life of honey could be affected because it may improve the conditions of microbial survival and perhaps their multiplication and other food spoilage factors.

As much as decontamination is necessary, it is equally significant to maintain the physicochemical parameters to maintain or improve the shelf life and natural quality of honey. The parameters analyzed were Total reducing sugars, apparent sucrose, pH and Ash content.

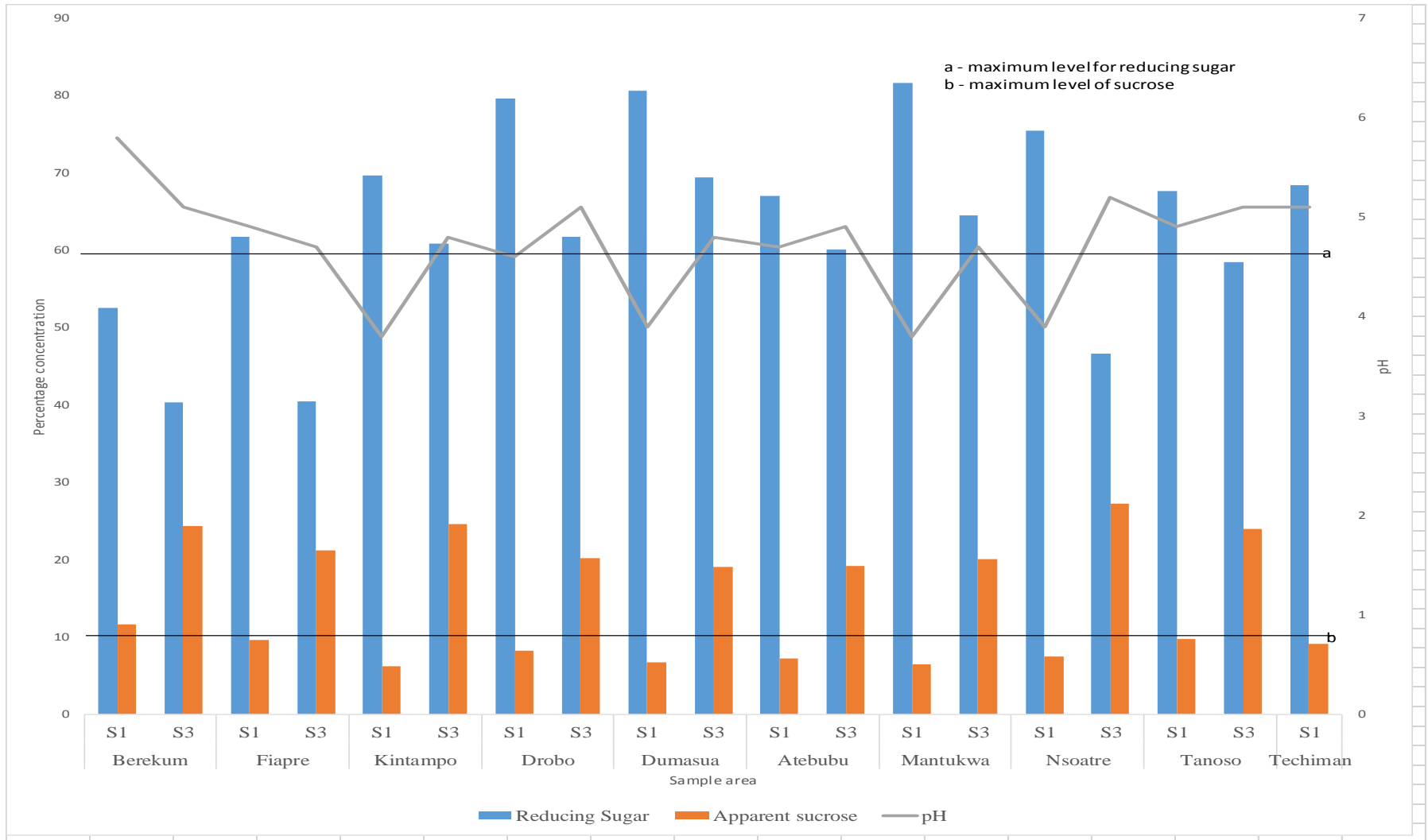


Fig.4.13: Pre-irradiation physicochemical parameters of samples from the Brong Ahafo Region

