UNIVERSITY FOR DEVELOPMENT STUDIES

DIETARY EXPOSURE TO GLYPHOSATE FROM HONEY IN WA, GHANA

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2020

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(UDS/MEM/0086/15)

THESIS SUBMITTED TO THE DEPARTMENT OF ENVIRONMENT AND RESOURCE STUDIES, FACULTY OF INTEGRATED DEVELOPMENT STUDIES, UNIVERSITY FOR DEVELOPMENT STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN ENVIRONMENT AND RESOURCE MANAGEMENT

MARCH, 2020

DECLARATION

Candidate's declaration

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this University or elsewhere:

Candidate's Signature: ………………………………….. Date: ……………...

Name: Cornelius K.A. Pienaah

Supervisor's declaration

We hereby declare that the preparation and presentation of this thesis was supervised in accordance with the guidelines on supervision of thesis laid down by the University for Development Studies.

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Co- Supervisor's Signature: ……………………………….Date: ………………...

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ABSTRACT

Glyphosate is widely used on crop lands by farmers to meet productivity in Wa, Ghana. It is considered a probable carcinogen by the World Health Organization's International Agency for Research on Cancer therefore raising concerns about its presence in food products. It is applied on farms and has the potential to contaminate with flower nectar of plants foraged by bees to form honey. The study therefore sought to analyze the level of Glyphosate in honey to characterize the health risk associated with the consumption of honey through a dietary exposure analysis. Some physicochemical qualities of honey were also determined. Ten samples of honey were purchased from Wa market for analysis. All laboratory tests were conducted at the Ghana Standards Authority. QuEChERS method for analysis of pesticide residues in low -fat matrix was used in the extraction procedure. Varian CP-3800 Gas Chromatograph with a CombiPAL Autosampler, equipped with pulse flame photometric detector by LC-MS/MS was used. All samples tested have levels below the LOD and LOQ. It is concluded that the honey samples contained Glyphosate at very low levels which may pose threat to human health. For other physicochemical tested, only one sample exceeded the standards set by Codex Alimentarius Commission. Despite the low limits of Glyphosate in the honey, there is still the need for Ghana Standard Authority to prevent potential exposure to it in honey due to the increasing using of agrochemicals by educating honey producers on the need to certify honey before sale, educating the public on the need to purchase certified honey and ensuring that honey is certified before consumption and sale.

ACKNOWLEDGEMENTS

My outmost gratitude goes to the Almighty God for His grace, strength and wisdom in the completion of this research work. I will like to extend my profound gratitude to my two supervisors; Dr. Dzigbodi Adzo Doke and Dr. Paul Armah Aryee for their guidance and constructive criticism throughout the process of conducting this research. My unreserved thanks and appreciation goes to Dr. Raymond Aabeyir who internally supervised this research work.

Mention to Amanda, Ignatius, Mary and all members of my family, I sincerely thank each and every one of you from the bottom of my heart for continuous love and support for making me the person I am today.

All I say is God bless you all.

DEDICATION

This research is dedicated to my beloved parents Mr. Douglas D. Pienaah and Mrs. Comfort Badii Pienaah, my dear wife Amanda Taabazuing and son Delson Pienaah as well as my entire family especially Shaddrack Pienaah for their support and love in all my life journey.

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LIST OF ACRONYMS

ADI	Acceptable Daily Intake
AMP	Aminomethyl-phosphonic acid
CF	Conversion Factor
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
EU	European Union
g	Grams
G.C	Gas Chromatography
EU	European Union
FAO	Food and Agriculture Organization
GlyBH	Glyphosate based herbicides
GSA	Ghana Standards Authority
GSS	Ghana Statistical Service
IUPAC	International Union of Pure and Applied Chemistry
Km	Kilometers
ml	Milliters
µg	Micrograms
MRL	Maximum Residue Limits
MS	Mass spectrometer
MOFA	Ministry of Food and Agriculture
NOAEL	No Observed Adverse Effect Level

Ppm	Parts per million
ppb	parts per billion
SPE	Solid Phase Extraction
UWR	Upper West Region
W.H.O	World Health Organization
QuEChERS	"Quick, Easy, Cheap, Effective, Rugged, and Safe".
EU	European Union
USA	United States of America

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Glyphosate (N-(phosphonomethyl) glycine) is a broad-spectrum, post-emergent, non-selective, systemic herbicide, which effectively kills or suppresses all plant types, including grasses, perennials, vines, shrubs, and trees (Dill et al., 2010). Glyphosate-based herbicides (GlyBH) are the most widely used pesticides worldwide (Coupe et al., 2011, European Commission, 2007; US EPA, 2012). It is used worldwide in various applications for weed and vegetation control in the past 40 years (Van Bruggen et al. 2018) including its introduction on the market (Sebiomo et al., 2011). Globally, more than 746 million kg of Glyphosate was used in 2014, roughly 17 times the amount used in 1994 (Benbrook, 2016). The increase in Glyphosate application is also partly due to the increasing development and cultivation of Glyphosate-tolerant crops (Rose et al., 2016, Duke and Powles, 2008). GlyBH use is still increasing every year (Benbrook, 2012).

Glyphosate is the active ingredient of more than 750 different broad-spectrum herbicides (Guyton et al., 2015). It acts by inhibiting 5 enolpyruvyl shikimate- 3-phosphate synthase (EPSPS) - an enzyme in the shikimate pathway involved in the production of aromatic amino acids and other secondary metabolites (Franz et al., 1997). Glyphosate use has become very popular in with 84% of rice areas treated with Glyphosate as a result of its cheap price (Ragasa et al., 2013). As the effectiveness of these Glyphosates is realized, farmers increased its application proportionately to meet their production target without giving due cognizance to the side effect (Sebiomo et al., 2011).

Glyphosate is indiscriminately applied to crops throughout the bee foraging period which has the tendency to remain in significant amount on these crops been visited by bees (Vázquez et al., 2018). It is systemic and persistent in plants with as much as 80% accumulating in plant tissues such as flowers and buds visited by bees and is found in honey collected by bees from contaminated flowers (Vázquez et al., 2018).

Concentration of Glyphosate in food and water has become a public health concern due to its increasing usage (Myers et al., 2016). The real contamination of populations by Glyphosate residues is poorly characterized. Based on limited studies using small populates in U.S for example, it is estimated that Glyphosate is regularly found in urine at levels corresponding to a dietary daily intake of around 0.1-3.3 µg/kg bw/d (Niemann et al., 2015). Glyphosate has also been found above Levels of Concern in some food products like honey, maize, sorghum, Wheat and Bread (Rubio et al., 2014) across the world.

Reviews on Glyphosate health effects have been performed by governmental agencies (EPA, 1993; European Commission, 2002), by scientists on behalf of companies selling Glyphosate (Greim et al., 2015; Mink et al., 2011; Williams et al., 2012; Williams et al., 2000), or by independent academics (Antoniou, 2012; Astiz et al., 2009; López et al., 2012; Székács, 2012). All these review report conflicting opinions, especially for long-term effects of Glyphosate on human health and its commercial formulations. Glyphosate has been confirmed to affect the cardiovascular system at acute doses (Gress et al., 2014).

Consumption of foods with high levels of Glyphosate may have significant negative health implication in adults and children including many cancers (Myers et al., 2016). In Ghana, Glyphosate is widely used on virtually all farms including maize and legume farms (MOFA, 2015). It is estimated that 87% of farmers in Ghana use agrochemicals to control pest and disease on vegetable farms (Dinham, 2003).

Farmers are also encouraged to undertake bee keeping as an alternative livelihood activity to reduce the over reliance on crop farming for food security. In Ghana, honey is a commodity used in several ways as a food, sweetener and flavoring in beverages and confectionary, cosmetic agent and as a medicine (Akangaamkum et al., 2010) study has revealed that, honey has the potential to prevent cancer (Beretta et al., 2007).

Besides these uses, honey is used in the treatment of wounds and eye defects (Kwapong et al., 2013) and has some other medicinal applications. Honey is good for consumption for its usefulness of an antimicrobial agent and antioxidant which reduces skin inflammation, edema and exudation as well as promotes wound healing, diminishes scar size, and stimulates tissue regeneration, used in sweetening drugs for children and adults, and also a known fact that honey improves cardiovascular risk factors (Alvarez-Suarez et al., 2010). It is often eaten by pupils, students and the aged for the purpose of developing intellectual ability or recovery of memory and prevention of the incidence of diabetes or augmenting drug therapy used by pharmaceutical industries in drug formulation (Alvarez-Suarez et al., 2010).

Generally, utilization of honey in Ghana is on the increase hence its quality needs to be checked before consumption due to the potential of Glyphosate contamination by foraging activities of bee.

However, consumers in Ghana enjoy honey without knowing the quality. This study is therefore to contribute to an estimation of health risk from Glyphosate. This would help in the development of guidelines for the safe use of the chemical in Ghana. The study is carried out in Wa, in the Upper West Region of Ghana.

1.2 Statement of the Problem

The past four decades have seen a large influx of Glyphosate herbicides being introduced into the market as pre and post-emergent herbicide in many parts of the world (Sebiomo et al., 2011). Increased agro-chemical use is ought to increase productivity, reduce food insecurity and poverty levels among smallholder farmers (Aloyce at el., 2014). As the effectiveness of these herbicides is realized, farmers increase its application consistently to meet their production target without giving due cognizance to the side effect of the herbicide to the environment and human health (Wumbei et al., 2019, Stanley et al., 2013; Myers et al., 2016). Farmers do not apply safety measures, adequate knowledge on safe handling and application of Glyphosate herbicide and other pesticides (Fianko et al., 2011). Glyphosate herbicide and other agrochemical mishandling and use constitutes one of the most several farm operation hazards confronting farmers, their produce, and the environment (Nikolaidis et al., 2007; Tekwa et al., 2010).

In recent times, the demand for Glyphosate and other pesticides residue-free honey, organic honey and other bee products continue to increase rapidly (Akangaamkum et al., 2010). In February 2002, the world honey market was strongly affected by a European Union (EU) ban on Chinese honey, following the identification of antibiotics in samples of Chinese honey (Apiconsult, 2003).

Glyphosate has been found above Levels of concern in some food products including honey, Sugar cane, Yam, Maize, Wheat, Rice, Beans, Soybeans, and Mushrooms amongst others (Wumbei et., al 2019, Rubio et al., 2014) and in urine of some consumers in U.S (Niemann et al., 2015).

In Ghana, Glyphosate is among the most used of all pesticides categories and the leading herbicide used in crop production (Environmental Protection Agency (EPA), Ghana, 2013). The herbicide has been used by farmers in the Wa traditional area in particular and throughout all communities in the municipality for land clearing and weed control on their farms over the last years (MOFA, 2015). Like in other parts of the world, the use of Glyphosate products by the farmers has led to increasing concerns about residues and food safety implications. Glyphosate when sprayed on farms has the potentials of accumulating and mixing with nectar of flowers and plants that the worker bees collect to form honey (Vázquez et al., 2018). Honey is a product formed through the foraging activities of bees which are the main pollinators in agricultural crop fields (Andrews et al., 2004; Bardy et al., 2008).

Glyphosate is considered as a probable carcinogen by the WHO's International Agency for Research on Cancer (IARC, 2015) therefore raising concerns about its presence in food. In the developed countries, there is a permissible and accepted level of Glyphosate in honey with label slips on honey containers before sale for consumption e.g E.U ADI (0.3mg/kg/day). In Ghana, the honey we buy do not have labeled slip or prescription. Consumers of honey enjoy it without knowing the quality. Consumption of honey with high level of Glyphosate can have a significant human health risk in adults and children including many cancers (Myers et al., 2016), (Samsel & Seneff, 2015) and affect the cardiovascular system at acute doses (Gress et al., 2014). Therefore, honey must be free from any chemical contamination and be safe for human consumption hence the need to study on honey quality before sale and consumption.

1.3 General Research Question

Are consumers at risk through consumption of honey in the Wa municipality?

1.3.1 Specific Research Questions

1. What levels of Glyphosate exist in honey sold on Wa market?
2. What levels of physicochemical exist in honey sold on Wa market?
3. What is the average dose of Glyphosate received through consumption?
4. At what risk are consumers exposed to the concentration levels of Glyphosate found in honey?
5. What level of knowledge do Consumers, Vendors and Processors of honey have regarding Glyphosate in honey?

1.4 General Research Objective

The main objective of this study is to examine the health risk associated with the dietary exposure to Glyphosate from honey consumption in the Wa municipality.

1.4.1 Specific Research Objectives.

1. To estimate the levels of Glyphosate in honey sold on the Wa market.
2. To determine the physicochemical quality of honey sold on the Wa market.
3. To estimate the average dose of Glyphosate received through dietary exposure.
4. To estimate the health risks associated with the concentration levels of Glyphosate found in honey.

5. To assess the knowledge of honey consumers, vendors and processors regarding Glyphosate in honey in Wa municipality.

1.5 Significance of the Study

This study would contribute to the estimation of health risk from Glyphosate present on honey. This would help in the development of guidelines for the safe use of Glyphosate in Ghana. This will inform consumers to check on the quality of honey before consumption as well as the health risk associated with consumption that effect human life and the environment since very limited data is available in Wa and Ghana as a whole. The essence therefore is to create awareness to the need to be mindful of quality and minimize consumption to reduce risks.

Finally, the research findings could be the basis for intervention initiatives by government and nongovernmental organizations (NGOs) to enhance quality checks standards on honey before sale to public like in the develop world.

1.6 Scope of the study

This research was conducted in the Wa Municipal of Upper West Region of Ghana. The foremost purpose of the study is to estimate dietary exposure to Glyphosate from honey consumption so as to inform guidelines on honey quality and the environmental fate of Glyphosate use in Ghana. The focused of the research is on dietary quality checks and consumption levels of honey in the Wa municipality.

The research adopted a mixed method approach; Laboratory analysis of honey samples (Glyphosate herbicide and physicochemical features), and collecting honey consumption data from respondents to estimate the average dose of Glyphosate received through consumption, estimating the health risks associated with the concentration levels of Glyphosate found in honey and to examine the knowledge of honey consumers, vendors and processors regarding Glyphosate in honey in Wa municipal. These parameters are direct indicators of honey quality in terms of shelf-life, fermentation and adulteration. The conduct of this study spanned between academic years (2015/16 and 2019/20); from proposal stage till the final research report was developed.

1.7 Ethical Consideration

Before the research was undertaken, a letter of introduction from the Department of Environment and Resource Studies was used for official sourcing of relevant information. Community entry was done in the selected communities, through the representatives of the community leaders in the communities. Oral consents were sought from consumers, vendors and processors of honey before interviews were conducted. They were all assured that any information given was purely for academic purpose and that their confidentiality was highly guaranteed.

Community entry protocols, observance to confidentiality, privacy and avoidance of harm of the respondents were ethically observed to gather relevant information from respondents. The respondents were well informed about the objectives of the study and their participation in the study was purely voluntary and as such they could choose to partake or not. Information provided by respondents was not to be given out to third party without prior approval of respondents.

1.8 Organization of the Research

The research report is structured under five (5) main chapters. Chapter one (1) presents the background to the study, the problem statement, the research questions and objectives, significance of the study, scope of the study, and the ethical considerations of the research.

The second chapter of the study focuses on examining secondary data and relevant concepts on the research objectives. The literature review conducted was based on the objectives of the study and this enabled the study to be grounded on empirical evidence in the literature so that convincing findings and conclusion were drawn based on the stands of existing literature.

In Chapter three, the research adopted a mixed method design including laboratory work and surveys on honey consumers, vendors and processors. Again, this chapter explains and justifies the research paradigm under which the methods for the study were selected. It also covers sources of data, laboratory analysis, sampling techniques and the instrumentation, the study population and the limitations of the study in addition to the data gathering procedure. This methodological chapter indicates the appropriateness of the methods to ensure a systematic approach that a scientific study of this stature demands.

In Chapter four (4), the results are presented and discussed, presents findings together with the discussions; this enables readers to follow the connection between the objectives of the study and research questions, the literature review, conceptual framework, laboratory experiments and the responses from respondents and in Chapter five (5), the findings, conclusions, and recommendations have been presented.

CHAPTER TWO

LITERATURE REVIEW

2.1. Introduction

Chapter two entails a detailed discussion on the key concepts that guide the study, literature review and the conceptual framework underpinning the study. Data were gathered from scientific publications identified using PubMed, a web-based database that comprises over 23 million citations for biomedical literature and other publications. There are three sections; section one discussed the key concepts, section two discussed relevant literature and section three presents the conceptual framework of the study. In the first sections, seven key concepts are discussed (Honey, Beekeeping, Food Consumption, physicochemical features of honey, Glyphosate, Dietary Exposure and Health Risk Characterization). In the second section, literatures has been reviewed on five main themes, in line with the research objectives, and in the last section, the conceptual frame work have been presented and discussed.

2.2 Honey

Honey is a natural food produced by bee (*Apis mellifera*) from the nectar of blossom (nectar honey) or from the secretions of living plants or excretions of plant sucking insects of the living part of plant (honeydew honey) that transform and combine this with specific substances of their own, and leave it in the honey comb to ripen and mature (Alvarez-Suarez et al., 2010). It is composed primarily by a mixture of sugars (85–95%) and water (16–18%) approximately, and minority compounds (proteins, free amino acids, organics acids, phenolic compounds, vitamins and minerals) (Sanz et al., 2005; Pires et al., 2009; Kahraman et al., 2010; Castro-Vázquez et al., 2010). Honey bees collect nectar from flowers and turn it into a product considered to be a delicious food and known to be a healthier nutritional choice than sugar (Bilandžić et al., 2012). It is widely used for both nutritional and medicinal purposes (Al-Waili et al., 2012). Honey matrix has different components such as sugars, organic acids and insoluble matter. It contains significant amounts of mineral matter, vitamins and enzymes (Tahboub et al., 2006).

2.3 Types and Classification of honey

Bees commonly forage on flowers within two kilometers of their nest, although some can forage about 14.4 kilometers away from their home, and foraging distances of five kilometers are common (Ratnieks et al,. 2002). Bees forage for nectar to produce honey. Honeys are classified into two main types: apiary honey and forest honey (Manyi-Loh et al., 2011). Apiary honeys are produced by the honeybees *Apis cerana indica* and *Apis mellifera*, in apiaries and collected by modern extraction methods. They are processed to be transparent and free from foreign materials. Forest honey are produced by rock bee *Apis dorsata* or from wild nest bee's *A. cerana indica* in forests and are collected by the crude method of squeezing the comb. They are turbid due to the profusion of pollen, wax, brood (bee larvae), parts of bees, and plant materials and therefore essential to filter to separate the suspended particles (Manyi-Loh et al., 2011).

Other classifications exit. These include blossom honey, honeydew honey, monofloral honey and polyfloral honey (O"Todle and Raw, 1991; Ouchemouk et al., 2007). Also, honey is classified as nectar honey and honeydew. Nectar is a sugar solution of varying concentrations: from 5–80%. About 95% of the honey dew dry substances are sugars, the rest are amino acids (0.05%), minerals (0.02–0.45%), small amounts of organic acids, vitamins and aroma compounds. The sugar value ranges widely, from 0.0005mg to 8 mg. The sugars composition is also typical for each plant species, the primary sugar being sucrose, glucose and fructose. Most plants have nectars consisting predominantly of fructose and glucose (60–85%); but in some plants the nectar is mainly sucrose (e.g. acacia clover, lavender) (Lazaridou et al., 2004).

Temperature plays also a very important role. Most advantageous temperatures are 10 to 30 ºC. Strong winds decrease nectar secretion. The nectar secretion depends also on the time of the day. Therefore it is not likely to anticipate nectar production. Utmost secretion is at noon and during early afternoons. Bees desire nectar with higher sugar contents, e.g. around 50% and will not forage if it is below 5%. Bees gather nectar for their energy needs. The higher the sugar value of a plant, the more it is visited by bees for foraging (Weryszko et al., 2007; Bentabol et al., 2011).

2.4 Mineral Composition of Honey

Centered on the mineral composition data, it is possible to record the influence of elements on the distribution of particular object samples and classify food products according to their country of origin, type, and genetic classification. Several authors have applied chemo metrical procedures on elemental composition data in order to classify honeys in view of their botanical and geographical provenance (Bogdanov et al., 2005). Glucose is one of the major constituents of honey and when this crystallizes the honey becomes solid, known as granulated honey. Granulation is a natural process and there is no difference in nutritional value between solid and liquid honey. This process may be likened to ice and water liquid honey and granulated honey is the same substance but in a different form. Some honeys are much more prone to granulation than others are, and almost all honey will granulate if its temperature is reduced.

As with the color of honey, diverse people favor different qualities: some prefer granulated honey while others take liquid honey. If honey is required in the granulated form, but it is slow to granulate, it is possible to start the granulation process by 'seeding' it by adding some finely granulated honey and stirring this in until it is evenly distributed. The honey will now granulate if kept at a low temperature. If a jar of granulated honey is required in the liquid form, stand it up to its neck in a container of warm water (60 °C) it should soon liquefy. However, heating honey always reduces its quality by destroying its enzymes, evaporating volatile compounds and therefore reducing the flavor (Alves et al., 2013).

2.5 Honey as a food product

Honey is basically used in baked products, confectionary, candy, marmalades, jams, spreads, breakfast cereals, beverages, milk products and many preserved products (Krell, 1996). Kime and Lee (1987) used honey as a source of fermentable sugars in alcoholic beverages (honey wines, fruit honey wine, fruit wine and beers) and in juice clarification (a property related to protein-phenolic compound interaction). Tong et al., (2010) also suggested the use of honey powder in bread dough formulation to increase overall nutritional, sensory and keeping quality of bread. Honey is spoken of by all religious books, and accepted by all generations, traditions and civilizations, both ancient and modern as very nutritious food (Ajibola et al., 2012). Honey has numerous uses and functional applications worldwide such as in food systems and religious ceremonies as well as in human and veterinary medicine (Nigussie et al., 2012). Honey has a significant place in traditional food preparation in various societies. Its many applications in processed foods and pharmaceuticals make honey versatile and its use on the rise.

2.6 Honey grading

According to United States Department of Agriculture (USDA) standards, honey is graded based upon a number of factors, including water content, flavor and aroma, absence of defects and clarity. Honey is also classified by color though it is not a factor in the grading scale USDA (1985)**.** Refer to Table 2.1 below.