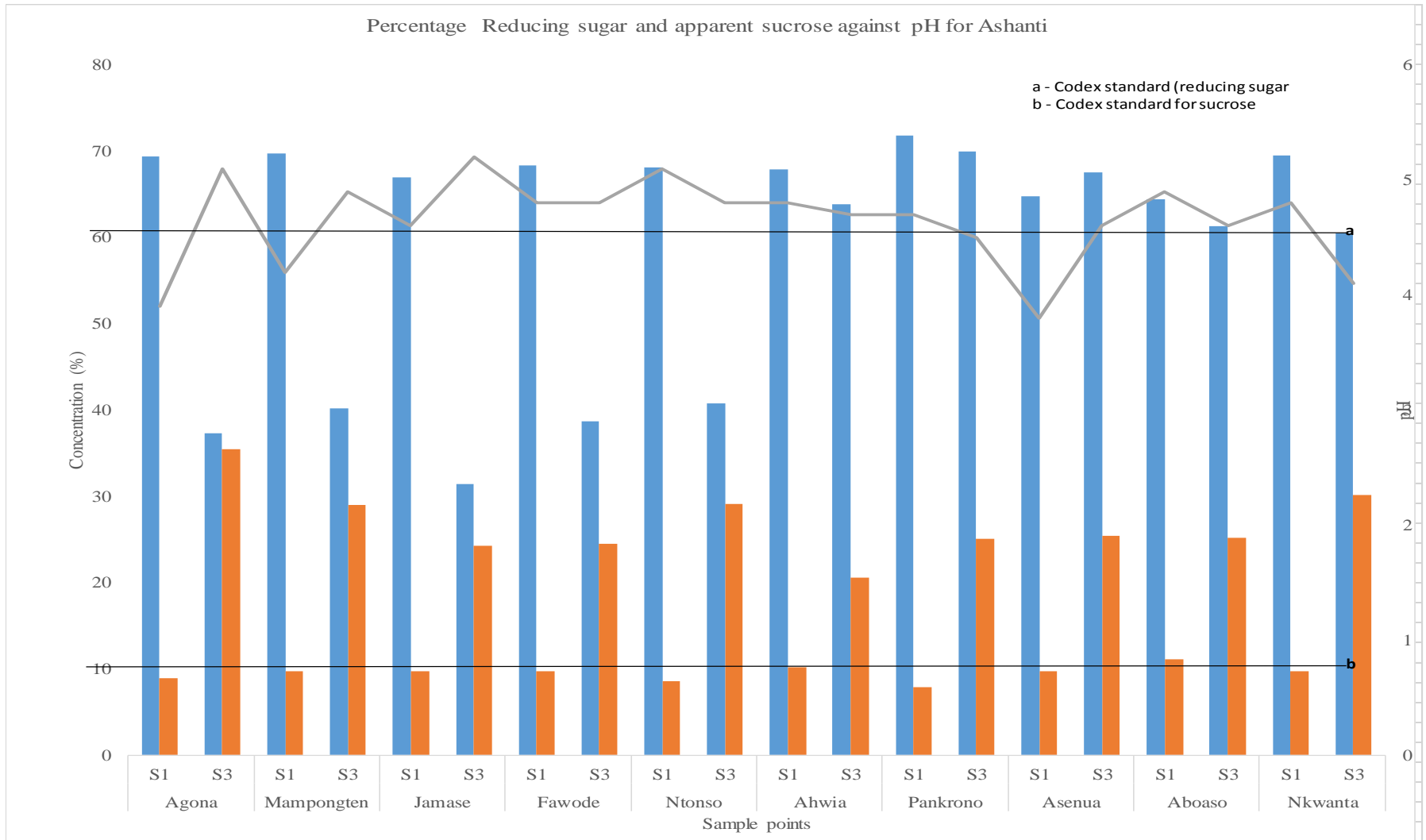


Fig. 4.14: Pre-irradiation physicochemical parameters of samples from the Ashanti Region

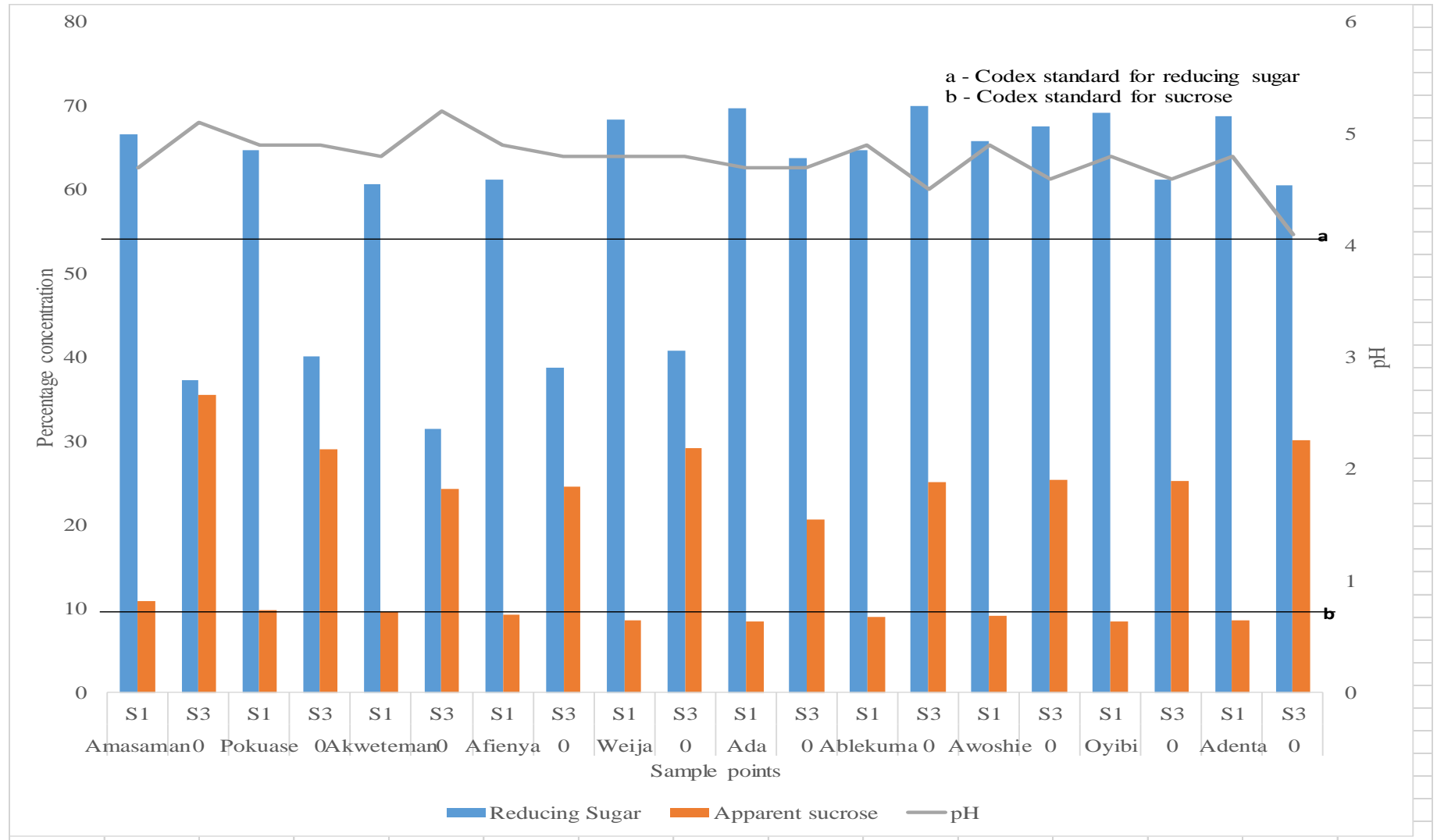


Fig.4.15: Pre-irradiation physicochemical parameters of samples from the Greater Accra Region.

The graphs (Fig.4.13, Fig.4.14, and Fig.4.15) show the concentration (in percentage) of reducing sugar and apparent sucrose in the honey sampled from the 30 sample points. The sucrose concentrations of the Honey Comb samples (S1) from all the sample points in the Brong Ahafo region were found to be within the Codex standards for honey ($\geq 60\%$ for Reducing Sugar concentration and $\leq 10\%$ for Apparent Sucrose concentration), except for the Berekum sample (12.24% for Apparent Sucrose and 54.16% for Reducing Sugar).

Apart from the sample from Aboaso in the Ashanti Region, all the Honey Comb samples (S1) from this region had apparent sucrose concentrations within the acceptable range. About 20% of the Honey Comb samples from the Greater Accra Region had sucrose concentrations slightly beyond the required maximum by the Codex standards ($\leq 10\%$). The Codex standard gives precedence to a type of honey (honeydew honey) with respect to the reducing sugar and apparent sucrose concentrations with the reducing sugar concentration ($\geq 53\%$) and an apparent sucrose ($\leq 15\%$) hence these samples could still be deemed wholesome.

All the Retail Samples (S3) from the thirty (30) sample locations had sucrose concentrations significantly above the average the maximum of 10%. The reducing sugar concentrations in the Retail Samples compared to the Honey Comb samples reduced for all the samples analyzed. The pH for all the samples were within the Codex standards of 3.8 ± 1 to 6.0 ± 1 .

Table 4.4: Physicochemical composition of samples from the B/A before gamma irradiation

Parameter	Type of Honey Sample			
	Honey Comb Sample		Retail Sample	
	Range	Mean \pm St. Dev.	Range	Mean \pm St. Dev.
TRS (%)	52.6 - 81.6	70 \pm 9	40.34 - 69.45	54 \pm 10
AS (%)	6.2 - 11.6	8 \pm 2	19.09 - 27.46	23 \pm 4
AC (%)	0.04 - 0.27	0.1 \pm 0.05	0.06 - 0.27	0.16 \pm 0.06
pH	3.8 - 5.6	5.0 \pm 0.6	3.8 - 5.3	5.0 \pm 0.2

TRS – Total Reducing Sugar; AS – Apparent Sucrose; AC – Ash Content

The Reducing Sugar (fructose and glucose) for honey, by Codex standards and the directive of the European Commission (EC Directive 2001/110), should be ≥ 60 g/100g of sample (i.e. 60%) whereas the Apparent Sucrose concentration should be ≤ 10 g/100g (10%) of the sample. The pH of honey should fall within 3.6 ± 0.1 and 6.1 ± 0.1 and the values for ash content by international standards (EC directive 2001; CODEX STAN 12-1981) should not exceed the range of (0.6 – 1.2)%.

Table 4.4 shows the values for the physicochemical parameters measured for honey sampled from the Brong Ahafo Region. The Reducing Sugar values recorded for the Honey Comb Samples were mostly within the required standard with a mean value of 70% while the mean value recorded for the Apparent Sucrose for these samples was 8 % both falling within required values for wholesome honey. For the Retail Samples (S3), mean value for ‘Total Reducing Sugar’ content of 54% falls below the expected standard according to Codex ($\geq 60\%$), and with reference the Apparent Sucrose, the mean

value of 23 % is significantly higher than the expected range of $\leq 10\%$. The ash content for the consumer samples were all within the required standard values.

Table 4.5: Pre-irradiation physicochemical parameters of honey sampled from Ashanti Region

Parameters	Type of Honey Samples			
	Honey comb samples (S1)		Retail Samples (S3)	
	Range	Mean \pm St. Dev.	Range	Mean \pm St. Dev.
TRS (%)	61.28 - 71.85	68 \pm 3	30.87 - 70.50	51.0 \pm 6
AS (%)	7.45 - 10.23	9.0 \pm 0.8	21.66 - 35.46	26.0 \pm 4
AC (%)	0.04 - 0.15	0.1 \pm 0.07	0.11 - 0.75	0.2 \pm 0.04
pH	3.9 - 5.1	5.0 \pm 0.3	4.4 - 5.6	4.9 \pm 0.3

TRS – Total Reducing Sugar; AP – Apparent Sucrose; AC – Ash Content

Table 4.6: Pre-irradiation physicochemical parameters of honey sampled from Greater Accra Region

Parameters	Type of Honey Sample			
	Honey comb samples (S1)		Consumer Samples (S3)	
	Range	Mean \pm St. Dev	Range	Mean \pm St. Dev
TRS (%)	60.71 - 69.68	66 \pm 3	31.57 - 66.96	43 \pm 12
AC (%)	8.42 - 10.88	9.0 \pm 0.7	21.02 - 41.49	32 \pm 6
AC (%)	0.12 - 0.24	0.2 \pm 0.09	0.45 - 0.78	0.7 \pm 0.02
Ph	4.7 - 4.9	5.0 \pm 0.07	4.9 - 5.6	5.0 \pm 0.2

TRS – Total Reducing Sugar; AP – Apparent Sucrose; AC – Ash Content

Table 4.5 and Table 4.6 are representations of the physicochemical qualities of honey sampled from the Ashanti and Greater Accra Regions respectively. The trend of results were similar to those obtained for honey sampled in the Brong Ahafo Region. The mean values of the Total Reducing sugars for the Honey Comb samples were 68% and 66% for Ashanti and Greater Accra samples, respectively. The Apparent sucrose concentrations were 9.0% and 9% respectively. These results satisfy the Codex standards for honey quality. For the Retail Samples, there was a reduction in the Reducing sugar concentration and a significant increase in the Apparent Sucrose concentrations for both regions as can be seen in the Table 4.5 and Table 4.6. The Ash content for the honeycomb samples were within the range 0.1 – 0.2 (%) which falls below the codex range of 2% - 4%.

4.3.2 Results from Microbiological Analysis after Irradiation

The effect of gamma-irradiation on the microbiological decontamination of the honey sampled from the sample points in the Brong Ahafo, Ashanti and Greater Accra region are shown in tables 4.7, 4.8, and 4.9

Table 4.7: Post-irradiation microbial analyses of honey from the Brong Ahafo Region

Irradiation dose (kGy)	Microbes (CFU/g)							
	Total viable count		<i>Coliform sp.</i>		<i>Clostridium sp.</i>		<i>Staphylococcus sp.</i>	
	S2	S3	S2	S3	S2	S3	S2	S3
0	16	183	5	112	2	23	3	13
20	ND	ND	ND	ND	ND	ND	ND	ND
30	ND	ND	ND	ND	ND	ND	ND	ND
40	ND	ND	ND	ND	ND	ND	ND	ND

ND – No detection (negative); S2 – Extracted/Sieved Sample; S3 – Retail Samples

Table 4.8: Post-irradiation microbial analyses of native honey from the Ashanti Region

Irradiation dose (kGy)	Microbes (CFU/g)							
	<u>Total viable count</u>		<u><i>Coliform sp.</i></u>		<u><i>Clostridium sp.</i></u>		<u><i>Staphylococcus sp.</i></u>	
	S1	S3	S1	S3	S1	S3	S1	S3
0	16	148	12	110	6	12	4	17
20	ND	ND	ND	ND	ND	ND	ND	ND
30	ND	ND	ND	ND	ND	ND	ND	ND
40	ND	ND	ND	ND	ND	ND	ND	ND