Table 2.1: USDA inspection and grading of honey

Grade	Water content	Flavor and aroma	Absence of defects	Clarity
A	< 18.6%	Good-has a good, normal flavor and aroma for the main floral source and is free from caramelization, smoke, fermentation, chemicals and other odor causes	Practically free- practically no defects that affect appearance or edibility	Clear-may contain air bubbles that do not materially affect the appearance; may contain a trace of pollen grains or other finely divided particles of suspended material that do not affect appearance
B	< 18.6%	Reasonably good—practically free from caramelization; free from smoke, fermentation, chemicals, and other causes	Reasonably free—do not materially affect appearance or edibility	Reasonably clear—may contain air bubbles, pollen grains, or other finely divided particles of suspended material that do not materially affect appearance
C	< 20.0%	Fairly good—reasonably free from caramelization; free from smoke, fermentation, chemicals, and other causes	Fairly free—do not seriously affect the appearance or edibility	Fairly clear—may contain air bubbles, pollen grains, or other finely divided particles of suspended material that do not seriously affect appearance
Substance	> 20.0%	Fails Grade C	Fails Grade C	Fails Grade C

Source: United States Department of Agriculture, 1985

According to the grading system of the United States Department of Agriculture (USDA, 1985), honey with total soluble solids greater or equal to 81.4% is considered of higher grade (A and B), while that falling between 80% and 81.3% is considered to be of lower grade C and honey with less than 80% soluble solids is likely to ferment during storage.

Other countries may have differing standards on the grading of honey. In Ghana, there are not fixed standards of both physical and chemical parameters of honey sold on the market. Ghana has adopted the International Honey Commission (IHC), and Codex Alimmentarius Commission standards (CAC) of honey grading and quality checks. According to the definition of Codex Alimentarius Commission Standards (2001), honey shall not have added any food ingredient other than honey, nor shall any particular constituent be removed from it. Honey shall not have any objectionable matter, flavor aroma or taint from foreign matter during its processing and storage with no fermentation or effervescence. No pollen or constituent particular to honey may not be removed except where this is unavoidable in the removal of foreign or organic matter. Honey shall not be heated or processed to such an extent that its essential composition is changed and/or its quality impaired.

Certain quality parameters are used to determinate honey quality. Countries strictly following these quality standards gain an appreciable amount of foreign exchange through honey export. The most important is the water-sugar relationship due to its effect on silt against fermentation and granulation (White, 1978). Sugars are the principal constituents of honey, which aside from determining its nutritious and energetic value, also influences some of its important physical characteristics such as crystallization, hygroscopic and viscosity. Ash value indicates the botanical origin; the blossom honey has lower mineral content than honeydew honey. Temperature effect is recognized by the production of 5-hydroxymethyl furfural (HMF). The HMF is inversely proportional to the quality of honey, which depends on pH, heat process after harvesting and storage temperature.

A study conducted in Ghana by (Akpabli-Tsigbe, 2015) adopted the standards of CAC and IHC standards and concluded on the results as; all artisanal honey samples analyzed were acidic in nature, with pH values ranging from 3.79 to 5.39 and the mean of 4.5 ± 0.5, Total soluble solids (sugar content) of the artisanal honey sample ranged from 76.3 % to 82.9 % for the samples with the mean TSS was 79 ± 2 %, average total solids value of the artisanal honey samples analyzed in the present study was 81 ± 2 g/100 g ranging from 77.8 g/100 g to 84.2 g/100 g, Moisture contents ranged from 15.8 to 22.2 %, with an average of 19 ± 2 % which the difference in moisture content was significant ($p < 0.05$) between all the samples analyzed and the refractive index of the samples ranged from 1.481 to 1.497 with an average of 1.489 ± 0.005.

2.7 Honey production and Bee keeping

Bee Keeping has very significant role in improving biodiversity and increasing crop production via pollination. Bees pollinate agricultural crops, home gardens, orchards and wildlife habitat. As bees travel from flower to flower in search of nectar, they transfer pollen from plant to plant, therefore fertilizing the plants and supporting them to bear fruit. There is also ready market both locally and internationally for bee products with underlying market opportunities given to producers to explore (Paterson, 2006). Even though there is little emphasis on the production of beeswax and other by-products in Ghana because the domestic market is underdeveloped, beeswax alone has over 120 industrial uses (Ahmed, 2014) with ready markets in Ghana and abroad(Paterson, 2006; Ahmed, 2014).

In 2011 the European Union (EU) certified Ghana to join other African countries accredited to export honey to the EU market (Ahmed, 2014). This implies that the international market for honey is expanding and Ghana is expected to meet the supply gap by increasing the volume of trade and quality of production. However, the type of beehives used in honey production and methods of harvesting, processing and packaging play a vital role in the quantity and quality of honey. The quality of honey is dependent on the source of collection (Akpabli-Tsigbe, 2015), either from wild hunters or beekeepers and method of extraction (traditional or modern methods) (Akpabli-Tsigbe, 2015). The Movable-Frame Hive (MFH) are the most advanced hive design that is used in large-scale commercial beekeeping throughout the world (Assefa, 2009).

The national estimated average yield of pure honey from MFH in Ethiopia is 19.92kg per hive/year and the amount of beeswax produced is 1-2% per kilogram of honey yield. An annual estimated production capacity of 35kg per hive/annum is reported in Ghana (Akangaamkum et al., 2010). They also require high investment cost and trained man power and are recommended for experienced beekeepers that want to optimize outputs. Tessega (2009) suggested that an intensive training is needed for beekeepers with no formal education before distributing movable frame hives.

According to Subbey (2009) study on production technology of honey, the study concluded that majority of the respondents in the Brong Ahafo (77.5%), Northern (39.1%), Upper East (79.7%) and Upper West (72.2%) regions use Kenya Top bar, followed by Saltpond Top bar in the Brong Ahafo (14.0%) and Northern (19.5%) regions with Borassus (8.1%) and clay pot (9.9%) following in the Upper East and Upper West regions respectively. Again, the study reported that on production levels of honey, all the regions shows increasing trend of honey production in 2007 to 2008 as well as increasing projected trend figures in honey production for 2009 and 2010. Total production of honey (gallons) in the Brong Ahafo region, Northern, Upper East, Upper West and Ashanti regions for 2008 were 10,584; 4,262; 1,533; 1,746 and 7,423 gallons per annum respectively.

2.8 Empirical Studies on Honey and Wax Production from Beehive Types

It is argued that the traditional beehives are not efficient in sustaining continuous and high honey production due to minimal protection from wind, rainfall and invaders such as ants and lizards which steal and kill the bees and though the cost of such hives is low, productivity is also low with less than 13kg of honey per annum (Paterson, 2006). Honey from traditional and top-bar hives is reported to have higher moisture content than honey collected from improved beehives (Paterson, 2006). A report on national honey production in Ethiopia is estimated at an average of 8.94kg of honey and 0.95kg wax per hive per year (Yetimwork et al., 2014) and an average of 6kg per hive/year is reported by Assefa (2009).

The annual average honey yield from the Volta Region of Ghana was 4kg per beehive whilst the national average yield per beehive in Ghana was estimated at 14kg per/hive/annum (Akangaamkum et al., 2010). Subbey (2009) in a study of the honey industry in Ghana reported that a beekeeper using one hive with extractors and beekeeping equipment would obtain 3 gallons (21kg) of honey from the second year but no reference was made to any specific beehive type. The design of KTBH is relatively simple with lifespan of 20 years and production between 20-26kg of honey per year in an ideal condition (Paterson, 2006). An average amount of 10.66kg per hive/year of crude honey is estimated and 8% of beeswax/kg of honey from KTBH in Ethiopia (Yetimwork et al., 2014). An annual estimated honey production capacity per KTBH in Ghana is 21kg and 2.6kg beeswax (Akangaamkum et al., 2010) and 34kg/beehive in transitional zone (Ahmed, 2014).

2.9 Global production, information and demand for honey

Honey is the major product of apiculture/bee keeping industry worldwide and produced in nearly all countries. In general, honey production has been well-known as one of the most profitable venture in various parts of the world, so much in use and as a result in demand that it can be termed a money spinner. According to the United Nations, Food and Agriculture Organization, Statistics Division (FAOSTAT), 2010, in 2016, global production of honey was 1.8 million tonnes, led by China with 27% of the world total production and other major producers were Turkey, United States, and Russia. According to Akangaamkum et al. (2010), world honey production is estimated at 1,394,000 MT and is growing between 2 % and 3 % per annum.

Bogdanov (2014) reported that the annual world honey production at present is about 1.2 million tons, which is less than 1 % of the total sugar production. Argentina is the second largest producer after China and accounts for 20 % of the world production, with an average of 350,000,000 kg per year (Isla et al., 2011). Turkey was the second biggest honey producer in the world with annual production of 81,115 tonnes and provides a convenience apicultural environment in terms of flowers (FAOSTAT, 2010). According to Kahraman et al. (2010), the total production of honey in Turkey makes a contribution of 5.7 % to the total world honey production (Getachew et al., 2014). According to Serem & Bester (2012), only 1,814 MT a year is produced in the southern Africa region compared to Kenya, the 17th top honey producer in the world, producing 25100 MT of honey a year.

In Ghana, there is general lack of records and documentation on honey production resulting into data gaps. Akangaamkum et al., (2010) however suggested an increasing trend in honey production in Ghana. The domestic production and demand gap in Ghana is consequently being bridged by high foreign imports of honey. There is therefore huge domestic market for honey in Ghana which needs to be exploited. Fasasi (2012) reported that ninety per cent (90 %) of the estimated annual world production of honey is consumed as honey, while the remaining ten per cent (10 %) is used industrially.

Bogdanov (2014) revealed that the annual honey consumption in the major honey producing and exporting countries (China and Argentina) is small: 0.1 to 0.2 kg per capita and higher in developed countries, where the home production does not always cover the market needs. He also reported that in the European Union (which is both a major honey importer and producer) the annual consumption per capita varies from medium (0.3 – 0.4 kg) in Italy, France, Great Britain, Denmark, Portugal to high (1 – 1.8 kg) in Germany, Austria, Switzerland, Portugal, Hungary, Greece; while in overseas countries such as USA, Canada and Australia the average per capita consumption is 0.6 to 0.8 kg/year. However, consumption has been relatively stable over the last five (5) years. The domestic production and demand gap in Ghana is consequently being bridged by high foreign imports of honey. There is therefore huge domestic market for honey in Ghana which needs to be exploited.

Honey is consumed all over the world. But USA is recognized as one of the largest consumer, with around 153,000 tonnes, followed by China with 123,000 tonnes and Germany, approximately 90,000 tonnes per annum. Fasasi (2012) reported that ninety per cent (90 %) of the estimated annual world production of honey is consumed as honey, while the remaining ten per cent (10 %) is used industrially. Bogdanov (2014) revealed that the annual honey consumption in the major honey producing and exporting countries (China and Argentina) is small: 0.1 to 0.2 kg per capita and higher in developed countries, where the home production does not always cover the market needs. The same author also reported that in the European Union (which is both a major honey importer and producer) the annual consumption per capita varies from medium (0.3 – 0.4 kg) in Italy, France, Great Britain, Denmark, Portugal to high (1 – 1.8 kg) in Germany, Austria, Switzerland, Portugal, Hungary, Greece; while in overseas countries such as USA, Canada and Australia the average per capita consumption is 0.6 to 0.8 kg/year.

Recognition of the significance of the honey subsector in the agricultural economies of African countries has been slow. In Africa, though reliable production and trade statistics on honey do not exist, it is believed that the consumption of honey on the continent far outstrips production (Akangaamkum et al., 2010). Ethiopia produces 45,300 MT of honey per annum, making the country to rank first honey producer in Africa and ninth in the world. Akangaamkum et al., *(*2010) studied the Honey Industry in Ghana and through Stitching Nederland's Vrijwilligers (SNV) Ghana observed that, honey wax production has been increase in the last five years. For example in the Greater Accra Region honey production level increase from 7,400kg in 2004 to 15,300kg in 2008. Production level of honey in the Central Region increased from 236,795kg in 2007 to 428,836kg in 2008.

2.10 Honey and Beekeeping in Ghana

In Ghana, beekeeping has been a tradition before other farming systems practiced. Even though it is one of the important and the oldest farming activities in country, there are no available records, which confirm when and where beekeeping was started. Beekeeping started with the hunting and robbing of wild colonies in hollow cavities in tree and rocks until 19th century when sugar cane (which was available) was refine as another sweetening agent (Akangaamkum et al., 2010).

Ghana's agro-ecological conditions are considered suitable for the production of honey in all regions especially the West African honeybee; *Apis mellifera adansonii* which is better adapted to the tropical conditions of the country (Akangaamkum et al., 2010). Since 1970, significant strides have been made by NGOs in the honey sub-sector through various interventions like the introduction of modern beekeeping technologies, training and the provision of beekeeping equipment. Therefore, the number of people involved in modern beekeeping has been improving resulting in an increase in the quantities of honey production. Akangaamkum et al., (2010) have reported that honey production in Ghana has been increasing over the years from 236,795kg in 2007 to 428,836kg in 2008 and total beeswax production also increased from 34,552kg to 60,031kg during the same period with about 52,883 beehives. Subbey (2009) has also observed a general increase in the farm gate price of honey per gallon in all the regions investigated in Ghana (Ashanti, B/A, Northern, Upper East and West) from GH₵18.00 in 2005 to GH₵20.00 in 2006, GH₵22.00 in 2007 and GH₵24.00 in 2008. The total farm gate income from honey production also increased from US$619,455 in 2007 to US$1,076,378 in 2008 (Akangaamkum et al., 2010). As a result, average contribution of honey production to beekeeper's annual income increased from 23% in 2008 to about 37% in 2010 (Akangaamkum et al., 2010).

2.11 Importance of beekeeping

The prospect for helping beekeepers of third world and raising their living standard through the development of beekeeping activities are bright (Akangaamkum et al., 2010). Beekeeping has many advantages that help beekeepers to improve their wellbeing. Its advantages can be itemized for the socio-economic impact of beekeeping. For instance, successful beekeepers raise their socio-economic standing in areas with subsistence agriculture, and farmers in developing countries can substantially supplement the family income, sometimes even double it (Akangaamkum et al., 2010). This means the family is food secured. Furthermore, some of the relative advantages and importance of beekeeping are the following:

1. Bees are cosmopolitan: they adapt to wide range of environment. In much of low land areas, where cattle production may be severely constrained due to tsetse fly, livestock disease and other reasons, harvest could be obtained from beekeeping (Akangaamkum et al., 2010).
2. Smallholders and landless people can practice beekeeping. The hive requires little land and bees can collect nectar and pollen from anywhere they can get. Thus, wild, cultivated and wasteland areas all have value for bee keeping (Akangaamkum et al., 2010).
3. Beekeeping doesn't compete for resources with other agricultural endeavors and can be run integrating with other agricultural activities.
4. Bee culture does not disturb ecological balance, as many cultivation of crops and practices of animal husbandry (Akangaamkum et al., 2010).

2.11.1 Major Constraints in Beekeeping

Like any other livestock sector, this sub sector has been hold by complicated constraints. The prevailing production constraints in the beekeeping sub sector of the country would vary depending on the agro-ecology of the areas where the activities is carried out (Akangaamkum et al., 2010). Variations of production constraints also extend in socio-economic conditions, cultural practices, climate (seasons of the year) and behaviors of the bees. The major constraints in the beekeeping sub sector are the following: the unpleasant behaviors of bees (aggressiveness, swarming tendency, and absconding behaviors); lack of skilled manpower and training institutions; low level of technology used; high price of improved beekeeping technologies; drought and deforestation of natural vegetation; poor post-harvest management of beehive products and marketing constraints; indiscriminate application of agrochemicals; honeybee disease, pest and predators; poor extension services; absence of coordination between research, extension and farmers; absence of policy in apiculture; shortage of records and up-to- date information; and inadequate research institutions to address the problems. But all these problems may not be constraints to all parts of the country and may not be equally pressing to every place. So it requires characterizing the constraints in their respective places to take an appropriate development measure (Akangaamkum et al., 2010).

The beekeeping research so far conducted in the country although encouraging is not satisfactory because one center could not address all parts of the country. Most of the research work is still being carried out on-station with modern technology and management systems. However, the great majority of beekeeping production is based on traditional production systems where the results of on-station research may not often be applicable to the local conditions (Akangaamkum et al., 2010). An introduction of improved hives and working tools to the rural community are beyond the pockets of farmers and not so easily available even for those who could afford it. Many beekeeping projects that were implemented by government and various organizations to boost honey and beeswax production were not successful mainly due to inadequate management and above all the beekeepers lack of awareness and interest. The potentiality of apiculture could be backed up by research and the beekeepers' indigenous knowledge which should be assessed. In this regard it is important and right time to conduct apicultural research in order to assess the situation at the grass-root level: to identify the system of honey production practices, the available marketing channels and quality status of the product.

2.12 Economic Importance of Honey

Globally, honey production is of high economic importance. It is a source of employment, provides income to people, a source of recreation, ecotourism, foreign exchange earnings, among others. It is an important commodity in the international market; serving as foreign exchange earner for many countries (Buba et al., 2013). The countries with the highest honey exports are Mexico, China and Argentina. Ethiopia earns US$ 76.6 (€57.6) million from honey export and is the largest producer and exporter of honey in Africa (Famuyide et al., 2014). However, to the best of my knowledge, there is no documented information on the contribution of honey to the Ghanaian economy.

According to Subbey (2009) baseline studies on honey sub sector in Ashanti, Brong Ahafo, Upper West, Upper East and Northern Regions shows that on employment, the study revealed that the Brong Ahafo region has 31 Associations/groups in the 9 survey districts with a total number of 5,748 beekeepers (3,536 Male, 2,212 Female), the Northern region has 36 Associations/groups in the 9 survey districts with a total number of 3,572 beekeepers (2,372 Male, 1,200 Female), the Upper East region has 20 Associations/groups in the 5 survey districts with a total number of 1,488 beekeepers (916 Male, 572 Female) and the Upper West region has 13 Associations/groups in the 5 survey districts with a total number of 1,788 beekeepers (1,140 Male, 648 Female).

2.12.1 Source of Income to Household.

Honey is an important income generating commodity with high potential for improving incomes, especially for communities living close to forests and woodlands. It is an essential source of extra income for people. Ajibola et al. (2012) reported that it is also a low risk business venture. According to Apiconsult (2003), honey production offers great potential for development and is comparatively less demanding in terms of investment, labor and time. It serves as a source of cash incomes for many households (Getachew et al., 2014) and gives men, women and youth room to make their own income. Honey production is to a large extent, a supplementary economic activity. It is a means of creating jobs specifically for the unemployed youth and poor rural population. Honey production through bee keeping has become popular among small scale farmers in current years and seen correctly as a reliable key in reducing poverty and malnutrition (Famuyide et al., 2014).

According to Subbey (2009) profitability of Honey and Wax Production, analysis of honey and wax production indicates that generally they are profitable. The study confirms that, a beekeeper using one hive, group shared extractors and beekeeping equipment will incur a loss of GH¢1.00 and make profits of GH ¢9.00 and GH ¢49.00 in the second and third years of operations when three gallons of honey are produced and sold at GH ¢20.00 each. For a beekeeper with 3 hives and producing 9 gallons of honey at a selling price of GH ¢20.00 per gallon of honey, a profit of GH ¢18.50 will be made in the first year.

In the second and third years, profit levels of GH ¢168.50 and GH ¢168.50 will be generated. Five hives generate 15 gallons of honey and applying same price as above, a profit of GH ¢46.50 and GH ¢288.20 will be made respectively in the first and second years. Profit level rises slightly in the third year to GH ¢288.30. The break-even years are 2 years for one hive, 1 year for 3, 5 and 10 hives. The minimum number of hives that generate the best economic returns is 3 hives. Subbey (2009) reported on marketing of Honey products that, all the honey produced is sold out by the close of the year. The market for honey may be categorized broadly into two, the domestic and export market, Subbey (2009). The domestic market can further be subdivided into rural and the Urban market. Concerning the domestic market, price trends indicate that there is no integration between them. This implies that prices in local/ rural market do not influence prices in urban market. The integration of these two markets will very much depend on the development of rural market, Subbey (2009).

2.13 Characteristics of Quality Honey

According to Subbey (2009) study on quality standards and packaging, majority of respondents in the Brong Ahafo region (75.5%) packed their honey for the market in plastic new containers, whiles 63.7% of respondents in the Northern, 92.5% in Upper East and 78.6% in Upper West regions packed their honey for the market in recycled plastic containers. A match of the above packaging statistics with price trends in the survey regions show a markedly strong correlation with poor packaging attracting low product prices.

In a study, White acknowledged the diversity of the physical characteristics of honey to be dependent on the nectar and pollen of the original plant, color, flavor, moisture and contents of proteins and sugars. Honey quality assessment was done using different analytical techniques or methods, such as, isotopic, chromatographic, thermal analysis, and nuclear magnetic resonance. However, these methods of honey quality assessment have been demonstrated to be time-consuming, destructive and sometimes expensive. The most widely used method to assess the quality of honey is high performance liquid chromatography (HPLC) but gas chromatograph/mass spectrometry (GC/MS) technique is a more precise methodology that can be used to assess honey quality (Kazemi, 2012)

2.13.1 Viscosity of honey

The rheological behavior of honey has been investigated for in shelf-life, proper handling, packing and processing issues (Ahmed et al., 2007). The honey viscosity depends on the water content, floral source, amount and size of crystals and, finally, the temperature (Zaitoun et al., 2001; Yanniotis et al., 2006). Honeys with higher water contents flow faster than those with lower ones (Goméz-Díaz et al., 2009). The composition of honey generally has some effects on honey viscosity.

Some studies, have reported honey as are Newtonian liquid (Junzheng and Changying, 1998; Abu-Jdayil et al., 2002; Lazaridou et al., 2004). However, there are a few honeys which show different characteristics regarding viscosity: heather (Calluna vulgaris), buckwheat (Fagopyrum esculentum), white clover (Trifolium ripens) and manuka (Leptospermum scoparium) honeys are described as thyrotrophic which means they are gel–like (extremely viscous) when standing still and turn liquid when agitated or stirred; while dilatancy has been detected in Nigerian honey and several eucalyptus honeys (e.g. Eucalyptus fisifolia) (Yanniotis et al., 2006; Bogdanov, 2009). The viscosity of honey decreases rapidly as its temperature rises. A 1% change in moisture content has been shown to have the same effect on viscosity as a 3.5 ºC change in temperature (Zaitoun et al., 2001). They concluded that, the carbohydrate concentration is the major factor contributing to unifloral Australian honey´s viscosity.

2.13.2 pH of Honey.

Generally, pH is a physical characteristic of honey which is of great importance. It influences the texture, stability and shelf-life of honey during extraction and storage (Terrab et al., 2002). IHC (2002) recommended an average pH value of 3.9. The acidity of honey is due to the presence of gluconic acid, formic acid, oxalic acid and lactic acid (Nanda *et al*., 2003). Gluconic acid is the main or predominant acid in honey (Manyi-Loh *et al*., 2011), however, it is present as a lactone and does not contribute to honey's active acidity. According to Bardy *et al*. (2008), most honey is acidic with pH ranging from 3.2 to 4.5 which is inhibitory to most neutrophilic bacteria. Honey has amino acids of about 18 free amino acids, but they are present in small amounts with little nutritional significance. Honey contains 0.05-0.1% amino acids with Proline being the most abundant. These amino acids are important in honey for the following reasons. (Bardy et al., 2008),

The low pH of honey inhibits the presence and growth of microorganisms. Its low pH makes honey compatible with many food products in terms of pH and acidity. Acids are an important component of the flavor and aroma of monofloral honeys. Acids also contribute to a manufactured product's flavor profile. These numbers of organic acids are known to occur in honey, including acetic, butyric, citric, formic, gluconic, lactic, malic, pyroglutamic, and succinic. The major organic acid is gluconic acid. Gluconic acid is produced in honey by the action of the enzyme glucose-oxidase on glucose (Bardy et al., 2008),

2.13.3 Moisture of Honey.

A number of factors influence the final value of moisture parameter in honey produced by honeybee colonies, such as low air humidity, medium abundance of nectar flow, good colony strength and ventilation of the beehive. Moisture in honey is an essential quality criterion in honey processing, as the probability of fermentation of honey over storage increases with moisture content (Codex Alimentarius Commission Standards for honey 2001). The refractive index is a measure that proportionally decreases with increasing water content, and therefore is used to determine the moisture content in honey. When processing honey, the moisture level is important to consider. Most beekeepers simply let the bees tell them when the moisture level is around 17-18% (Codex Alimentarius Commission Standards for honey 2001). This is when the bees cap off the comb to prevent the honey from absorbing any moisture to prevent honey fermentation (Codex Alimentarius Commission Standards for honey 2001)

The control of the water content is an important requirement. (Codex Alimentarius Commission Standards for honey 2001), which sets an upper limit for moisture of 21% for honey in general which is harmonized with the EU Directive (EU Council, 2002), fixes a maximum moisture content in honey sold in the market at 20 %. Low moisture content in honey may result in increase in sugar content in dry season with low humidity. An increase in the moisture content of honey is an indicator of adulteration which affects other properties like density, specific gravity, refractive index, viscosity and optical properties, and also plays an important role in the preservation of honey.

If the moisture content exceeds twenty-two per cent (22 %), honey is likely to ferment (Attri, 2011). Honey moisture content depends on the environmental conditions and the manipulation from beekeepers at the harvest period, and it can vary from year to year (Larsen et al., 2014). According to (Conti and Botre, 2001), the water content generally depends on the botanical origin of the sample, the processing techniques and the storage conditions.

2.13.4 Refractive index (RI) of honey

The refractive index of honey is said to be a rapid, accurate and simple measure of its moisture content. The refractive index measurement was done with an abbe refractometer. The refractometer‘s sample compartment were made at room temperature (200C). The electrical conductivity measurements was done at 250C using PH/Conductivity meter model 20 (Denyer instrument). The instrument was calibrated using 0.01m Kcl (potassium chloride solution). The refractive index of the honey has codex standard of 1.4000 – 1.9000. The moisture content and the total soluble solids (TSS) in honey are determined by measuring its refractive index (RI) using a refractometer at 20 ºC (normally about value of 1.49) and the corresponding moisture content (%) is calculated using the relationship between the refractive index and the water content (Bogdanov, 2002). In general, the RI increases with the increase in the solid content (Idris et al., 2011). This helped prevent microbial contamination and activities. Good Refractive Index of honey has a stable shelf life without crystallization and fermentation.

2.13.5 Total soluble solids (TSS) of honey

IHC, (2002) set Total Soluble Solids minimum at 80%. Total soluble solids (sugar content) when high in content suggests that the honey is ripped and matured in the honey combs before harvested. High sugar content also makes the honey hygroscopic.

2.13.6 Total solids (TS) of honey

Total solid is a measure of dissolved solids in the honey samples. According to the grading system (USDA, 1985), honey with total solids greater or equal to 81.4% is considered of higher grade (A and B), while that falling between 80% and 81.3% is considered to be of lower grade C. Generally, honey with high total solids indicates low moisture content, and high shelf life stability. High total solid content indicates high mineral content and other soluble solids (sugars).

2.13.7 Color of honey

Honey color is the single most important factor determining import and wholesale prices. The price of honey depends to a great extent on honey color, light honeys like acacia and citrus generally achieving the highest prices (Alvarez-Suarez et al., 2012; Al-waili et al., 2012; Alqarni, 2012). Bogdanov et al. (2004) reported that in Germany, Austria and Switzerland, dark honeydew honeys are especially appreciated and Murphy et al., (2000) concluded that the Irish consumer appreciated dark honeys. Bogdanov et al. (2005) and Terrab et al., (2002) reported that the color of honey is one of the parameters of higher variability and is mainly determined by the botanical origin, but also depends on other factors for example ash content, conditions of processing (temperature) and conditions of storage (temperature and time). Most instances liquid honey color varies from clear and colorless to dark amber or black. Honeydew honey are mostly dark-brown whereas floral honey vary from clear, colorless to brown and may darken if stored for long even at 27 °C (White et al., 1975).

According to the USDA classifications (Wiest, Giroud, Fratta, Amic, Pouliquen, & Arnaudguilhem, 2011), the most important aspect of honey color lies in its value for marketing and determination of its end use. In many countries with a large honey market, the color of honey determines consumer preferences, thus, used as an important quality index (Mathew, 2004). The lighter honeys are milder in flavor while the darker honeys are stronger in flavor and higher in minerals and proteins (Patricia, 1996).

2.14 Changes in Honey during storage

Honey is considered to be a moderately stable foodstuff, with only slight changes in flavor, color visual aspect (crystallization) and composition taking place during several years of storage (White, 1975). The rate with which these changes occur depends on several different factors (e.g. temperature, light, oxygen, and composition of honey) (White, 1975). There are two parameters that can be used to establish the freshness of honey or can be used to judge processing and storage conditions; those are the diastase activity and the hydroxymethylfurfural content (Blasco et al., 2011; Bogdanov et al., 2008).

The use of metallic containers and some of its physicochemical properties, which are related to the floral source from which the honey has been extracted (at pH 5 or lower, total acidity, mineral content, UV light), presence of organic acids and low water activity (Wunderlin et al., 1998) observed that different metal ions had different effects in the production of HMF; for example, manganese had the highest effect, as opposed zinc, magnesium and iron. Also, this effect is greater at higher temperatures. Therefore, the use of metallic containers made from these metals can accelerate the HMF production during storage, which might lead to an excess of the recommended maximum limit. During long shipping or during long storage, darkening of honey may occur, and parallel changes in its organoleptic properties have detrimental effects on its quality, masking its original aroma, which promotes the loss of competitiveness in the World market.

The rate of darkening has been related to the composition of honey and to the storage temperature. Of the compositional factors, the ratio of glucose to fructose, the nitrogen content, free amino acids, and moisture content have been cited as possible factors determining the rate of darkening (Gonzalez et al., 2005). The darkening that occurs in honey could be due to: (a) an increment of melanoidins (Maillard reaction), (b) a combination of tannates and other oxidized polyphenols with ferrum salts; (c) the instability of fructose (caramelization reaction).

The activity of diastase is closely related to its structure and can be modified by denaturation, brought about by heating. Denaturation may be considered as a discontinuous phenomenon with various intermediate or transition states between the natural or native state and the completely denatured state (Cheftel et al., 1989). White et al., (1963) evaluated the diastase and invertase activities in honeys subjected to different temperatures. The results led them to conclude that invertase is more heat-sensitive than diastase and that the storage time has the same effect as the heating on both enzymes activity. Also, the best conditions for storing raw honey would seem to be at below 10 ºC. Sancho et al. (2001) analyzed the invertase activities of Spanish honeys over two years. Samples were stored in darkness at room temperature for up 24 months. Results showed that invertase activity had decreased with time, according to exponential (56% of samples), linear (25%), logarithmic (11%), inverse (5%) and quadratic models (3%).

One other change that can occur is spontaneous fermentation induced by osmophilic yeasts. This process depends on the initial load of microorganisms, the conditions of storage (temperature/time), and moisture content of the honey Conti and Botre, 2001). Castro-Vazquez et al. (2007) evaluated the effect of shelf-life on physicochemical parameters of sidder honeys from Pakistan. The results obtained showed that after one year at room temperature the pH, total acidity, diastase activity, HMF, proline content, electrical conductivity, invertase activity values fell within the limits prescribed for good quality, but there were significant changes.

Moreira et al. (2010) performed a study of the effect of storage under tropical conditions (temperatures ranging 35 to 40 ºC for 3 and 6 months) on in the volatile compounds of Brazilian honeys. During storage time a number of changes were evidenced, namely reduction of the carbohydrate concentration, an increment of alcohol concentration (e.g. octadecanol and benzenemethanol) due to the degradation of lipid oxidative products or by aldehyde reduction processes catalyzed by enzymes; an increment and formation of furan derivate from Maillard reaction was also reported. As previously commented, honey has several bioactive compounds with antioxidant and antibacterial properties. After 2 years the results showed a drastic reduction in the antibacterial capacities against Escherichia coli and Bacillus subtilis resistance to honey action. This reduction related to the diminished/concentrations of active phytochemicals due their sensitivities to storage conditions. The antioxidant property changes have been analyzed after of storage. Wang et al. (2015) evaluated the antioxidant capacities of clover and buckwheat honey samples after being storage for 6 months at 4 ºC and at -20 ºC. The results obtained showed that the antioxidant capacity of honeys was reduced after that storage period, with no impact of storage temperature or container type detected at the end point of the storage period. However, this does not include the impact that might have occurred during early time periods of storage.

One of the most important changes that may occur during storage is crystallization. Crystallization is a complex phenomenon, being a matter of interest of beekeepers, honey handlers, and processors. When crystallization occurs during storage in a undesirable and uncontrolled fashion, it causes the product to be cloudy and, therefore, less appealing to consumers, but it is possible to obtain a desirable product through controlled crystallization, as "creamed honey", in which there are a large number of crystals of very small size, so that they will not be perceived by the palate. There are many factors that affect crystallization, such as composition, physicochemical parameters (moisture, water activity) and the range temperature, thus between 13–23 ºC (Blasco et al., 2011). Impurities such as dust, dirt as well as air bubbles, pollen grains and bee wax particles have been reported to influence the nucleation of honey.

The rate of nucleation and crystal growth depends on temperature, with lower temperatures producing smaller crystal sizes, due to the limited mobility of the molecules (Conforti et al., 2006). A study made by Bonvehi & Coll (2003) attempted to predict the crystallization of honey with respect to the glucose composition and correlated the coefficient of super saturation (CS) of glucose in honey with crystallization. He defined the CS as the ratio of the concentration of solute (w/w) in water at a given temperature to the concentration of solute in saturated solution at the same temperature, with the value varying from 1.8 to 2.6 in honey. According to this author, honey with a CS < 1.8 will remain liquid for a long time, whereas honey with a value > 2.6 will crystallize very quickly.

2.15 Glyphosate

Glyphosate is a post-emergent, systemic and non-selective (or broad-spectrum) herbicide widely used in both agricultural and non-agricultural areas to suppress annual and perennial weeds (Coupe et al., 2011). It is effective against more than 100 annual broadleaf weed and grass species, and more than 60 perennial weed species (Dill et al., 2010). Glyphosate is reported to be manufactured by at least 91 producers in 20 countries, including 53 in China, 9 in India, 5 in the USA, and others in Australia, Canada, Cyprus, Egypt, Germany, Guatemala, Hungary, Israel, Malaysia, Mexico, Singapore, Spain, Taiwan (China), Thailand, Turkey, the United Kingdom, and Venezuela (Farm Chemicals International, 2015). It was registered in over 130 countries as of 2010 and is probably the most heavily used herbicide in the world, with an annual global production volume estimated at approximately 600 000 tonnes in 2008, rising to about 650 000 tonnes in 2011, and to 720 000 tonnes in 2012 (Dill et al., 2010; CCM International, 2011; Hilton, 2012; Transparency Market Research, 2014).

IARC's classification of Glyphosate as a probable human carcinogen means that it now meets the criteria for a Highly Hazardous Pesticide as defined by PAN International (2016) and by FAO/WHO Joint Meeting on Pesticide Management as implemented by FAO in Mozambique (Come et al., 2013). Its use in Ghana has become very popular to control weeds and 84% of rice farms are treated with herbicides as a result of its cheap price (Ragasa et al., 2013). Herbicide products containing Glyphosate is currently labeled in the United States and around the world for many purposes (Giesy et al., 2000). Reviews of Glyphosate health effects have been performed by governmental agencies

(EPA, 1993; European Commission, 2002), or by independent academics (López et al., 2012; Székács, 2012). All these reviews report conflicting opinions, especially for long-term effects of Glyphosate and its commercial formulations.

In Europe, the new Glyphosate threshold for long-term toxicity (established on rats) is 350 mg/kg bw/d, based on liver dysfunctions (Germany Rapporteur Member State, 2015). The no-observed-adverse-effect level (NOAEL) was 100 mg/kg bw/d. The new proposed Acceptable Daily Intake (ADI) was calculated from the lowest NOAEL in rabbit developmental studies (50 mg/kg bw/d). Taking into account a safety factor of 100 (10 for interspecies and 10 for interspecies variability), ADI has been calculated at 0.5 mg/kg bw/d. From the same data, the USA equivalent of the ADI, the reference dose (RfD), was calculated at 1.75 mg/kg bw/d (EPA. 2009). In this case, the LOAEL was considered to be 350 mg/kg bw/d (the NOAEL being 175 mg/kg bw/d) from rabbit teratogenicity studies.

It should be emphasized that doses used in regulatory toxicity experiments, generally ranging from 10 to 1000 mg/kg/d, are not representative of human environmental exposures, which occur at the level of µg/kg bw/d (Niemann et al., 2015). They performed a review of effects of Glyphosate and its formulations on laboratory mammals below these regulatory limits, taking into consideration all data relative to mammalian Glyphosate and Glyphosate toxicities. A literature review was performed on Science Direct and PubMed databases using the keywords "Glyphosate", "N-(phosphonomethyl) glycine" and "Roundup" (until April 2015). They also used our personal bibliography database generated by a 10-year scientific literature follow-up. We did not report short-term studies or studies with doses resulting in acute effects, in other words with doses above the regulatory threshold for long-term toxicity (350 mg/kg bw/d), because they are not a matter of debate. Indeed, ADI or RfD is clearly exceeded in some accidental and intentional exposures. This is often through handling accidents or suicide attempts by farmers. These generally one-off exposures are in the range of acute intoxication doses. The most common symptom recorded after 4000 Glyphosate accidental exposures is a mild transient gastrointestinal impairment (Roberts *et al.*, 2010). Glyphosate also affect the cardiovascular system at acute doses (Gress et al., 2014), the underlying electrophysiological mechanisms have been studied (Gress et al., 2015).

Death was strongly associated with greater age, larger ingestions and high plasma Glyphosate concentrations on admission (>734 µg/mL) (Roberts et al., 2010). Extreme exposure (around 100-200 ml of the pure formulation ingested) resulted in respiratory, heart and hepatorenal damage (Bradberry et al., 2004). In intentional ingestions (suicide attempts), up to 500 ml are ingested (Bradberry et al., 2004). In order to include some results of regulatory tests on Glyphosate alone, they used regulatory reports that served as a basis for Glyphosate commercial authorization in Europe and USA. However, we were limited by the unpublished status and confidentiality of the pre commercialization tests included in these reports. They asked the French agency for food, environmental, and occupational health and safety (ANSES) for the raw data for the health assessment of Glyphosate based herbicide and Glyphosate. Also, data on the short and long-term effects of Roundup consumption on blood parameters were lacking (Séralini et al., 2014).

For Europe, they used the German authorities' draft assessment report (DAR) on the industry studies (Germany Rapporteur Member State, 2015). As studies and raw data summarized in the DAR were not publicly available, they were not able to independently assess the studies; thus they have considered summary data. Health evaluation in the DAR was mostly based on studies provided by the Glyphosate Task Force (25 companies joining resources in order to renew the European Glyphosate registration). Some were amended by deletion of redundant parts and corrections of obvious errors (Germany Rapporteur Member State, 2015). Each new study was commented. Studies that were part of the previous EU evaluation were also subjected to reassessment according to current quality standards. A wide range of technical databases have been used for the literature search to create the DAR. This is thus the most comprehensive regulatory report, grouping results from 150 new toxicological studies and considering results of 900 publications from scientific journals, among which 200 publications were reviewed in detail. For the USA, we used the 2011 US Forest Service risk assessment on Glyphosate and the US EPA 1993 Re-registration eligibility Decision (RED) Fact Sheet (US.EPA, 1993)

Poisoning incidences of Glyphosate include**:** Many of the observations of adverse effects from exposure to Glyphosate have come from Latin America, where populations have been repeatedly exposed to the herbicide from aerial spraying campaigns to eradicate coca in Colombia and along its border with Ecuador since 1997 (Solomon et al., 2009), or for weed control in GM soybean fields in Argentina. Symptoms observed after direct exposures from aerial spraying included red eyes, dizziness, vomiting, diarrhea, abdominal pain, gastrointestinal infections, itchy skin, skin rashes and infections (particularly prevalent in children), respiratory infections, headaches, and fever. One baby was observed to have blood in its urine and kidney problems 3 months after the spraying (Oldham & Massey 2002).

In February 2001, the Health Department in Putumayo published a preliminary report on health effects in the municipalities of Orito, Valle del Guamuez, and San Miguel, which had been sprayed between December 22, 2000 and February 2, 2001. Three local hospitals reported "increased visits due to skin problems such as dermatitis, impetigo, and abscesses, as well as abdominal pain, diarrhea, gastrointestinal infections, acute respiratory infection, and conjunctivitis following spraying in the rural areas surrounding their respective municipalities"

In Argentina, numerous health effects have been linked to exposures to Glyphosate resulting from the aerial spraying of GM soybean fields, over the last 5 years. These include cancers, birth defects, lupus, kidney disease, and respiratory and skin ailments (Kruger et al., 2013). The State of California registered 202 cases of Glyphosate-related illness on their website, for the years 2000-2007. Of these, only 10 were from ingestion, the rest being unintentional occupational or bystander exposure; 94 were caused by non-agricultural uses.

2.15.1 Glyphosate Use/Application

The use of herbicides in agriculture has over the years contributed tremendously to both food and cash crop production all over the world of which Ghana is not an exception. But one of the challenges undermining the farming business (Ntow et al., 2006), has been the invasion of many common weed species due to favorable environmental conditions such as abundance of rainfall, adequate sunlight, fertile soil etc. in Ghana. It is estimated that Glyphosate use has increased globally almost 15-fold between 1994 and 2014 (Table 2.2), with the increasing usage even more evident in the USA. See table 2.3. Benbrook (2016) showed that the amount of Glyphosate active ingredient (a.i.) applied ranged between 0.53 kg a.i. /ha on cropland globally and 1.0 kg a.i. /ha in crop-land in the U.S.

Table 2.2 Global application of Glyphosate (active ingredient)

	1994	1995	2000	2005	2010	2012	2014
Glyphosate use (tonnes)	56,296	67,078	193,485	402,350	652,486	718,600	825,804
Agriculture	42,868	51,078	155,367	339,790	578,124	648,638	746,580
Non-Agriculture	13,428	16,000	38,118	62,560	74,362	69,962	79,224

Data was cited by Benbrook (2016)

Table 2.3 Application of Glyphosate (active ingredient) in the United States

	1974	1982	1990	1995	2000	2005	2010	2012	2014
Glyphosate use (tonnes)	635	3,538	5,761	18,144	44,679	81,506	118,298	118,753	125,384
Agriculture	363	2268	3357	12,274	35,720	71,441	106,963	107,192	113,356
Non-Agriculture	272	1270	2404	5,670	8,958	10,065	11,335	11,562	12,029

Data from Benbrook (2016). Sourced from National Agriculture Statistical Service Pesticide use data and the Environmental Protection Agency Pesticide Industry and use reports.

Table 2.4 Types of Glyphosate in Wa market

PESTICIDE CATEGORY	NON SELECTIVE
HERBICIDE	GLYPHOSATE 1. Sunphosate 2. Glyphader 480 (LDC) 3. Cutout 4. Flysate 5. Force-up 6. Landlord 7. Rival 8. Kondem 9. Sarosate 10. Borizaa 11. Aqua Wura 12. Adwuma Wura 13. Destroyer 14. Sharp 15. Kalach 16. Forceup 17. Flysate 18. Adwumaye 19. Glystar 20. Adwura 21. Roundup

Source: Researcher Wa Market Survey 2017

Table 2.5: Glyphosate found in honey samples collected from the local market around the world

Samples	Glyphosate found(ng/g)a	Source
Wild flower honey	Trace	GA, USA
Organic honey	17	Brazil
Orange blossom honey	Trace	FL, USA
Clover honey	26	GA, USA
Orange blossom honey	21	USA
Clover honey	40	USA
Clover honey	Trace	Canada
Wild flower honey/miel	46	Canada
Bonne Maman honey	Trace	Unknown
Munaka honey	Trace	New Zealand
Honey	Trace	France
Billy bee honey	19	Canada
Honey	Trace	Ivory Coast
Honey blend with fructose/flavor	Trace	Taiwan
Local honey	Trace	GA, USA
Organic honey	Trace	TX, USA
Honey from Florida	24	FL, USA
Honey from Los Angeles	121	LA, USA
Honey from Iowa	35	IA, USA

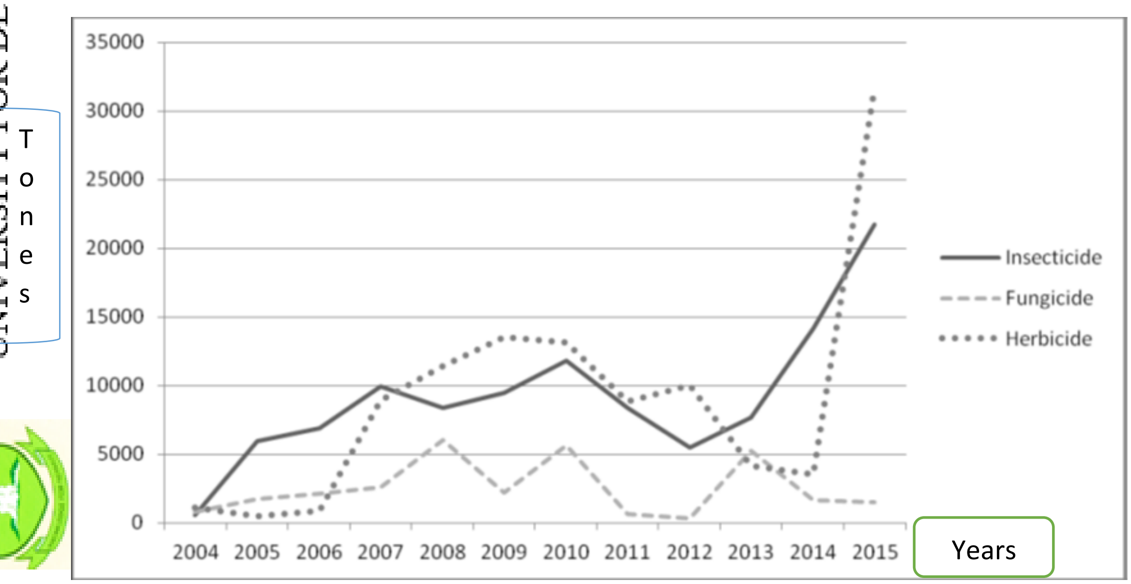
A trace= amount found less 16 ng/g (estimated LOQ)

Source: U.S. Food and Drug Administration, 2015

2.15.2 Trends in volumes of pesticides imported into Ghana

From 2004 to 2015, Ghana imported an average of 9,216 tons of insecticides, 8,986 tons of herbicides and 2,545 tons of fungicides (Figure 2.1). From 2004 to 2007, imported herbicides and fungicides were lower than that of insecticides at the same period (Fig 2.1). From 2007, import of herbicides has increased and exceeded that of insecticides and fungicides and continues to progress with declining imports noticed from 2012 to 2014. The import of fungicides is lower than that of insecticides and herbicides throughout the period 2004 to 2015. The increase in the importation of herbicides could be explained mainly by the reduction of labor force for agriculture in general and the mastery of the application of plant protection products by farmers.