ND – No detection (negative)

Table 4.9: Post-irradiation microbial analyses of native honey from the Greater Accra Region

Irradiation dose (kGy)	Microbes (CFU/g)							
	<u>Total viable count</u>		<u>Coliform sp.</u>		<u>Clostridium sp.</u>		<u>Staphylococcus sp.</u>	
	<u>S1</u>	<u>S3</u>	<u>S1</u>	<u>S3</u>	<u>S1</u>	<u>S3</u>	<u>S1</u>	<u>S3</u>
0	22	271	8	183	5	63	4	72
20	ND	ND	ND	ND	ND	ND	ND	ND
30	ND	ND	ND	ND	ND	ND	ND	ND
40	ND	ND	ND	ND	ND	ND	ND	ND

ND – No detection (negative)

All the honey samples irradiated tested negative for microorganisms. The gamma radiation at a dose of 20 kGy, 30 kGy and 40 kGy were enough to decontaminate the honey samples.

4.3.3 Physicochemical results after gamma-irradiation

The table below is a representation of the physicochemical results of the irradiated samples.

Table 4.10: Post-irradiation physicochemical analyses

Dose (kGy)	Reducing Sugar			Apparent Sucrose			Ash Content			pH		
	BA	ASH	GAR	BA	ASH	GAR	BA	ASH	BAR	BA	ASH	GAR
Pre-irradiation	54.3	51.13	42.70	22.16	26.23	32.25	0.16	0.22	0.67	4.8	4.9	4.8
20	55.2	51.46	43.10	22.62	26.25	32.31	0.15	0.23	0.67	4.7	4.8	4.8
30	54.7	51.25	42.74	22.40	27.10	32.11	0.14	0.25	0.66	4.8	4.9	4.9
40	52.1	50.3	40.41	20.20	27.9	34.65	0.16	0.23	0.66	4.4	4.3	4.4

There were no significant changes to the Reducing Sugar, Apparent Sucrose, Ash and pH of the honey samples after irradiation with gamma energy from a Cobalt – 60 source.

4.4 RESULTS COMPARED TO STANDARDS

The results of the microbiological analyses were compared with the results of similar research undertaken by Finola *et al.* 2005 in Argentina; and Sereia *et al.*, 2013 in Brazil. These researchers reported that microbial contaminants in honey were within “MERCOSUR stipulated values” of $10^1 - 10^2$ CFU/g of honey.

Going by this standard, the mean viable counts in microbes detected in the honey comb samples (S1) from all the sample points were in the range 30 – 35 CFU/g which falls within the MERCOSUR stipulated value. The mean count for *Faecal* sp. in all the honey comb samples were below 10 CFU/g. The count for sulfite-reducing *Clostridium* sp. and *Staphylococcus* sp. were all below 10 CFU/g. About 40% (12 out of 30) of the honey comb samples from all the three (3) regions sampled were contaminated with sulfite-reducing *Clostridia* sp. whereas approximately 43% (13 out of 30) of these honey comb samples were contaminated with *Staphylococcus* sp. Most of these contaminations were due to the locations of the beehives and also due to lack of proper maintenance of the hives.



Fig. 4.16: Beehive in unfavorable condition at a sample point in the Ashanti Region

However, from the microbiological point of view, the relatively low microbial count in the honey comb samples is an indicative of the proper management of the beehive by a majority of the honeybee keepers.



Fig. 4.17: Beehive in a 'healthy' condition at a sample point in the Ashanti Region

The microbial count values in samples harvested and treated by the farmers (S2) were relatively higher for all the thirty (30) samples analyzed, compared to the honey comb samples. About 90% (27 out of 30) of these samples were contaminated with *Faecal* sp. among which approximately 18% of the samples were from the Ashanti Region (the highest among the three regions) for *Faecal* sp. Sulfite-reducing clostridium were detected in all the contaminated samples as well as *Staphylococcus* sp. Soil is the main source of *Clostridium* sp., although dust, equipment, buildings and the environment could also contain this genus of microbe. The presence of these microorganism indicates contamination or pollution. To decrease the chances of making honey impure, the chain of manufacturing and maturity at harvest should be monitored.

Most of the beekeepers used their bare hands to remove the comb from the beehive, very little attention is given to the possibility of contamination during harvesting. The combs are kept in plastic containers, sometimes in metallic containers and left overnight to drain. In the day, the containers with the honey are brought out into the sun so as to heat-drain exposing the honey to dust and other particles.

Microorganisms were detected in all the thirty (30) honey sampled from the retailers. The mean count of *Faecal* sp. in the samples were 111 CFU/g, 124 CFU/g and 183 CFU/g for Brong Ahafo, Ashanti and Greater Accra Regions respectively. These count values were higher than the maximum values as stipulated by the MERCOSUR regulations which is 10^2 CFU/g. Samples from the Greater Accra region had the highest recorded values for microbial counts. The mean values for *Clostridium* sp. and *Staphylococcus* sp. were 53 CFU/g and 72 CFU/g. Comparing the areas sampled in the Greater Accra Region to the areas sampled in the other two regions, the Greater Accra Region has the highest population density hence human activities is higher in this region than the other regions. This is a major factor to the contribution of contaminants into any food sample, honey not an exception. All the samples were negative for *Salmonella* sp.

Mean values for *Clostridium* sp. and *Staphylococcus* sp. for the Ashanti Region were 25 and 19 CFU/g respectively whereas the mean values of *Clostridium* sp. and *Staphylococcus* sp. detected in samples from the Brong Ahafo Region were 24 and 13 CFU/g respectively. Honey sampled from the Brong Ahafo Region can be said to be the most wholesome for human consumption.

Honey from the honey combs sampled from all the regions had reducing sugar values within the required concentrations as in the standards of the International Honey Commission ($\geq 60\%$) and also the CODEX Alimentarius Commission.

About 70% of the sugars in the honey combs samples from the Brong Ahafo Region were reducing sugars and also contained the required concentrations of sucrose (average; 8%) according to the Codex standards.

The results showed relatively high concentrations of sucrose in the honey sampled from the retailers. The mean reducing sugar concentration for the honey comb samples from the Ashanti Region was 68% also within the accepted concentration range, and an average of 9% for sucrose.

Honey sampled from the retailers in the Greater Accra Region had the highest mean concentration of sucrose (34%) compared to 26% and 22% in the samples from the Ashanti and Brong Ahafo regions, respectively. Relative to the Codex, EC directive and the standards from the International Honey Commission, these results were above the required concentration of sucrose in natural honey. The great disparity in apparent sucrose concentration in the honey comb samples (S1) relative to the retail samples (S3) is an indication of adulteration of honey with white sugar. This inference is with respect to the percentage increase, for the samples from the Brong Ahafo Region,

The ash content helps to interpret the origin of honey and also indicates the foraging area of the honeybees. Ash content standards are set at a minimum of 0.6 g/100 g of sample and a maximum of 1.2 g/100 g (i.e. 0.6% - 1.2%) of honey by the Codex Alimentarius commission, the IHC, and the EC directive among other standards all of which follow a

similar quality assurance criteria. The ash content of all the honey sampled and analyzed were within the required standard with an average of 0.16% in the honey comb samples and average of 0.62% for the retail samples.

The results of this research showed that the honey sampled from all the sample areas were found to have an acidic character. The pH values ranged from 3.8 to 5.6 in samples from the Brong Ahafo Region (Table 4.7), 3.9 to 5.6 in samples from the Ashanti Region (Table 4.8) and from 4.7 to 5.6 in the samples from the Greater Accra Region. These results conform to the standard values. The results also showed that the pH in the retail samples were higher than the pH values in the honey comb samples. This could have contributed to the less detection values of microorganisms in the honey comb samples. The pH, meet the values reported by Bera *et al.* (2009), with pH values in the range 3.8 to 4.2, and the Codex standard range of 3.8 ± 1 to 6.0 ± 1 .

Gamma irradiation at a temperature of 25°C at doses ranging from 20 kGy to 30 kGy had no significant effect on the physical and chemical qualities of the honey. The mean reducing sugar concentration reduced insignificantly after irradiating at 40 kGy relative to the average concentration before irradiation. The change in apparent sucrose concentration, on average, was insignificant as the Figures in Table 4.13 show, whereas there was a slight reduction in pH after irradiation at 40 kGy.

CHAPTER FIVE (5)

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The principal objective of this work was to investigate ways of improving upon the quality of honey produced in Ghana. To achieve this, ninety (90) honey samples taken from three regions were analyzed for total aerobic bacteria, namely *Faecal* sp., *Clostridium* sp., *Staphylococcus* sp. and *Salmonella* sp. The samples were also analysed for some physicochemical parameters including Ash content, Reducing Sugar content, Apparent Sucrose content and pH.

The 90 honey samples were negative for *Salmonella* sp. after incubation at 37 °C for 48 hours in required media. About 80% of honey sampled directly from the honey comb (S1) was free from total aerobic bacteria whereas about 96.7% of honey sampled after the farmer had harvested and extracted the honey from the comb (S2) was contaminated with total aerobic bacteria. All of the honey sampled from the retailers were contaminated with Total Aerobic and Anaerobic bacteria, *Faecal* sp., *Clostridium* sp. and *Staphylococcus* sp. Spore forming *Clostridium* sp. were in the range (7 – 53 CFU/g) and *Staphylococcus* sp. were in the range (7 – 72 CFU/g). These values are within the required range of (10 – 100 CFU/g), as stipulated by MERCOSUR, for all the samples. *Faecal* sp. values were in the range of 110 to 183 CFU/g which was significantly higher than the required range of 10^1 – 10^2 CFU/g for most of the honey sampled from the retailers.

The notable observations in this work was that honey gets contaminated as it leaves the honey comb, through processing and packaging before it gets to the consumer. The

contamination was mainly by secondary sources, the method of harvesting and extraction as well as from the comb, the materials used in extraction and the containers used in storing the honey.

The apparent sucrose concentrations in honey sampled directly from the honey comb were within the maximum allowed in wholesome honey according to the Codex standards (i.e. $\leq 10\%$). However, honey sampled from the retailers had apparent sucrose concentrations significantly higher than the required maximum. The values for apparent reducing sugar for the retail samples were within the range of 20% - 34%. The honey on sale in the areas where the research was conducted had significantly higher amounts of sucrose.

Honey from the honey comb is wholesome for human consumption, however, the processes through which it is harvested and extracted, and the tools used in harvesting and preparing the honey for the market introduces contaminants into it.

Gamma radiation dose of 20 kGy from a ^{60}Co source was enough to achieve a high efficiency in sterilizing honey and making it wholesome for public consumption. The reducing sugar concentration, sucrose concentration and pH were not ominously affected by gamma energy at 20 kGy hence at this dose rate, the honey quality was preserved.

5.2 Recommendations

Based on the results obtained through this research, the following recommendations have been proposed:

- (i) A comprehensive microbiological quality of Ghanaian honey should be conducted to cover all the ten (10) regions of Ghana.

- (ii) Extensive research into the other quality determinants which were not covered in this study (e.g. pesticides, toxicology, geographical origin, among others), should be considered in future.
- (iii) Honey meant for human consumption must undergo gamma radiation.
- (iv) The Ministry of Food and Agriculture should enhance the education on apiculture technology.
- (v) A National Honey Commission should be established. This Commission should have an oversight responsibility for standard/ wholesome honey production in Ghana.

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APPENDICES**APPENDIX 1****SAMPLE QUESTIONNAIRES****PERSONAL INFORMATION****GENDER:** Male Female **AGE RANGE:** 10– 20 years 21 – 30 years 31 – 40years 41 – 50
years 51 – 60 years 61– 70 years 71– 80 years 81– 90 years **LEVEL OF EDUCATION:** Basic Secondary Tertiary Post
Graduate None **OCCUPATION:.....REGION.....DISTRICT.....**

Commercial bee keeping..... Bee hunting.....

1) How long have you been in the honey business?