Figure 2.1 Quantity (tons) of pesticides imported into Ghana from 2004 to 2015



Source: EPA, PPRSD / MOFA, 2015

2.15.3 The concept of Glyphosate contamination in honey through consumption.

In recent years, the worldwide consumption of all kinds of honey has increased due the potentially beneficial compounds that it contains according to the floral origin. Improvements in taste and consumer habits are also important. Honey production is carried out throughout the country, but the highest concentration of hives is in the South area of Ghana. Honey is a product formed through the foraging activities of bees which are the main pollinators in agricultural crop fields (Andrews et al., 2004; Bardy et al., 2008).

Bees make honey from nectar that they collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature from flowers and other plants from crop fields. After visiting a flower, the foraging honeybee flies back to her nest that may be in a hollow tree or other natural cavity, or inside a man-made hive. Bees commonly forage on flowers within two kilometers of their nest, although they can travel much further. Bees have been recorded foraging 14.4 kilometers from their home, and foraging distances of five kilometers are common (Ratnieks, 2002). Glyphosate is obtained from the market for application mainly on crop farms. After spraying, crops and water bodies may be contaminated with Glyphosate. The worker bee that visits the flowers of the crops for nectar is then exposed to the chemical Glyphosate. The ripped honey when harvested is consumed directly as spread, eating raw, and beverage or indirectly as additive in other food and drugs products.

According to a recent study, in which 69 honey samples were analyzed, 59% contained Glyphosate levels above the limit of quantitation (17-163 µg/kg, mean 61 µg/kg). Furthermore, 22 samples (32%) exceed the EU limit for Glyphosate for non-organic honey (50 µg/kg). (Rubio et al., 2014)

In other words, every third honey in that study contained too high amounts of Glyphosate and would not be marketable in the EU. Regarding other measurements in which 103 honey samples were analyzed, 55.3% contained Glyphosate levels above the limit of quantitation (LOQ: 10 µg/kg). Furthermore 25% exceed the EU limit (Rubio et al., 2014)

2.16 Estimation of average dose of Glyphosate received through consumption of honey

Different countries have established a range of "acceptable" daily intake levels of Glyphosate-herbicide exposures for humans, generally referred to in the U.S. as the chronic Reference Dose (cRfD), or in the E.U. as the Acceptable Daily Intake (ADI). The current U.S. Environmental Protection Agency (EPA) cRfD is 1.75 mg of Glyphosate per kilogram body weight per day (mg/kg/day). In contrast, the current E.U. ADI is more than 5-fold lower at 0.3 mg/kg/day, a level adopted in 2002 (Myers et al., 2016).

The data upon which these exposure thresholds are based were supplied by manufacturers during the registration process, are considered proprietary, and are typically not available for independent review. The German Federal Institute for Risk Assessment is the lead regulatory authority currently conducting an E.U.-wide reassessment of GBHs. Their renewal assessment report calls for an increase of the E.U. ADI from 0.3 mg/kg/day to 0.5 mg/kg/day. However, from an analysis of their assessment, it is difficult to understand the basis on which the German regulators are making this recommendation, since they still rely on the same proprietary, industry-supplied dataset that led to setting a lower ADI (0.3 mg/kg/day) in 2002 (Myers et al., 2016). In contrast, an international team of independent scientists concluded that the current E.U. ADI is probably at least three-fold too high, based on a transparent, fully documented review of the same dataset.

In December 2009, the U.S. EPA's re-registration review of Glyphosate identified a number of issues of ongoing concern, as well as GBH data gaps in particular, it noted that data relating to the effects of GBHs on the immune and neurological systems were limited and announced that future registrants would be required to conduct both neurotoxicity and immune toxicity studies. The U.S. EPA's updated risk assessment and final re-registration decision on GBHs is scheduled to be completed in 2015–2016.

In the EFSA Conclusion on the peer review for iprodione (EFSA, 2016) new toxicological reference values were established. The ADI was lowered from 0.06 mg/kg bw to 0.02 mg/kg bw and a new Acute Reference Dose (ARfD) of 0.06 mg/kg bw was established which previously was not deemed necessary. An updated review report with the new endpoints has been presented to the Standing Committee on Plants, Animals, Food and Feed (PAFF) section pesticides legislation on 22 March 2018 for endorsement. As noted above, most GBH use has occurred in the last 10 years, while most studies considered by regulatory agencies for the assessment of GBHs focused just on the active ingredient, and were conducted in the 1970s through mid-1980s. Since the late 1980s, only a few studies relevant to identifying and quantifying human health risks have been submitted to the U.S. EPA and incorporated in the agency's GBH human-health risk assessment.

2.17 Adulteration of Honey

Honey is one of humankind's oldest food products. It contains a number of nutritionally important substances that support good health and recovery. It is a characteristic sugary foodstuff; according to current regulations, apart from other forms of honey no other substances or additives can be added to it (Codex Alimentarius 2001). The fact that honey contains antioxidants, minerals, vitamins and proteins makes it an appealing ingredient compared to artificial sweeteners (Serem & Bester, 2012). This has triggered a demand for honey amongst health consciousness consumers (Serem & Bester, 2012).

There is also a small number of small and medium enterprises (SMEs) that use honey as an ingredient in the making of lotions, hair pomade, soap, baby products, yogurts and drinks (ITC, 2015). The demand for honey is based on a range of taste and preference criteria set in the mind of consumers who either reject or accept particular types of honey (Ghorbani and Khajehroshanaee, 2009; Ahmed et al., 2013). Honey adulteration is a topical issue because increasingly sophisticated adulteration methods are constantly being developed and the official (legislative) determination of the quality indicators of honey is unable to detect most methods of honey adulteration.

In addition, while the popularity among consumers is constantly growing, the worldwide production of honey is unstable. Increasing environmental pollution and spread of diseases has led to a decrease in global honeybee populations. This fact coupled with a higher demand means that honey is becoming an increasingly scarce commodity and consequently, honey adulteration is on the rise. In a study on consumer consumption patterns influencing honey demand, honey quality was identified as a major driver of demand (Serem and Bester, 2012). Quality was described based on characteristics such as sweetness, smoothness, taste, granulated, color and presence or absence of impurities (Serem and Bester, 2012).

Food quality for honey means being healthy and safe for consumption, with high nutritional value and quality packaging (Serem and Bester, 2012). Honey adulteration can be direct or indirect. Direct adulteration means that a substance is added directly to honey. Indirect adulteration happens when honeybees are fed adulterating substance. Plants that are sources of substances used for honey adulteration can be classified as C3 or C4 plants, based on their carbon metabolism. The C3 plants fix atmospheric CO2 using the Calvin (C3) cycle, and they have a lower 13C/12C ratio than C4 plants that fix CO2 using the Hatch-Slack (C4) cycle. Most of the honey-contributing plants like rice, wheat and beet are C3 plants whereas maize and sugarcane are C4 plants.

In Ghana, consumer's perception to adulteration of honey is that, producers or venders mainly adulterates honey using sugars from C4 plants like sugar from sugar cane and many others including melting of mattresses with table sugar.

Direct adulteration is the addition of foreign substances directly to honey. Methods of detecting direct adulteration is often the traditional analyses of chemical composition and physical properties of honey are commonly used to detect direct adulteration. They are routinely applied in the honey trade but these analytical methods are relatively time-consuming and require tedious preparation of the samples as well as complex analytical equipment. Honey adulteration can also be detected using several modern methods such as measuring stable carbon-isotope ratios, NMR or differential calorimetry. Much attention has been paid to measuring major sugars in honey with gas chromatography (GC) and liquid chromatography coupled to various types of detectors (Bogdanov et al. 2005). Indirect adulteration of honey is accomplished by feeding honeybees with industrial sugars at the stage when broods become naturally available. Such indirect adulteration is extremely difficult to detect.

In Ghana, there is general lack of records and documentation on honey production resulting into data gaps. Akangaamkum *et al.* (2010) however suggested an increasing trend in honey production in Ghana. The domestic production and demand gap in Ghana is consequently being bridged by high foreign imports of honey. There is therefore huge domestic market for honey in Ghana which needs to be exploited.

2.18 Characterization of Glyphosate in honey and its associated health risk

Risk assessment: A scientifically based process consisting of the following steps: 1) hazard identification, 2) hazard characterization, 3) exposure assessment and 4) risk characterization. (IPCS, 2004). Risk Characterization is the final phase of the health risk assessment process. It integrates the three phases; Hazard Identification, Dose-Response Assessment, and Exposure Assessment. For there to be a risk there must be both the hazard and the exposure to that hazard present at the same time. Risk = hazard + exposure. Risk assessment is a process by which scientists evaluate the potential for adverse health or environmental effects from exposure to naturally occurring or synthetic agents.

These agents include; chemicals such as those that occur in food naturally, food additives, drugs, and environmental contaminants, and physical agents, such as radiation or electromagnetic fields (IPCS, 2004). Risk Assessments are used to characterize the nature and magnitude of health risks to humans; e.g., residents, workers, recreational visitors) and ecological receptors e.g., birds, fish, wildlife. Risk managers use this information to help them decide how to protect humans and the environment from stressors or contaminants Alqarni et al., (2012).

2.18.1 Hazard identification

As asserted by Alqarni et al., (2012) is the process of determining whether exposure to a stressor can cause an increase in the incidence of specific adverse health effects; e.g., cancer, birth defects, asthma, cardiovascular disease and whether the adverse health effect is likely to occur in humans. Hazard Identification determines the types of health problems a chemical could cause by reviewing studies of its effects in humans and laboratory animals.

2.18.2 Dose-Response

Dose–response assessment approaches generally take one of two forms: 1) analyses that provide a quantitative (or sometimes just qualitative) estimation of risk and 2) analyses that establish health-based guidance values, such as an acceptable daily intake (ADI) or tolerable daily intake (TDI), which are levels of human exposure considered to be without appreciable health risk. The latter approach, which is often described as "safety assessment", is used more often in cases where exposure can be controlled, such as for food additives and residues of pesticides and veterinary drugs in foods (IPSC, 2009). One of the primary criteria of a risk assessment is determination of the presence or absence of a cause–effect relationship. If there is sufficient plausibility for the presence of such a relationship, then dose– response data are essential, and dose–response analysis is a major part of the hazard characterization within the risk assessment paradigm. This is determined by evaluating scientific data on how a response occurs (IPSC, 2009). The response determines the nature of the extrapolation used in the second step of the process discussed above, either through non-linear or linear dose-response assessment. Reference Dose (RfD) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfD is generally expressed: mg/kg/day (IPCS, 2009)

A reference concentration (RfC) is used to assess inhalation risks, where concentration refers to levels in the air mg/m3: RfD = NOAEL (or LOAEL or BMDL) / UFs. The estimated daily intake (EDI – mg/kg bw/day): It provides an estimate of expected dietary exposure and is calculated as follows: EDI = Σ Fi x STMRi/ bw, Fi = intake of a given food commodity (kg/person/day) and STMRi = standard trial median residue corresponding to that food commodity (mg/kg), bw = body weight (60kg usually chosen). Acceptable daily intake (ADI – mg/kg bw/day) for a given compound is derived from the toxicological database. The ADI is based on the lowest no effect concentration of the most sensitive species from a range of sub-chronic/chronic studies, to which appropriate safety factors are applied.

2.18.3 Exposure Assessment

Exposure assessment is an essential element for quantifying risk. The role of dietary exposure assessment has been central to the work of the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in performing risk assessments on chemicals in foods. The Codex Alimentarius Commission's (CAC) Procedural Manual (FAO/WHO, 2008) defines exposure assessment as "the qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant". This chapter deals with the assessment of dietary exposure to chemicals present in food (i.e. food additives, contaminants, processing aids, nutrients and residues of pesticides and veterinary drugs). Dietary exposure assessment combines food consumption data with data on the concentration of chemicals in food. The resulting dietary exposure estimate may then be compared with the relevant health based guidance value for the food chemical of concern, if available, as part of the risk characterization. Assessments may be undertaken for acute or chronic exposures, where acute exposure covers a period of up to 24 h and long-term exposure covers average daily exposure over the entire lifetime.

The general equation for both acute and chronic dietary exposure is:

$$\text{Dietary exposure} = \frac{\Sigma\,(\text{Concentration of chemical in food} \times \text{Food consumption})}{\text{Body weight (kg)}}$$

This is done to determine how long people were exposed, how much of the chemical they were exposed, whether the exposure was continuous or intermittent; and how people were exposed. All of this information is combined with factors such as breathing rates, water consumption, and daily activity patterns to estimate how much of the chemical was taken into the bodies of those exposed.

2.18.4 Risk Characterizations

Risk characterization is the fourth step of the risk assessment process, integrating information from the hazard characterization and the exposure assessment to produce scientific advice for risk managers (Renwick et al., 2003). The Codex Alimentarius Commission (CAC) has defined risk characterization as "The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment" (FAO/WHO, 2008).

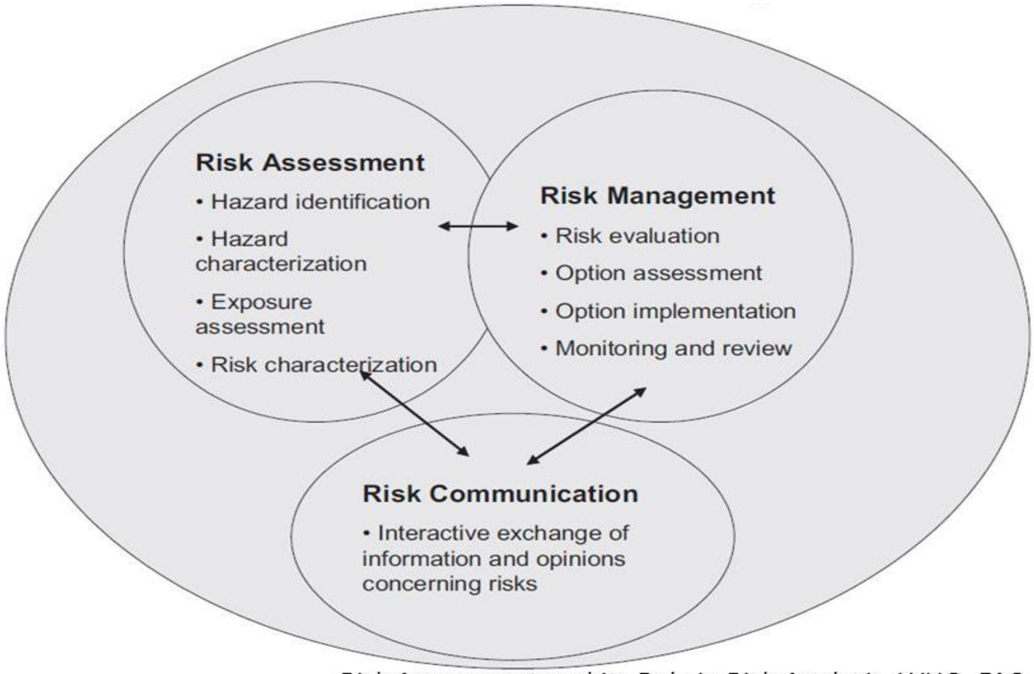
Transparency, Clarity, Consistency, and Reasonableness are key to consider in risk. In risk characterization all relevant information pertaining to the decision at hand, including such factors as the Nature and weight of evidence for each step of the process, the estimated uncertainty of the component parts, and the distribution of risk across various sectors of the population, the assumptions contained within the estimates and others are necessary to manage risk.

2.19 Conceptual Framework

The framework provides the pillar on which this study has been fixed, just as blocks sit on the foundation of a building. The framework helps to shape and guide the research to a logical conclusion. The linkages among these key concepts help to quantify and qualify the risk to exposure.

Risk analysis has been defined by Codex Alimentarius Commission (CAC) as "a process consisting of three components: risk assessment, risk management and risk communication", which are themselves defined as follows (FAO/WHO, 2008).

Figure 2.2: Risk Analysis Model



Risk Assessment and its Role in Risk Analysis, WHO, FAO, 2009

Risk assessment (in particular in the food context, also often called "safety assessment"), comprise four steps of hazard identification, hazard characterization (including dose–response assessment), exposure assessment and risk characterization. In this monograph, the terms "risk assessment" and "safety assessment" are used interchangeably.

Risk assessment can include a key component in which the probability of harm is estimated. As a probability calculation, a risk assessment will include both a statement of the nature of the harm and the basis for the assertion that the harm may occur (i.e. the probability). The risk assessment is followed by either a risk management decision or a request for further analysis, which may influence any further research that is conducted. The record produced by a risk assessment stands as a scientific basis for any risk management decision at that time. However, the risk assessment or risk analysis may be reopened for example, if additional information becomes available.

Hazard identification is defined as follows (IPCS, 2004): The identification of the type and nature of adverse effects that an agent has an inherent capacity to cause in an organism, system, or (sub) population. Hazard identification is the first stage in hazard assessment and the first of four steps in risk assessment. The purpose of food chemical hazard identification is to evaluate the weight of evidence for adverse health effects, based on assessment of all available data on toxicity and mode of action. It is designed to primarily address two questions: 1) the nature of any health hazard to humans that an agent may pose and 2) the circumstances under which an identified hazard may be expressed. Hazard identification is based on analyses of a variety of data, ranging from observations in humans or domestic animals and studies in laboratory animals and in vitro laboratory studies through to analysis of structure–activity relationships. From the range of studies and observations available, the nature of any toxicity or adverse health effects occurring and the affected target organs or target tissues are identified.

Hazard characterization is defined as follows (IPCS, 2004): The qualitative and, wherever possible, quantitative description of the inherent properties of an agent or situation having the potential to cause adverse effects. This should, where possible, include a dose–response assessment and its attendant uncertainties. Hazard characterization is the second stage in the process of hazard assessment and the second of four steps in risk assessment. Hazard characterization describes the relationship between the administered dose of, or exposure to, a chemical and the incidence of an adverse health effect. The critical effect that is, the first adverse effect observed as the dose or exposure is increased is determined. In cases where the toxic effect is assumed to have a threshold, hazard characterization usually results in the establishment of health based guidance values for example, an acceptable daily intake (ADI) for additives or residues or a tolerable intake (TI) for contaminants. For some substances used as food additives, the ADI may not need to be specified; in other words, no numerical ADI is considered necessary. This may be the case when a substance is assessed to be of very low toxicity, based on the biological and toxicological data, and the total dietary intake of the substance, arising from the levels used in foods to achieve the desired function, does not represent a hazard.

Exposure assessment is defined by IPCS (2004) as follows: "Evaluation of the exposure of an organism, system, or (sub) population to an agent (and its derivatives). Exposure assessment is the third step in the process of risk assessment." According to CAC, the exposure assessment of food chemicals may be described more narrowly as "The qualitative and/or quantitative evaluation of the likely intake of chemical agents via food as well as exposure from other sources if relevant" (FAO/WHO, 2008). In the case of food chemicals, dietary exposure assessment takes into consideration the occurrence and concentrations of the chemical in the diet, the consumption patterns of the foods containing the chemical and the likelihood of consumers eating large amounts of the foods in question (high consumers) and of the chemical being present in these foods at high levels. Usually a range of intake or exposure estimates will be provided (e.g. for average consumers and for high consumers), and estimates may be broken down by subgroup of the population (e.g. infants, children, adults).

Risk characterization is defined by IPCS (2004) as follows: The qualitative and, wherever possible, quantitative determination, including attendant uncertainties, of the probability of occurrence of known and potential adverse effects of an agent in a given organism, system, or (sub)- population, under defined exposure conditions. Risk characterization is the fourth step in the risk assessment process.

This definition of the final step of risk assessment is, if restricted to the population of consumers only, practically identical to the one agreed to and used by Codex (FAO/WHO, 2008). In risk characterization, the information from the intake or exposure assessment and the hazard characterization is integrated into advice suitable for decision-making in risk management. Risk characterization provides estimates of the potential risk to human health under different exposure scenarios. It should include all key assumptions and describe the nature, relevance and magnitude of any risks to human health.

The information and advice provided to risk managers may be qualitative or quantitative. Qualitative information may include:

● Statements or evidence that the chemical is of no toxicological concern owing to the absence of toxicity even at high exposure levels;

● Statements or evidence that the chemical is safe in the context of specified uses; and

● Recommendations to avoid, minimize or reduce exposure. Quantitative information may include:

● Comparison of dietary exposures with health-based guidance values; estimates of risks at different levels of dietary exposure;

● Risks at minimum and maximum dietary intakes (e.g. nutrients); and

● Margins of exposure.

The risk characterization statement should include a clear explanation of any uncertainties in the risk assessment resulting from gaps in the science base. It should also include, where relevant, information on susceptible subpopulations, including those with greater potential exposure or specific predisposing physiological conditions or genetic factors. The advice to risk managers can be in the form of a comparison of the relative risks among risk management options.

Codex has adopted the following definitions for hazard and risk in relation to food that cover not only chemical agents, but also biological and physical agents:

- Hazard: A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect.
- Risk: A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food.

Risk management: The process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices and, if needed, selecting appropriate prevention and control options (IPCS, 2004).

Risk communication: The interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions (IPCS, 2004). Communication during emergencies is often very different from communication under non-emergency circumstances. Communication needs to occur frequently during an emergency because there is usually an urgent demand from various stakeholders for timely up-to-date situation reports. Often such communication messages need to be developed in a very short time frame and in consultation with a wider range of agencies than in normal situations. All risk communication should be coordinated through one individual or office, to ensure consistency of messaging and to avoid confusion.