- (a) 0-5 years
- (b) 6-10 years
- (c) 11-20 years
- (d) 21-30 years
- (e) Above 30 years

2) How many colonies do you have? (where applicable)

- 3) Which people do you normally sell your products to?
- (a) Individuals
 - (b) Traders
 - (c) Both
 - (d) Others, e.g.
- 4) What is the location of your honey farm (Apiaries)?
- (a) Close to an industry
 - (b) Close to a tarred road/ filling station
 - (c) Few meters away from human settlement
 - (d) Other
- 5) What equipment do you usually use during honey extraction
- (a) Wood
 - (b) Metal
 - (c) Both (wood and metal)
 - (d) others
- 6) What kind of safety equipment do you use/Measures taken to reduce contamination
- (a) Gloves
 - (b) Goggles
 - (c) Head cover
 - (d) Others

- 7) Containers for keeping the products
- (a) New plastic containers
 - (b) Used plastic containers
 - (c) Metal containers
- 8) Treatment of Containers before honey storage
- (a) Rinsing
 - (b) Thorough washing with soap or detergents
 - (c) No washing or rinsing
 - (d) others
- 9) What is the color of your honey?
- (a) Brown
 - (b) Colourless
 - (c) Dark brown
 - (d) Other
- 10) Honey's consistency?
- (a) Fluid
 - (b) Viscous
 - (c) Partly to entirely crystallized

11) Aroma or flavor of the honey?

- (a) Sweet scented
- (b) Foul smell
- (c) Fermented
- (d) Other

12) What treatment method do you use after extraction?

- (a) Filtering
- (b) Removal of contaminants
- (c) Heating
- (d) Sieving
- (e) Other

13) Do you add any additives before selling them?

- (a) Yes
- (b) No

14) If yes what is the additive?

15) (If yes), why do you add them?

- (a) To improve the flavor
- (b) To change the colour
- (c) To increase the volume
- (d) To change honey's consistency
- (e) Others

16) Do you have particular days and times that you usually do the extraction?

(a) Yes

(b) No

17) (If yes), why?.....

18) How are the bees deployed for pollination?

(a) On a small scale

(b) On commercial basis.

(c) Others

19) What food do you normally feed the bee colonies on?

(a) Protein supplements

(b) Pollen supplements

(c) Others

20) What methods are employed for feeding such foods to the bee colonies?

(a) You collect pollen from flowers and bring back to the hive

(b) From nitrogenous food-stuffs provided by the beekeeper

(c) Others

21) How do you control Pest and disease, and the chemical employed?

- (a) Use of oxytetracycline and fumagillin as aids in the control and prevention of bee diseases
- (b) Diagnosis and treatment of disease in the laboratory
- (c) Others

22) Bees' foraging activities and the size of the area normally covered.

- (a) On a stand of honeysuckle in the farm
- (b) On brightly colored flowers in the farm
- (c) Other

23) Which place do you normally sell the honey?

- (a) Hawking
- (b) Market
- (c) Home
- (d) Roadside
- (e) Schools
- (f) Other

APPENDIX 2
PHYSICOCHEMICAL PARAMETERS OF HONEY SAMPLED IN THE
GREATER ACCRA REGION

SAMPLE AREA		REDUCING SUGAR		APPARENT SUCROSE		ASH		pH	
		^a 1	^b 2	1	2	1	2	1	2
Amasaman	S1	67.60	65.56	10.85	10.91	0.11	0.13	4.7	4.7
	S3	66.74	64.46	40.88	42.10	0.47	0.43	4.8	4.8
Pokuase	S1	65.69	63.69	9.9	9.8	0.14	0.14	4.8	5.0
	S3	67.69	66.23	35.42	35.52	0.79	0.76	4.8	5.0
Akweteman	S1	61.72	59.70	9.5	9.9	0.20	0.16	4.9	4.9
	S3	36.89	38.85	34.30	36.20	0.70	0.64	5.4	5.4
Afienea	S1	62.30	60.26	9.4	9.2	0.25	0.23	4.8	5.0
	S3	38.17	36.15	21.15	20.89	0.68	0.60	5.6	5.6
Weija	S1	69.38	67.36	8.50	8.56	0.11	0.13	4.8	4.8
	S3	37.11	36.79	33.65	31.61	0.61	0.61	5.0	5.2
Ada	S1	69.91	69.45	8.40	8.44	0.20	0.20	4.6	4.8
	S3	41.75	39.55	26.9	26.84	0.78	0.72	5.1	4.9
Ablekuma	S1	65.71	63.73	9.03	9.09	0.26	0.20	4.9	4.9
	S3	33.08	39.68	31.95	31.93	0.70	0.67	5.4	5.6
Awoshie	S1	66.61	65.03	9.20	8.9	0.19	0.16	4.9	4.8
	S3	31.55	31.59	36.50	36.56	0.73	0.77	5.2	5.2
Oyibi	S1	69.18	69.12	8.46	8.46	0.17	0.15	4.8	4.8
	S3	33.98	33.40	34.65	34.30	0.76	0.78	5.1	5.1
Adenta	S1	68.71	68.75	8.61	8.64	0.20	0.18	4.8	4.8
	S3	41.20	39.04	26.74	26.40	0.50	0.56	5.0	5.0

KEY:

- ✓ All values were in percentage except for pH values
- ✓ ^a – value after first analyses
- ✓ ^b – value after second analyses

APPENDIX 3
PHYSICO CHEMICAL PARAMETERS OF HONEY SAMPLED IN THE ASHANTI REGION

SAMPLE AREA		REDUCING SUGAR		APPARENT SUCROSE		ASH		pH	
		^a 1	^b 2	1	2	1	2	1	2
Agona	S1	68.93	67.79	9.61	9.69	0.40	0.20	3.7	4.1
	S3	31.98	29.76	36.51	9.61	0.76	0.74	5.1	5.1
Mampongten	S1	68.65	41.25	9.12	9.24	0.05	0.03	4.1	4.3
	S3	70.85	39.05	28.19	26.93	0.06	0.08	4.9	4.9
Jamase	S1	67.95	65.97	9.12	9.36	0.09	0.07	4.4	4.8
	S3	32.41	30.39	23.45	22.71	0.15	0.11	5.2	5.2
Fawode	S1	68.45	68.35	9.12	9.26	0.13	0.14	4.8	4.8
	S3	39.74	37.72	24.12	22.48	0.29	0.31	4.7	4.9
Ntonso	S1	68.15	68.11	7.67	8.59	0.06	0.12	5.0	5.2
	S3	40.61	40.89	30.41	30.93	0.25	0.35	5.0	5.1
Ahwiaa	S1	68.19	67.59	9.85	9.51	0.07	0.09	4.6	4.9
	S3	63.74	63.89	10.56	9.82	0.15	0.10	4.6	4.7
Pankrono	S1	72.87	70.83	7.89	7.79	0.06	0.04	4.7	4.7
	S3	66.97	72.92	7.49	7.49	0.17	0.13	4.4	4.6
Asenua	S1	61.98	67.5	9.71	8.71	0.06	0.06	3.9	3.7
	S3	66.95	68.23	25.23	23.03	0.10	0.12	4.6	4.5
Aboaso	S1	63.28	59.18	10.36	10.9	0.07	0.09	4.9	4.9
	S3	65.48	63.56	25.23	23.19	0.13	0.11	4.5	4.7
Nkwanta	S1	68.45	70.49	9.10	9.30	0.10	0.10	3.9	3.8
	S3	71.6	69.40	28.7	28.5	0.14	0.16	4.7	4.9