CHAPTER THREE

METHODOLOGY

3.1 Introduction

Chapter three has two central segments; segment one discussed the profile of Wa municipality, the sampled communities and segment two discussed the procedural issues involved in the study. It begins by defining the broad paradigm within which the study is situated. The methodology entails detailed discussion how, when, and which data was collected, analyzed and presented. This section further discusses the approach that was used to address the research questions. These methods are captured as the research approach, design, sampling and sampling technique, sample size, instrumentation, and source of data, data collection procedure and analysis.

3.2 Profile of Wa municipal.

The Upper West Region is situated in the north-western part of Ghana. It lies between longitude 1º 25'W and 2º 45' W and latitudes 9º 30' N and 11º N. It is bordered to the south by the Northern region, to the north and West by Burkina Faso, to the east by the Upper East region. It covers a geographical area of 18.476 sq. km, which constitutes 12.7 percent of the total land area of Ghana (GSS, 2010).

Wa Municipality is located in Upper West Region. It is one of the eleven administrative municipals/districts in the Upper West Region (UWR) of Ghana. The Wa Municipality shares administrative boundaries with Nadowli-Kaleo District to the north, Wa East District to the east and to the west and the south Wa- West District. It lies within latitudes 9º30'N to 2º28'N and longitudes 2º33''W to 10º20'W. Wa Municipality has its capital as Wa, which also serves as the Regional capital of Upper West Region. It has a land area of approximately 579.86 square kilometers, which is about 6.4% of the Region. The population of Wa Municipal, according to the 2010 Population and Housing Census, is 107,214 representing 15.3 percent of the region's total population. Males constitute 49.7 percent and females represent 50.6 percent. About 34+ percent of the population reside in rural localities (GSS, 2010).

About 54.8 percent of the population aged 15 years and older are economically active and 45.2 percent are economically not active. Of the economically active population, 91.5 percent are employed while 8.5 percent are unemployed. For those who are economically not active, a larger percentage of them are students (66.6%), 14.8% perform household duties and 9.9 percent are either too young or old to work. Five out of ten (51.5%) of unemployed persons in the Municipal are seeking work for the first time (GSS, Population and Housing Census, 2010). On occupation, of the employed population, the highest proportion (29.3%) is engaged as skilled agricultural, forestry and fishery workers. About 25.7 percent are engaged in service and sales; 18.5 percent in craft and related trades, 8.5 percent are engaged as professionals (GSS, 2010).

The 2010 Population and Housing Census show that 80.4 percent of the people in the Wa Municipality belong to the Mole-Dagbani group which comprises the Waalas who are the indigenous people, Dagaabas and the Sissalas. There have been considerable inter-marriages between the Waalas, Dagaabas and the Sissalas. Other ethnic groups found in the Municipality include the Frafra, Akan, Ewe, Ga, Dagomba, Grushi, Gonja and Moshies who are engaged in secular work and commercial activities. The total age dependency ratio for the Municipality is 65.1, the age dependency ratio for rural localities is higher (77.5) than that of urban localities (59.4) (GSS, Population and Housing Census, 2010).

About 30.9 percent of households in the Municipality are engage in agriculture. In the rural localities, about 58 percent of households are agricultural households while in the urban localities, 20.3 percent of households are into agriculture. Most (82.9%) of the agricultural households in the Municipality are involved in crop farming (GSS, Population and Housing Census, 2010). Under the agriculture sector, most of the farmers are engaged in peasant farming and the main crops grown include millet, sorghum, maize, rice, cowpea and groundnut cultivated on subsistence basis. However, soya beans, groundnuts, Bambara beans are produced as cash crops.

The vegetation is one of the guinea savannah grassland type, made up of short trees with little or no canopy and shrubs of varying heights and luxuriance, with grass ground cover in the wet season. Commonly occurring trees are Shea, Dawadawa, Kapok and Baobab. Cashew and Mango are exotic species that grow well in the area making the bee keeping encouraging (GSS, 2010). Wa Municipality has two marked seasons, namely, the wet and dry seasons. The South Western Monsoon winds from the Atlantic Ocean bring rains between April and October, while the North-Eastern Trade winds from the Sahara Desert bring the long dry season between November and March (GSS, 2010).

Wa Municipality has over twenty communities with one urban town (Wa), there are other peri-urban communities (Kperisi, Bamahu, Kpongu and Charia) where this study was undertaken. Wa town was selected because of the larger population size that has most consumers in the municipal. It has the largest market of honey with many businesses. These communities were selected for consumption of honey study due to the cosmopolitan nature and the purchasing power of honey consumers revealed in preliminary field study. These communities are classified as peri-urban in the Wa Municipality with Wa being the only city in the municipality (GSS, 2010).

Consumption of honey is on rise in the peri-urban and urban centers due to their economic status. These five communities have most population of the municipal including tertiary and other students, public and private sector workers as well as most literate populates in the municipality who consume honey. The municipal is described as cosmopolitan due to the numerous educational, economic, agricultural, public and private activities ongoing there with many different people living there. This study revealed that production of honey if done in the rural areas with less consumption there because; most beekeepers and processors prefer to sell their produce for income in the urban and peri-urban communities in the municipality.

The Fadama market is a subsidiary of the Wa market where ten different honey samples were bought. Five honey samples were from apiary source sold in the market and the rest were from wild source. These samples were collected in both rainy and dry seasons in the Wa and Fadama markets area in March and June. The origin of the ten different samples include; Sagu, Sombo, Jonga, Danko, Chansa, Busa, Piisi, Sing, Dandafuoro and Buosooyiri) (See Figure 3.1 which shows the map of the Wa municipal).

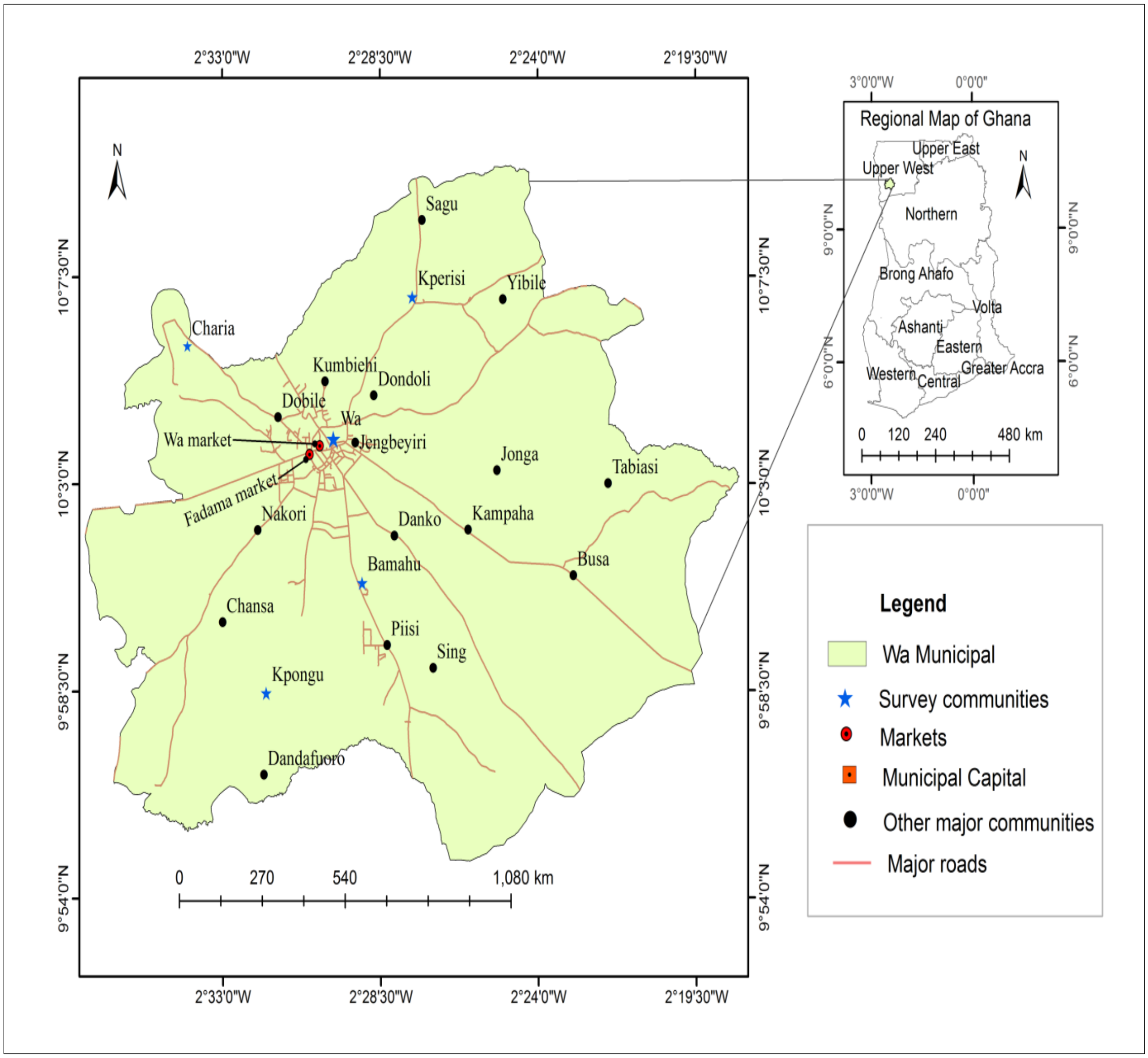


Figure 3.1: The Map of Wa Municipality

Source: GIS Revolution and I.T Consult, (2017)

3.3. Research Approach

The study adopted a mixed method approach of two main phases. The first phase of the study is on laboratory experimental analysis of honey sold on Wa market. This involves testing for Glyphosate residue and physicochemical feature of honey obtained. Ten (10) different honey vendors were identified through preliminary field survey. A sample each was purchased from each vendor labelled (A, B, C, D, E, F, G, H, I, & J) and analyzed. All the samples (Five wild and five apiary honeys) obtained from vendors were from different locations of the Wa Municipal and at particular season, and type. The five (5) apiary honeys were obtained from beekeepers through honey vendors in the market. The other five (5) wild honeys were directly obtained from vendors in the market after verifying from them on its type.

The second phase involved a survey on honey consumers, processors and vendors on honey consumption, determining consumer knowledge and awareness of Glyphosate herbicide in honey. Apart from that, this phase also estimates the amount of honey consumed in the Wa Municipality so as to provide data to help in the characterization of health risks. Information sought included consumer's age, diet history (especially honey), geographic location and participation in activities that might increase chances of exposure to Glyphosate herbicide.

3.4 Sampling

There are two categories of respondents in this study. The first category involved consumers of honey in both peri-urban communities (Kperisi, Kpongu, Bamahu and Charia) and urban (Wa) community of Wa municipality. A total number of 107,214 people represent the target population from Ghana Statistical Service population census (GSS, 2010) for Wa Municipal representing 15.3

percent of the region's total population. Males constituted 49.7 percent and females represent 50.3 percent.

This is employed as the sample frame for the study in the municipal. Furthermore, a mathematical method by Yamane (1967) was employed to determine the sample size. Sample size (n) = N/ (1+Ne²). n=sample size, N=107,214, e=0.05, confidence level of 95% n= (400). A total number of 400 respondents were sampled using accidental sampling method with Yamane (1967) formula to obtained consumers of honey after validating their consumption status with a check list. This represented the sample size of the survey.

The second category of respondents comprises honey vendors and processors. From preliminary field data which indicated ten (10) vendors in the Wa market, ten samples of 500ml each were obtained from these vendors in the Wa Fadama and Wa Kejetia markets in the months of March and June, 2017 through a snow balling method. Five wild honey samples were obtained from Fadama and other five apiary honey were obtained from Kejetia. All samples were prepared for laboratory analysis at Ghana Standards Authority, Accra

Table 3.1. Classification of Sampled Honey

Sample	Vendor	Market	Location/Source	Month obtained	Season	Honey type	Quantity bought
A	V1	Wa Fadama	Sagu	March	Dry	Wild	1 Bottle (500ml)
B	V2	Wa Fadama	Sombo	March	Dry	Wild	1 Bottle (500ml)
C	V3	Wa Fadama	Jonga	March	Dry	Wild	1 Bottle (500ml)
D	V4	Wa Fadama	Danko	March	Dry	Wild	1 Bottle (500ml)
E	V5	Wa Fadama	Chansa	March	Dry	Wild	1 Bottle (500ml)
F	V6	Wa Kejetia	Busa	June	Rainy	Apiary	1 Bottle (500ml)
G	V6	Wa Kejetia	Piisi	June	Rainy	Apiary	1 Bottle (500ml)
H	V8	Wa Kejetia	Sing	June	Rainy	Apiary	1 Bottle (500ml)
I	V9	Wa Kejetia	Dandafuoro	June	Rainy	Apiary	1 Bottle (500ml)
J	V10	Wa Kejetia	Buosooyiri	June	Rainy	Apiary	1 Bottle (500ml)

Source: Survey Field Data on Classification of Sampled Honey, 2017

3.5. Methods of Data Collection

Two main methods of data collection were employed. Snow balling to purchase honey samples for laboratory analysis and administration of survey questionnaires (Semi-structured) to consumers, vendors and processors. In each community, data collection started by accidentally selecting consumers of honey for interview after verifying respondents eligibility from a check list. During observations, pictures were taken to support the analysis of data. Four main tools/instruments were used in the data collection process; interview guide, field notebooks, digital camera and audio tape recorder. A pretested Interview guides was used to facilitate all surveys (See appendix I). Also, tape recorders were used to record some key open ended questions during interviews for later transcription. Key notes were also taken alongside the audio recordings to ensure data security.

Finally, field pictures were taken with a digital camera to support the data analysis and discussions. Creswell (2013) recommends the use of flexible tools/instruments such as interview guides/checklist for gathering in-depth qualitative data, as they can be modified at each stage of the process to incorporate field experiences and observation for the purpose of collecting relevant in-depth information.

Questionnaires were administered to the consumers, processors and vendors and data obtained on consumption of honey, diet history in order to check quality of honey consumed. The questionnaire was structured based on the research questions, which is reflective of the objectives of the study and provided primary data from the sampled consumers, processors and vendors for both quantitative and qualitative sections of the study.

Data were gathered from scientific publications identified using PubMed, a web-based database that comprises over 23 million citations for biomedical literature as secondary source of data

3.6. Laboratory Procedures

Ten honey samples were bought directly from different honey vendors in 2017, to test for some Glyphosate residues in honey and some physicochemical features. Five out of the ten were from wild source and the other from bee keepers (apiary honey) in either the dry season or rainy season. Basically the samples were labeled (A, B, C, D, E, F, G, H, I, J). Samples were transferred into sterilized and plastic bottles containers at room temperature (20°C). Before the samples were transferred into the sterilized plastic bottles, dead bees and other debris large and visible enough were removed by hand picking. All samples were analyzed in duplicates with the average recorded for analysis on physicochemical parameters (pH, Moisture, Refractive Index, Total Soluble Solids and Total Solid) of honey.

3.7 Analysis of Glyphosate

3.7.1. Gas Chromatography

The samples obtained were extracted and analyzed for Glyphosate pesticides residues using standard method. Varian CP-3800 Gas Chromatograph with a CombiPAL Auto sampler with Pulse Flame Photometric Detector because it has been proposed that it is among the most efficient technique available to analysts (Wiest et al., 2011). The limit of detection (LOD) and the limit of quantification (LOQ) for Glyphosate analyzed on the LC-MS/MS were 0.01 mg kg−1 and 0.03 mg kg−1, respectively. The limit of quantification (LOQ) was determined with a linear range. It is demonstrated that this method is reliable and sensitive for the analysis of Glyphosate and other pesticides with low concentrations in foods (honey).

3.7.2. Honey Sample Preparation for Glyphosate analysis

Ten (10) honey samples from Wa market were obtained from apiary as well as wild source from vendors. The samples were put in plastic bottles and wrapped with aluminium foil before they were taken to the laboratory. All samples were kept at ambient temperature until the analysis

3.7.3. Extraction Procedure for Glyphosate analysis

In order to analyze a number of pesticide residues in honey, QuECHER method, a multi residue method for the analysis of pesticide residue in low fat matrix was used. This method consists in two steps, liquid-liquid extraction and purification by dispersive Solid Phase Extraction. QuEChERS is an acronym which refers to Quick, Easy, Cheap, Efficient, Rugged and Safe (Wiest et al., 2011). The QuEChERS Method is a streamlined approach that makes it easier and less expensive for analytical chemists to examine pesticides residues in food sample preparation technique followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed and initially validated according to the SANTE/11813/2017 guidance "Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed". A 5 g of the homogenized honey sample was weighed in an Erlenmeyer flask and spiked with 100 µL of the internal pesticide standard solution and mixed with 10 ml of water and homogenized by shaking to reduce its viscosity and facilitate its handling. The sample was mixed with 10 ml of acetonitrile solvents tested and subjected to extraction by agitating for 3 minutes. A mixture of salts (1 g sodium chloride, 1 g disodium hydrogencitrate sesquihydrate, 0.5 g trisodium citrate dehydrate and 4 g anhydrous magnesium sulphate) were added to the sample and vortex for 3 minutes for extraction with separation.

The organic phase was separated from the inorganic phase by centrifugation at 3000 rpm for 5 minutes. The supernatant was collected and the residue was re-extracted with 10 ml of acetonitrile. The extract was transferred into PP single use centrifugation tube, which contained 25 mg PSA and 150 mg $MgSO_4$ per ml. The addition of $MgSO_4$ was to absorb the residual water. The tube was vortex for 1 min followed by centrifugation at 3000 rpm for 5 minutes. After the centrifugation, the cleaned extract was transferred into a screw cap vial and the pH was quickly adjusted to ca.5 by adding a 5 % formic acid solution in acetonitrile (vol/vol) (pro mL extract ca. 10 µL). The pH adjusted extract in filled into vials for gas chromatography and is used for further analysis (Basavarajappa & Raghunandan, 2013). For honey fortification 5 g of the control sample was heated in a water bath at 40^{o}C for 20 min and spiked by adding an appropriate volume of standard working solution to reach the concentrations 0.02 and 0.20 mg/kg. The mixture was mechanically stirred in a blender to ensure homogenization and then subjected to the extraction step (Basavarajappa & Raghunandan, 2013).

3.7.4. Chromatographic conditions of Glyphosate Pesticides Residue in Honey Using GC-PFPD

All compounds were determined and quantified with the aid of a gas chromatograph equipped with pulse flame phosphorus detector (GC-PFPD), an autosampler and a split-splitless injector, 30 m x 0.25 mm internal diameter fused silica capillary coated with VF-1701ms (0.25 µm film) from Varian Inc. The carrier gas was nitrogen at a flow rate of 2 ml/min at constant flow rate. Oven temperature was maintained initially at 70 ^{O}C for 2 min, increased at 25 ^{O}C/min to 200 ^{O}C/1 min, then at 20 ^{O}C/min to 250 ^{O}C. The injection volume was 1µL, injected in splitless mode at injection temperature of 270 ^{O}C whilst the detector temperature was 280 ^{O}C.

3.7.5. Limits of Detection of the Glyphosate

The detection limits of the GC coupled with either (ECD/PFPD) were determined for Glyphosate pesticide category by successive dilution of the standard mixed pesticide solution followed by injection into the GC-volume several times. Serial dilution experiments provided the necessary information to calculate the detection limits. The detection limits for all the pesticide (Glyphosate) categories were found to be 0.01 mg/kg.

3.7.6. Quality Control on Glyphosate analysis

The quality control for the analysis of Glyphosate in honey consisted of two samples, one honey spiked and one blank spike with five calibration standards (ranging from 0.010 to 2.00 mg/kg of mixed pesticide solution standards), a calibration check standard and ethyl acetate rinses. The honey spike was selected from a set of several free pesticide samples and consisted in fortifying the honey with a mixed pesticide spike standard. The honey and blank samples were fortified at 0.020 mg/kg and analyzed from 60% to 130%. The positive results in the honey samples were confirmed by comparing the retention time and identifying the main ions in relation to those of a pesticide standard. Retention times were within ±0.20 min of the expected retention times (Basavarajappa & Raghunandan, 2013).

3.8 Determining honey physicochemical features purchased from the Wa market

3.8.1 Determining pH of honey samples

The pH was determined by direct measurement, using a pH meter standardized. The apparatus/equipment used for this analysis includes: pH meter, Thermometer, Volumetric flask, 100mL capacity and Beakers. The analysis procedure involves the following steps:

Step 1**:** Standardize pH meter by immersing the electrode in standard buffer of pH 7 was carried out. If the pH meter reads pH 7 no asymmetry potential adjustment is required. If the reading is not exactly pH 7 the necessary asymmetry potential adjustment is made to get the instrument to read pH 7; the temperature reading was noted for further analysis.

Step 2: The electrode was removed from the buffer solution and rinsed with a jet of distilled water from the wash bottle.

Step 3: The electrode was gently wiped with a soft clean dry tissue paper and immersed in a buffer of pH 4 solution. If the reading is not pH 4 adjust the slope % to get the instrument read pH 4.

Step 4: Recheck the pH meter against the buffer solution of pH 7 and pH 4 any time an adjustment is made.

Step: 5 finally, the electrode was rinsed, dry it and immerse it in degassed sample and read its pH, noting the temperature of the malt. Between successive measurements rinse the electrode with distilled water and recheck the pH meter against the standard buffer after series of sample pH measurements. All results were recorded to the nearest 0.05 pH. All measurements were done in duplicates with the average value as final measure of the pH of honey, GSA. (2017)

3.8.2 Determining Moisture and Refractive Index of honey

The determination of moisture was determined using Digital refractometer. All measurements were performed at 20°C, after waiting for 6 minutes of equilibration.

The corresponding % moisture (g/100 g honey) was obtained from the refractive index of the honey sample by consulting a standard table by International Honey Commission (2009) for the purpose. The moisture content of honey samples was estimated by determining the refractive index of the sample with the use of refractometer. The samples were directly smeared on the surface of the prism evenly; after two minutes the reading of refractive index was recorded. Each sample was measured twice and averages of two readings recorded and corresponding value for moisture content recorded, GSA (2017).

3.8.3 Determining Total Soluble Solids of honey

The refractive index of a test solution is measured at 20^{o}C ± 0.5 $^{\circ}$C using a refractometer by GS ISO 2173: 2003. The refractive index is correlated with the amount of soluble solids (expressed as sucrose concentration) using tables, or by direct reading on the refractometer of mass fraction of soluble solids. The Equipment and reagents used include; Refractometer and Distilled Water. The procedure for analysis involves reparation of test solution; a clear liquid product is thoroughly mixed in the laboratory sample and uses it directly for the determination. Semi-thick products (purees, etc.); thoroughly mix the laboratory sample. Press a part of the sample through gauze folded in four, rejecting the first drops of the liquid and reserving the remainder of the liquid for the determination.

Measurement; Distilled water should give a reading of zero. If not and where possible, the refractometer must be adjusted to read zero. The prism plate is wiped dry with a soft tissue free from fluffs. Several drops of distilled water are placed on the prism surface. An equal number of drops from the fruit juice are placed on the refractometer prism plate.

The reading on the prism scale is noted to one decimal place. After each test the prism plate must be cleaned with distilled water and wiped dry. Record results in analyst's notebook (GSA-QR-T08). The test report contain at least the following data: All information necessary for the identification of the sample includes; a reference to the method used, the results and the units in which the results have been expressed, date and type of sampling (if known), date of receipt of the laboratory sample, date of test, any particular points observed in the course of the test, any operations not specified in the method or regarded as optional which might have affected the results. The results obtained from the total soluble solids on the ten honey samples include; all samples were analyzed in duplicates and the percentage mean recorded GSA. (2017).

3.8.4 Determining Total Solids of honey

The principle involves a known mass of sample is evaporated to complete dryness on a steam water bath and further dried at 105±1°C to a constant weight in a thermostatically controlled oven. The apparatus/equipment use include; an analytical balance, capable of weighing to an accuracy of 0.1mg, Desiccator, provided with drying agent and hydro-metric indicator**,** Oven thermostatically controlled at 100°C - 110°C**,** Aluminum can, Steam water bath and Beaker – 250ml capacity. The procedure for Analyses involves**,** Dry two clean Aluminum cans in an oven at a temperature of 105°C between 15 – 20mins in an oven cool them in a desiccators. Weigh 10g of sample into the cans and place them on the water bath which is heated to about 100°C. Remove cans after the sample is evaporated to dryness. Place them in the oven for 30 min. Remove from oven, cool in a desiccators and weigh. Analysis is done in duplicate per each sample and the mean value taken.

The percentage Total Solids is Calculated as;

$$\% \text{ Total Solids} = \frac{M1 \times 100}{M2}$$

Where

M1 = weight of solids (weight difference between can and sample after evaporation and weight of empty can) M2 = weight of sample taken for analysis.

The analysis was done on ten honey samples GSA. (2017).

3.8.5 Determining Refractive Index of honey

The refractive index measurement was done with an abbe refractometer. The refractometer's sample compartment were made at room temperature (20C). The electrical conductivity measurements was done at 25C using PH/Conductivity meter model 20 (Denyer instrument). The instrument was calibrated using 0.01m Kcl (potassium chloride solution).

3.9 Consumers, vendors and Processors sample size determination

3.9.1 Proportional sample size Distribution (Urban Communities) sampled

Sample size (SS) =Total Population of Urban Communities (TPU) multiple by the Actual sample size (n) of the Wa Municipal calculated divide by the total population (TP) of the whole Municipal.

$$SS = \frac{TPU \times n}{TP}$$

Where

TPU=71340

n=400

TP=107214

By substituting the values, SS =**265**

3.9.2 Rural communities (All other communities) sampled

Calculation of Rural Communities (Wa) sample size:

Sample size (SS) =Total Population of other communities (TPO) multiply by the Actual sample size (n) of the Wa Municipal calculated divided by the total population (TP) of the whole Municipal.

$$SS = \frac{TPO \times n}{TP}$$

Where SS=

TPO=35874

n=400

TP=107214.

By substituting the values, **SS =135**

3.10 Statistical analysis

Statistical analyses were performed using Microsoft Excel and Statistical Package for Social Sciences (SPSS) version 21 Windows Professional. For health risks estimation a life time average daily dose divided by the slope factor (SF) for Glyphosate was used to estimate cancer risk.

3.11 Internal and External Validity Measures in the Research Design

Internal validity was enhanced through a number of measures. The questionnaire was reviewed by two internal supervisors and then pre-tested in two different communities (Nakore and Busa) within the municipality. This followed a long term engagement in Laboratory work and survey for a continuous analysis and reflection of the emerging findings.

By engaging consumers, vendors and processors in long term discussions which afforded a tap into the long term experiences of respondents. It also enabled a cross-check for consistency and evaluation of preliminary findings.

3.12 Limitation of the Study

Honey samples were obtained from different locations within the Wa Municipality which makes floral origin of nectar collected by bees to make honey difficult to trace. This could possibly be the fact that bees might have collected nectar from plants nectar that is not contaminated with Glyphosate though the chemical use is of rise in the region on most farms.

Production and consumption data on honey in the study area are limited which does not provide more trends of evidence on both wild and apiary source of honey obtained. Even though equal quantities of both wild and apiary honey samples were obtained for the analysis, the total numbers obtained were smaller and it is only limited to the study area.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

This chapter is devoted to the results and discussions of the data obtained mainly from the laboratory and survey. In this chapter, I present and discuss the results of the research. As reported earlier in the preceding chapter, a review of the data identified five main themes; The levels of Glyphosate in honey sold on the Wa market, the levels of physicochemical in honey sold on the Wa market, the average dose of Glyphosate received through consumption, the health risks associated with the concentration levels of Glyphosate found in honey and the knowledge level of consumers, vendors and processors of honey regarding Glyphosate in honey as well as its health effects.

4.2 Levels of Glyphosate in honey sold in Wa market.

This section examines objective one of the study which seeks to determine the levels of Glyphosate in honey sold in the Wa market. The limit of detection (LOD) and the limit of quantification (LOQ) for Glyphosate analyzed on the LC-MS/MS were 0.0mg/kg and 0.03mg/kg respectively. The results on sample (A, B, C, D, E, F, G, H, I, J) obtained are; A (0.00013mg/kg/day), B (0.0000mg/kg/day), C (0.00010mg/kg/day), D (0.00032mg/kg/day), E (0.0000mg/kg/day), F (0.00017mg/kg/day), G (0.0000mg/kg/day), H (0.0000mg/kg/day), I (0.00041mg/kg/day) and J (0.0000mg/kg/day). The mean concentration of Glyphosate contained in the ten samples is 0.000761mg/kg/day.

The results obtained from the ten (10) honey samples (wild and apiary honey) from the Wa Market were below level of detection and not above the E.U MRLs (0.3mg/kg/day), (Rubio *et al.*, 2014) adapted by Ghana. The results obtained are similar to a recent study of Zoller et al. (2018) in which foodstuffs were analyzed for Glyphosate using a LC-MS/MS method with relatively low LOQ in the range of 0.0005–0.0025 mg kg^{-1}. The results are quite different from that of Wumbei et al. (2019) research on Glyphosate in Yam. The limit of detection (LOD) and the limit of quantification (LOQ) for Glyphosate analyzed on the LC-MS/MS were 0.04 mg kg^{-1} and 0.12 mg kg^{-1}, respectively in their study. It was concluded that the Glyphosate residues at very low levels may not pose threat to human health.

To account for Glyphosate residue levels in honey that were below limit of method detection, three assumptions are were arrived at. The first assumption that content of Glyphosate not detected have been assumed to be 0 (zero). The limitation of this model is that the calculated results might be underestimated, since non-detected residues are ignored. The second assumption is that actual concentrations of Non-detect (ND) samples were equal to limit of detection. This model tends to overestimate concentrations if there were no contamination of the sampled honey. The third assumption that if a pesticide has not been detected at all in honey, then non-detect samples of that commodity are assumed to be zero. If however, pesticide has been detected in some samples of the commodity, then the non-detect samples are assumed to be 50% of the limit of detection (Petersen et al., 2013). A correction factor is sometimes applied to the third assumption since it is assumed that a small margin of overestimation can still occur because the summation of very small frequencies of detection that may sometimes occur may still be high enough to be significant. The correction factor is normally applied when the food commodity of interest undergoes processing before consumption. In this study concentration means, rather than summation are used and means computed based on the second or third models/approaches will underestimate detected levels hence the first assumption adopted. Furthermore, there is no need for correction factor since honey is consumed whole and only the edible part of melon is extracted.

This findings are different from that of Rubio et al., (2014), who in a study found Glyphosate above Levels of the (ADI 0.3 mg/kg/day) in some food products including honey, Sugar cane, Maize, Wheat, Rice, Beans, Soybeans, and Mushrooms amongst others. Based on limited studies using U.S population for example, Glyphosate was found in urine at levels corresponding to a dietary daily intake of around 0.1-3.3 µg/kg bw/d (Niemann et al., 2015) and Wumbei et al. (2019) who recently found Glyphosate in Yam from Northern Region, Ghana.

4.3. Physicochemical Quality of Honey

The study focused on five physicochemical features of honey sold in the Wa market. These include; pH, Moisture, Total Soluble Solids (TSS), Total Solids (TS) and Refractive index.

4.3.1 pH of honey

The pH test acidity of honey and it is an indicator of honey quality confirming the durability of honey to fermentation. Figure 4.1 presents the pH of honey in Wa market. The results of the pH of the honey from the ten (10) samples are described herein.

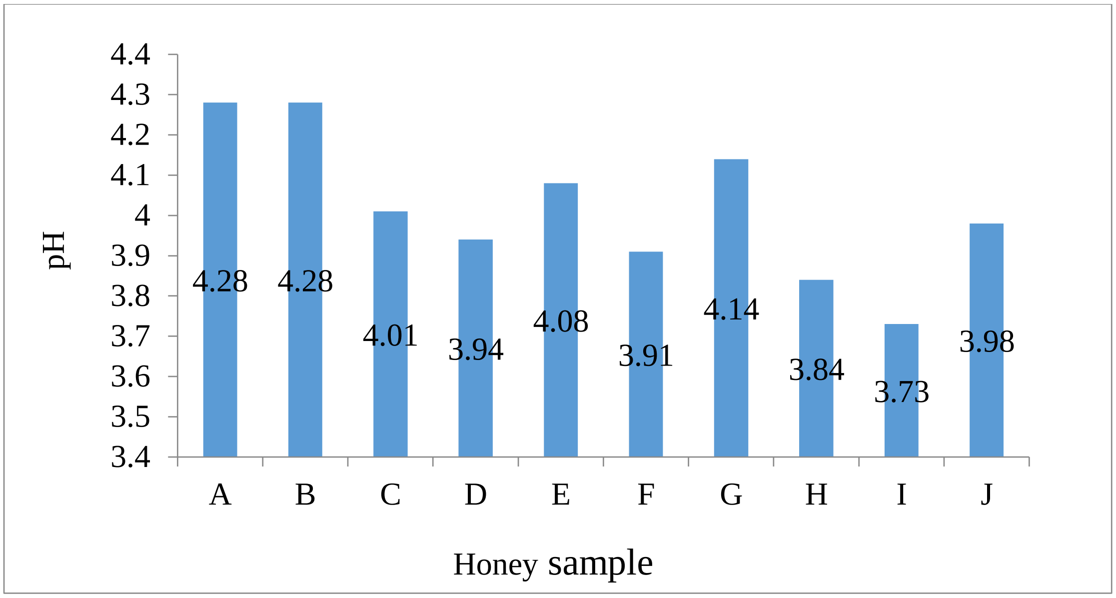


Figure 4.1: pH of honey on Wa market.

Source: GSA Laboratory, 2017

The IHC (2002) set pH of 3.2 to 4.5 (moles per liter) as the required standard for honey considered safe for consumption. The test results obtained from this study conforms to the International Honey Commission (IHC) set standard. The honey that had the highest pH was that of honey sample A (4.28) and B (4.28) from the market. These samples are from wild source collected from Sagu and Sombo communities in Wa municipal respectively. The results of the pH of honey revealed that both wild and apiary beehive type did not have a substantial effect on this quality characteristic. The pH values for all samples ranged from 3.73 to 4.28 (moles per liter) of honey sold in Wa market. Sample "I" recorded an average pH of 3.73 as the lowest. The result is similar with study by Bardy et al. (2004) on wild and apiary honey recorded a pH range between 3.2 and 4.5 (moles per liter).

These values compared well with the 3.52 – 5.13 reported by Chakir et al. (2012) and 3.87 – 5.12 by Serem and Bester (2012) have quit significant difference. Low pH of honey inhibits the presence and growth of micro-organisms and makes honey compatible with many food products in terms of pH and acidity. This parameter is of great importance during the extraction and storage of honey as it influences the texture, stability and shelf life, of honey (Gomes et al., 2010). Also, Gomes *et al*. (2010), reported that pH of honey has not been legislated. However, Olugbemi et al. (2013) stated 3.7–4.2 to be the pH limits.

4.3.2 Moisture content of honey

The moisture content from the ten different honey samples ranged from 15.8 to 21.8% illustrated in (Figure 4.2). The control of the water content is an important requirement set by (Codex Alimentarius Commission, 2001) at an upper limit of moisture at 21% for honey in general.

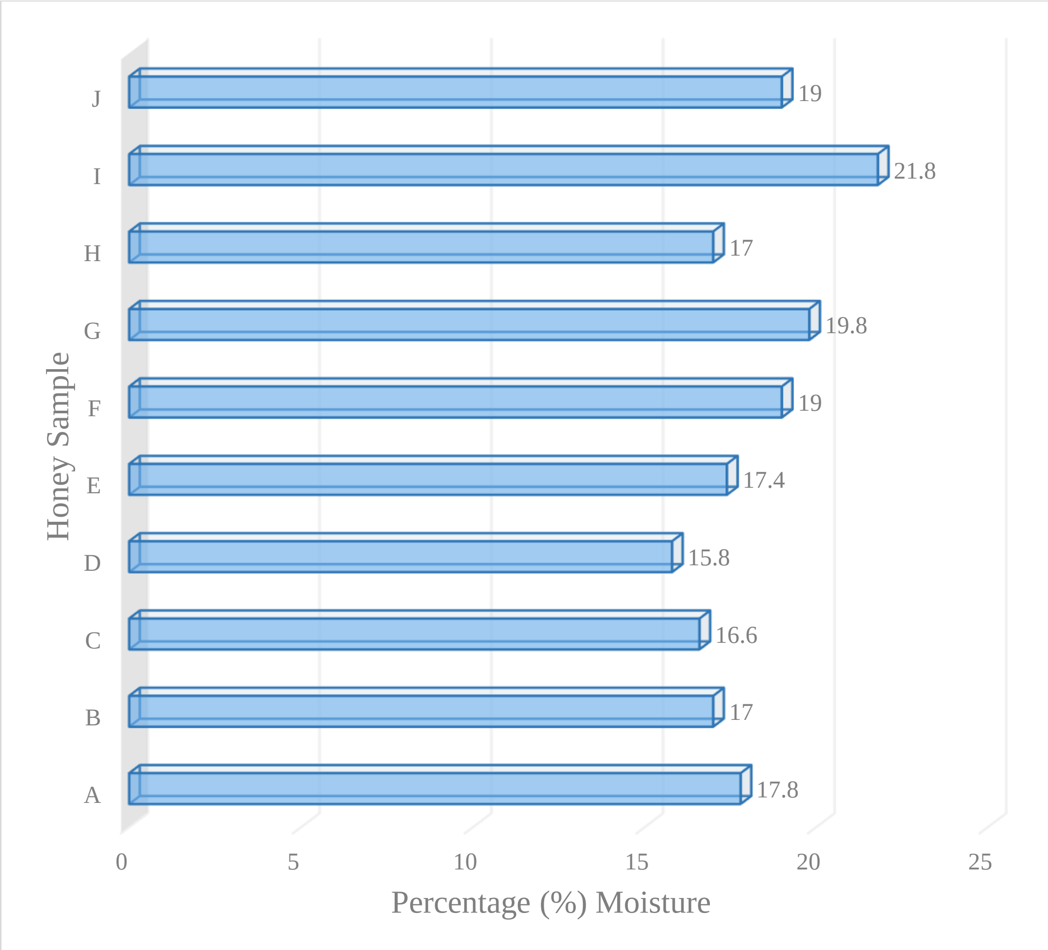


Figure 4.2 Moisture of honey on Wa market.

Source: GSA Laboratory, 2017

Sample "I" recorded 21.8% as the highest moisture content of the study. This limit observed in the sample is above the (Codex Alimentarius Commission, 2001) and EU Directive (EU Council, 2002), fixes a maximum moisture content in honey sold in the market at 21% and 20% respectively. Almost all of the honey samples analyzed had close percentage moisture content compared to the limit set by the Codex Alimentarius Standard (Serem & Bester, 2012) and EU Directive (EU Council, 2002). The low moisture content observed may have been due to the increase in sugar content of the samples as the harvest season was dry with low humidity.

An increase in the moisture content of honey is an indicator of adulteration and, therefore, the values obtained from this study showed that the honey was of good quality with regard to the moisture content except honey sample "I". Also, according to Attri (2011), moisture content of honey is a very important physical quality characteristic, as it affects other properties like density, specific gravity, refractive index, viscosity and optical properties, and also plays an important role in the preservation of honey. If the moisture content exceeds twenty-two per cent (22 %), honey is likely to ferment (Attri, 2011). Honey moisture content depends on the environmental conditions and the manipulation from beekeepers at the harvest period, and it can vary from year to year (Larsen et al., 2014). The water content generally depends on the botanical origin of the sample, the processing techniques and the storage conditions.

Water content is strictly related to climatic conditions and the degree of maturity; anomalous values may be an index of adulterations. This confirms the reason why moisture content of honey samples sold in the market are little higher. Although differences in moisture content of the honey samples were observed, they were not due to the beehive types as their effect on the moisture content of the honey was found to be minor.

4.3.3 Total Soluble Solids (TSS) of honey

Total Soluble Solids (sugar content) of the honey sample ranged from 77.6% to 83.9% for the samples. The mean TSS was 79 ± 2 %. Generally, all the samples showed high total soluble solids (sugar content) values and all values measured agree with those reported by Alqarni et al. (2012) as Total Soluble Solids value ranged between 70%-85% sugars. Sample "I" (77.6%) recorded the lowest TSS which is below the set standard by IHC, (2002) at 80% minimum.

The high sugar content of the honey analyzed suggests that the honey samples were ripped and matured in the honey combs before harvested. The high sugar content also makes the honey samples hygroscopic.

This property exhibited by the honey analyzed might make them vital both in processing and for final use and antimicrobial in nature, contributing to their high antimicrobial activity exhibited as stated above. (See figure 4.3 below)

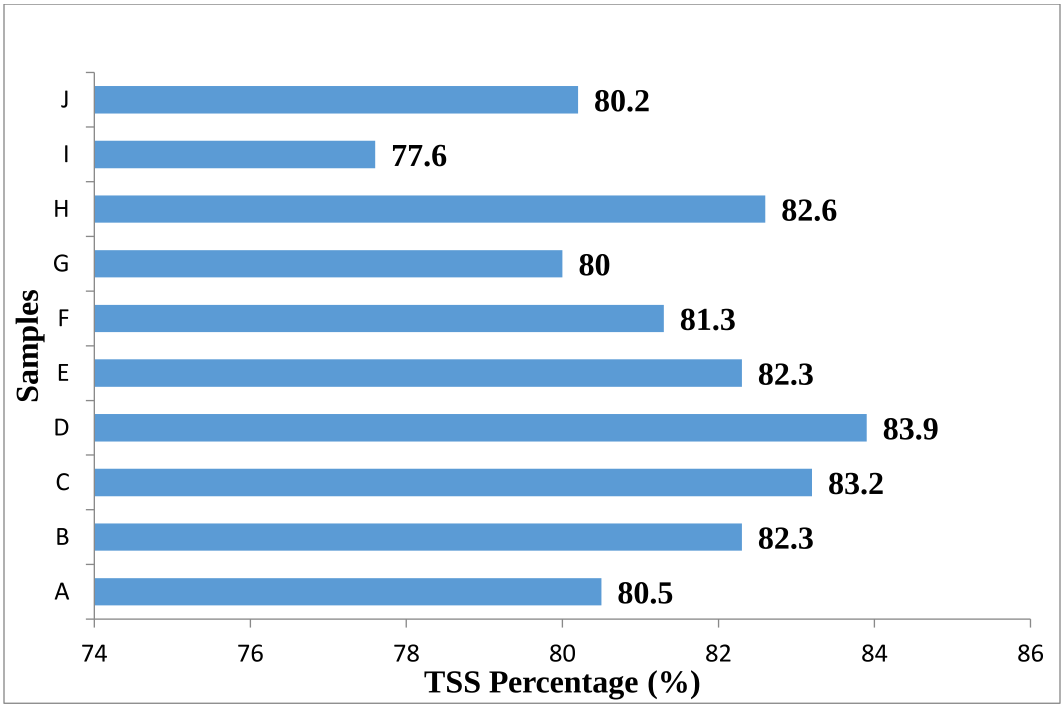


Figure 4.3 TSS of honey in Wa market

Source: GSS laboratory, 2017

4.3.4 Total Solids (TS) of honey

The results of the soluble sugars of the honey harvested from different beehive types are illustrated in Figure 4.4. The soluble sugar content ranged from 78.30 to 86.99% and the beehive type had no effect on the amount of soluble sugars of the honey. Total solid is a measure of dissolved solids in the honey samples.

According to the grading system of the United States Department of Agriculture (USDA), honey with Total Solids greater or equal to 81.4% is considered of higher grade (A and B), while that falling between 80% and 81.3% is considered to be of lower grade C. Thus, the honey investigated in this study can be considered stable with regard to fermentation upon storage and thus of high grade except sample "I". For all the honey samples, total soluble solids were generally more than 81% except sample "I" (78.30) and can be considered of high grade and highly stable upon storage.

On the other hand, honey with less than 81% soluble solids (Sample "I") with 78.30 is likely to ferment during storage. The average total solids value of the honey samples analyzed in the study was 81 ± 2 g/100 g ranging from 78.30 g/100 g to 86.99g/100 g. Ninety nine (99%) of the honey samples (A, B, C, D, E, F, G, H and J) had total solids values that agreed with those (78.4 – 82.8 %) reported by Saxena et al. (2010) and Alvarez-Suarez et al., 2010). while sample "I" 78.30 g/100 g did not conform with Sexena et al. (2010) but slightly equal to it. The high the total solid content, the low the moisture content and vice versa. Generally, all the honey analyzed had high total solids depicting low moisture content, and high shelf life stability.

The high total solid content might also be an indication of high mineral content and other soluble solids (sugars).