KEY:

- ✓ All values were in percentage except for pH values
- ✓ ^a – value after first analyses
- ✓ ^b – value after second analyses

APPENDIX 4
PHYSICO CHEMICAL PARAMETERS OF HONEY SAMPLED IN THE
BRONG AHAFO REGION

SAMPLE AREA		REDUCING SUGAR		APPARENT SUCROSE		ASH		pH	
		^a 1	^b 2	1	2	1	2	1	2
Berekum	S1	51.06	54.12	11.02	12.24	0.19	0.13	5.7	5.9
	S3	41.25	39.43	23.17	25.41	0.11	0.25	5.2	5.0
Fiapre	S1	61.29	62.29	9.41	9.63	0.11	0.07	4.9	4.8
	S3	41.37	39.63	19.21	21.21	0.08	0.12	5.3	5.0
Dumasua	S1	81.56	79.72	6.71	5.65	0.14	0.10	3.9	3.7
	S3	68.37	70.53	23.48	25.26	0.18	0.16	4.5	4.8
Kintampo	S1	70.65	68.79	8.51	7.95	0.09	0.07	4.8	4.4
	S3	61.76	59.94	21.02	19.34	0.06	0.10	5.4	4.8
Drobo	S1	81.75	77.61	6.63	6.79	0.07	0.05	4.1	3.9
	S3	62.76	60.78	19.91	18.13	0.14	0.18	5.1	5.0
Atebubu	S1	67.85	66.01	6.62	6.82	0.03	0.05	4.6	4.8
	S3	59.15	61.15	18.14	20.02	0.07	0.05	4.8	5.0
Mantukwa	S1	79.15	84.05	6.43	6.43	0.11	0.13	3.9	3.7
	S3	65.12	63.92	20.13	19.89	0.17	0.19	4.6	4.8
Nsoatre	S1	74.15	76.75	7.43	7.47	0.12	0.28	3.8	4.0
	S3	46.15	47.16	28.12	26.24	0.29	0.25	5.1	5.3
Tanoso	S1	68.52	66.92	9.62	9.76	0.04	0.07	4.9	4.8
	S3	58.41	58.39	24.81	23.01	0.12	0.08	5.2	5.0
Techiman	S1	67.39	69.41	9.01	9.05	0.08	0.01	4.1	4.3
	S3	39.33	41.35	28.16	26.70	0.15	0.17	4.7	4.9s

KEY:

- ✓ All values were in percentage except for pH values
- ✓ ^a – value after first analyses
- ✓ ^b – value after second analyses

APPENDIX 5
MIRCROBIOLOGICAL ANALYSIS - PRE-IRRADIATION RESULTS

This is a representation of a summary of the data obtained from the plate count (CFU/g)

BRONG AHAFO REGION

SAMPLE SITE	MICROBES	INCUBATION PERIOD	S1	S2	S3
Berekum	Total viable count	48hrs	175	191	*
	<i>Faecal sp.</i>		98	103	*
	<i>Clostridium sp.</i>		23	45	157
	<i>Staphylococcus sp.</i>		31	22	107
	<i>Salmonella sp.</i>		0	0	0
Fiapre	Total viable count	48hrs	0	20	196
	Faecal		0	7	83
	Clostridium		0	5	47
	Staphylococcus sp.		0	6	33
	Salmonella		Negative	Negative	Negative
Kintampo	Total viable count	48hrs	6	13	23
	Faecal sp.		3	2	9
	Clostridium sp.		1	1	5
	Staphylococcus sp.		2	1	7
	Salmonella sp.		Negative	Negative	Negative
Drobo	Total viable count	48hrs	0	15	20
	Faecal sp.		0	2	8
	Clostridium sp.		0	0	6
	Staphylococcus sp.		0	0	5
	Salmonella		Negative	Negative	Negative
Dumasua	Total viable count	48hrs	0	17	21
	Faecal sp.		0	3	7
	Clostridium sp.		0	1	4
	Staphylococcus sp.		0	0	1
	Salmonella sp.		Negative	Negative	Negative
Atebubu	Total viable count	48hrs	5	16	30
	Faecal sp.		1	9	11
	Clostridium sp.		0	1	3
	Staphylococcus sp.		0	0	1
	Salmonella sp.		Negative	Negative	Negative
Mantukwa	Total viable count	48hrs	7	17	43
	Faecal sp.		3	4	12
	Staphylococcus sp.		1	2	6
	Clostridium sp.		2	3	6
	Salmonella sp.		Negative	Negative	Negative
Nsoatre	Total viable count	48hrs	0	0	23
	Faecal sp.		0	0	11
	Clostridium sp.		0	0	4
	Staphylococcus sp.		0	0	1
	Salmonella sp.		Negative	Negative	Negative
Tanoso	Total viable count	48hrs	0	11	37
	Faecal sp.		0	3	15
	Clostridium sp.		0	1	9
	Staphylococcus sp.		0	1	5
	Salmonella sp.		Negative	Negative	Negative
Techiman	Total viable count	48hrs	12	25	69
	Faecal sp.		5	7	39
	Clostridium sp.		4	7	16
	Staphylococcus sp.		4	10	9
	Salmonella sp.		Negative	Negative	Negative