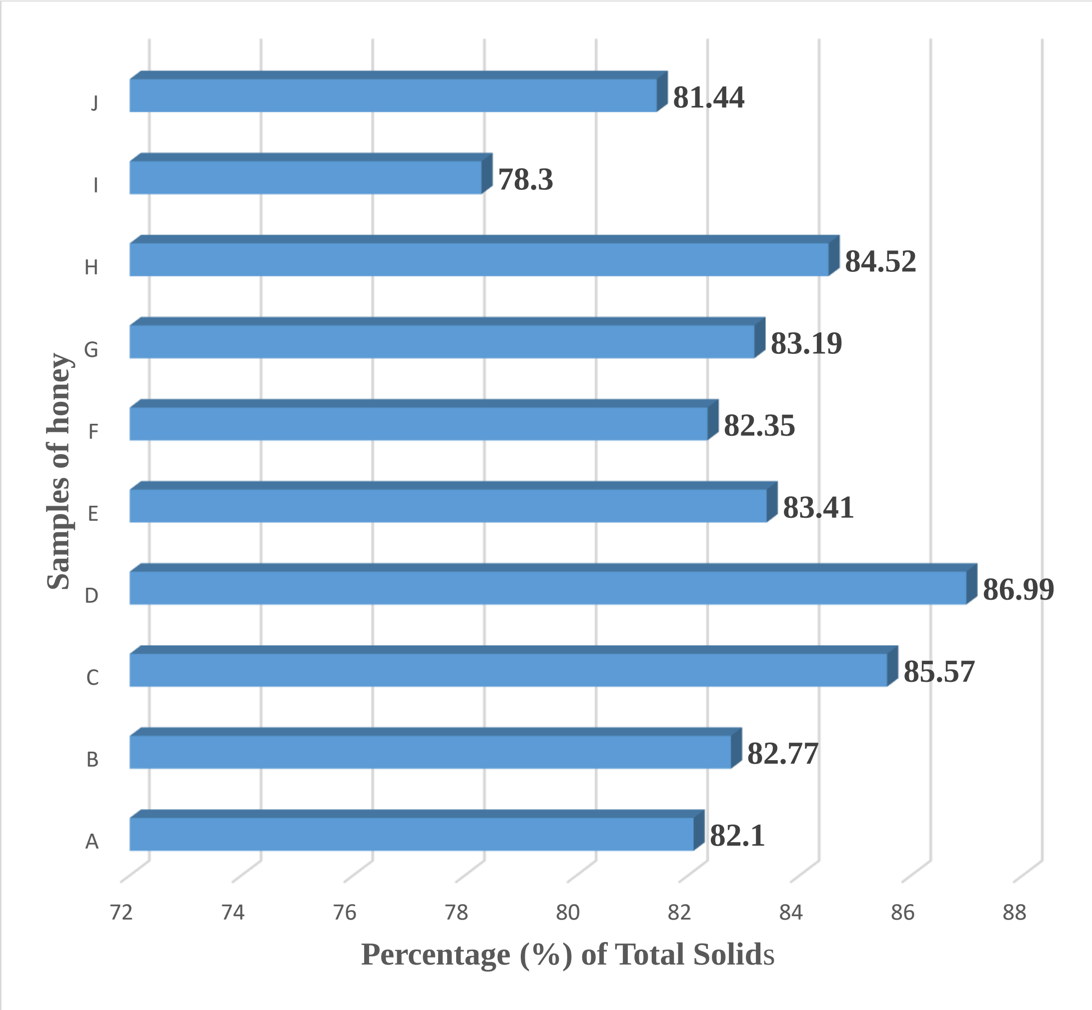


Figure 4.4 Total Solids of honey in Wa market.

Source: GSA Laboratory, 2017

4.3.5 Refractive Index of honey

The refractive index of the samples ranged from 1.489 to 1.499 with an average of 1.489 ± 0.005. The measured values (A,B,C,D,E,F,G,H,I,J) 1.494, 1.496, 1.497, 1.499, 1.495, 1.491, 1.489, 1.496, 1.484 and 1.491 respectively agree with those reported by Alqarni *et al*. (2012) and fell within those standardized for American honey. The refractive index of honey is said to be a rapid, accurate and simple measure of its moisture content (White, 1975).

The less variation in the refractive index values of the honey analyzed confirms less variation in their moisture content. This helped prevent microbial contamination and activities stated.

The honey samples in this study therefore might have a stable shelf life without crystallization and fermentation.

4.4. Estimation of the average dose of Glyphosate received through dietary exposure.

This section of the study addresses objective three of the study which seeks to estimate the average dose of Glyphosate received through dietary exposure. To achieve this objective, a dietary exposure was undertaken. Survey on honey consumer in Wa Municipality was conducted on 400 consumers to establish the quantity on average daily consumption of honey. As earlier mentioned, the concentration levels of Glyphosate in honey was ascertained through laboratory test. A stepwise approach was used to obtain realistic dietary exposure assessments. For the purposes of dietary exposure estimates, food consumption data is presented such that individual consumer body weights are applied to the consumption figures for each consumer. The average body weights for the target population (e.g. adults, GEMS/Food Consumption Cluster Diet C) was used (FAO/WHO, 2011). Average body weights of 60 kg for adults and 15 kg for children are assumed for most populations in the world adopted by this study (FAO/WHO, 2011). If the default 60-kg adult body weight underestimates the actual individual body weights, the dietary exposure estimate on a per kilogram body weight basis will be overestimated. Likewise, if the default 60-kg adult body weight overestimates the actual individual body weights, the dietary exposure estimate on a per kilogram body weight basis will be underestimated (FAO/WHO, 2011).

4.4.1 Quantity of honey consumed in Wa Municipal

The consumption of honey was measured in five different frames based on multiple response taken from 400 consumers of honey which represented the sample size; daily, weekly, monthly, yearly and rarely ranging from >5ml -21ml measurement.

Overall, the results show that the largest proportion of respondents (24%) consumes honey weekly, followed closely by monthly with 23%, and the least being rarely (15%). Only 18% consume honey daily. However, there were significant variations with regards to the quantities. The overall average of quantity of honey consumed per person in in Wa Municipality was calculated at 1.7 ml. The largest proportion of respondents (62%) consumes between 5-10ml of honey per month. This was followed by 53% of respondents consuming between 11-20ml per month, 47% consuming between 5-10 ml per week, 43% in the same category consuming less than 5ml and about 45% of respondents consuming between 11-20ml, and above 21 ml per year. The least proportion of respondents consumes about 10% of honey monthly and yearly.

Table 4.1. Quantity of honey consumed by respondents in Wa Municipality.

Duration	**Consumption of honey among respondents**				
	Less than 5ml (%)	**5ml-10ml (%)**	**11ml-20ml (%)**	**21ml above (%)**	**Total (%)**
Daily	40.0	25.0	43.3	13.3	**18.3**
Weekly	43.3	46.7	36.7	31.7	**23.7**
Monthly	10.0	61.7	53.3	25.0	**22.5**
Yearly	10.0	36.7	45.0	45.0	**20.5**
Rarely	23.3	35.0	20.0	21.7	**15.0**

Source: Field survey Quantity of honey consumed, 2017

The average dose of dietary exposure to Glyphosate in the study for both adults and children are estimated.

4.4.2 Dietary Exposure to Adult consumers of honey in Wa Municipality

$$Dietary\ Exposure = \frac{Food\ Chemical\ Concentration \times Daily\ Food\ Consumption}{Body\ Weight}$$

Where: Dietary exposure (mg/kg/day) =? Food chemical (Glyphosate) concentration (mg/kg) = 0.000761mg/kg and Daily Food consumption=1.7 mg/kg/day and Average Adult Body weight = 60kg.

By substituting the numbers into the formula, the Dietary Exposure of Adult = -0.0000216mg/kg.

4.4.3 Dietary Exposure to Children consumers of honey in Wa Municipality.

Where: Dietary exposure (mg/kg/day) =? Average Food chemical (Glyphosate) concentration (mg/kg) = 0.000761mg/kg and Daily Food consumption=1.7 mg/kg/day and Average Children Body weight = 15kg.

By substituting the numbers into the formula, the Dietary Exposure of Adult = -0.000086mg/kg.

4.5 Estimation of health risks associated with the concentration levels of Glyphosate in honey.

This section of the study seeks to address objective four. This section helped to characterize the health effects and risk associated with the concentration levels of Glyphosate found in honey.

4.5.1 Dietary exposure and Health Risk Estimation

Dietary exposure to pesticides is calculated in order to assess the chronic (long-term) consumer health risk. The Codex Alimentarius Commission Procedural Manual (Codex Alimentarius Commission, 2006) defines exposure assessment as “the qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant”. For each type of exposure, the estimated lifetime exposure dose (mg/kg/day) is obtained by multiplying the residual pesticide concentration (mg/kg) in the food of interest by the daily food consumption rate (per capita consumption) (kg/day), and dividing the product by the body weight (kg). Hazard index (HI) is used as a measure of the risk associated with the exposure. In HI assessment, the estimated dietary exposure is compared with the toxicological reference value, Acceptable Daily Intake (ADI) or reference dose (RfD). The acceptable daily intake (ADI) is a level of intake of a chemical that can be ingested daily over an entire lifetime without any appreciable risk to health (WHO, 2001). HI = Exposure dose ADI

Two hazard Indices were calculated; one based on national per capita consumption and the other based on the estimated per capita consumption in the study area. Hazard indices were also determined for two categories of people: children at an average of 15kg and 60-kg for adults. In both cases, the risk assessment may focus on two different time frames of the exposure:

- Long-term (chronic) exposure or
- Short-term (acute) exposure (usually the exposure related to a single meal or over a day).

In the long-term (chronic) risk assessment, the estimated chronic dietary exposure is compared with the acceptable daily intake (ADI). The Acceptable Daily Intake (ADI) of a combination of Glyphosate and certain metabolites (AMPA, N-acetyl Glyphosate, and N-acetyl AMPA) for humans is 1.0 mg/kg value which gives the concentration of a chemical that can be consumed over a long period without unacceptable negative health effects. For the short-term (acute) risk assessment, the Acute Reference Dose (ARfD) for Glyphosate is 0.3 mg/kg/day is used to identify possible consumer health risks. The ARfD gives the concentration of a chemical that can be ingested over a short period of time (one meal, one day) without appreciable risks.

For estimating the potential impact of Glyphosate residue intake on human health, two parameters have to be taken into consideration: The actual level of pesticide residues in the diet (dietary exposure) and the acceptable daily intake of the pesticide (based on toxicological properties of the compound).

4.5.2 Risk Characterization for adult consumers of honey

Estimated Daily Intake (EDI) = ΣC×F/D×W Where C is the mean of pesticide residues concentration in honey (0.000761µg·kg-1), F is mean annual intake of honey per person (1.7ml per person), D is number of days in a year (365), and W is mean body weight (60 kg)

EDI= 0.000761×1.7/365×60

EDI=0.001294/21900

EDI= (-0.000000059)

4.5.3 Hazard Index for adult consumers of honey

Hazard index (HI) = (EDI/ADI, %)

HI= (-0.000000059/0.3)

HI= (-0.000000197)

4.5.4 Risk Characterization for children consumers of honey

EDI = ΣC×F/D×W, Where C is the mean of pesticide residues concentration in honey (0.000761μg·kg-1), F is mean annual intake of honey per person (1.7ml per person), D is number of days in a year (365), and W is mean body weight (15 kg)

EDI= 0.000761×1.7/365×15

EDI=0.001294/5475

EDI= (-0.0000000236)

4.5.5 Hazard Index for children consumers of honey

Hazard index (HI) = (EDI/ADI, %)

HI= (-0.0000000236/0.3)

HI= (-0.000000788)

The study reveals that an average daily consumption of honey for the general population in Wa municipal is 1.7ml. Again, the maximum residue level corresponding to that Glyphosate in honey (mg/kg) by E.U /reference dose (RfD) for Glyphosate is 0.3 mg/kg/day. The EDI figure provides a more realistic yet still conservative estimate of exposure, as it assumes that all the consumed food is treated, but that it contains a median rather than maximum level of residues.

4.5.6 Risk assessment

The estimated daily intakes of Glyphosate in all samples were below detection levels set which shows that honey consumption has no toxicological effect in consumption per this study. These findings are in coincidence with those obtained by Irene-Nyangoge (2012), Jomo Kenyatta University of Agriculture. The consumer is considered to be adequately protected if the hazard index of a pesticide residue does not exceed standard limit. The hazard index values show that all the intakes of Glyphosate residue remains clearly below the safe limit. It should be emphasized that dietary pesticide intakes estimated in this study considered only exposures from honey and did not include the food products such as grains, vegetables, fruits, dairy, fish, and meats. As such, estimates are not considered as total dietary exposure to the pesticides, nor do we consider drinking water, residential, or occupational exposures. On the other part, if Glyphosate residue were found in the honey samples analyzed, the EDI wound have been calculated as seen below

4.5.7 Non-cancer risk assessment

To estimate the potential noncancerous health risk associated with Glyphosate exposure via honey consumption by Ghanaians, the hazard index (HQ) was estimated for level of Glyphosate exposure for an adult and children consumers in the Wa Municipality. The results were compared to the EU ADI of 0.3mg/kg. Of the honey samples identified in the studies, 100% of the honey had below or no detection level of Glyphosate concentration. No honey samples produced an HI of > 1 from the Wa market.

4.6 Assessment of consumers, vendors and processors of honey knowledge regarding Glyphosate in honey.

This section detailed objective five of the study.

4.6.1 Socio-Demographic characteristics of survey respondents

This section presents demographic features of surveyed respondents. These include; the age, sex, occupation and educational status of respondents. This will enable readers to appreciate the contextual and socio-demographic background of the unit of analysis. The survey gathered views of 400 respondents (consumers, processors and vendors).

Table 4.2: Socio-demographic characteristics of respondents

Parameters	Category	Frequency (n=	Percentage (%)
Gender	Male	231	57.8
	Female	169	42.3
Age group	Less than 20 years	31	7.8
	20-29 years	135	33.8
	30-39 years	141	35.3
	40-49 years	60	15
	50-59 years	20	5
	60 years above	13	3.3
Occupation	Farming	83	20.8
	Trade/Business	101	25.3
	Private sector	58	14.5
	Public sector	105	26.3
	Others	53	13.3
Education	No formal education	92	23
	Basic education	67	16.8
	Secondary/Tech/Voc	83	20.8
	Tertiary	158	39.5

Source: Survey field data on **Socio-demographic characteristics, 2017**

4.6.1.1 Sex of respondents

Overall, the study constituted 231 males and 169 females. The study revealed that, males have purchasing ability than females in the Wa municipality. Economically, men are sound than females in the Wa municipality (Ghana living standard survey, 2014). Preliminary field investigation shows that, only ten honey vendors are engage in honey sale. Eight of these vendors were women representing 80% of the total vendors whilst the remaining two were males representing 20% of the total number in Wa municipal. From this finding, women were the main vendors of honey in the Wa market. The survey indicates that all honey vendors are into business/trade as their main occupation and not engaged in other activities including farming, private sector employment and public sector employment.

This confirms that, honey vending serves as a source of cash incomes for many households (Getachew *et al*., 2014) and gives men, women and youth room to make their own income since women are the larger population in the municipal. The study also revealed ten beekeepers in the municipal as at the study period. All these beekeepers in the study area are males. This means that, men are the main beekeepers in the Wa municipal.

4.6.1.2 Age of respondents

In terms of age, the survey results shows that majority (about 68%) of the respondents were within the age brackets of 20-39 years. The largest cohort of respondents are however within the age brackets of 30-39 years, representing 35%, followed by those within ages 20-29 years, which constitute about 33%. This result suggest majority of honey 80% consumers, 5% processors and 5% vendors in the Wa Municipality are young people within the age category of 20-39 years in the Wa municipal. The other 10% of processors and vendors are older age categories in the study.

4.6.1.3 Occupation of respondents

The results indicate a significant proportion of respondents (41%) are gainfully employed in the waged employment sectors (public & private sectors).

This is followed by those employed in the trade and business sector (25%), farming (20%) and others- students, Artisans, Electrician, Carpenter and apprentices (13%). Again, this result reflects a typical urban environment, which is often dominated by commerce and waged employment. The results thus reflect the urban dynamics of the Wa Township (the capital of the upper west region).

From the economic perspective, this result suggests a nexus between peoples' purchasing power (ability to afford) and consumption of honey. Therefore, the consumption of honey directly correlates with the purchasing ability of the consumers. In this sense, those in waged employment sector are better placed to consume honey than farmers because they are unable to afford honey and at times will want to consume honey produced by them due to adulteration perception in the markets.

4.6.1.4 Education level of respondents

Overall, 77% of the respondents in the study have had some form of education (basic, secondary or tertiary). In specific terms, a greater proportion of respondents (40%) have had tertiary level qualification, and about 38% (combined) have basic and secondary school education. The high level of education among respondents accounts for equally high proportion of respondents gainfully employed in the waged labor sectors which was 41%.

However, those without any form of formal education are still significant, representing about 23%. From the results, out of every 10 respondents in the survey, only three (3) are not educated. Although this result looks impressive, the overall educational status in Ghana and the Upper West Region in particular is not encouraging. In the 2010 population and housing census report, 6 in every 10 people (60%) in the upper west region do not have any form of education (GSS, 2010).

This figure is twice higher than the national average illiteracy of about 26%. Although this result does not reflect national statistics, the results portray a unique characteristic of honey consumers in the Wa municipality, which indicate majority of consumers of honey, are educated and gainfully employed in wage labor employment.

4.6.2 Brands of honey sold on the Wa market.

The results also show there are two main brands of honey in the study area. The honeys collected for the laboratory analysis were 100% local brand. The results also show that local brand of honey constitutes the majority (82%) whiles external brands constitute only 9% when consumers were asked to identify the source of honey they consume. Another 9% of respondents do not know the brand of honey they consumed. These include; local and external brands. Local brand refers to honey produced and processed within the upper west region. On the contrary, honey produced or processed elsewhere either than the upper west region are classified as external brands.

This classification is important for the researcher to be able to identify the various sources of honey and to be able to identify the class of honey prone to Glyphosate contamination in the study area.

4.6.3 Source of honey to consumers in Wa Municipality

In terms of access points, the results shows that significant proportion of honey is purchased directly from processors and distributors (70%) and the least from market place (0.8%) (Table 4.8). This means that majority of honey is procured from the farm gate (source of production), and this could have implications for quality and pricing of honey.

This also means that honey is not often sold in the open market like other goods. Therefore, this results shows that majority of honey in the Wa municipality is sold in the 'informal' market, where consumers purchase honey directly from vendors and distributors There is the belief that consumption of honey gives retentive memory or high intelligent quotient (IQ) for excellent academic performance. Most of the consumers buy honey with the perception that it's of good quality and not adulterated as well as very accessible to consumers. Some consumers buy honey as a result of hygienic practices, more quantity and cost less from processors and distributors. See table 4.3 below

Table 4.3 Main source of honey to consumers

Source	Frequency	Percent
Super markets	97	24.3
Processors	146	36.5
Distributors	130	32.5
Pharmaceutical shops	24	6
Others	3	0.8
Total	400	100

Source: Field survey on source of honey to consumers, 2017

4.6.4 Quantity of honey purchased and type of packaging preference by consumers

In term of quantities purchased, most respondents were unable to specify the exact quantities they purchased. However, results indicate that honey is often purchased in three main types of containers. These include; plastic bottles (estimated at about 375ml), less beer bottles (estimated at about 750 ml) and medium size gallons (estimated at 1875ml). Subsequently, respondents were asked with regards to their preference and satisfaction with regards to these storage containers. Only 22% of respondents expressed their satisfaction with regards to the packaging containers in which honey was sold to them. A half of respondents (50%) were unsatisfied that honey is stored in plastic bottles. Consequently, about 46% of the respondents wished that honey is packaged, labeled and stored in glass bottles for sale. See the table below.

Table 4.4. Packaging of honey

Storage containers	Frequency	Percent
Glass bottle	181	45.3
Plastic/Rubber bottle	187	46.8
Recycled drinking water bottle	32	8
Total	400	100

Source: Field survey on Storage/packaging of honey, 2017

4.6.5 Features consumers consider when purchasing honey for consumption at home

Quality is one major focus of this study. Respondents' perception of quality of honey was therefore assessed.

The results show that majority of respondents have issues with the quality of honey sold in the Wa market and therefore endeavor to look out for a number of quality feature, when buying honey. Consequently, about 47% of respondents look out for 'thickness, 25% look out for taste, whiles only 13 and 14% look out for color and flavor respectively (figure 4.5). Both honey vendors and consumers in the Wa municipality commonly used four quality characteristics (flavor, taste, color and thickness) in procuring honey supply and consuming respectively. They believed that these physical characteristics are all-inclusive in determining the quality of honey and stretches an upright conclusion of honey quality.

Honey vendors in the Wa market ranked thickness, taste, color, and flavor respectively as the most important physical characteristics for obtaining honey for sale. It is not different from the consumers look out features for obtaining honey from vendors. Also, many vendors recommended examining moisture adulteration by testing ability of paper or cotton wool dipped in honey and been able to burn. Again, a match stick dipped in honey to kindle fire as the technique used for guaranteeing quality of honey on the markets. They specified they used this technique frequently because it measures the moisture content of honey, an essential quality index which determines the stability and shelf-life as well as the grade of honey.

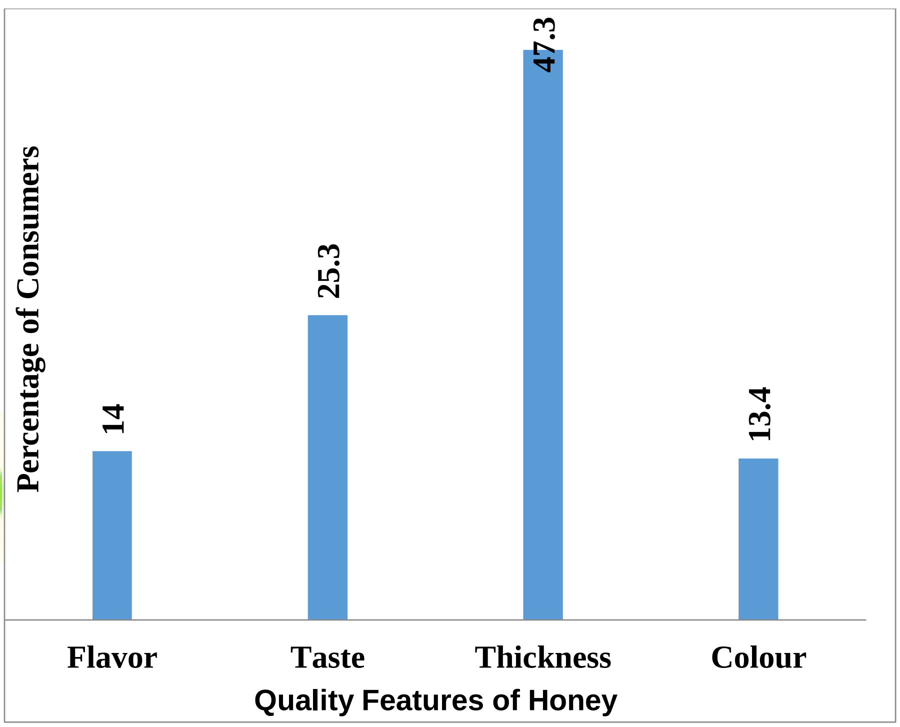


Figure 4.5: Quality feature of honey to consumers on purchase

Source: Field survey on Quality feature of honey to consumers on purchase, 2017

4.6.6 Access and reasons for consumers purchasing honey

With regards to access, honey in Wa is largely purchased from venders or sellers and processors. The results show that most respondents have their own preferred honey vendors or sellers from whom they buy honey. There are several reasons that influence one's decision to procure honey from a particular vendor or source. For most respondents, a major factor is the quality, and followed by accessibility of the vendor. As much as 36% of respondents' reports that they prefer to buy honey from their current source because of its quality. Another 24% ascribed the reason of accessibility of the vendor in their purchasing decision. On the contrary, only 5% of respondents reports that they buy honey from any vendor who comes around to their doorstep (Figure 4.6).

The results suggest a significant majority of honey consumers have their own preferred vendors from which they often buy honey from, and majority have come to stick to a particular vendor because those vendors have quality honey and are easily accessible to customers. Perhaps, this informs the reason why majority of respondents indicated earlier that they often look out for thickness in honey as a sign of quality.

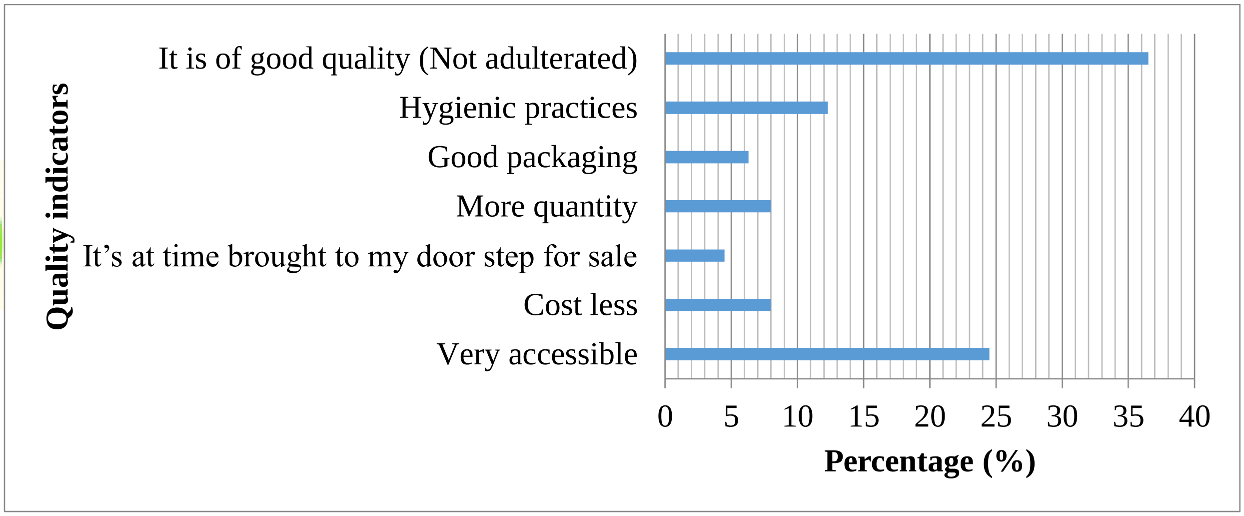


Figure 4.6: Reasons for purchasing honey

Source: Field survey Reasons for purchasing honey, 2017

4.6.7 Use of Honey by Consumers in Wa Municipality.

Although honey have several uses aside home consumption, majority of respondents (64%) are unaware of this, and only 36% of respondents reports that they also use honey for other purposes aside home consumptions. This means that majority of honey consumers in Wa are unaware of the other uses and importance of honey. The commonest additional uses of honey include; nutrition, medical, cultural and spiritual purposes.

Table 4.5. Use of Honey

Use	Frequency	Percent
Consumption as food only	254	64.0
Consumption & Others purposes	146	36.5
Total	400	100

Source: Field survey on Awareness and use of Honey, 2017

4.6.8 Health and nutritional benefit of consuming honey

Respondent's views were sorted on health and nutritional benefits of honey to consumers. These responses were given as Only 7.4% indicated that honey is serves as antimicrobial agent, 9% indicated honey serves as anti-inflammatory, 8.4% indicated that honey aids digestion, 9% indicated honey boost the human immune system, 8% indicated honey is rich in micro nutrients, 8% indicated honey builds human brain, 7.4% indicated honey reduces the risk of getting heart disease, 9% indicated honey controls sugar/insulin related diseases, 8.5% indicated honey cures catarrh and cold, 9.1% indicated honey heals wounds and scars, 8.1% indicated honey is a sleep promoter and finally 8.1% of the survey respondents indicated that honey cures ulcer and other allergies.

4.6.9 The knowledge of consumers, vendors and processors of honey regarding Glyphosate presence in honey.

This section of objective four present the survey results on respondents' knowledge/awareness of Glyphosate in honey, the sources and uses of honey, as well as respondents' consumptions statistics of honey.

A greater majority of respondents are unaware of Glyphosate or its presence in honey. Three related question were posed to respondents about Glyphosate likelihood presence in honey and the importance of it. From the results, an overall 68% of surveyed respondents did not know or have any idea of the herbicide Glyphosate and its possible danger associated with consumption of diet. A further 70% of respondents were also unaware that Glyphosate could be found in honey and could link up any possible route of human exposure to Glyphosate from honey consumption. Another 75% of respondents suggested that Glyphosate advantages far outweigh the disadvantages in the sense that, it's mostly used by farmers in the region to meet their crops production needs. These results means that majority of respondents lack adequate information about Glyphosate and especially the possibility of it being found in honey and the likely dangers it pose for their health. Hence, a significant majority of honey consumers in Wa municipality have low knowledge about Glyphosate.

This in itself poses greater health risk to consumers, considering that Glyphosate have considerable risk to human health. Considering that the average pH content of honey in the Wa municipality is 4.9 and contains 1.5 Mg/kg/bw amount of Glyphosate (above EU MRLs) it thus means that majority of honey consumers in the area stand greater chances of consuming contaminated honey, which comes with health risk to the consumer. The consequential impacts on health and socioeconomic wellbeing of people could be devastating.

Table 4.6 Respondents awareness and knowledge regarding Glyphosate

Response	Awareness of Glyphosate	Percentage (%)	Glyphosate is important	Percentage (%)	Glyphosate presence in honey	Percentage (%)
Yes	120	**30**	298	**75**	92	**22**
No	270	**68**	98	**24**	278	**70**
No response	10	**2**	4	**1**	30	**8**

Source: Field survey on awareness and knowledge regarding Glyphosate, 2017

4.6.10 Major complaints of consumers about honey.

The major concerns respondents expressed about honey were centered on three main themes, including; quality, pricing, and packaging. Almost all respondents have express at least one concern or more about one or a combination of issue across these three themes. From the findings, about 83% of respondents have concerns about the price of honey, 84% with quality, and 78% have concern about the packaging of honey. In terms of quality, about 99% of all respondents were concern that there is ineffective government legislation on honey standards to enhance quality of honey. Another 97% of respondents were emphatic about the adulteration of honey (dilution of honey with material such as foam mattress), and a further 86% were concern about the environmental conditions under which honey is sold. With regards to storage, a half of respondents (about 50%) were unhappy with the current state of storage of honey. Specifically, 46% were unsatisfied with honey being stored in plastic bottles and recommended glass bottles for storage instead.

Surprisingly, only 15% of respondents were concern about the high cost/price of honey. This perhaps corroborates economic theories regarding abnormal goods (which demand does not depend on price).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Introduction

This chapter presents a summary of the major research findings, conclusions and recommendations.

5.2 Summary of Key Findings of the Research

The research findings have been summarized in five main points, covering the main research objectives which include the level of Glyphosate in the honey sold in the Wa municipality, physicochemical parameters of honey, average dose of Glyphosate received through honey consumption among the people in the municipality, estimating the health risk associated with honey consumption contaminated with Glyphosate and the to assess the knowledge of honey consumers, vendors and processors regarding Glyphosate levels in honey in Wa Municipality.

5.2.1. Levels of Glyphosate in honey sold on the market in Wa

The mean concentration of Glyphosate contained in the ten samples is 0.000761mg/kg/day. The results obtained from the ten (10) honey samples (wild and apiary honey) from the Wa Market were below level of detection and not above the E.U MRLs (0.3mg/kg/day), (Rubio et al., 2014) adapted by Ghana. The results obtained are similar to a recent study of Zoller et al. (2018) in which foodstuffs were analyzed for Glyphosate using a LC-MS/MS method with relatively low LOQ in the range of 0.0005–0.0025 mg kg^{-1}. Additionally, the results showed that the acceptable daily intake (ADI) is quite favorable in line with the toxicological properties of the compound.

5.2.2. Physicochemical Parameters of honey sold in the Wa market.

Physicochemical parameters of honey including; pH, Moisture content, Total Solids, Total Soluble Solids and Refractive Index were tested on all the honey samples. The test results from Ghana Standards Authority (GSA) indicated that 99% of the samples tested agreed with Codes Alimentarius Commission and IHC, (2001) standards. This indicates that honey in the municipality are safe for consumption. The 1% honey sample indicates adulteration due to increase in moisture content, decrease total solids and total soluble solids.

5.2.3 Estimation of the average dose of Glyphosate received through dietary exposure.

The consumption of honey was measured in five different frames based on multiple response taken from 400 consumers of honey which represented the sample size; daily, weekly, monthly, yearly and rarely ranging from >5ml -21ml was measurement. The daily average of quantity of honey consumed per person in Wa Municipality is 1.7 ml revealed from the survey.

A dietary exposure of an adult consumer with an average body weight of 60kg and children with an average body of 15kg were calculated to be 0.0000216mg/kg and 0.000086mg/kg respectively. These values were then compared with the allowable daily intake of Glyphosate that has no health effect by EU ADI (0.3mg/kg). The daily exposure obtained was far below the EU ADI standard adopted by Ghana hence both category of consumers were not at high risk. The result conforms to other studies (EFSA 2015; JMPR 2016; Tarazona et al. 2017) in which glyphosate residues in food products were below LOQ. However, the accumulation of this herbicide in honey may result in cancerous human health. Consumption of honey with high level of Glyphosate can have significant human health risk (Myers et al., 2016;Samsel and Seneff, 2015) since Glyphosate is considered as a probable carcinogen by the WHO's International Agency for Research on Cancer (IARC, 2015) therefore raising concerns about its presence in food.

5.2.4 Estimation of health risks associated with the concentration levels of Glyphosate in honey.

The hazard index and the allowable daily intake for both adult and children consumers of honey in the Wa Municipality values indicated that, all the intakes of Glyphosate residue remains clearly far below the safe limit. To estimate the potential noncancerous health risk associated with Glyphosate exposure via honey consumption by Ghanaians, the hazard index (HQ) was estimated for level of Glyphosate exposure for an adult and children consumers in the Wa Municipality. No honey samples produced an HI of > 1 from the Wa market.

5.2.5 Knowledge assessment of consumers, vendors and processors of honey regarding Glyphosate

The results show that majority of respondents have issues with the quality of honey sold in the Wa market and therefore endeavor to look out for a number of quality feature, when buying honey. Consequently, about 47% of respondents look out for ‘thickness, 25% look out for taste, whiles only 13 and 14% look out for color and flavor respectively. Both honey vendors and consumers in the Wa municipality commonly used four quality characteristics (flavor, taste, color and thickness) in obtaining honey for sale and consumption respectively. They believed that these physical characteristics are all-inclusive in determining the quality of honey and stretches an upright conclusion of honey quality.

Additions of water, sugar and melted mattresses as well as grave honey are the adulteration perceptions raised by respondents. Also, many vendors recommended examining moisture and other features adulteration by testing ability of paper or cotton wool dipped in honey and its ability to burn. They specified they used this technique frequently because it measures the moisture content of honey, an essential quality index which determines the stability and shelf-life as well as the grade of honey. The study also reveals that honey consumption is high in towns than in the villages. This according to the study may be as a result of economic status of consumers and perceived adulteration and honey coming from human graves site. The study equally revealed that consumption of honey is as a result of health grounds, belief systems and its nutritional benefits.

The results showed that a greater majority of respondents are unaware of Glyphosate or its presence in honey. An overall 68% of surveyed respondents were completely unaware of Glyphosate. A further 70% of the respondents were also unaware that Glyphosate could be found in honey. These results mean that majority of respondents lack adequate information about Glyphosate and especially the possibility of it being found in honey and the likely dangers it pose for their health. Hence, a significant majority of honey consumers in Wa municipality have low knowledge about Glyphosate and could be consuming it if present in honey.

5.3 Conclusion

This analysis was carried out to characterize health risk on dietary exposure to Glyphosate from honey through consumption as well as consumer knowledge about Glyphosate. From the analysis, the limit of detection (LOD) and the limit of quantification (LOQ) for Glyphosate analyzed on the LC-MS/MS were 0.01 mg/kg and 0.03 mg/kg respectively. Based on the samples tested for

Glyphosate, the computed dietary exposure, ADI and HI were far below the EU set standards which may not pose any threat to human health. The Physicochemical qualities (99%) were within the IHC, (2001) set standards except one sample. Based on the parameters tested, the honey samples were safe for consumption without any health threats. The study also concluded on an average daily consumption of honey in Wa Municipality as 1.7ml per day which was used to calculate the ADI and HI for adult and children consumers of honey that indicated safe limits to their health. Honey consumers, processors and vendors all lack knowledge on the presence of Glyphosate in honey and this poses a major threat to public health in Wa municipality.

5.4 Recommendations

The study made its recommendations based on the findings obtained from the analysis of the data gathered from the field. Due to the potential health risk associated with Glyphosate residues in honey, the study recommends that,

1. The study recommends that the Food and Drugs Authority, Ghana Standard Authority, Environmental Protection Agency (EPA) and other relevant institutions should set Ghanaian honey standards for the production, processing and sale of honey and its related products to the public.
2. There is knowledge gap on honey safety among vendors, processors, and consumers with regards to Glyphosate contamination in the Wa municipality. Accordingly, the study recommends that the Food and Drugs Authority in collaboration with the Municipal Health Service organize regular seminars and workshops to educate and sensitize vendors,

processors and consumers on safety of honey and the consequences of consuming unacceptable level of Glyphosate to the health of consumers.

3. A proportion of people in the Wa municipality express interest in the consumption of honey and its related products. This provides an economic opportunity for people to improve their livelihoods. However, the study revealed less producers of honey in the study area. Accordingly, the study recommends that the Ministry of Food and Agriculture should roll out policies and programmes that will encourage and support people who have the desire to go into Bee keeping for honey production.

4. There is lack of control over the activities of the honey industry though the industry is fast growing. There is therefore the need to regulate the honey industry. Therefore, the study recommends that a National Honey Commission should be established with an oversight responsibility for regulating the production, processing and sale of honey and its related products. The commission should also be tasked with the responsibility of establishing the required standards necessary for producing wholesome honey production in in the Wa Municipality and Ghana at large.

5. Lastly, the study recommends that future researchers should consider researching into the various factors motivating honey processors and vendors to engage in unhygienic practices that compromises the safety of honey in the Wa Municipality and Ghana as a whole as well as further studies into determining what level of exposure occurs to bees visiting flowers sprayed with Glyphosate.

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APPENDICE

APPENDIX I

UNIVERSITY FOR DEVELOPMENT STUDIES, TAMALE

FACULTY OF INTEGRATED DEVELOPMENT STUDIES

DEPARTMENT OF ENVIRONMENT AND RESOURCE STUDIES

Study: Dietary exposure to Glyphosate from honey in Wa, Ghana

Questionnaire for honey consumers and vendors in Wa municipality

Cherished respondent, this questionnaire seeks to solicit information on honey consumption in Wa municipality, as part of an MPhil Environment and Resources Management thesis on the Dietary Exposure to Glyphosate from Honey in Wa, Ghana. The information you provide in this document will be treated as trustworthy and used for academic purposes only. Thank you.

Gentle circle the responses that apply to you. Where applicable, write out your own responses in the spaces provided

CHECKLIST FOR RESPONDENTS

1. Do you eat honey?

   a. If yes continue
   b. If no, stop the interview

2. Do you live (Resident) in Wa municipal?

   a. If yes continue
   b. If no, stop the interview

3. Would you participate in this study?

   a. If yes continue
   b. If no, stop the interview

4. Which area do you get your honey from in the Upper West Region? Location ………………………… (Eg. Kunzokala in Nandom, UWR) Note: If not Wa municipal, stop

DEMOGRAPHIC DATA:

1. Gender: a). Male b).Female

2. Major Occupation: a). Farming b).Trade/ Business. c). Private sector d). Public Sector e). Others specify …………………….

3. Which of the age group below do you belong?

a) Less than 20 years b) 20 – 29 years c) 30 – 39 years d) 40 – 49 years e) 50 – 59 years f) 60 years and above

4. Educational status (formal)

a) No formal education b) Basic c) Secondary d) Tertiary

CONSUMPTION AND CONCERNS ON HONEY:

5. How long have you been eating honey?

a) Less a year b) 1 – 5 years c) 6 – 10 years d) More than 10 years e) Other ....

6. Do you use honey for other purposes apart from eating or not?

a) Yes b) No, if yes specify

………………………………………………………………………………………

7. Which category best defines you?

a) Consumer b) Both consumer and seller b) both processor and consumer d) Seller e) both processor and seller

8. How often do you eat honey? a) Daily b) weekly c) Monthly d) Yearly e) Rarely

9. Specify the quantity you consume in a meal per tea spoon in milliliters (5ml)

…………………………………………………….

10. Where do you buy your honey? (You can circle more than one)

a) Super markets b) Processors c) Distributors d) Pharmaceutical shops e) I don't buy

e) Other, specify...

11. Why do you buy honey from this/these source(s)?

....

....

12. How do vendors handle honey during the period of sale?

a) Packaged in sealed container b) Packaged in plastic/rubber bottle

c) Packaged in glass bottle d) Packaged in recycled drinking water bottle

e) Packaged in recycled alcoholic and/or soft drink bottles

13. What is the main quality feature you look out for in the honey that you are vending or going to buy for consumption?

a) Flavor b) Taste c) Thickness d) Appearance (color)

e) Other, specify..

14. Is adulteration of honey a known problem in consumption in Wa municipal? a) Yes b) No

15. Do buyers/customers complain of adulteration? a) Yes b) No

16. Mention some perceptions people associate with adulteration?

a)..

b)..

c)..

d)..

e)..

17. What are the major concerns you have about honey? Note probe to circle (Multiple)

1. Ineffective government legislation on quality checks by Food and Drug Board Authority etc.
2. Adulteration of honey
3. Wild harvesting of honey with fire which may temper with its quality
4. Honey are sold at open places in the market
5. Some vendors/processors melt mattresses, sugar and honey together and sell to the public
6. Processing honey under unhygienic environment
7. Poor packaging
8. Poor storage of honey during processing and sale
9. The price of honey is high /No value for money
10. Very few People are into honey production
11. Employment generation is less due to difficulty in apicultural activities
12. Avoid bush fire and practice apiculture
13. Others

18. Give suggestions to address these concerns. Note probe to circle (Multiple)

1. Government agencies should set honey quantity standards before sale to the public
2. Effective sensitization on honey adulteration and its negative effects to public health

3. Modern honey harvesting methods, equipment and materials should be used by processors
4. Honey should be sold in enclosed hygienic environment
5. Legislative sanctions should be applied to culprits in melting sugar, mattresses and honey sold to the public
6. Hygienic practices should be enforced and checked during processing and sale
7. Honey should be properly packaged in clean and hygienic containers branded nicely.
8. Honey should be stored in cool dry place without any threats of contamination
9. Honey should be well branded and given fixed prices
10. Bee keeping should be encouraged for more honey production
11. Apiculture should be made an effective sustainable livelihood activity for poverty reduction
12. People should avoid bush fires to encourage apiculture
13. Others

19. What volume/quantity of honey do you buy? a) Bear bottles b) Gallons c) Plastic bottles

Specify in quantity if a vendor……………………………………..

20. How often does it take you to consume the honey you purchased? a) Daily b) weekly c) Monthly d) Yearly e) Rarely e) Others specify...............................

21. Why do you store your honey?

....

....

22. How do you use honey?

a) As a sweetener b) As a spread c) As an ingredient for food processing

d) As a beverage e) other, specify...

23. Was the consumption of honey recommended by a health practitioner as a result of ill health?

a) Yes b) No

24. Which of these categories best describe you?

a) I have insulin related diseases b)I eat honey to build my immune system and brain c) I eat honey as a result of my belief system d)I just consume it without any taught e) Other specify…………………………………..............................

BENEFITS OF HONEY:

25. Please list the benefits of honey with regards to medicinal and nutritional. Note probe to circle (Multiple)

Nutritional Benefits

1. It serves as antimicrobial
2. It serves as anti-inflammatory
3. It aids digestion
4. It boost the immune system
5. It is rich in micronutrients
6. It build the human brain
7. Others ………………………………………………………………………………

Medicinal Benefits

1. It reduces the risk of getting heart disease
2. It controls sugar/insulin related diseases

3. It cures catarrh/cold

4. It heals wounds and scars

5. It is a sleep promoter

6. It cures ulcer and other allergies

7.

Others…………………………………………………………………………………………

………………………………………………………………………………………………

PACKAGING AND STORAGE OF HONEY:

26. Is the honey that you buy packaged? a) Yes b) No

27. In what package do you buy your honey?

a) Glass bottle b) Plastic/Rubber bottle c) Metallic can d) Recycled drinking water bottle e) Recycled alcoholic or soft drink bottle

f) Other, specify...

28. What type of packaging materials do you want processors/vendors to use?

a) Glass b) Plastic/Rubber c) Metal d) Other, specify...

29. How do you store your honey?

a. Cool dry place in my kitchen
b. Cool dry place in my bed room
c. Cool dry place in my living room
d. In refrigerator

30. The honey I consume is? a) Locally produced b) Imported brand c) Both d) Don’t know

If both, which do you prefer?

……………………………………………………………………………………………

KNOWLEDGE AND AWARENESS OF GLYPHOSATE

31. Do you know about Glyphosate and its importance? a) Yes b) No

32. Would you say Glyphosate importance outweighs their disadvantages? a) Yes b) No

33. Do you think honey may contain contaminants like Glyphosate? a) Yes b) No

34. Would you prefer honey to sugar as a result of health reasons/conditions or reason? a) Yes b) No

35. Would you recommend the consumption of honey to another consumer due to its benefits?

a) Yes b) No

36. Would you recommend the intensify use of Glyphosate? a) Yes b) No

37. Do you think Glyphosate are harmful to human and living organisms' health?

a) Yes b) No c) Don't know

Thank you.

APPENDIX II

Picture of Glyphosate herbicide sold in Wa market

Source: Field work (June, 2017)

APPENDIX III

Honey samples collected from Wa Market

Source: Field work (June, 2017)

APPENDIX IV: Laboratory analysis some quality features of honey

Source: Researcher Laboratory Work, 2017

APPENDIX V: Glyphosate Pesticides analysis with Gas Chromatograph

Source: Researcher Laboratory Work, 2017

APENDIX VI: Consumers Survey

Source: Field Survey data, 2017

APENDIX VI: Vendors and processors survey

Source: Field Survey data, 2017