APPENDIX 6 - ASHANTI REGION

SAMPLE SITE	MICROBES	INCUBATION PERIOD	S1	S2	S3
Agona	Total viable count	48hrs	0	69	173
	Faecal sp.		0	39	151
	Clostridium sp.		0	11	48
	Bacillus sp.		0	9	62
	Salmonella sp.		Negative	Negative	Negative
Mampongten	Total viable count	48hrs	11	155	*
	Faecal sp.		6	102	*
	Clostridium sp.		2	36	54
	Staphylococcus sp.		3	21	66
	Salmonella sp.		Negative	Negative	Negative
Jamase	Total viable count	48hrs	13	61	121
	Faecal sp.		5	33	65
	Clostridium sp.		2	14	22
	Staphylococcus sp.		3	11	39
	Salmonella sp.		Negative	Negative	Negative
Fawode	Total viable count	48hrs	0	7	8
	Faecal sp.		0	4	4
	Clostridium sp.		0	2	3
	Staphylococcus sp.		0	2	2
	Salmonella sp.		Negative	Negative	Negative
Ntonso	Total viable count	48hrs	1	7	23
	Faecal sp.		0	4	6
	Clostridium sp.		0	1	3
	Staphylococcus sp.		0	1	4
	Salmonella sp.		Negative	Negative	Negative
Ahwiaa	Total viable count	48hrs	5	15	22
	Faecal sp.		1	3	9
	Clostridium sp.		0	1	2
	Staphylococcus sp.		0	4	3
	Salmonella sp.		Negative	Negative	Negative
Pankrono	Total viable count	48hrs	0	6	21
	Faecal sp.		0	0	4
	Staphylococcus sp.		0	0	1
	Clostridium sp.		0	0	3
	Salmonella sp.		Negative	Negative	Negative
Asenua	Total viable count	48hrs	0	7	25
	Faecal sp.		0	2	6
	Clostridium sp.		0	1	1
	Staphylococcus sp.		0	1	4
	Salmonella sp.		Negative	Negative	Negative
Aboaso	Total viable count	48hrs	5	16	34
	Faecal sp.		1	4	12
	Clostridium sp.		0	1	5
	Staphylococcus sp.		1	3	9
	Salmonella sp.		Negative	Negative	Negative
Nkwanta	Total viable count	48hrs	11	29	78
	Faecal sp.		3	11	35
	Clostridium sp.		1	6	11
	Staphylococcus sp.		3	8	23
	Salmonella sp.		Negative	Negative	Negative

APPENDIX 7
GREATER ACCRA REGION

SAMPLE SITE	MICROBES	INCUBATION PERIOD	S1	S2	S3
Amasaman	Total viable count	48hrs	4	12	*
	Faecal sp.		2	5	446
	Clostridium sp.		1	4	61
	Staphylococcus sp.		1	4	53
	Salmonella sp.		Negative	Negative	Negative
Pokuase	Total viable count	48hrs	3	13	84
	Faecal sp.		0	7	13
	Clostridium		1	3	9
	Staphylococcus sp.		1	2	7
	Salmonella sp.		Negative	Negative	Negative
Akweteman	Total viable count	48HRS	14	31	67
	Faecal sp.		7	11	21
	Clostridium sp.		4	6	12
	Staphylococcus sp.		3	5	9
	Salmonella sp.		Negative	Negative	Negative
Afiencya	Total viable count	48hrs	13	46	121
	Faecal sp.		5	15	36
	Clostridium sp.		4	7	21
	Staphylococcus sp.		4	6	15
	Salmonella sp.		Negative	0	0
Weija	Total viable count	48hrs	0	22	33
	Faecal sp.		0	6	11
	Clostridium sp.		0	5	8
	Staphylococcus sp.		0	5	8
	Salmonella sp.		Negative	Negative	Negative
Ada	Total viable count	48hrs	0	12	20
	Faecal sp.		0	2	9
	Clostridium sp.		0	6	4
	Staphylococcus sp.		0	1	3
	Salmonella sp.		Negative	Negative	Negative
Ablekuma	Total viable count	48hrs	12	25	*
	Faecal sp.		3	12	146
	Clostridium sp.		3	6	73
	Staphylococcus sp.		1	4	56
	Salmonella sp.		Negative	Negative	Negative
Awoshie	Total viable count	48hrs	0	27	77
	Faecal sp.		0	13	35
	Clostridium sp.		0	7	21
	Staphylococcus sp.		0	4	14
	Salmonella sp.		Negative	Negative	Negative
Oyibi	Total viable count	48hrs	0	18	*
	Faecal sp.		0	7	123
	Clostridium sp.		0	6	56
	Staphylococcus sp.		0	4	38
	Salmonella sp.		Negative	Negative	Negative
Adenta	Total viable count	48hrs	0	18	38
	Faecal sp.		0	9	15
	Clostridium sp.		0	5	9
	Staphylococcus sp.		0	2	4
	Salmonella sp.		Negative	Negative	Negative

APPENDIX 8
TABLE OF RESULTS FOR QUESTIONNAIRE

How old are you?		
	Frequency	Percent
10-20years	1	3.3
21-30years	5	16.7
31-40years	1	3.3
41-50years	7	23.3
51-60years	7	23.3
51-60years	7	23.3
61-70years	8	26.7
81-90years	1	3.3
Total	30	100.0

How long have you been in the Honey business?

	Frequency	Percent
(0-5 years	4	13.3
6-10 years	9	30.0
11-20 years	11	36.7
21-30 years	3	10.0
More than 30 years	3	10.0
Total	30	100.0

Equipment used in honey's extraction

	Frequency	Percent
Wood	13	43.3
Metal	3	10.0
Both (wood and metal)	14	46.7
Total	30	100.0

Measures taken to reduce contamination

	Frequency	Percent
Gloves only	8	26.7
Head cover only	3	10.0
Head cover and gloves	17	56.7
Other	2	6.7
Total	30	100.0

Containers for keeping or storing honey

	Frequency	Percent
New Plastic containers	6	20.0
Used plastic containers	13	43.3
Metal containers	6	20.0
Glass bottles	5	16.7
Total	30	100.0

Treatment of containers prior to storage

	Frequency	Percent
Rinsing	19	63.3
Thorough washing with soap or detergents	7	23.3
No washing or rinsing	4	13.3
Total	30	100.0

Treatment method do used after extraction?

	Frequency	Percent
Filtering	21	70.0
Heating	7	23.3
Other	2	6.7

Additives?

	Frequency	Percent
Yes	4	13.3
No	26	86.7
Total	30	100.0

Why add them?

	Frequency	Percent
To increase volume	2	6.7
To change honey's consistency	2	6.7
Total	4	13.3
System(missing)	26	86.7
Total	30	100.0

What additive?

	Frequency	Percent
water	4	13.3
none	26	86.7
Total	30	100.0

Selling Place?

	Frequency	Percent
hawking	8	26.7
market	7	23.3
home	4	13.3
roadside	7	23.3
schools	4	13.3
Total	30	100.0

Apiary's location?

	Frequency	Percent
Close to an industry	2	6.7
Close to a tarred road/ filling stations	8	26.7
Few meters away from human settlement	16	53.3
others	4	13.3
Total	30	100.0